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Effect of light intensity, light duration and photoperiods in the performance of an outdoor photobioreactor for urban wastewater treatment

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ABSTRACT

A series of eight experiments were carried out to analyse the effects of light intensity, light duration and photoperiods on a microalgae culture for treating AnMBR effluent at an outdoor photobioreactor (PBR) plant.

Improved performance was achieved in terms of nutrient recovery rates, biomass productivity and effluent nutrient concentrations at a higher net photon flux. However, the higher irradiance was also responsible for lower biomass productivity:light irradiance ratios.

None of the experiments with different lighting regimes and the same net photon flux showed any significant differences. The data obtained suggest that microalgae performance in this system did not depend on the time of day when light was applied or the length of the photoperiods, but on the net photon flux. No photoinhibiton was

observed in any of the experiments, probably because of the significant shadow effect on the microalgae in the PBRs.

1. Introduction

Discharging nutrients such as nitrogen and phosphorus into sensitive water bodies can cause the eutrophication and deterioration of water ecosystems [1]. In this respect, microalgae-based processes have recently been receiving increasing attention [2] due to their high capacity to recover nitrogen and phosphorus from wastewater streams [3] while producing valuable microalgae biomass [4].

Anaerobic membrane bioreactor (AnMBR) effluents emerge as an ideal source of nutrients for microalgae growth, since they contain fairly high amounts of nutrients [5]. Nutrient recovery by microalgae from AnMBR effluents has several advantages over other conventional treatments [6]: i) nitrogen and phosphorus can be removed from the AnMBR effluent without adding either extra chemical reagents or an additional source of organic carbon [7]; ii) the discharged effluent is oxygenated; and iii) the microalgae biomass cultivated in the process can be digested for biogas production [8]. In this case, the digested sludge would be nutrient-enriched and have enhanced fertiliser properties [9,10]. Combining microalgae cultivation with AnMBR effluents therefore makes it possible to recover both nutrients and energy from sewage, thus reducing the process's carbon footprint [10].

Microalgae can be cultivated in open ponds or closed photobioreactors (PBRs) [11,12,13]. Open ponds generally present less operating costs than closed systems [14,15]. However, the biological process is more difficult to control in open reactors since they are remarkably more affected by ambient factors than closed PBRs [11]. Furthermore, part of the nitrogen (up to 73% according to Romero-Villegas et al. [6]) is

lost in open systems due to ammonia stripping [16]. Similarly, carbon dioxide would also be stripped in case of adding CO_2 for pH control [16]. On the other hand, closed PBRs are designed to enhance the photosynthetic efficiency of microalgae, which allows to increasing the biomass productivity and nutrient recovery [14,15,17]. In this respect, De Vree et al. [18] reported a photosynthetic efficiency of 2.7-3.8% in flat-panel PBRs, while for open ponds it only accounted for 0.5-1.5%.

Light is a key parameter in microalgae cultivation [19,20,21,22,23,24]. In fact, light intensity, light frequency and photoperiods have been reported to influence microalgae productivity and nutrient removal efficiency [25,26]. Microalgae growth is proportional to light intensity until reaching a saturation point at which the photosynthetic activity of microalgae achieves their maximum value [27]. When it falls below this optimal value, microalgae growth will be limited [22,28]. On the other hand, if the light intensity values exceed the optimum, photosystem I (PSI) and photosystem II (PSII) will be damaged, causing microalgae photoinhibition [26,29]. Photoinhibition can be reduced by combining periods of high light irradiance with periods of darkness [27]. Since algae have been reported to respond to light intensity almost instantaneously [28], the temporary lack of light is considered to allow the dark reactions of photosynthesis, which are slower than the light reactions [30], to use the stored energy from light reactions [25] without the addition of extra photons that cannot be used for photosynthesis. In fact, the excess of photons absorbed by microalgae is emitted as heat or fluorescence and reduce photosynthetic efficiency [11,22,31]. In this context, the use of appropriate light-dark (L:D) photoperiods has been reported to reduce the light energy demand with similar or even higher productivity [32,33]. Nevertheless, longer than optimum dark periods could result in lower mass productivity [20].

Photoperiods can be divided into three main groups: i) long-term photoperiods, which refer to L:D cycles in hours [32]; ii) frequency photoperiods, which go through several L:D cycles per day [34]; and iii) short photoperiods, also known as the flashing light effect (FLE), which involve L:D cycles of seconds or even milliseconds [25,33]. Although different L:D cycles can lead to variations in photosynthetic performance [35], the studies available in the literature provide conflicting reports (Table 1).

Table 1. Biomass productivities and growth rates obtained at different L:D cycles in lab-scale experiments.

Microalgae	**Intensity ($\mu mol \cdot m^{-2} \cdot s^{-1}$)**	**L:D cycle (h:h)**	**Productivity ($mg \cdot L^{-1} \cdot d^{-1}$)**	**Growth rate (d^{-1})**	**Ref.**
Aphanothece microscopica Nägeli	150	24:0 16:8 12:12	770 240 301	-	[32]
Chlorella kessleri	45	24:0 12:12	-	~ 0.1 ~ 0.1	[36]
Chlorella pyrenoidosa	128* 90* 69*	24:0* 16:8* 12:12*	~ 85 ~ 77 ~ 60	-	[37]
Chlorella vulgaris	200	24:0 16:8 12:12	-	1.18 1.20 1.15	[38]

*Constant energy consumption of 0.8 $Kwh \cdot d^{-1}$.

Solar light is the most economical option for outdoor microalgae cultivation [24,39], but variations in the weather, day:night cycles and seasonal changes affect light intensity

and its spectrum [40], which can negatively affect microalgae [29,41]. In addition, in high-dense microalgae cultures, the light is not uniformly distributed [27]. The cells close to the PBR surface receive high light intensities that can reach up to 1800 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at midday [13] and hence are likely to suffer from photoinhibition [27,42]. Also, the cells near the surface absorb most of the applied light irradiance, causing a dark zone where photosynthesis is limited [30,43], known as the shadow effect or self-shading [28,33,41]. The volume of the dark zone depends on the microalgae biomass concentration, microalgae pigments, light intensity, light path, culture turbidity and PBR opacity [22,25,28,44]. The shadow effect also affects the amount of pigments (such as chlorophyll) in microalgae. Chlorophyll is not synthesised in complete darkness, but when the microalgae is illuminated inside a PBR, the pigment concentration increases at low light intensities to take advantage of the photons available to reach the cells [22,45].

Mixing of the microalgae culture can help to mitigate this shadow effect since it involves the movement of algae from the highly illuminated areas of the reactor to dark zones [30], therefore reducing photoinhibition [46] and applying a random FLE to the culture [47]. In contrast, mixing is usually poor within open systems [30].

Light attenuation caused by the shadow effect can also be overcome by applying additional artificial lighting to the microalgae culture. This way, higher nutrient recovery efficiencies and biomass productivities can be achieved in shorter retention times [1,48]. Although artificial illumination can better regulate the light photons and photoperiods which can enhance photosynthesis performance [25], it also requires large amounts of energy. The illumination regime should therefore be used efficiently, with the appropriate L:D cycles.

The criteria used for selecting the artificial light source include electric energy efficiency, low heat dissipation, high reliability, long durability, low cost and emissions within the microalgae spectrum [19]. Table 1 in [49] briefly summarises the main advantages and disadvantages of different artificial light sources, in which LED lamps seem to be the most beneficial artificial light source for microalgae growth.

The effects of light intensity, photoperiods and light wavelength have been extensively reported under lab conditions [43,45,50]. Other studies describe design proposals for new PBR prototypes to simulate an FLE in the microalgae culture [25,47] or to increase the light available to the culture [27]. However, the transition from prototypes (or lab scale PBRs) to outdoor microalgae cultivation has not been successfully studied [21] because of the complexity produced by the variations in natural light [25] and the difficulty of decoupling the light effect from the other parameters which influence outdoor microalgae growth, such as ambient temperature [13].

In this context, the goal of the present study was to examine the effects of light intensity, light duration and photoperiods on an outdoor microalgae culture which treated AnMBR effluent. PBR performance was evaluated by considering nutrient recovery rates, effluent nutrient concentrations and microalgae biomass productivity.

2. Material and methods

2.1. Microalgae culture and substrate

The microalgae used in this study consisted of an indigenous mixed culture, originally collected from the walls of the secondary clarifier of the Carraixet WWTP (39º30’04.0’’N 0º20’00.1’’W, Valencia, Spain).

The microalgae were mainly composed of green algae *Scenedesmus* and *Chlorella*; although diatoms, cyanobacteria, heterotrophic and autotrophic bacteria were also present in lower concentrations.

The microalgae substrate consisted of the nutrient-rich effluent from an AnMBR plant that treated real sewage [5] with high nutrient concentrations; i.e., 56.6 ± 9.7 mg $N \cdot L^{-1}$ (n = 99) for nitrogen (mainly in the form of ammonium) and 6.5 ± 1.3 mg $P \cdot L^{-1}$ (n = 99) for phosphorus.

The AnMBR effluent also had low COD values (92 ± 32 mg $COD \cdot L^{-1}$, n = 34), mainly non-biodegradable, and a negligible suspended solids concentration. The substrate was previously aerated to oxidise the large amounts of sulphide (around 112.7 ± 13.8 mg $S \cdot L^{-1}$, n = 34) to sulphate, as described in González-Camejo et al. [51].

2.2. Photobioreactors

Microalgae were cultivated in two outdoor flat-panel 1.25-m high x 2-m wide x 0.25-m deep methacrylate PBRs (PBR-A and PBR-B) with a working volume of 550 L each, continuously stirred by an airflow of 0.10 vvm and sparged by two perforated pipes on the PBR floor. This setup provided nutrient and light homogenisation, lowered thermal stratification [11] and reduced wall fouling. Pure CO_2 (99.9%) was added to the airflow through an automatic valve whenever the pH value went over 7.5 to avoid undesirable phenomena such as ammonia volatilisation and phosphorus precipitation [52].

An irradiation sensor (Apogee Quantum SQ-200) on the surface of PBR-A continuously measured photosynthetically active radiation (PAR). In addition to natural light, an artificial light source was used consisting of twelve LED lamps (Unique Led IP65 WS-TP4S-40W-ME). Six of them were cold white (6500K) and the other six were neutral white (4500K). They were installed at the back of the tanks to illuminate the PBR

surface that did not receive any sunlight. When all the lamps were on, an average light irradiance of 300 $\mu E \cdot m^{-2} \cdot s^{-1}$ was measured on the surface but this dropped to 150 $\mu E \cdot m^{-2} \cdot s^{-1}$ when only half the lamps were in action.

2.3. Operating conditions

Eight different experiments were carried out (Table 2) in which both PBRs were inoculated with the same inoculum and substrate concentration. The PBR start-up phase (not included in the data analysis) was as described in González-Camejo et al. [53] and was designed to obtain a consistent initial microalgae biomass concentration. Both PBRs were then fed in semi-continuous operation with the same nutrient load, maintaining a hydraulic retention time (HRT) of 8 days. Temperature was in the range of 18-27ºC, which is within the optimum range for green algae *Scenedesmus* and *Chlorella*: 15-25ºC [54,55].

As the PBR pilot plant was operated outdoors, which meant that solar light intensity was variable, the different experiments could not be compared with one another, although the two PBRs used in each experiment were oriented in the same direction, so that they only differed in the artificial lighting regime, which varied the total net photon flux as shown in Table 2. Three different effects were studied: i) light intensity; ii) light duration (and the time of day when artificial light was applied, i.e. day or night); and iii) light photoperiods.

Light intensity was studied in Experiments 1 and 2, which were designed to determine whether the addition of artificial light would improve the PBR performance. Three different artificial light intensities were evaluated: 0, 150 and 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$.

Light duration and the time of day when light was applied were assessed in Experiments 3, 4 and 5. Experiment 3 included different L:D cycles of 12:12 h and 24:0 h at the

same light intensity. In Experiment 4, the same L:D cycles (12:12 h) and same light intensity were applied, but PBR-A was illuminated at night and PBR-B was lit during the day. Different L:D cycles and different light intensities were chosen in Experiment 5 (Table 2).

The light photoperiods were studied in Experiments 6, 7 and 8. Three different on:off photoperiod cycles (which represented the total time that the artificial lamps were continuously on and off) were tested: 1.5:1.5 h, 0.75:0.75 h and 1:2 h, in Experiments 6, 7 and 8, respectively. These photoperiods were compared to continuous illumination with the same quantity of photons per day, which were L:D cycles of 12:12, 12:12 and 8:16 h, respectively (Table 2).

Table 2. Outdoor and artificial lighting conditions of PBR-A and PBR-B in each experiment.

Exp.	**Days**	**Solar PAR ($\mu mol \cdot m^{-2} \cdot s^{-1}$)**	**Artificial light**							
			Intensity ($\mu mol \cdot m^{-2} \cdot s^{-1}$)		**L:D cycle (h:h)[1]**		**on:off cycle (h:h)[2]**		**Time of illumination[3]**	
			PBR-A	*PBR-B*	*PBR-A*	*PBR-B*	*PBR-A*	*PBR-B*	*PBR-A*	*PBR-B*
1	25	277 ± 146	300	0	24:0	0:24	24:0	0:24	D-N	-
2A	14	99 ± 12	300	150	24:0	24:0	24:0	24:0	D-N	D-N
2B	28	107 ± 20	150	150	24:0	24:0	24:0	24:0	D-N	D-N
3	30	89 ± 15	300	300	24:0	12:12	24:0	12:12	D-N	N
4	26	124 ± 23	300	300	12:12	12:12	12:12	12:12	N	D
5	32	109 ± 53	300	150	12:12	24:0	12:12	24:0	N	D-N
6	15	120 ± 54	300	300	12:12	12:12	1.5:1.5	12:12	D-N	N
7	20	132 ± 56	300	300	12:12	12:12	0.75:0.75	12:12	D-N	N
8	27	124 ± 44	300	300	8:16	8:16	1:2	8:16	D-N	N

[1]L:D cycles represent the number of total hours a day that artificial lights are either in light or dark.

[2]On:off cycles represent the maximum consecutive time that lights are either on or off.

[3]D: Artificial lights on during daylight hours; N: Artificial lights on during night hours; D-N: Artificial lights on during day and night.

2.4. Sampling and Analytical Methods

Grab samples were collected from the PBR influent and effluent streams as well as from the microalgae culture three times a week. The soluble fraction of the sample was obtained by vacuum filtration with 0.45 mm pore size filters (Millipore). Ammonium (NH_4), nitrite (NO_2), nitrate (NO_3), and phosphate (PO_4) were analysed according to Standard Methods [56]: 4500-NH3-G, 4500-NO2-B, 4500-NO3-H and 4500-P-F, respectively, in a Smartchem 200 automatic analyser (Westco Scientific Instruments, Westco). The sum of NH_4, NO_2 and NO_3 concentrations was considered to be equivalent to total soluble nitrogen (Ns). Volatile suspended solids (VSS) were determined according to Standard Method 2540-E [56].

COD and sulphide concentrations of the influent, as well as total eukaryotic cell (TEC) and chlorophyll concentrations of the culture were measured once a week. COD and sulphide were performed according to Standard Methods [56] 522-COD-D and 4500-S^{2-}D, respectively. TEC was counted by epifluorescence [57] and chlorophyll content was determined by the tricromatic method based on visible spectroscopy [56]. Jeffrey and Humphrey equations [58] were used to obtain chlorophyll concentration. Pigment was extracted with acetone 90%.

Maximum quantum efficiency (F_v/F_m) was measured in-situ three times a week with a portable fluorometer AquaPen-C AP-C 100 (Photon Systems Instruments, Czech Republic) after the samples had remained in the dark for ten minutes [31].

All measurements were done in duplicate.

2.5. Calculations

It was assumed that all the nutrient reduction from wastewater was recovered by the microalgae biomass. Nitrogen recovery rate (NRR) (mg $N \cdot L^{-1} \cdot d^{-1}$), phosphorus recovery rate (PRR) (mg $P \cdot L^{-1} \cdot d^{-1}$) and biomass productivity (BP) (mg $VSS \cdot L^{-1} \cdot d^{-1}$) were calculated according to Eqs. 1, 2 and 3, respectively:

$$NRR = \frac{(N_i - N_e) \cdot F}{V_{PBR}} \quad \text{(Eq. 1)}$$

where N_i is the nitrogen concentration of the influent (mg $N \cdot L^{-1}$), N_e is the nitrogen concentration of the effluent of PBR-A or PBR-B (mg $N \cdot L^{-1}$), F is the flow rate of the substrate ($L \cdot d^{-1}$), and V_{PBR} is the culture volume in the PBRs (L).

$$PRR = \frac{(P_i - P_e) \cdot F}{V_{PBR}} \quad \text{(Eq. 2)}$$

where P_i is the phosphorus concentration of the influent (mg $P \cdot L^{-1}$) and P_e is the phosphorus concentration of the effluent of PBR-A or PBR-B (mg $P \cdot L^{-1}$).

$$BP = \frac{VSS \cdot V_p}{V_{PBR}} \quad \text{(Eq. 3)}$$

where VSS (mg $VSS \cdot L^{-1}$) is the volatile suspended solids concentration in the PBRs and Vp is the volume of the microalgae culture purged ($L \cdot d^{-1}$).

The biomass productivity:light irradiance ratio (BP:I, g $VSS \cdot mol^{-1}$) was calculated according to Eq. 4 (modified from [59]).

$$BP{:}I = \frac{BP \cdot V_{PBR} \cdot 1000}{TP \cdot t \cdot S \cdot 24 \cdot 3600} \quad \text{(Eq. 4)}$$

where TP is the total photon flux applied to the PBR surface (i.e. the sum of solar irradiance plus artificial lighting, $\mu mol \cdot m^{-2} \cdot s^{-1}$); t is the period of time considered (d) and S is the PBR surface (m^2).

Similarly, the nitrogen and phosphorus recovery rate:light irradiance ratios (NRR:I and PRR:I) were calculated with Eq. 5 and Eq. 6, respectively:

$$NRR{:}\,I = \frac{NRR \cdot V_{PBR} \cdot 10^6}{TP \cdot S \cdot 24 \cdot 3600} \qquad \text{(Eq. 5)}$$

$$PRR{:}\,I = \frac{PRR \cdot V_{PBR} \cdot 10^6}{TP \cdot S \cdot 24 \cdot 3600} \qquad \text{(Eq. 6)}$$

2.6. Statistical analysis

All the values were expressed as the mean ± standard deviation. The data were analysed on Statgraphics Centurion XVII statistical software. Statistically significant differences were considered with p-values < 0.05.

3. Results

3.1. Effect of light intensity

In Experiment 1, PBR-B was lit by natural light only. In PBR-A, one surface received sunlight (277 ± 146 μmol·m^{-2}·s^{-1}, n = 25), while the other was lit artificially at an intensity of 300 μmol·m^{-2}·s^{-1}. This artificial light source was not considered to cause some photochemical stress to microalgae since F_v/F_m remained at high values in both PBRs [60]; i.e., 0.76 ± 0.03 in PBR-A and 0.75 ± 0.01 in PBR-B (p-value 0.20; n = 12). As can be seen in Table 3, in Experiment 1 PBR-A achieved 37.5% higher NRR and 58.4% higher PRR than PBR-B, which indicated lower effluent nutrient concentrations in PBR-A than in PBR-B (Figure 1a). PBR-A also reached higher biomass productivity (Table 3) due to the significantly higher biomass concentration: 538 ± 101 mg VSS·L^{-1} and 333 ± 86 mg VSS·L^{-1} for PBR-A and PBR-B, respectively (p-value = 0.01; n = 12), indicating 63.9% more microalgae biomass in the artificially lit PBR. However, the efficiency in the use of light was higher in PBR-B since PBR-A presented lower BP:I than PBR-B; i.e., 0.48 ± 0.15 g VSS·mol^{-1} and 0.61 ± 0.20 g VSS·mol^{-1}, respectively (p-value = 0.02; n = 12). These values are in the range of those reported by Morales-

Amaral et al. [61], who obtained values of BP:I in the range of 0.2-0.6 g VSS·mol^{-1} for a *Scenedesmus* sp. culture.

Table 3. Nutrient recovery rates and biomass productivities obtained in PBR-A and PBR-B in each experiment.

	NRR (mg N·L^{-1}·d^{-1})			**PRR (mg P·L^{-1}·d^{-1})**			**BP (mg VSS·L^{-1}·d^{-1})**		
Exp	**PBR-A**	**PBR-B**	**p-value**	**PBR-A**	**PBR-B**	**p-value**	**PBR-A**	**PBR-B**	**p-value**
1	7.7±1.6	5.6±2.2	0.00*	1.03±0.21	0.65±0.24	0.00*	100±32	61±20	0.01*
2A	5.0±1.2	3.1±1.5	0.09*	0.71±0.14	0.47±0.13	0.05*	55±6	42±5	0.00*
2B	2.3±1.0	2.2±0.5	0.70	0.31±0.21	0.29±0.18	0.82	27±7	25±7	0.59
3	3.5±1.8	2.2±1.1	0.03*	0.50±0.19	0.35±0.23	0.09*	34±6	26±5	0.00*
4	2.7±0.7	3.0±0.9	0.47	0.31±0.14	0.33±0.11	0.68	30±2	29±2	0.19
5	3.2±1.8	3.2±1.7	0.99	0.46±0.18	0.49±0.24	0.73	31±9	34±9	0.44
6	2.7±1.0	3.3±1.2	0.31	0.29±0.11	0.31±0.13	0.81	27±6	23±6	0.26
7	3.7±1.5	3.5±1.1	0.80	0.53±0.17	0.50±0.15	0.76	46±7	46±8	0.93
8	1.7±1.1	1.5±0.7	0.55	0.32±0.18	0.26±0.13	0.46	27±4	25±2	0.20

*Showed statistically significant differences.

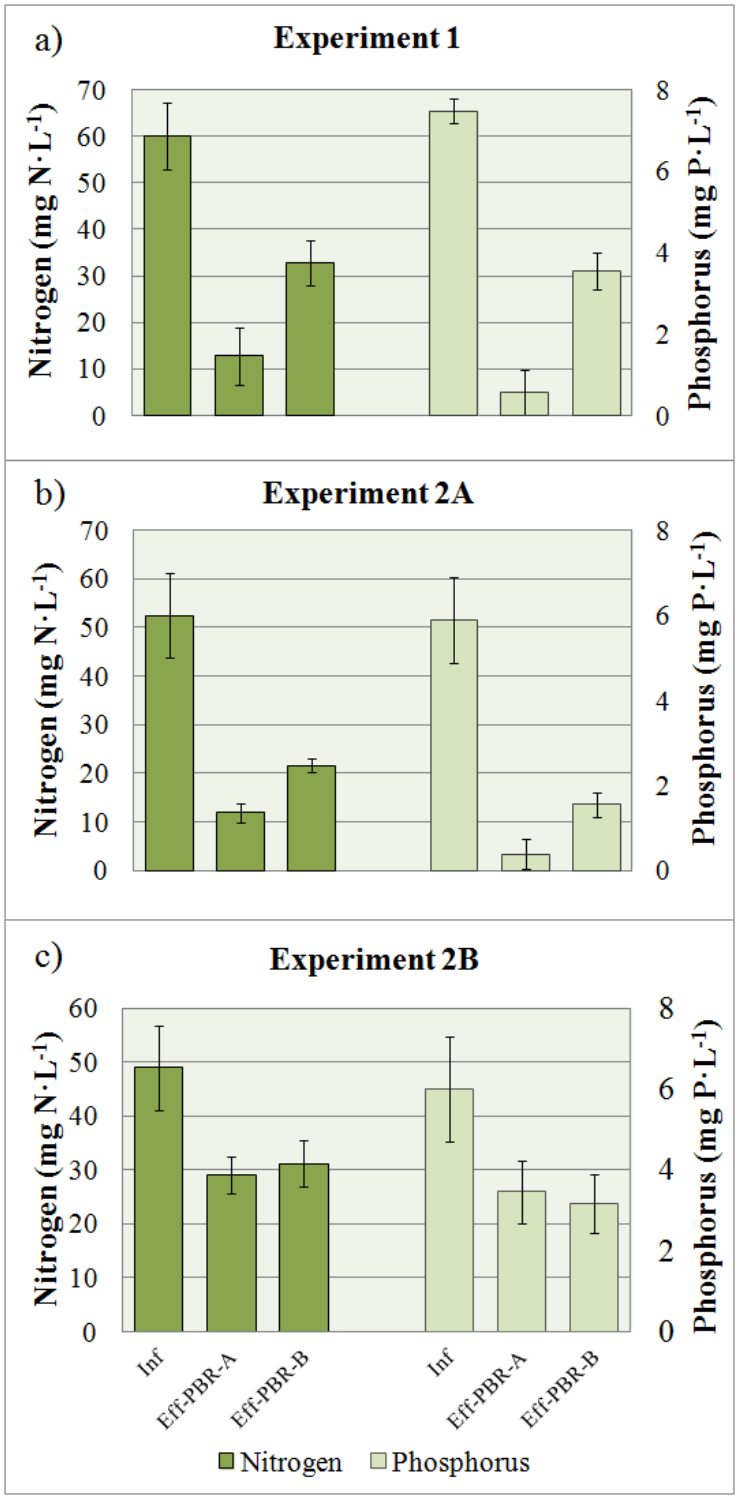

Figure 1. Effect of light intensity. Average measures (and standard deviation) of nitrogen and phosphorus concentration in the influent (Inf) and effluent of PBR-A (Eff-PBR-A) and PBR-B (Eff-PBR-B) in: a) Experiment 1; b) Experiment 2A; and c) Experiment 2B.

It should be also noted that, despite the different VSS concentration in the PBRs during Experiment 1, TEC concentration in PBR-A was not significantly higher than in PBR-B: $7.33 \cdot 10^9 \pm 1.21 \cdot 10^9$ cells·L^{-1} and $6.27 \cdot 10^9 \pm 1.63 \cdot 10^9$ cells·L^{-1}, respectively (p-value = 0.27; n = 5), both having a similar strain distribution; i.e. around 90% of the TEC was *Scenedesmus* and around 10% was *Chlorella*.

Regarding nutrient recovery:light irradiance rates, PBR-A attained lower NRR:I than PBR-B (37.3 ± 7.7 mg N·mol^{-1} and 55.9 ± 22.0 mg N·mol^{-1}, respectively; p-value = 0.00; n = 7). PRR:I was also lower in PBR-A than in PBR-B (5.3 ± 1.0 mg P·$mol^{-1}$and 6.5 ± 2.4 mg P·mol^{-1}, respectively; p-value = 0.00; n = 7).

With respect to photosynthetic pigments, PBR-A achieved higher intracellular chlorophyll content than PBR-B (6.35 ± 2.35 mg chl·g VSS^{-1} and 5.72 ± 1.83 mg chl·g VSS^{-1}, respectively). Although this difference was not statistically significant (p-value = 0.83; n = 5), the chlorophyll content per microalgae cell was significantly higher for PBR-A ($5.34 \pm 1.43 \cdot 10^{-10}$ mg chl·$cell^{-1}$) than for PBR-B; i.e., $2.43 \pm 0.74 \cdot 10^{-10}$ mg chl·$cell^{-1}$ (p-value = 0.00; n = 5).

Experiment 2 was divided into two: 2A and 2B. In Experiment 2A, PBR-A remained at an artificial light intensity of 300 μmol·m^{-2}·s^{-1}; while PBR-B was continuously lit artificially at an intensity of 150 μmol·m^{-2}·s^{-1}; i.e. half of the net photon flux emitted by LED lamps. The aim of this period was therefore to assess whether the continuous artificial light intensity of 300 μmol·m^{-2}·s^{-1} was excessive for optimum microalgae growth since the photoinhibition point has been reported to be at light irradiances of around 200 μmol·m^{-2}·s^{-1} [27].

According to the results shown in Table 3, in Experiment 2 PBR-A showed significantly higher NRR, PRR and biomass productivity than PBR-B. Consequently,

PBR-A presented significantly lower effluent nutrient concentrations than PBR-B (Figure 1b).

As in Experiment 1, the TEC concentration was not significantly different in Experiment 2A in both PBRs: $8.75 \cdot 10^9 \pm 1.86 \cdot 10^9$ cells$\cdot L^{-1}$ and $7.54 \cdot 10^9 \pm 2.17 \cdot 10^9$ cells$\cdot L^{-1}$, for PBR-A and PBR-B, respectively (p-value = 0.48; n = 4), even though the VSS concentrations were statistically different: 410 ± 58 mg VSS$\cdot L^{-1}$ and 320 ± 28 mg VSS$\cdot L^{-1}$, respectively (p-value = 0.01; n = 5). Since genera distribution was similar in both PBRs (around 30% of TEC was *Scenedesmus* and around 70% *Chlorella*), cell size might have been different in both PBRs [43].

Similarly to Experiment 1, PBR-B in Experiment 2A was more efficient as regards biomass production:light irradiance ratios than PBR-A: 0.46 ± 0.04 g VSS$\cdot mol^{-1}$ and 0.38 ± 0.03 g VSS$\cdot mol^{-1}$, respectively (p-value = 0.02; n = 5). On the other hand, both PBRs showed similar nutrient recovery rates:light irradiance ratios (i.e. NRR:I 30.8 ± 6.0 mg N$\cdot mol^{-1}$ and 32.4 ± 11.7 mg N$\cdot mol^{-1}$ for PBR-A and PBR-B, respectively (p-value = 0.99; n = 5), while PRR:I 6.3 ± 0.7 mg P$\cdot mol^{-1}$ and 5.4 ± 1.2 mg P$\cdot mol^{-1}$ were measured in PBR-A and PBR-B, respectively (p-value = 0.39; n = 5).

In Experiment 2B the light intensity in PBR-A was reduced to 150 μmol$\cdot m^{-2} \cdot s^{-1}$. As can be seen, even though PBR-A started at lower effluent nutrient concentrations, its performance tended to be similar to PBR-B, meeting stable operations with similar effluent nutrient concentrations (Figure 2b). In the case of microalgae biomass, PBR-A started Experiment 2B at a concentration of 400 mg VSS$\cdot L^{-1}$, while PBR-B started with a biomass concentration of 285 mg VSS$\cdot L^{-1}$. However, from day 19 until the end of Experiment 2B, the microalgae biomass concentration was similar in both PBRs, so that both reached significantly similar NRR, PRR and biomass productivity (Table 3).

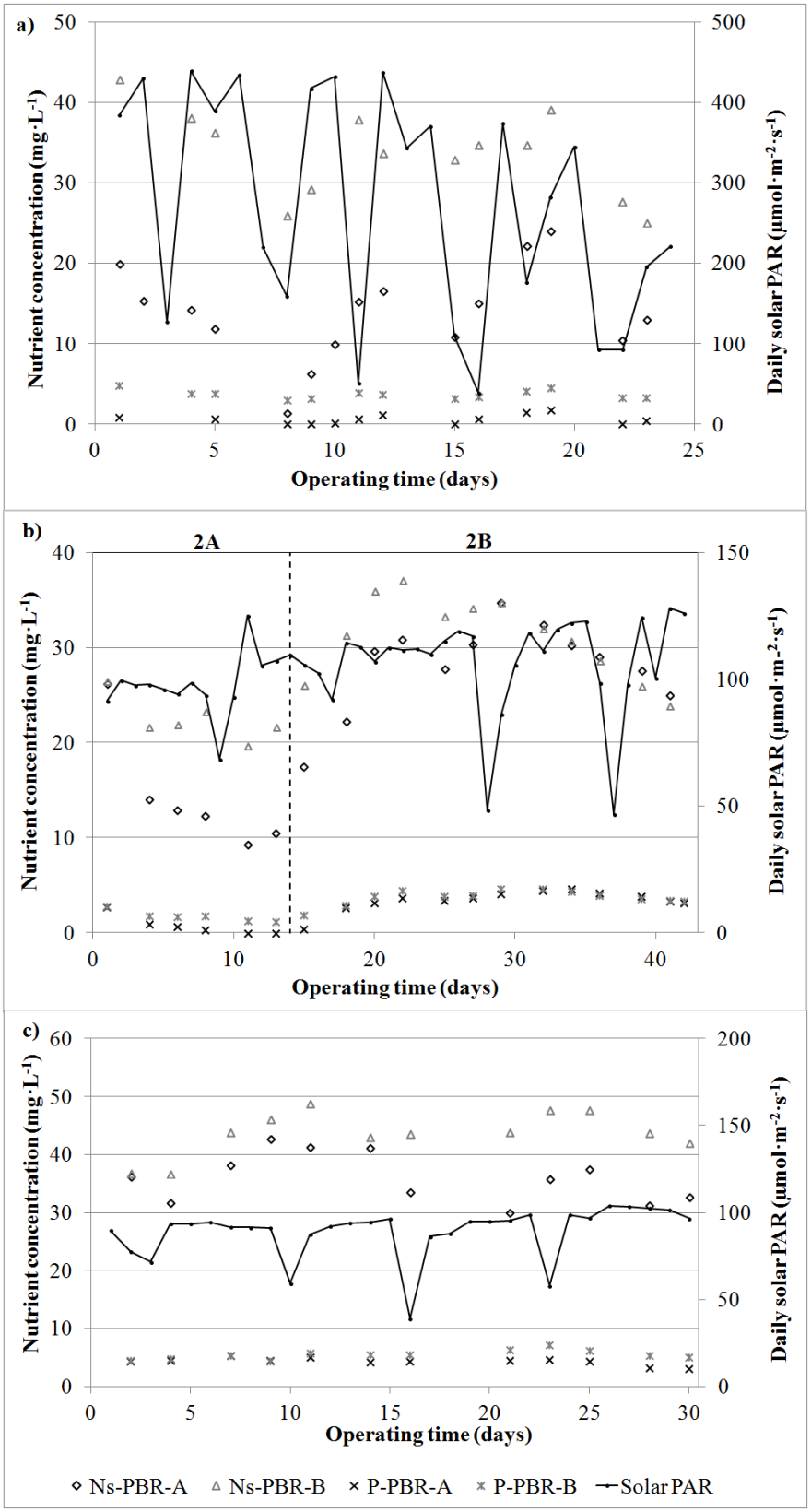

Figure 2. Evolution of nitrogen (Ns) and phosphorus (P) effluent concentrations and daily average solar PAR in: a) Experiment 1; b) Experiment 2; c) Experiment 3.

3.2. Effect of light duration

Different L:D cycles of artificial light were tested in Experiment 3. PBR-A was operated with continuous artificial lighting and PBR-B was only lit during the hours of darkness (L:D cycle of 12:12 h), so that PBR-A received twice as much artificial photon flux than PBR-B. As a result, PBR-A performance was significantly higher than PBR-B in terms of NRR, PRR and biomass productivity (Table 3). The PBR-A effluent nutrient concentrations were therefore lower than in PBR-B (Figure 3a).

With respect to light efficiency, BP:I of PBR-B in Experiment 3 was higher than in PBR-A: 0.59 ± 0.06 g VSS·mol^{-1} and 0.24 ± 0.03 g VSS·mol^{-1}, respectively (p-value = 0.00; n = 13), but the nutrient recovery rate:light irradiance ratios were similar for both PBRs. PBR-A showed NRR:I and PRR:I of 25.0 ± 10.0 mg N·mol^{-1} and 3.5 ± 0.6 mg P·mol^{-1}, respectively; while PBR-B obtained 25.0 ± 10.1 mg N·mol^{-1} and 3.3 ± 1.5 mg P·mol^{-1}, respectively (p-values = 0.99 and 0.76, respectively; n = 13).

Unlike Experiments 1 and 2A, the higher biomass concentration obtained in PBR-A (277 ± 39 mg VSS·L^{-1}) than in PBR-B; i.e., 208 ± 41 mg VSS·L^{-1} (p-value = 0.00; n = 13), was related to a higher TEC concentration in PBR-A in comparison to PBR-B: $9.96 \cdot 10^{9} \pm 6.10 \cdot 10^{8}$ cells·L^{-1} and $4.50 \cdot 10^{9} \pm 2.38 \cdot 10^{9}$ cells·L^{-1}, respectively (p-value = 0.01; n = 6); although the strain distribution was similar, i.e. 85% of TEC consisted of *Chlorella* and 15% was *Scenedesmus* in PBR-A, while 80% of the TEC consisted of *Chlorella* and 20% was *Scenedesmus* in PBR-B. On the other hand, the chlorophyll content in PBR-A (which received a higher photon flux) was noticeably lower than PBR-B: 4.48 ± 1.12 mg chl·g VSS^{-1} and 6.7 ± 2.04 mg chl·g VSS^{-1}, respectively (p-value = 0.04; n = 6). This also occurred with the chlorophyll content per cell; i.e. $1.01 \pm 0.25 \cdot 10^{-10}$ mg chl·cell^{-1} for PBR-A and $1.74 \pm 0.32 \cdot 10^{-10}$ mg chl·cell^{-1} for PBR-B (p-value = 0.01; n = 6).

Experiment 4 evaluated the effect of artificially illumination during day or night. PBR-A (which was illuminated at night with a 12:12 h L:D cycle) obtained similar nutrient effluent concentrations than PBR-B (which was lit during daylight with the same L:D cycle and was therefore in complete darkness at night) (Figure 3b). Neither did the NRR, PRR and biomass productivity (Table 3) nor chlorophyll content show any significant differences: 11.97 ± 0.37 mg chl·g VSS^{-1} and 11.28 ± 0.30 mg chl·g VSS^{-1}, respectively (p-value = 0.18; n = 4).

The goal of Experiment 5 was to assess the most efficient artificial light regime for the culture; i.e. with a high-intensity 12:12 h L:D cycle during the night (300 μmol·m^{-2}·s^{-1}): PBR-A, or with continuous low-intensity illumination (150 μmol·m^{-2}·s^{-1}): PBR-B, both with the same net photon flux. The results of this experiment did not show any statistically significant differences between both PBRs (Table 3 and Figure 3c).

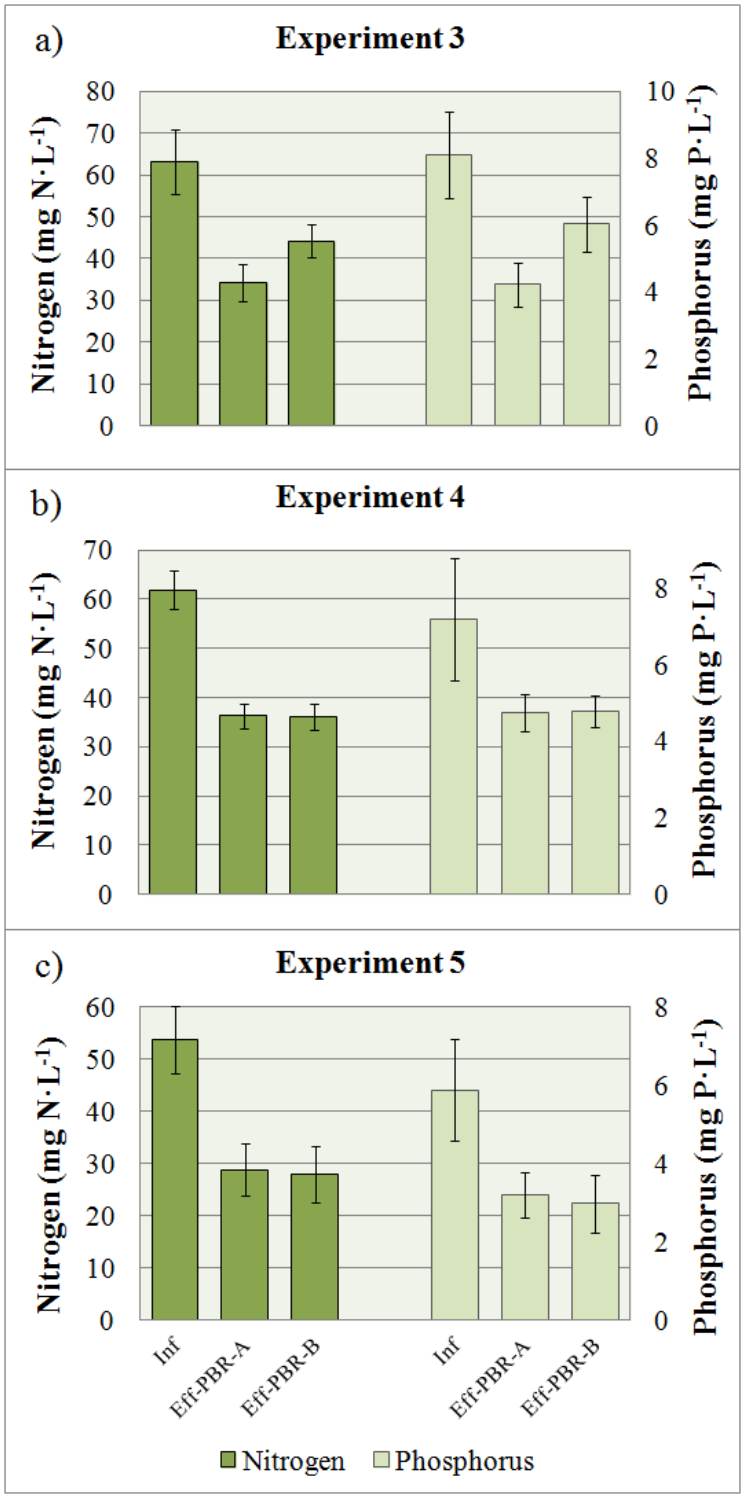

Figure 3. Effect of light duration. Average measures (and standard deviation) of nitrogen and phosphorus concentration in the influent (Inf) and effluent of PBR-A (Eff-PBR-A) and PBR-B (Eff-PBR-B) in: a) Experiment 3; b) Experiment 4; and c) Experiment 5.

3.3. Effect of photoperiods

The long-term photoperiods and frequency photoperiods [32,34] were compared in Experiments 6, 7 and 8. In all three experiments, PBR-B was continuously illuminated at night, i.e. the on:off cycles (which is the maximum period of time when artificial lights were on and off) were equal to the L:D cycles. In Experiments 6 and 7, PBR-B was operated with 12:12 h L:D cycles, while in Experiment 8 the L:D cycle was reduced to 8:16 h. PBR-A was operated under the same L:D cycles as PBR-B, but with different on:off cycles: in Experiment 6, this cycle was 1.5:1.5 h and in Experiment 7 this frequency was reduced to 0.75:0.75 h. In Experiment 8 the lights were left on for 1 h and switched off for 2 h.

The effluent nutrient concentrations in both PBRs showed no significant differences throughout Experiments 6, 7 and 8 (Figure 4). Neither were the differences in terms of nutrient recovery rates and biomass productivity statistically significant (Table 3). Similar behaviour was observed in the chlorophyll content of microalgae, obtaining: i) in Experiment 6, 8.29 ± 1.06 mg chl·g VSS^{-1} and 9.38 ± 2.23 mg chl·g VSS^{-1}, for PBR-A and PBR-B, respectively (p-value = 0.41; n = 4); ii) in Experiment 7, 6.64 ± 1.08 mg chl·g VSS^{-1} and 7.08 ± 0.55 mg chl·g VSS^{-1}, for PBR-A and PBR-B, respectively (p-value = 0.49; n = 5); and, iii) in Experiment 8, 7.59 ± 2.01 mg chl·g VSS^{-1} and 8.29 ± 2.52 mg chl·g VSS^{-1}, for PBR-A and PBR-B, respectively (p-value = 0.61; n = 6).

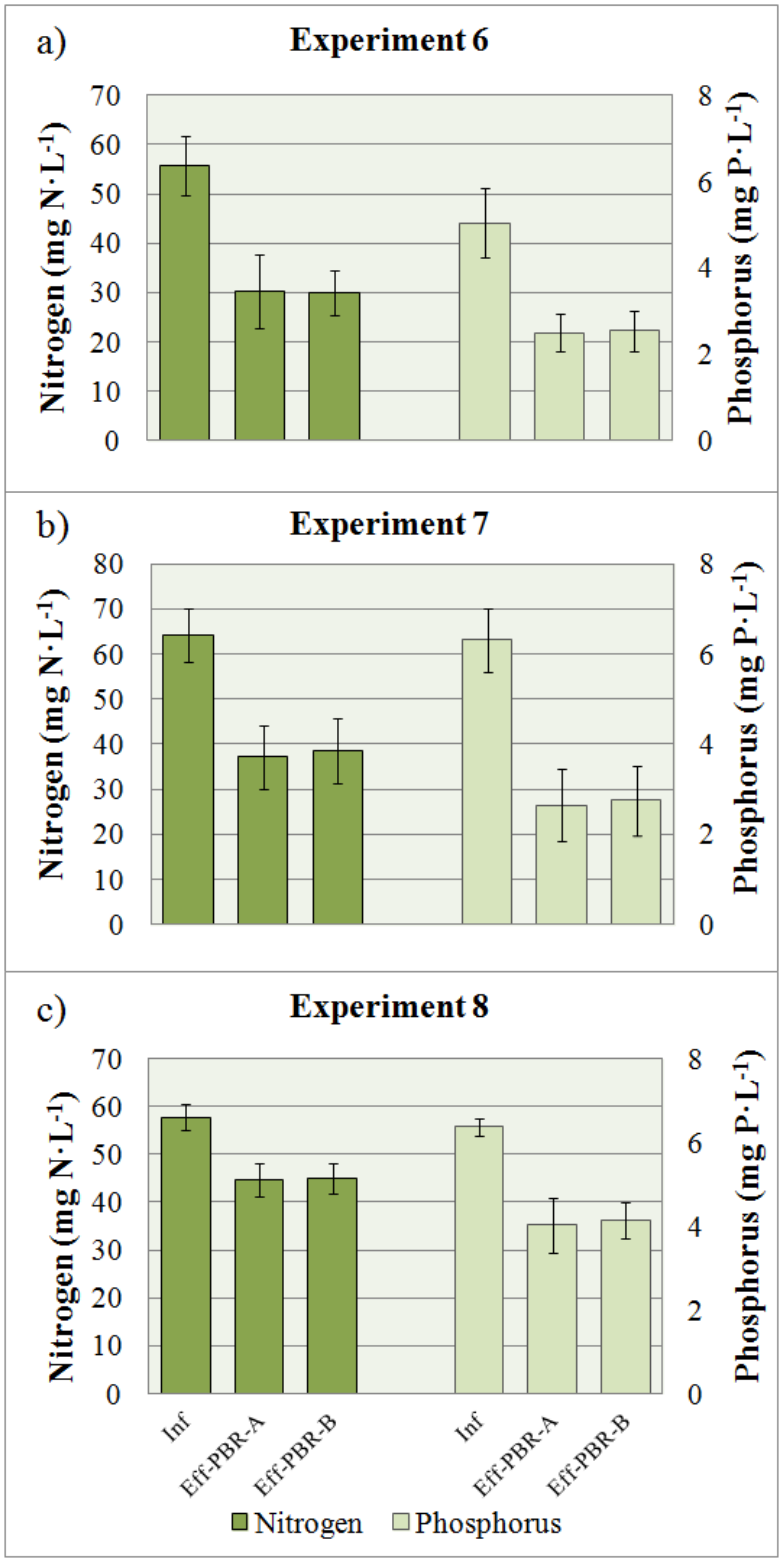

Figure 4. Effect of photoperiods. Average measures (and standard deviation) of nitrogen and phosphorus concentration in the influent and effluent of PBR-A and PBR-B, in: a) Experiment 6; b) Experiment 7; and c) Experiment 8.

4. Discussion

The results have been discussed according to the two different situations in the Experiments evaluated: i) the net photon flux was higher in PBR-A than in PBR-B (Experiments 1, 2A and 3); and ii) the net photon flux was the same for both PBR-A and PBR-B (Experiments 2B, 4, 5, 6, 7 and 8).

It must be highlighted that factors which influence microalgae growth such as solar irradiance [24;39], temperature [54,55], nutrient loading rates [8,53] and culture mixing [17,30] were the same for PBR-A and PBR-B in each experiment, only differing in the artificial lighting regime. In addition, nutrients were maintained in replete conditions (i.e., nitrogen higher than 10 mg $N \cdot L^{-1}$ and phosphorus above negligible concentration as explained in Pachés et al. [62]) during all the Experiments except for 1 and 2A (Figure 2a). Hence, microalgae were only considered to be nutrient-limited in PBR-A during Experiments 1 and 2A.

4.1. *Different net photon flux*

When PBR-A was lit by a higher photon flux than PBR-B (i.e., in Experiments 1, 2A and 3), it achieved higher performance in terms of nutrient effluent concentrations, nutrient recovery rates and biomass productivities. It can thus be concluded that the highest artificial lighting (300 $\mu mol \cdot m^{-2} \cdot s^{-1}$) increased the nutrient recovery capacity and biomass production of the PBRs, which suggested that the system was light-limited. Other lab-scale experiment showed different results. For instance, Gris et al. [43] did not observe any enhancement in the growth rate of *Scenedesmus obliquus* at light intensities over 150 $\mu mol \cdot m^{-2} \cdot s^{-1}$, while Deng et al. [43] obtained optimal daily average irradiances of 90 $\mu mol \cdot m^{-2} \cdot s^{-1}$ for *Chlorella kessleri*. In these lab-scale photobioreactors, microalgae were expected to suffer from photoinhibition since it

usually occurs at light irradiances of around 200 μmol·m^{-2}·s^{-1} [27]. However, the light path of those lab-scale photobioreactors was short (lower than 10 cm). On the contrary, PBRs of this study presented a considerably wide light path (i.e., 25 cm). Consequently, the shadow effect in this PBR [49] might be more significant than those of lab-scale studies in spite of receiving higher light irradiance, leaving a significant volume of the PBR in darkness [30,49], hence reducing the light availability in the pilot-scale PBRs. The PBR light path therefore plays a significant role in making light available to the culture [25,44]. Indeed, there is a current tendency to reduce PBR depth in order to increase the algae's photosynthetic efficiency, although this requires a larger area [40]. Further research needs to be done to find the best PBR width without excessively increasing the surface area required for microalgae cultivation.

It must be also noted that in Experiments 1, 2A and 3, the efficiency in the use of light for biomass production (i.e., BP:I) was always higher in PBR-B, where less artificial photon flux was supplied than in PBR-A (artificial light intensity of 300 μmol·m^{-2}·s^{-1}). This indicated a lower photosynthetic efficiency at higher photon fluxes [63,64]. One hypothesis for this behaviour is that most of the algae in the culture were acclimatised to poor lighting because of the large dark volume of the PBRs [49]. In these light-limited conditions, microalgae tend to assemble a larger photosynthetic antenna which forces the poorly light-adapted cells to absorb excessive photons when lit [27,65,66], reducing their efficiency [12]. This effect could be expected to be greater in PBR-A.

With respect to the efficiency of light use for nutrient recovery (i.e., NRR:I and PRR:I), PBR-B also showed higher values than PBR-A, but only during Experiment 1, when none artificial light source was applied to PBR-B. This could have been related to the fact that microalgae can assimilate nutrients in dark conditions until reaching maximum biomass nutrient content, although they cannot synthesise new algae biomass [20,67].

Nutrient consumption in Experiment 1 could also have been influenced by the limited nitrogen and phosphorus in PBR-A, since this has been reported to reduce nitrogen recovery rates [62,68]. On the contrary, when PBR-B was lit by artificial light intensity of 150 $\mu mol \cdot m^{-2} \cdot s^{-1}$, both PBRs showed similar results in NRR:I and PRR:I, possibly due to PBR-B not being in complete darkness at any time. Since the algae were continuously lit artificially in both PBRs during Experiments 2A and 3 (although at different net photon fluxes), they were always able to grow, preventing the extra accumulation of intracellular nutrients [67].

Regarding cell concentration, there were no significant differences between PBR-A and PBR-B in Experiments 1 and 2A, which suggests that the higher biomass productivity achieved in PBR-A during these Experiments was probably due to the larger cell size of its microalgae. In fact, microalgae can vary their size by up to 100% [22]. In this respect, Wu et al. [69] found that the inhibition of microalgae cell division was not directly related to light intensity, but to the availability of phosphorus in the culture, which is the main element in the synthesis of DNA and RNA [70]. In addition, Baroni et al. [71] reported a cell size increase when nitrogen was scarce since it prevented protein synthesis. Under nutrient starvation (as in the case of PBR-A in Experiments 1 and 2A, see Figure 2), there was probably limited synthesis of proteins and genetic materials in the microalgae cells, which could have led to less cell division. Nonetheless, the synthesis of other materials such as carbohydrates and lipids is not so seriously affected by a short-term scarcity of nutrients [69,71,72], so that they were able to increase in size in Experiments 1 and 2A. On the contrary, in Experiment 3 the microalgae culture was not nutrient-limited (Figure 2c) and algae were therefore able to synthesise new genetic material and proteins [69,71]. PBR-A therefore probably used the higher amount of light photons to produce new cells instead of increasing their cell

size, showing a higher cell concentration in PBR-A in comparison to PBR-B during Experiment 3 (section 3.2).

In the case of Experiment 1, the extra photons supplied by the artificial lighting could have triggered the chlorophyll synthesis in PBR-A, since chlorophyll was not synthesised in darkness. Consequently, higher chlorophyll concentration was obtained in PBR-A than in PBR-B. On the other hand, in Experiment 3, both PBRs were continuously lit, but at different photon flux; i.e., PBR-A had an artificial light L:D cycle of 24:0, while PBR-B alternated the natural radiation during daytime and artificial lighting at night time. In this situation, higher chlorophyll content was obtained in PBR-B, which agrees with Chen et al. [45]. These authors found that microalgae synthesise more chlorophyll under lower net photon flux in order to absorb as many photons as possible.

4.2. Same net photon flux

Experiments 4, 5, 6, 7 and 8 were carried out using different light duration or photoperiods, but maintaining the same net photon flux in both PBRs during each experiment. As a result, no significant differences between PBR-A and PBR-B were observed in the effluent nutrient concentrations (Figures 3 and 4), nutrient recovery rates, biomass productivities (Table 3) and chlorophyll content (section 3). This disagrees with the results of other authors obtained under lab conditions. For instance, Li et al. [37], under constant light energy consumption, observed higher microalgae productivity under continuous illumination than with 12:12 h L:D cycles. In addition, Abu-Ghosh et al. [25] and Park and Lee [33] reported an enhancement of the microalgae photosynthetic activity when dark periods were shortened.

A possible reason for the similar results obtained in this study could lie in the culture mixing. In mixed PBRs, microalgae cells rapidly move between the illuminated areas near the surface and the deeper dark zones [30], creating a random flashing light effect which can enhance photosynthetic efficiency [27,47]. According to Barceló-Villalobos et al. [30], the effect of this random flashing light effect on the photosynthetic rate of microalgae can be more significant than light intensity on the PBR surface. Hence, the theoretical benefits on microalgae performance caused by L:D cycles applied to the PBRs [33,36] seemed to be vanished by this random flashing light effect produced due to mixing.

On the other hand, nutrient recovery rates were significantly lower in Experiment 8 than in the rest of experiments, probably due to the lower light exposure (L:D cycles of 8:16 h) [26]. These results therefore suggest that microalgae performance depends on the net photon flux received, and not on the lighting regime or the time of day that this energy is received. In fact, in Experiment 2A and 3, in which PBR-B received the same photon flux with different lighting regime, an analogous behaviour with respect to PBR-A was observed (section 4.1).

Further studies will be required to assess the long-term feasibility of adding an artificial light source to treat AnMBR effluents and/or designing PBRs with enhanced light availability. Raising the net photon flux by an artificial light source would increase nutrient recovery rates and biomass productivity. Higher nutrient recoveries would enable shorter operating HRT, thus reducing total PBR volume, while high biomass productivities would increase biofuel production from the microalgae biomass [8], although this would involve higher operating costs.

Results from Experiment 2B suggest the initial state of the microalgae culture did not have a significant influence on the performance of microalgae in this system. Similar

behaviour was found in lab conditions by [1]; when they cultivated microalgae with initial concentrations of 200, 500 and 800 mg $VSS \cdot L^{-1}$, NRR increased with higher initial concentration, from 5.4 to 10.8 mg $N \cdot L^{-1} \cdot d^{-1}$ in batch experiments which lasted a maximum of 9 days. When the batch experiments were lengthened to 14 days, NRR were similar: 4.4-4.8 mg $N \cdot L^{-1} \cdot d^{-1}$. On the other hand, in a previous study in an outdoor membrane photobioreactor (MPBR) plant [53], 60.3% higher NRR was obtained with higher initial biomass (270 mg $VSS \cdot L^{-1}$ in comparison to 160 mg $VSS \cdot L^{-1}$). The initial concentration of 160 mg $VSS \cdot L^{-1}$ obtained in this previous study was unlikely to be consistent enough to obtain optimum performance. However, in the present work, PBR-B started Period 2B with a consistent concentration of 300 mg $VSS \cdot L^{-1}$.

5. Conclusions

The PBR in an outdoor operation of a mixed microalgae culture treating AnMBR effluent supplied with higher net photon flux (either higher light intensity or duration) obtained better results in terms of nutrient recovery and biomass productivity. Maximum NRR, PRR and biomass productivity of 7.7 ± 1.6 mg $N \cdot L^{-1} \cdot d^{-1}$, 1.03 ± 0.21 mg $P \cdot L^{-1} \cdot d^{-1}$ and 100 ± 32 mg $VSS \cdot L^{-1} \cdot d^{-1}$, respectively, were obtained under continuous artificial illumination with an average light intensity of 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$. No photoinhibiton was observed at the highest net photon flux, probably because of the significant shadow effect on the microalgae inside the PBRs. The system thus appeared to be light-limited. However, the biomass productivity:light irradiance ratios were higher with reduced net photon flux, indicating that the higher net photon flux entailed lower light-use efficiency.

When the system was phosphorus-limited, the increase in microalgae biomass was seen to be due to larger cell size and not to higher cell numbers.

None of the experiments with the same net photon flux showed any significant differences, showing that the microalgae performance in this outdoor PBR in the operating conditions evaluated did not depend on the time of day when light was supplied or the length of the photoperiods, but on the net photon flux.

The mixing rate of the PBR and the significant PBR light path (25 cm) were probably responsible for creating a random flashing light effect, which could have outweighed the effects of the frequency photoperiods.

Further studies on PBR width and on the light supply inside the culture will be required to improve photosynthetic efficiency. This would provide higher nutrient recovery and biomass productivity in outdoor microalgae cultivation treating AnMBR effluent.

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Authors' contribution

J. González-Camejo, M.V. Ruano and R. Barat designed the experiments. J. González-Camejo and A. Viruela carried out the maintenance of the pilot plant and the analysis of the samples. J. González-Camejo interpreted the data and elaborated the numerical information (i.e., graphics, tables, statistical information, etc). J. González-Camejo, M.V. Ruano and R. Barat were in charge of writing the manuscript. A. Seco and J.

Ferrer supervised the work and were involved in the deep discussion and revision of the manuscript.

Conflict of interest statement

The authors declare that there are no financial and personal relationships with other people or organizations that could influence their work.

Statement of informed consent, human/animal rights

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

Declaration of authors' agreement

The authors agree to submit this research manuscript for publication in Algal Research. No other people have been involved in this study.

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