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Outdoor flat-panel membrane photobioreactor to treat the effluent of an anaerobic membrane bioreactor. Influence of operating, design, and environmental conditions.

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Abstract

As microalgae have the ability to simultaneously remove nutrients from wastewater streams while producing valuable biomass, microalgae-based wastewater treatment is a win-win strategy. Although recent advances have been made in this field in lab conditions, the transition to outdoor conditions on an industrial scale must be further investigated. In this work, an outdoor pilot-scale membrane photobioreactor plant was operated for tertiary sewage treatment. The effect of different parameters on microalgae performance were studied, including: temperature, light irradiance (solar and artificial irradiance), hydraulic retention time (HRT), biomass retention time (BRT), air sparging system, and influent nutrient concentration. In addition, the competition between

microalgae and ammonium oxidising bacteria for ammonium was also evaluated. Maximum nitrogen and phosphorus removal rates of 12.5 ± 4.2 $mgN \cdot L^{-1} \cdot d^{-1}$ and 1.5 ± 0.4 $mgP \cdot L^{-1} \cdot d^{-1}$, respectively, were achieved at a BRT of 4.5 days and HRT of 2.5 days, while a maximum biomass productivity of 78 ± 13 $mgVSS \cdot L^{-1} \cdot d^{-1}$ was reached. While the results obtained so far are promising, they need to be improved to make the transition to industrial scale operations feasible.

Keywords: flat-panel; membrane photobioreactor; microalgae; outdoor; pilot plant; wastewater treatment.

Introduction

Microalgae are microorganisms that carry out photosynthesis and thus require inorganic carbon and light (energy source) to grow. They also require nutrients (mainly nitrogen and phosphorus), which can be obtained from wastewater streams (Ledda et al. 2015), avoiding eutrophication of natural water bodies.

Algae based wastewater treatment has some interesting advantages over other classical technologies: i) it produces valuable biomass; ii) reduces chemicals, and iii) reduces sludge production (Gao et al. 2016). Green microalgae seem to be more appropriate for wastewater treatment than other types of microalgae such as cyanobacteria (Arias et al. 2017). In this respect, green algae *Chlorella* and *Scenedesmus* have been extensively reported as ideal for wastewater treatment because of their adaptability to such media (Xu et al. 2015; Wu et al. 2017).

Many authors have studied pure microalgae cultures in highly controlled lab conditions looking for fast-growth strains. However, single-genus cultures are difficult to maintain on a large scale under outdoor conditions. On the other hand, polycultures can increase microalgae performance, since they are more robust before contamination by other microorganisms (Gouveia et al. 2016). Microalgae can be used to treat different types of wastewater streams: urban (raw wastewater, primary and secondary effluents, centrate), aquaculture, etc. Each type has different characteristics which can affect microalgae growth positively or negatively. In this regard, Ledda et al. (2015) reported that the organic matter was the main factor affecting microalgae growth, as it was directly related with turbidity and that nutrient content did not affect the microalgae process, while Gao et al. (2016) found that high nutrient concentrations are needed to maintain high microalgae growth rates.

There are two main groups of microalgae cultivation systems: open ponds and closed photobioreactors (PBRs). Open ponds allow CO_2 uptake by microalgae directly from the atmosphere, but CO_2 can also be supplied by an aerator. Although they have lower investment and operational costs than PBRs, they also have disadvantages: large surface areas are required; contamination by predators; high CO_2 diffusion to the atmosphere; ineffective light distribution from the surface to the bottom of the reactor and high evaporative losses. PBRs are designed to improve photosynthesis efficiency by increasing the light available to the microalgae culture. While they are perfectly mixed to avoid wall fouling and enable light and nutrient homogenisation, their investment and maintenance costs are high. Moreover, photoinhibition, overheating, biofouling and oxygen accumulation can cause microalgae growth inhibition (Arbib et al.

2013). Table 1 summarises the results of different microalgae cultivation systems which treated wastewater under outdoor conditions.

Table 1. Results of algae based wastewater treatment studies under outdoor conditions.

Type of PBR	Type of wastewater	HRT (d)	N-Feed ($mgN \cdot L^{-1}$)	P-Feed ($mgP \cdot L^{-1}$)	Productivity ($mgVSS \cdot L^{-1} \cdot d^{-1}$)	NRE (%)	PRE (%)	Reference
Vertical PBR	Primary effluent	13(1)	133	8.3	100	84	95	Gouveia et al. 2016
HRAP	Secondary effluent	8	25.7	2.2	30	56.3	86.5	Arbib et al. 2013
Rectangular PBR	Municipal wastewater	15(1)	30.5	2.6	-	96	99	Woertz et al. 2009
Rectangular PBR	ADAS(2) + Secondary effluent	21(1)	259.7	42.6	109	73.3	66.5	Tan et al. 2016
Flat-panel PBR	AnMBR effluent	8	44.7	5.2	23.4	41.6	36.1	Viruela et al. 2016
Flat-panel PBR	AnMBR effluent	14	81.5	9.2	13.8	50.9	50.9	Viruela et al. 2016

(1) Batch operation. HRT indicates the length of the study; (2) ADAS: Anaerobically digested activated sludge.

Generally, closed PBRs obtained high nitrogen (NRE) and phosphorus removal efficiencies (PRE) (around 80-100%), while open ponds are less efficient. Moreover, Table 1 shows that the highest productivities and nutrient removal efficiencies were obtained in batch experiments. However, both batch and high HRT operations would imply considerably high surface areas to treat wastewater at industrial scale. Thus, algae based wastewater treatment technologies must operate at minimum HRT. In this respect, membrane photobioreactors (MPBR), which are the combination of PBRs and membrane technology, appear as an ideal solution for microalgae cultivation to treat wastewater. Membranes separate the microalgae biomass from the water

effluent, so that high nutrient loads can be maintained while microalgae biomass wash-out is avoided (Gao et al. 2016).

This paper summarises the results obtained from an outdoor MPBR pilot plant under different environmental, design, and operating conditions. This plant was fed by the effluent of an anaerobic membrane bioreactor (AnMBR) treating sewage. The aim of the MPBR plant was to simultaneously reduce the nutrient load in the AnMBR effluent and to produce microalgae biomass.

Material and methods

The substrate

The microalgae substrate consisted of the nutrient-rich effluent from an AnMBR plant that treated real sewage (Giménez et al. 2011). Its nutrient concentration varied in the range of 40-80 $mgN \cdot L^{-1}$ and 4-10 $mgP \cdot L^{-1}$ due to variations on wastewater characteristics and AnMBR performance. The substrate also contained large amounts of sulphide (around 100-120 $mgS \cdot L^{-1}$), which inhibit microalgae growth (González-Camejo et al. 2017). The substrate was therefore aerated before feeding the PBRs to oxidise the sulphide to sulphate (González-Camejo et al. 2017). Moreover, the AnMBR effluent presented a COD concentration of 72 ± 37 $mgCOD \cdot L^{-1}$ (mostly non-biodegradable) and an alkalinity of 370 ± 67 $CaCO_3 \cdot L^{-1}$.

Pilot plant

The MPBR pilot plant was located in the Carraixet WWTP (Valencia, Spain), and consisted of two outdoor flat-panel PBRs connected to a filtration system.

Each PBR had a working volume of 550 L: 2.00 m long x 1.10 m high x 0.25 m wide. The aeration system consisted of two perforated pipes (5 mm diameter) placed on the bottom of the PBRs, which continuously introduced air at a flow rate of 0.09 vvm. This way, microalgae settling and wall fouling were minimised. Whenever the pH value of the culture was over 7.5 (set point), pure CO_2 (99.9%) was introduced into the air system, reaching a maximum percentage of CO_2 in the air flow of 4%. This way, phenomena such as ammonia volatilisation and phosphorus precipitation were considered negligible (Whitton *et al.,* 2016). Both PBRs had twelve white LED lamps (Unique Led IP65 WS-TP4S-40W-ME) installed at the back, offering a continuous light irradiance of 300 $\mu E \cdot m^{-2} \cdot s^{-1}$ (Light:Dark cycle of 24:0 h).

Both PBRs were connected to a filtration system, which mainly consisted of two membrane tanks which included industrial hollow-fibre ultrafiltration membrane units (PURON® Koch Membrane Systems (PUR-PSH31), 0.03 µm pore size), with a working volume of 38 L and filtering area of 6.8 m^2. They were stirred by the same CO_2-enriched air flow as the PBRs to reduce cake formation and avoid undesirable phenomena.

During the experiments with inhibition of nitrification, a concentration of 5 $mg \cdot L^{-1}$ of allylthiourea (ATU) was maintained in the PBRs to inhibit AOB growth (Table 2).

Experimental periods

Before each operating period, the MPBR plant went through a start-up phase, consisting of: i) adding 10% of the working volume with microalgae biomass (300-500 $mgVSS \cdot L^{-1}$; mainly *Scenedesmus* and *Chlorella*; although bacteria

and cyanobacteria were also present) and 90% of the working volume with the aforementioned substrate; ii) batch mode until reaching a biomass concentration of around 250-400 mgVSS·L^{-1} (data not shown); and iii) continuous feeding maintaining the desired BRT and HRT.

The experimental set-up consisted of 4 periods in which the MPBR was operated under different environmental (temperature, solar irradiance and influent nutrient concentration), operating (BRT and HRT) and design (bubble size of the air sparging system and operating the MPBR plant without membrane filtration, i.e. as a PBR system) conditions. Moreover, artificial light and ATU addition were also modified (Table 2).

Period 1 was operated without microalgae biomass filtration so that BRT was equal to HRT (PBR system). No additional artificial light source was used. It was divided into 4 sub-periods: 1) 1A was operated at HRT of 8 days and ATU was continuously added; 2) 1B was operated at the same HRT without ATU; 3) in sub-period 1C, HRT was increased to 14 days without ATU. 4) In 1D, an initial ATU dose of 5 mg·L^{-1} was added. The rest of the sub-period was operated at HRT of 14 days without further ATU addition.

In period 2, the pilot plant was also operated as a PBR system (without membranes), maintaining HRT (i.e. BRT) at 8 days. A neoprene diffuser with 0.5 mm pore size was installed in PBR1. In PBR2, the same air sparging system (5 mm pore size) was maintained. The rest of the operating and outdoor conditions were the same for both PBRs. Thus, only in this period, PBR1 and PBR2 were operated separately in order to compare the effect of different bubble size of the air sparging system.

In period 3, the plant was operated as an MPBR system at BRT of 4.5 days and variable HRT: 2.5, 2 and 3 days, for sub-periods 3A, 3B and 3C, respectively.

Period 4 was operated as an MPBR system at a BRT and HRT of 4.5 days and 2.5 days, respectively, but the period started with a microalgae biomass concentration of 160 mgVSS·L^{-1} (lower than the other periods).

Table 2. Operation and outdoor conditions of each period.

Sub-period	Days of operation	Daily average solar PAR (µE·m^{-2}·s^{-1})	Average artificial PAR (µE·m^{-2}·s^{-1})	Temperature (ºC)	BRT (d)	HRT (d)	NLR(1) (gN·d^{-1})	PLR(1) (gP·d^{-1})	ATU (mg·L^{-1})
1A	17	171 ± 55	0	28.0 ± 1.5	8	8	2.5 ± 0.2	0.3 ± 0.0	5
1B	13	164 ± 34	0	25.4 ± 1.9	8	8	3.0 ± 0.2	0.4 ± 0.0	0
1C	21	294 ± 100	0	24.4 ± 2.2	14	14	1.7 ± 0.3	0.2 ± 0.0	0
1D	33	249 ± 111	0	16.8 ± 2.3	14	14	2.2 ± 0.5	0.3 ± 0.1	5(2)
2(3)	24	119 ± 32	300	23.0 ± 1.1	8	8	3.9 ± 0.3	0.4 ± 0.1	5
3A	20	234 ± 19	300	23.5 ± 0.3	4.5	2.5	9.7 ± 2.3	1.3 ± 0.2	5
3B	22	259 ± 43	300	26.9 ± 4.0	4.5	2	14.4 ± 1.8	1.8 ± 0.1	5
3C	47	283 ± 75	300	24.8 ± 1.3	4.5	3	8.4 ± 1.1	1.1 ± 0.2	5
4	40	357 ± 105	300	23.2 ± 2.1	4.5	2.5	13.6 ± 2.0	1.4 ± 0.2	5

(1) Nutrient loading rate to each PBR; (2) single ATU dosage; (3) Smaller bubble size in PBR1 than PBR2.

Analytical Methods

Grab samples were collected in duplicate from the influent and effluent streams of the MPBR pilot plant three times a week. Ammonium, nitrite, nitrate, and phosphate were analysed in a Smartchem 200 automatic analyser (Westco

Scientific Instruments), according to Standard Methods (APHA et al. 2005).VSS was also analysed following APHA et al. (2005).

50 µL of sample were measured twice a week according to Pachés *et al*. (2012) to count (in duplicate) the total eukaryotic cells (TEC).

Calculations

Biomass productivity (mgVSS·L^{-1}·d^{-1}), nitrogen removal rate (NRR) (mgN·L^{-1}·d^{-1}), phosphorus removal rate (PRR) (mgP·L^{-1}·d^{-1}), nitrogen removal efficiency (NRE) (%) and phosphorus removal efficiency (PRE) (%) were calculated by the equations 1, 2, 3, 4, and 5, respectively:

$$\text{Biomass productivity} = \frac{X_{VSS}}{BRT} \quad \text{(Eq. 1)}$$

$$NRR = \frac{Q \cdot (N_i - N_e)}{V_{PBR}} \quad \text{(Eq. 2)}$$

$$PRR = \frac{Q \cdot (P_i - P_e)}{V_{PBR}} \quad \text{(Eq. 3)}$$

$$NRE = \frac{(N_i - N_e)}{N_i} \cdot 100 \quad \text{(Eq. 4)}$$

$$PRE = \frac{(P_i - P_e)}{P_i} \cdot 100 \quad \text{(Eq. 5)}$$

where X_{VSS} (mg VSS·L^{-1}) is the volatile suspended solids concentration in the PBRs, BRT is the biomass retention time (d), Q is the wastewater flow rate (L·d^{-1}), N_i is the nitrogen concentration of the influent (mgN·L^{-1}), N_e is the nitrogen concentration of the effluent (mgN·L^{-1}), P_i is the phosphorus concentration of the influent (mgP·L^{-1}), P_e is the phosphorus concentration of the effluent (mgP·L^{-1}) and V_{PBR} is the total volume of the PBRs (L).

In order to compare different operating periods with variations in solar irradiances, the NRR:light irradiance ratio (NRR:I) (mgN·mol photons^{-1}), and

PRR:light irradiance ratio (PRR:I) (mgP·mol photons^{-1}) were calculated by equations 6 and 7, respectively:

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

$$PRR{:}\,I = \frac{PRR \cdot V_{PBR} \cdot 10^6}{I \cdot S \cdot 24 \cdot 3600} \qquad \text{(Eq. 7)}$$

where I is the total light PAR irradiance on the PBR surface, i.e. the 24-hour average solar irradiance plus the light from the LED lamps (µmol photons·m^{-2}·s^{-1}) and S is the illuminated PBR surface (m^2).

Results

Period 1

In this period, in which *Scenedesmus* remained the main microalgae genus (>99% of TEC), the effect of different BRT (i.e. HRT) under different environmental conditions and the competition of microalgae and ammonium oxidising bacteria (AOB) for ammonium was evaluated.

In sub-period 1A, AOB growth was inhibited by ATU addition, so that nitrite and nitrate concentration remained at negligible values, although ammonium and phosphate stayed at high values during the entire sub-period (Figure 1a).

In sub-period 1B, ATU was not added, but nitrite and nitrate concentrations remained at negligible concentrations, which suggested that no nitrifying bacteria proliferation occurred. Biomass concentration dropped from 301 ± 15 mgVSS·L^{-1}in sub-period 1A to 213 ± 28 mgVSS·L^{-1} in 1B. Since temperature has been shown to have a direct effect on biomass productivity (Viruela et al. 2016), the biomass reduction was assumed to be due to the temperature dropping from 28.0 ± 1.5 ºC in sub-period 1A to 25.4 ± 1.9 ºC in 1B. This

temperature reduction could have also favoured microalgae over AOB (González-Camejo et al. 2018).

In sub-period 1C, the HRT (i.e. BRT) was raised from 8 to 14 days. In consequence, VSS concentration achieved a maximum concentration of 304 $mgVSS \cdot L^{-1}$ (Figure 1b). However, this increased biomass concentration could also have been related to a solar PAR increase from 164 ± 34 $\mu mol \cdot m^{-2} \cdot s^{-1}$ in sub-period 1B to 294 ± 100 $\mu mol \cdot m^{-2} \cdot s^{-1}$ in 1C. On the other hand, by the end of sub-period 1C, nitrite concentration reached a maximum value of 18.5 $mgN \cdot L^{-1}$ (Figure 1a), which indicated that an AOB proliferation occurred.

Lastly, a single ATU dose was added at the beginning of sub-period 1D to inhibit AOB growth. Consequently, nitrite concentration dropped due to the nitrate oxidising bacteria (NOB) proliferation, which oxidised nitrite to nitrate (Figure 1a). When the nitrite was exhausted, the NOB could no longer grow and nitrate concentration declined due to wash-out.

In terms of microalgae biomass, sub-period 1D started with a concentration of 360 $mgVSS \cdot L^{-1}$, but steadily decreased mainly due to a significant reduction in the culture temperature (Figure 1b).

It is worth mentioning that HRT was not directly related to nutrient loading rates due to both WWTP intake dynamics and AnMBR plant performance. For instance, sub-period 1A (HRT of 8 days) had a similar NLR and PLR to 1D (HRT of 14 days) (Table 2). Hence, NLR and PLR must also be considered as controlling parameter.

In this period, the highest biomass productivities were achieved in sub-periods 1A and 1B (Table 3), probably because the temperature was higher (Table 2). Similar results were obtained by Viruela et al. (2016). Moreover, the nutrient

removal rates in terms of NRR:I and PRR:I were also higher in sub-periods 1A and 1B, although the solar irradiances were considerably lower than in periods 1C and 1D (Table 2). Since nutrient removal rates have been reported to be directly related to light irradiance (Viruela et al., 2016), these results suggested that the culture could have been nutrient-limited in during sub-periods 1C and 1D. In fact, the ammonium concentration remained under 10 $mgN \cdot L^{-1}$ during days 49-63; i.e., in sub-periods 1C and 1D (Figure 1a). In this respect, ammonium values below 10 $mgN \cdot L^{-1}$ have been reported to limit ammonium absorption by microalgae (Ruiz-Martinez et al., 2014). This low ammonium concentration in sub-periods 1C and 1D was mainly due to an AOB proliferation, which competed with microalgae for ammonium (González-Camejo et al., 2018). Hence, the proliferation of AOB did not seem to be desirable, as the system can get nutrient-limited. Further research in this topic must be developed in order to better understand the operating conditions which favour microalgae growth over AOB.

When the system was non-nutrient-limited, the effluent nutrient concentration followed approximately the same trend as the influent (Figure 1a). This tendency was in agreement with Arbib et al. (2013), who reported higher effluent nutrient concentrations at higher influent nutrient concentrations in outdoor microalgae cultivation.

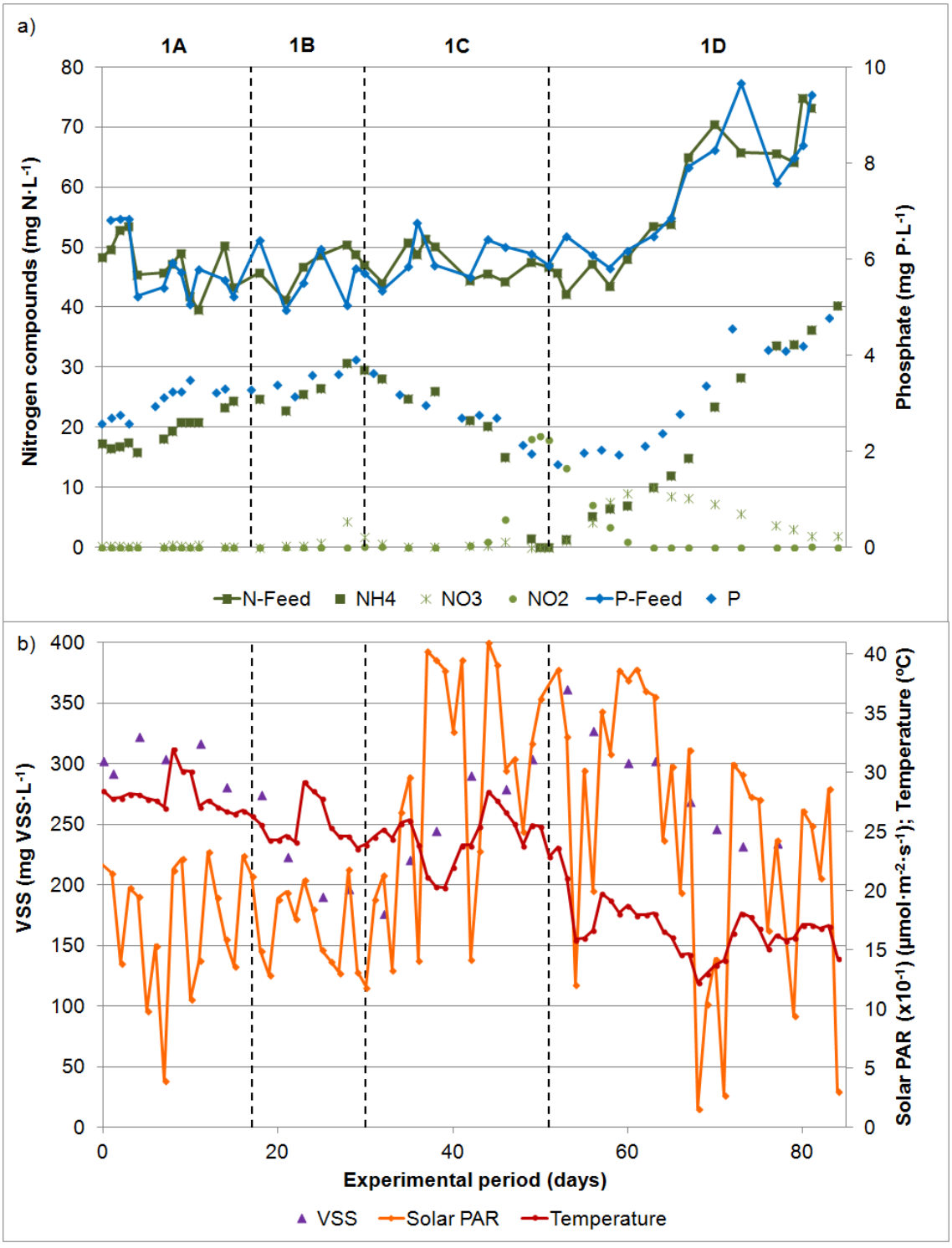


Figure 1. Evolution during Period 1 of: a) Effluent concentration of: ammonium (NH4); nitrite (NO2); nitrate (NO3) and soluble phosphorus (P); and feed concentration of nitrogen (N-feed) and phosphorus (P-Feed); b) VSS concentration, solar PAR and culture temperature.

Period 2

The effect of the bubble size of the air sparging system was studied in this period. Bubble diameter in PBR1 was reduced to 0.5 mm, while it remained at 5 mm in PBR2. PBR1 and PBR2 showed similar behaviour (Figure 2), reaching no significant differences between nutrient removal rates and biomass productivity (Table 3).

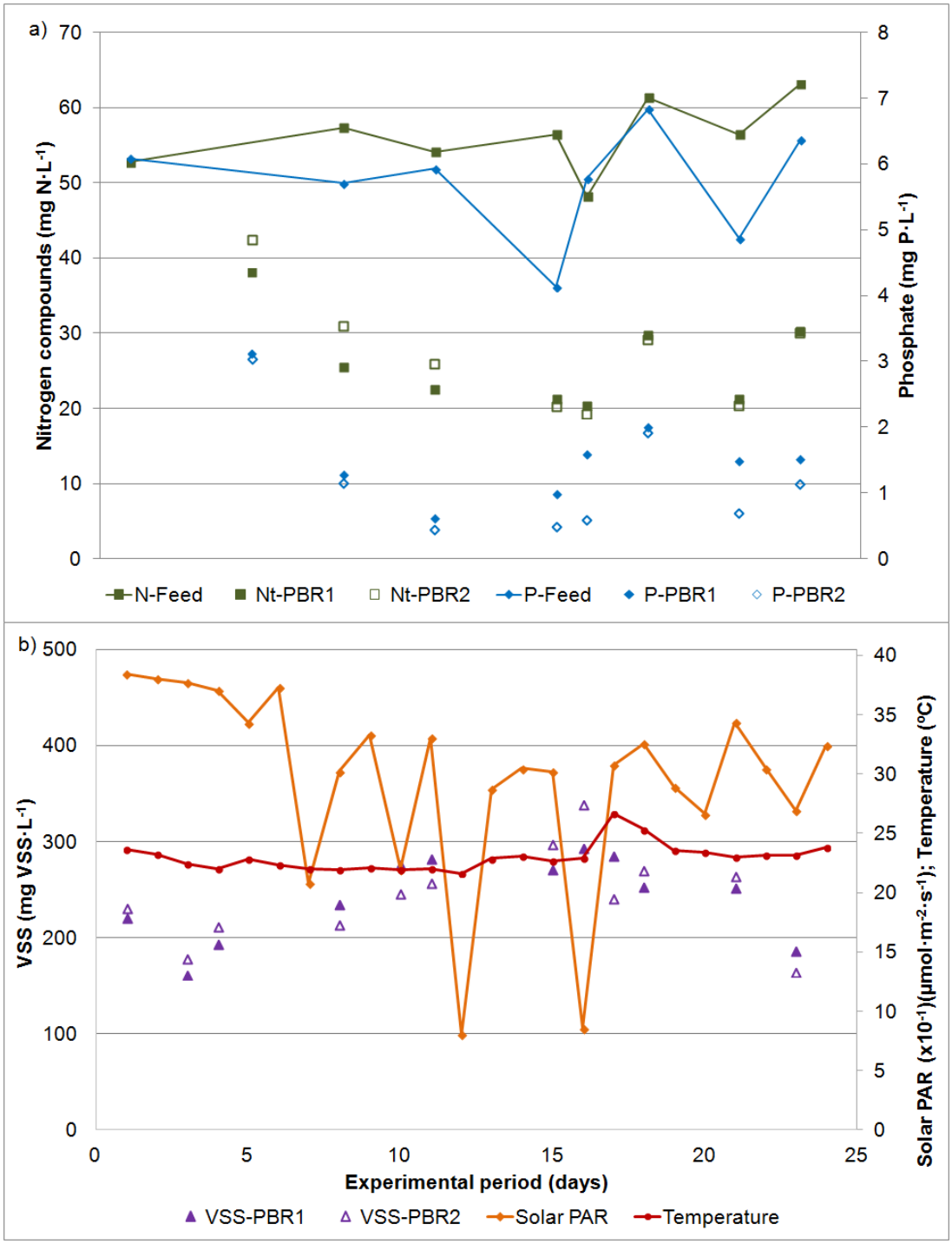


Figure 2. Evolution during Period 2 in PBR1 and PBR2 of: a) Effluent concentration of: soluble nitrogen (Nt) and soluble phosphorus (P); and feed concentration of nitrogen (N-feed) and phosphorus (P-feed); b) solar PAR, culture temperature and VSS concentration.

However, the genera distribution in the cultures was different; PBR1 had 40 % *Scenedesmus* and 55 % *Chlorella*, while PBR2 had 85 % *Scenedesmus* and 10% *Chlorella*. Moreover, by the end of period, the phosphorus concentration in PBR2 was slightly lower than in PBR1.These differences could have been related to a cyanobacteria proliferation observed in PBR1 at the end of period 2 (Figure 3). This agrees with Kin et al. (2014), who reported that small bubble size favours cyanobacteria growth over green algae. The proliferation of cyanobacteria is not desirable, as they have been reported to excrete some allelopathic substances that can damage green microalgae (Leão et al. 2009).

The results obtained in this period showed that nutrient removal rates and nutrient removal efficiencies were higher in period 2 than in period 1 (Table 3), mainly due to an additional light source that had not been used in period 1. Increasing the light irradiance on the PBRs was therefore considered beneficial for nutrient removal in outdoor conditions.

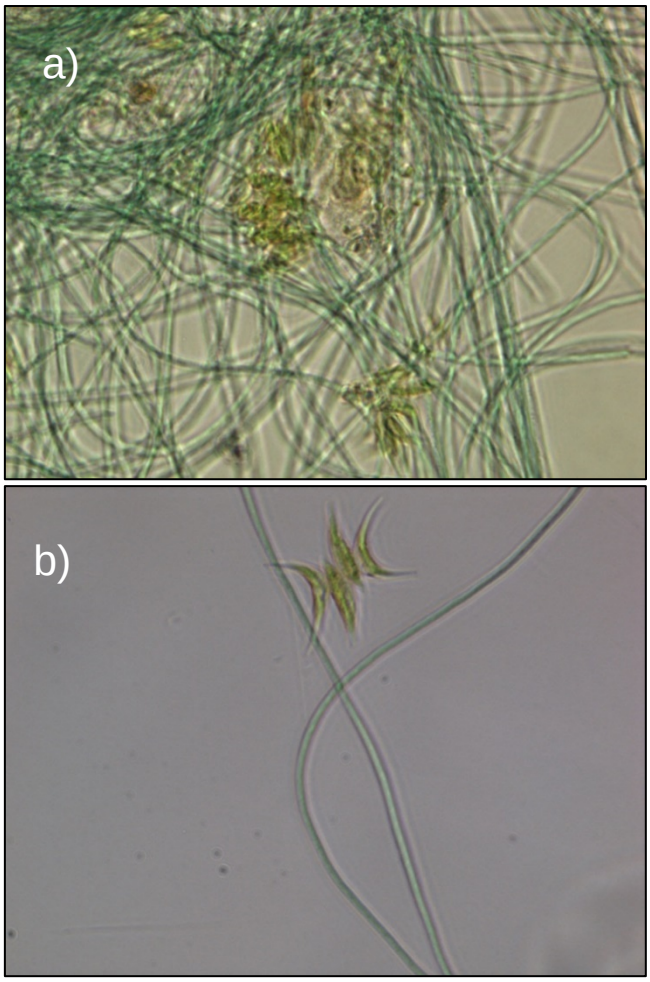


Figure 3. Samples observed under epifluorescence microscope (Leica DM2500/ DFC420c digital camera, 63x) in period 2 (day 21). a) PBR1: Cyanobacteria and green algae (mainly *Scenedesmus* and *Chlorella*) floc; b) PBR2: *Scenedesmus* in four-cell coenobia and a small amount of cyanobacteria.

Period 3

The use of the membrane system in this period enhanced the treatment capacity of the MPBR plant: HRT was significantly reduced from 8 (period 2) to 2.5 days (sub-period 3A). This means that nutrient loading rates were considerably higher during this period (Table 2), which has been reported to favour microalgae growth (Gao et al. 2016). In consequence, nutrient removal rates and biomass productivity were considerably higher in period 3 than in the

previous periods (Table 3), reaching maximum NRR, PPR and biomass productivity in sub-period 3A: 12.5 ± 4.2 $mgN \cdot L^{-1} \cdot d^{-1}$, 1.5 ± 0.4 $mgP \cdot L^{-1} \cdot d^{-1}$ and 78 ± 13 $mgVSS \cdot L^{-1} \cdot d^{-1}$, respectively. The light use efficiency of the microalgae improved in this period (operating as an MPBR system), since NRR:I and PRR:I values were around 2-fold and 3-fold higher than in the previous periods, in which the system operated as a PBR (Table 3).

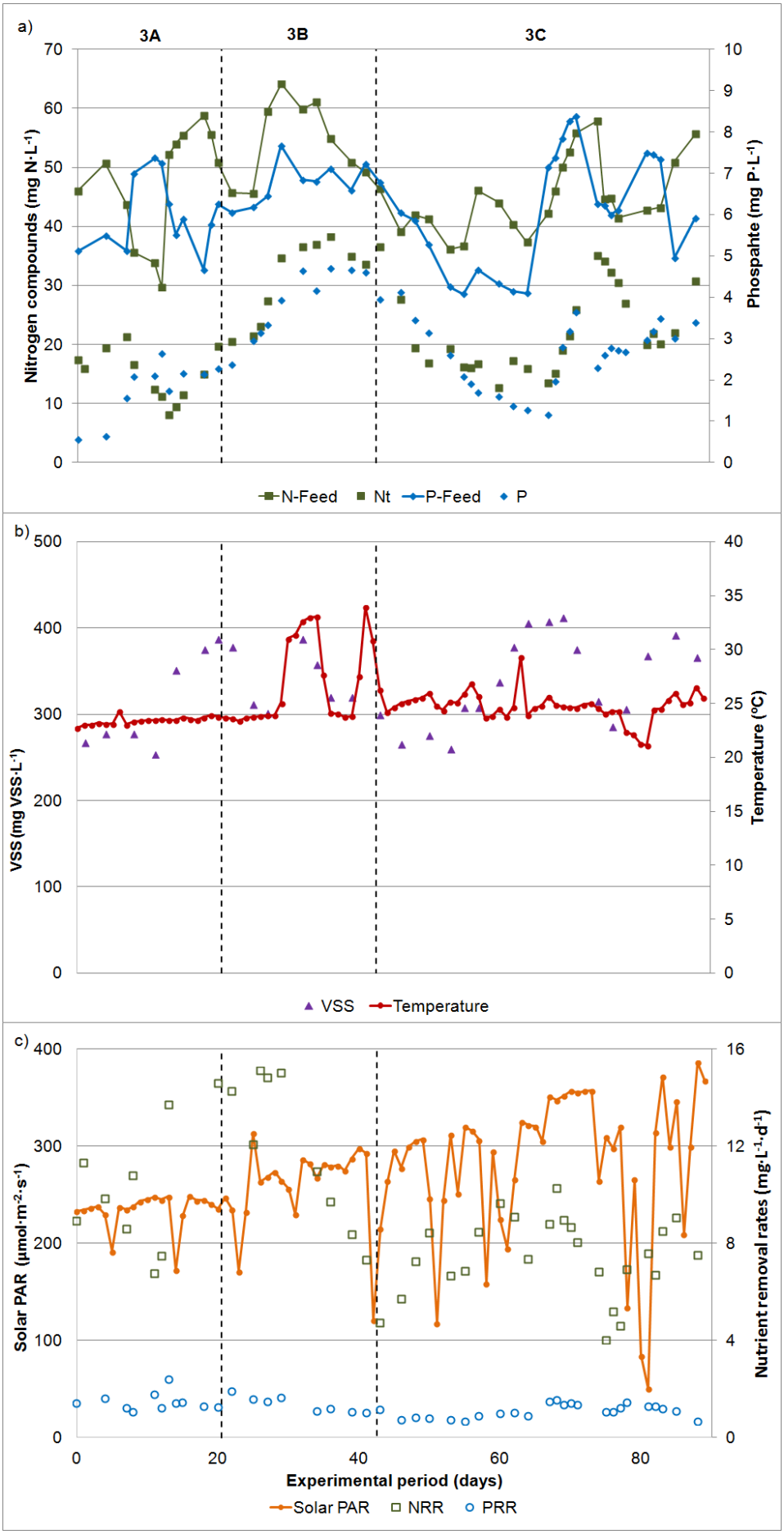
In sub-period 3B nutrient removal rates started at values around 15 $mgN \cdot L^{-1} \cdot d^{-1}$ and 1.7 $mgP \cdot L^{-1} \cdot d^{-1}$, but after day 30 they suddenly dropped to 7-10 $mgN \cdot L^{-1} \cdot d^{-1}$ and 1.0 $mgP \cdot L^{-1} \cdot d^{-1}$ and did not recover their high initial values (Figure 4c). This reduced nutrient removal rates could have been due to a significant increase in the culture temperature from around 25 to 33ºC in days 30-35 (Figure 4b). These high temperatures could have affected biomass productivity. Indeed, the biomass concentration dropped from around 400 to 300 $mgVSS \cdot L^{-1}$. Consequently, nutrient removal capacity also decreased.

In sub-period 3C, temperature stabilised and NRR was solar PAR-dependent (Figure 4c), which was in agreement with Viruela et al. (2016). However, NRR and PRR were lower in sub-period 3C than in sub-periods 3A and 3B, which could be explained by: i) after the high temperatures in sub-period 3B, the system took around two weeks to recover the initial microalgae biomass (Figure 4b), so that its nutrients removal capacity was reduced; ii) sub-period 3C had the lowest nutrient loading rates of period 3 (Table 2). Consequently, effluent nitrogen concentration (which was mainly ammonium) was reduced to values of 10-15 $mgN \cdot L^{-1}$ during days 55-68 (Figure 4a). Ruiz-Martinez et al. (2014) reported that NRR decreased whenever ammonium concentration in the culture was below 10-13 $mgN \cdot L^{-1}$. Hence, in sub-period 3C, the culture was considered

to be nutrient-limited; iii) in spite of having received a higher solar PAR in sub-period 3C (Table 3), this irradiance was more variable than in sub-periods 3A and 3B (Figure 4c). This means that the alternation of very sunny days, in which photoinhibition could have occurred, with photo-limited days could have negatively affected microalgae growth.

Throughout period 3, *Scenedesmus* remained as dominant genus (80-95% of TEC) and *Chlorella* only reached 5-20 % of TEC.

The best efficiencies of this period (67 ± 11% and 69 ± 9%, for nitrogen and phosphorus, respectively) were obtained at an HRT of 2.5 days, even though solar PAR in sub-period 3A was the lowest of the period (Table 2). On the other hand, in sub-period 3C, with the lowest nutrient loading rates, the culture could be nutrient-limited and therefore nutrient removal efficiencies were lower than in 3A (Table 3). NLR and PLR thus appear to be key parameters in assessing MPBR performance.


a)
3A
3B
3C
Nitrogen compounds (mg N·L-1)
Phospahte (mg P·L-1)
N-Feed
Nt
P-Feed
P
b)
VSS (mg VSS·L-1)
Temperature (°C)
VSS
Temperature
c)
Solar PAR (µmol·m-2·s-1)
Nutrient removal rates (mg·L-1·d-1)
Experimental period (days)
Solar PAR
NRR
PRR

1

Figure 4. Evolution during Period 3 of: a) Effluent concentration of: soluble nitrogen (Nt) and soluble phosphorus (P); and feed concentration of nitrogen (N-feed) and phosphorus (P-feed); and feed concentration of nitrogen and phosphorus; b) culture temperature and VSS concentration; c) solar PAR and nutrient removal rates.

Period 4

The same HRT and BRT were used in period 4 as in sub-period 3A, but at higher nutrient loading rates (Table 2), however the results obtained were significantly different (Table 3).

As Figure 5b shows, microalgae biomass concentration was under 250 $mgVSS \cdot L^{-1}$ for the entire period, while in sub-period 3A it always remained over 250 $mgVSS \cdot L^{-1}$ (Figure 4b), so that the nutrient removal capacity of the system diminished and nutrient removal rates were not as high as in sub-period 3A (Table 3). This lower biomass concentration could have been influenced by the lower initial microalgae concentration in the start-up period: 160 $mgVSS \cdot L^{-1}$ in period 4, while sub-period 3A started at 270 $mgVSS \cdot L^{-1}$. Su et al. (2012) also obtained higher NRR and PRR in the culture with a higher initial biomass concentration. Moreover, Feng et al. (2011) reported that cultures with denser initial biomass concentration achieved higher biomass productivity and adapted quickly to outdoor conditions.

Solar PAR, in spite of being higher than in sub-period 3A (Table 2), was quite variable in period 4 (Figure 5c) and, as in *Period 3,* could have negatively affected microalgae growth.

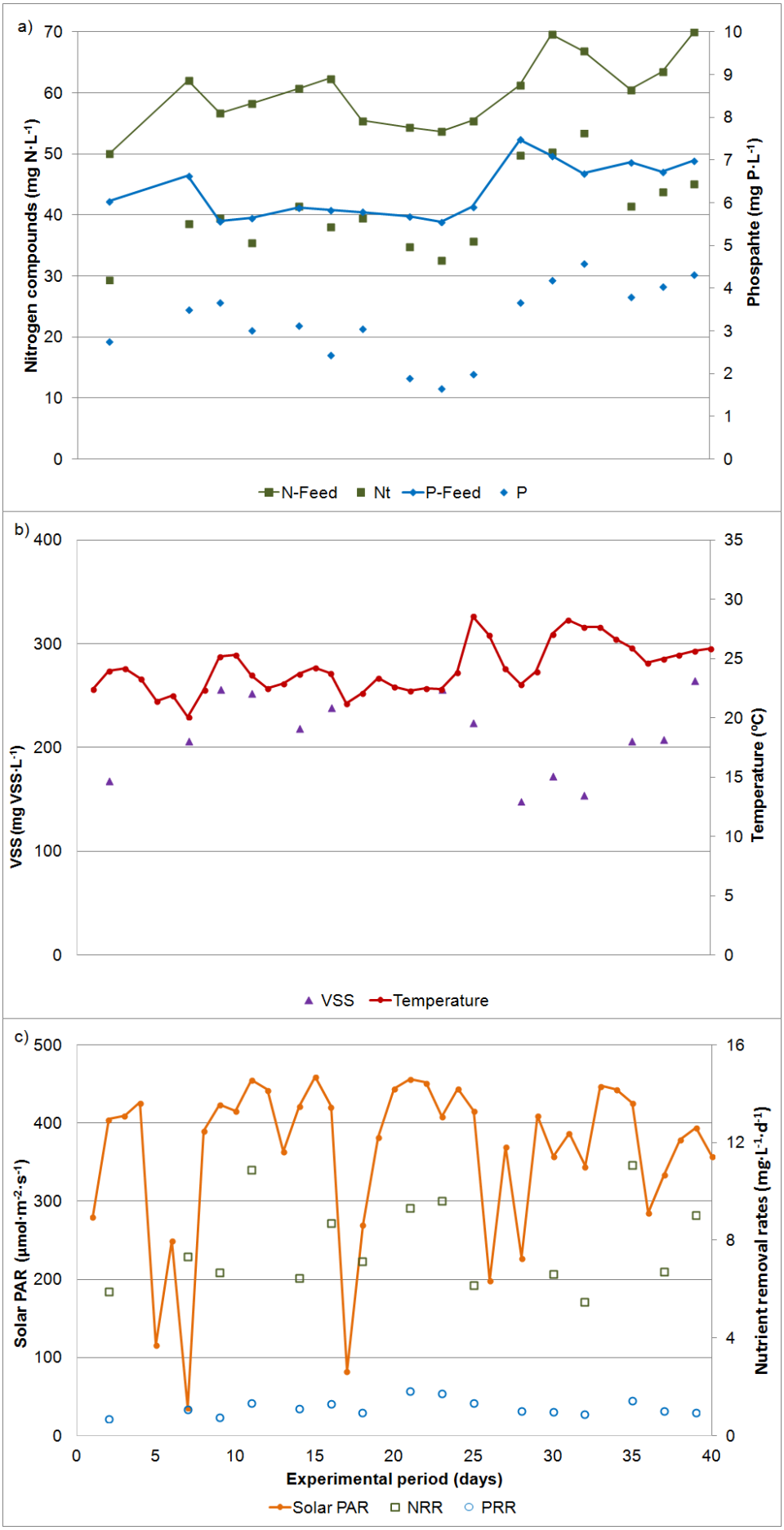
Nutrient removal rates could also have been influenced by a shift in the microalgae culture. In period 4 there was a proliferation of *Monoraphidium* (45 % TEC) which co-habited with *Scenedesmus* (50 % TEC). No significant amount of *Chlorella* was present.

Table 3. Results obtained in each sub-period.

Sub-period	Biomass productivity ($mgVSS \cdot L^{-1} \cdot d^{-1}$)	NRE (%)	PRE (%)	NRR ($mgN \cdot L^{-1} \cdot d^{-1}$)	PRR ($mgP \cdot L^{-1} \cdot d^{-1}$)	NRR:I ($mgN \cdot mol^{-1}$)	PRR:I ($mgP \cdot mol^{-1}$)
1A	38 ± 2	56 ± 9	46 ± 8	2.8 ± 1.0	0.3 ± 0.1	36.4 ± 9.5	4.0 ± 2.1
1B	27 ± 4	40 ± 6	38 ± 6	1.9 ± 1.4	0.2 ± 0.1	31.3 ± 25.8	3.8 ± 2.0
1C	19 ± 3	49 ± 7	52 ± 10	2.3 ± 0.5	0.3 ± 0.1	25.8 ± 12.7	3.5 ± 1.5
1D	20 ± 3	57 ± 8	60 ± 8	1.6 ± 0.7	0.2 ± 0.1	24.6 ± 13.7	3.6 ± 1.8
2-PBR1(1)	28 ± 6	57 ± 4	76 ± 7	3.3 ± 2.0	0.3 ± 0.2	37.1 ± 32.2	3.7 ± 2.3
2-PBR2	28 ± 6	56 ± 7	87 ± 10	3.1 ± 1.2	0.4 ± 0.1	31.7 ± 28.1	4.3 ± 3.4
3A	72 ± 8	67 ± 11	69 ± 9	12.5 ± 4.2	1.5 ± 0.4	64.2 ± 22.5	12.7 ± 3.4
3B	69 ± 5	43 ± 11	43 ± 10	11.5 ± 2.9	1.4 ± 0.3	56.4 ± 15.4	11.8 ± 2.9
3C	78 ± 13	50 ± 15	56 ± 12	7.5 ± 1.8	1.1 ± 0.3	36.3 ± 9.5	9.6 ± 2.5
4	53 ± 15	33 ± 7	49 ± 12	7.8 ± 2.5	1.2 ± 0.3	33.5 ± 9.9	9.5 ± 2.4

(1) Bubble diameter: 0.5 mm.

As happened in period 1, in period 4 the effluent nutrient concentrations followed the same trend as the influent nutrient concentrations (Figure 5a), since the system was not nutrient-limited. According to Arbib et al. (2013), in these conditions, microalgae are mainly limited by outdoor conditions.


a)
Nitrogen compounds (mg N·L-1)
Phospahte (mg P·L-1)
N-Feed
Nt
P-Feed
P
b)
VSS (mg VSS·L-1)
Temperature (°C)
VSS
Temperature
c)
Solar PAR (µmol·m-2·s-1)
Nutrient removal rates (mg·L-1·d-1)
Experimental period (days)
Solar PAR
NRR
PRR

1

Figure 5. Evolution during Period 4 of: a) Effluent concentration of: soluble nitrogen (Nt) and soluble phosphorus (P); and feed concentration of nitrogen (N-feed) and phosphorus (P-feed); and feed concentration of nitrogen and phosphorus; b) culture temperature and VSS concentration; c) solar PAR and nutrient removal rates.

Discussion

The performance of this outdoor MPBR pilot plant treating AnMBR effluent within a wide range of environmental, design, and operating conditions produced some interesting results, which deserve to be commented on.

When the plant was operated as a PBR system without membrane filtration (periods 1 and 2), the highest values in terms of nutrient removal and biomass productivity were obtained when HRT was 8 days (Table 3). When the plant was operated as an MPBR system (periods 3 and 4), the best results were achieved at a BRT and HRT of 4.5 days and 2.5 days, respectively (sub-period 3A, Table 3). In this respect, optimum BRT and HRT must be assessed to further improve MPBR performance.

Comparing PBR and MPBR performance, nutrient removal rates and biomass productivity were significantly higher in MPBR as the use of membranes to separate microalgae from water enabled to operate at lower HRT (i.e. higher nutrient loading rates), avoiding microalgae wash-out.

Generally, the plant performance was strongly dependent on outdoor conditions; solar irradiance seemed to be one of the main factors affecting nutrient removal, while temperature variations had a major impact on biomass productivity. The plant performance yields were reduced when the culture was

nutrient-limited, which meant that high nutrient loading rates were required to reach high nutrient removal rates. In this respect, the proliferation of AOB in the culture can worsen PBR performance since they compete with microalgae for ammonium consumption.

Increasing the light supply to the microalgae seemed to be beneficial for nutrient removal as nutrient removal rates were lower in period 1 with no artificial lighting (Table 3).

Small bubble size (0.5 mm diameter) in the air sparging system was not found to be suitable, as it favoured the proliferation of filamentous cyanobacteria, which could hinder green microalgae growth.

The initial biomass concentration appeared to have some influence on the plant performance, since higher biomass concentrations attained better results at quite similar operating conditions.

Overall, as the nutrient removal efficiencies achieved in this continuously-operated MPBR under outdoor conditions and using real anaerobically-treated sewage were not particularly high, some improvements need to be made to comply with legal requirements. Special efforts should be focused on increasing the efficiency of the light applied to the PBRs, lowering the plant HRT to further increase its treatment capacity, controlling BRT (and HRT when treatment capacity can be variable) to optimise microalgae productivity and nutrient removal, avoiding AOB growth without using chemical inhibitors, and reducing operating costs.

Conclusions

In this study, an MPBR plant was operated outdoors under different conditions: BRT, HRT, temperature, light irradiance, influent nutrient concentration, ATU addition, and bubble size of the air sparging system; reaching maximum biomass productivity and nitrogen and phosphorus removal rates of 78 ± 13 $mgVSS \cdot L^{-1} \cdot d^{-1}$, 12.5 ± 4.2 $mgN \cdot L^{-1} \cdot d^{-1}$ and 1.5 ± 0.4 $mgP \cdot L^{-1} \cdot d^{-1}$, respectively. Although these values are promising, further research needs to be carried out to make this technology feasible on an industrial scale. The main challenges to overcome include: increasing the efficiency of the light supplied to the PBRs, avoiding AOB growth, improving the plant's treatment capacity and reducing its operating costs.

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