Nutrients and biomass dynamics in photo-sequencing batch reactors treating wastewater with high nutrients loadings

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Abstract

The present study investigates different strategies for the treatment of a mixture of digestate from an anaerobic digester diluted and secondary effluent from a high rate algal pond. To this aim, the performance of two photo-sequencing batch reactors (PSBRs) operated at high nutrients loading rates and different solids retention times (SRTs) were compared with a semi-continuous photobioreactor (SC). Performances were evaluated in terms of wastewater treatment, biomass composition and biopolymers accumulation during 30 days of operation. PSBRs were operated at a hydraulic retention time (HRT) of 2 days and SRTs of 10 and 5 days ($PSBR_{2\text{-}10}$ and $PSBR_{2\text{-}5}$, respectively), whereas the semi-continuous reactor was operated at a coupled HRT/SRT of 10 days ($SC_{10\text{-}10}$). Results showed that $PSBR_{2\text{-}5}$ achieved the highest removal rates in terms of TN (6.7 mg $L^{-1}\cdot d^{-1}$), TP (0.31 mg $L^{-1}\cdot d^{-1}$), TOC (29.32 mg $L^{-1}\cdot d^{-1}$) and TIC (3.91 mg $L^{-1}\cdot d^{-1}$). These results were in general 3-6 times higher than the removal rates obtained in the $SC_{10\text{-}10}$ (TN 29.74 mg $L^{-1}\cdot d^{-1}$, TP 0.96 mg $L^{-1}\cdot d^{-1}$, TOC 29.32 mg $L^{-1}\cdot d^{-1}$ and TIC 3.91 mg $L^{-1}\cdot d^{-1}$). Furthermore, both PSBRs were able to produce biomass up to 0.09 g L^{-1} d^{-1}, more than twofold the biomass produced by the semi-continuous reactor (0.04 g L^{-1} d^{-1}), and achieved a biomass settleability of 86-92%. This study also demonstrated that the microbial composition could be controlled by the nutrients loads, since the three reactors were dominated by different species depending on the nutritional conditions. Concerning biopolymers accumulation, carbohydrates concentration achieved similar values in the three reactors (11%), whereas <0.5 % of polyhydrohybutyrates (PHB) was produced. These low values in biopolymers production could be related to the lack of microorganisms as cyanobacteria that are able to accumulate carbohydrates/PHB.

Keywords: Centrate, cyanobacteria, microalgae, biopolymers, secondary effluent

1. Introduction

Wastewater treatment with microalgae is regarded as an economical and environmentally friendly process with the additional advantage that the biomass produced can be reused, allowing an efficient nutrient recycling (Rawat et al., 2011; Honda, et al., 2012). In this process, microalgae work in association with aerobic heterotrophic bacteria so that photosynthetic microorganisms produce molecular oxygen that is used as electron acceptor by bacteria to degrade organic matter (Abed et al., 2009; Borde et al., 2003). In return, bacteria release carbon dioxide during the mineralization process and complete the photosynthetic cycle (Muñoz and Guieysse, 2006). This wastewater treatment process has been successfully used for a range of purposes such as removal of nutrients and other compounds (i.e. heavy metals) and also to reduce the load of organic matter (Abdel-Raouf et al., 2012; de Godos et al., 2009; Honda et al., 2012; Wang et al., 2010). Furthermore, wastewater is nowadays considered the only economically viable source of water and nutrients for the production of microalgae biomass that can then be used for valuable by-products generation (Pittman et al., 2011; Uggetti et al., 2014).

In spite of the benefits, microalgae-based wastewater treatment technologies face operational limitations and challenges, such as the high costs derived from biomass separation from the treated wastewater (Renuka et al., 2013; Trivedi et al., 2015; Udom et al., 2013). Indeed, an efficient separation requires the use of biomass harvesting processes which can increase the production cost by 20–30% (Molina-Grima et al., 2003; Renuka et al., 2013; Yaakob et al., 2014). Recently, several studies have proposed to include a

sedimentation period in the operational mode in order to increase spontaneous flocculation and the subsequent formation of large flocs (Valigore et al., 2012; Van Den Hende et al., 2016, 2014). This process can be carried out in a photo-sequencing batch reactor (PSBR), where hydraulic retention time (HRT) and solids retention time (SRT) are uncoupled, similarly to activated sludge systems (Wang et al., 2015). This way, the cells are forced to form flocs that settle faster, whereas unsettled cells are removed from the supernatant (Valigore et al., 2012). Contrary to conventional operations, which do not promote extensive spontaneous flocculation (i.e. continuous, semi-continuous and batch), this approach can avoid additional intensive harvesting process. In addition, uncoupled HRT/SRT could influence nutritional dynamics and biomass composition. This can cause biochemical changes in microalgal biomass, affecting the accumulation of valuable biopolymers such as carbohydrates, lipids and, in the case of cyanobacteria, polyhydroxybutyrates (PHBs) (Arcila and Buitrón, 2016; Arias et al., 2018a). All these compounds have obtained an increasing attention due to their potential use as biodiesel substrate and as bioplastics in the case of PHBs. The information of such promising alternative is still insufficient and all the aspects concerning nutrients dynamics in this kind of systems need to be addressed.

In a previous work by Arias et al., (2017), it was demonstrated that nutrients dynamics in a semi-continuous reactor used for a wastewater tertiary treatment played an important role in the biomass composition during a long term study. In that study, the use of digestate from an anaerobic digester diluted with secondary wastewater from a high rate algal pond proved to be suitable for the growth of a selective culture of cyanobacteria. All in all, the present study aims to evaluate the performance of different photo-sequencing batch reactors during

tertiary treatment of digestate diluted with secondary wastewater, comparing the dynamics with a conventional semi-continuous reactor (SC) in terms of wastewater treatment, biomass composition and biopolymers accumulation.

2 Material and methods

2.1 Inoculum

A mixed culture composed by green algae, cyanobacteria, bacteria, protozoa and small metazoa was used as inoculum. It was collected as thickened biomass (100 mL) from a harvesting tank connected to a pilot closed-photobioreactor (30 L) already used as tertiary wastewater treatment (Arias et al., 2017).

2.2 Experimental set-up

Experiments were performed at lab-scale in three photobioreactors consisting of a closed polymethacrylate cylinder with an inner diameter of 11 cm, a total volume of 3 L and a working volume of 2.5 L each. Experiments were carried out during 30 days, and all of them were submitted to light/dark cycles of 12 h each. Illumination during the light phase was supplied by two external halogen lamp (60W) placed at opposite sides of each reactor and providing 220 μmol m^{-2} s^{-1} of light. Reactors were continuously agitated (with the exception of settling periods) with a magnetic stirrer (Selecta, Spain) set at 250 rpm. Temperature was continuously measured by a probe inserted in the PBR (ABRA, Canada) and kept constant at 27 (±2) °C by means of a water jacket around the reactor. pH was continuously monitoring with a pH sensor (HI1001, HANNA, USA) and kept at 8.5 with a

pH controller (HI 8711, HANNA, USA) by the automated addition of HCl 0.1 N or NaOH 0.1 N. A diagram of the process of each reactor is presented in Fig. 1.

Two of the reactors were operated in a sequencing batch operation mode at a HRT of 2 days. One of these photo-sequencing batch reactors (PSBR), named $PSBR_{2\text{-}10}$, was operated at a SRT of 10 days. This means that 0.25 L of mixed liquor were discharged at the end of the dark phase, then the agitation was stopped and biomass was allowed to settle during 30 minutes. After this period, 1 L of the supernatant was withdrawn and then the total volume discharged (1.25 L) was replaced with the same volume of wastewater influent (Fig. 1a). The other sequencing batch reactor (named $PSBR_{2\text{-}5}$) was operated with a SRT of 5 days. Thus, 0.5 L of the mixed liquor were withdrawn at the end of the dark phase before the subsequent settling time of 30 minutes. After the settling period, 0.75 L of the supernatant was withdrawn and then the total volume retired (1.25 L) was replaced with the same volume of wastewater influent (Fig. 1b). The operation of these PSBRs was compared with that of a semi-continuous reactor named $SC_{10\text{-}10}$ (control reactor). This reactor was fed once a day and operated at a HRT and SRT of 10 days. This means that each day at the end of the dark phase, 0.2 L of the mixed liquor were withdrawn and subsequently this volume was replaced by 0.2 L of wastewater influent (Fig. 1c).

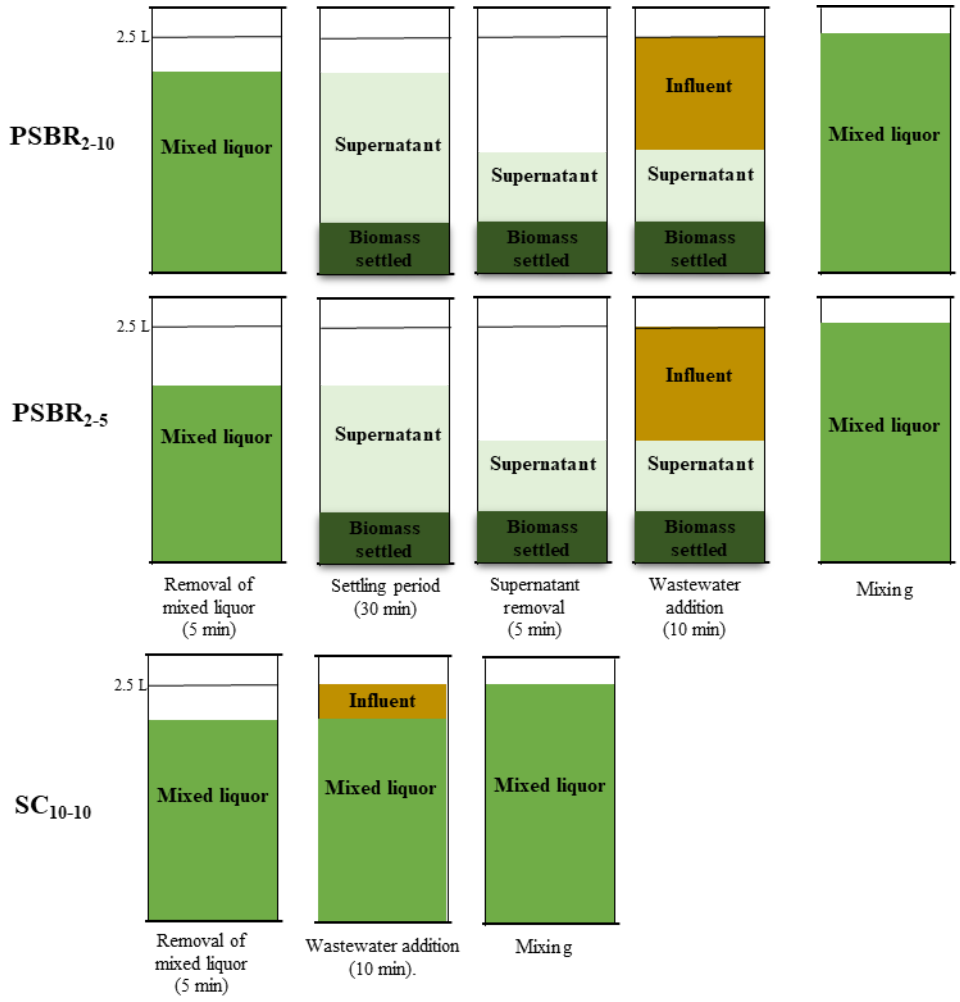

Fig. 1. Daily operation process of $PSBR_{2\text{-}10}$, $PSBR_{2\text{-}5}$ and $SC_{10\text{-}10}$.

The influent treated in the reactors consisted on uncentrifuged digestate diluted in secondary effluent in a ratio of 1:50 (characteristics are shown in Table 1). The secondary effluent was obtained from a pilot system treating municipal wastewater which comprised a primary settler, a high rate algal pond (HRAP) and a secondary settler (Gutiérrez et al., 2016). The digestate was obtained from lab-scale anaerobic digesters (1.5 L) that produced biogas from microalgae biomass harvested from the HRAP. A detailed description of the system may be found in (Arias et al., 2018b). Mixed liquor and supernatant withdrawal, and feeding were performed by the automatic peristaltic pumps.

Table 1. Average (standard deviation) of the main water quality parameters of digestate, secondary effluent and the influent wastewater (constituted by digestate diluted in a ratio 1:50 with secondary effluent) (n=4).

Parameter	Digestate	Secondary effluent	Influent wastewater
pH	-	-	7.1 (0.8)
SST [$g \cdot L^{-1}$]	21.85 (1.80)	-[a]	0.44 (0.04)
SSV [$g \cdot L^{-1}$]	17.90 (2.21)	-[a]	0.36 (0.04)
TC [$mg \cdot L^{-1}$]	20638.50 (1145.00)	38.54 (6.00)	413.23 (23.02)
TOC [$mg \cdot L^{-1}$]	16993.5 (382.30)	18.01 (3.20)	340.23 (7.71)
TIC [$mg \cdot L^{-1}$]	3645.00 (762.70)	20.53 (2.8)	73.31 (15.31)
TN [$mg \cdot L^{-1}$]	4685.41 (678.52)	25.51 (5.98)	83.35 (13.69)
TAN [$mg \cdot L^{-1}$]	1020.45 (233.99)	0.045 (0.00)	20.41 (4.68)
$N\text{-}NO_3^-$ [$mg \cdot L^{-1}$]	<LOD	8.99 (1.24)	8.99 (1.24)
$N\text{-}NO_2^-$ [$mg \cdot L^{-1}$]	<LOD	1.22 (0.29)	1.22 (0.29)
TIN [$mg \cdot L^{-1}$]	1020.45 (306.55)	10.25 (3.45)	30.62 (6.20)
TON [$mg \cdot L^{-1}$]	2644.51 (373.52)	5 (1)	52.99 (7.49)
TP [$mg \cdot L^{-1}$]	402 (115)	3.22 (1.02)	11.26 (1.63)
IP [$mg \cdot L^{-1}$]	<LOD	1.72 (0.13)	1.72 (0.13)
TOP [$mg \cdot L^{-1}$]	402 (115)	1.51 (0.60)	9.54 (2.35)

[a] TSS and VSS in the secondary effluent corresponded to values lower than 0.07 g L^{-1}.

2.3 Analytical methods

2.3.1 Nutrients concentrations

Nutrients monitoring was carried out by analyzing samples taken from the reactors at the end of the dark phase, after settling. All parameters were determined in triplicate and analyzed from the influent (mixed digestate and secondary effluent) and the supernatant of each reactor. Note that in the case of the reactor $SC_{10\text{-}10}$, the supernatant sample was taken from the mixed liquor withdrawn and submitted to a separation process. Samples from the influent were measured once per week, and samples of supernatant were analyzed three days per week.

Nitrogen was measured as total ammoniacal nitrogen (TAN), nitrite ($N\text{-}NO_2^-$), nitrate ($N\text{-}NO_3^-$), total nitrogen (TN) and total phosphorus (TP). TAN (sum of $N\text{-}NH_3$ and $N\text{-}NH_4^+$) was determined using the colorimetric method indicated in Solorzano (1969). $N\text{-}NO_2^-$ and $N\text{-}NO_3^-$ concentrations were analyzed using an ion chromatograph DIONEX ICS1000 (Thermo-scientific, USA), while TN was analyzed by using a C/N analyzer (21005,

Analytikjena, Germany). Total inorganic nitrogen (TIN) was calculated as the sum of N-NO_2^-, N-NO_3^- and TAN. Total organic nitrogen (TON) (in dissolved and particulate form) was calculated as the difference between TN and TIN.

Phosphorus compounds analyzed were inorganic phosphorus (IP) measured as orthophosphate (dissolved reactive phosphorus) (P-PO_4^{3-}) and total phosphorus (TP). IP concentrations were analyzed using an ion chromatograph DIONEX ICS1000 (Thermo-scientific, USA) and total phosphorus (TP) was analyzed following the methodology described in Standard Methods (APHA-AWWA-WPCF, 2001). Total organic phosphorus (TOP) forms (dissolved and particulate) were calculated as the difference between TP and IP.

Total organic carbon (TOC), Total inorganic carbon (TIC), soluble organic carbon (OC) and soluble inorganic carbon (IC) were measured from raw and filtered samples using a C/N analyzer (21005, Analytikjena, Germany).

The volumetric load (Lv-X) of each nutrient (TOC, TIC, TAN, NO_2^-, N-NO_3^-, TIN, TON, TN, IP, TOP and TP) was calculated in [mg X $L^{-1}d^{-1}$] as shown in eq. 1:

(1)

Where Q is the flow [$L^{-1}d^{-1}$], X is the nutrient influent concentration [mg X L^{-1}] and V [L^{-1}] is the volume of the reactor.

2.3.2 Biomass concentration

Total suspended solids (TSS) and volatile suspended solids (VSS) were measured in the mixed liquor at the end of the dark phase three days per week. In $PSBR_{2\text{-}10}$ and $PSBR_{2\text{-}5}$, two samples were taken; one from the mixed liquor right before stopping the agitation in order to evaluate the biomass production, and one from the supernatant after the sedimentation to evaluate the biomass settleability. Chlorophyll *a* was analyzed twice per week in the mixed liquor. Both analytical procedures were performed using the methodology described in Standard Methods (APHA-AWWA-WPCF, 2001).

Biomass production of each reactor in [g VSS $L^{-1}d^{-1}$] was estimated as follows:

(2)

where Q is the flow [$L^{-1}d^{-1}$], VSS is the biomass concentration in the reactor [g L^{-1}] and V [L^{-1}] is the volume of the reactor.

Settleability [%] was determinate according to the following formula:

(3)

Where TSS_m [mg L^{-1}] is the mixed liquor suspended solids concentration and TSS_s [mg L^{-1}] is the supernatant suspended solids concentration.

Microalgae composition was qualitatively evaluated by means of microscope observations twice per week using an optic microscope (Motic, China) equipped with a camera (Fi2, Nikon, Japan) connected to a computer (software NIS-Element viewer®). Cyanobacteria and microalgae species were identified *in vivo* using conventional taxonomic books

(Bourrelly, 1985; Palmer, 1962), as well as a database of Cyanobacteria genus (Komárek and Hauer, 2013).

2.3.3 Biopolymers quantification

Carbohydrates and polyhydroxybutyrates (PHB) content were measured twice per week in the biomass sampled from each reactor at the end the dark phase and before the settling period. Then, 50 mL of mixed liquor were collected and centrifuged (4200 rpm,10 min), frozen at −80 °C overnight in an ultra-freezer (Arctiko, Denmark) and finally freeze-dried for 24 h in a lyophilizer (−110 °C, 0.049 hPa) (Scanvac, Denmark). PHB and carbohydrates extraction and quantification was performing the methodology described in Arias et al. (2018a).

3. Results and discussion

3.1 Nutrients dynamics and removal efficiency

Due to the different HRT, nutrients volumetric load applied to $PSBR_{2\text{-}10}$ and $PSBR_{2\text{-}5}$ was five times higher than the load applied to $SC_{10\text{-}10}$ (Table 2). Furthermore, it is noticeable that the organic forms of nitrogen and phosphorus (TON and TOP) provided by the digestate (Table 1) were the main sources of nutrients. This fact influenced the TN and TP uptake and removals efficiencies.

Table 2. Nutrients volumetric load (Lv) in each reactor according to the hydraulic retention time (n=4).

Parameter	$SC_{10\text{-}10}$[a]	$PSBR_{2\text{-}10}$[b]	$PSBR_{2\text{-}5}$[c]
Lv-TC [$mg \cdot L^{-1} \cdot d^{-1}$]	41.35 (2.3)	186.10 (9.36)	186.10 (9.36)
Lv-TOC [$mg \cdot L^{-1} \cdot d^{-1}$]	34.02 (0.77)	153.11 (3.47)	153.11 (3.47)
Lv-TIC [$mg \cdot L^{-1} \cdot d^{-1}$]	7.33 (1.53)	32.99 (6.89)	32.99 (6.89)
Lv-TN [$mg \cdot L^{-1} \cdot d^{-1}$]	8.65 (1.99)	37.60 (8.95)	37.60 (8.95)
Lv-TAN [$mg \cdot L^{-1} \cdot d^{-1}$]	2.04 (0.47)	9.18 (2.10)	9.18 (2.10)
Lv-N-NO_3^- [$mg \cdot L^{-1} \cdot d^{-1}$]	0.90 (0.12)	4.04 (0.55)	4.04 (0.55)

Lv-N-NO_2^- [mg·L^{-1}·d^{-1}]	0.12 (0.03)	0.55 (0.13)	0.55 (0.13)
Lv-TIN [mg·L^{-1}·d^{-1}]	3.06 (0.62)	13.77 (2.79)	13.77 (2.79)
Lv-TON [mg·L^{-1}·d^{-1}]	5.29 (0.75)	23.82 (3.37)	23.82 (3.37)
Lv-TP [mg·L^{-1}·d^{-1}]	1.13 (0.16)	5.63 (0.82)	5.63 (0.82)
Lv-IP [mg·L^{-1}·d^{-1}]	0.17 (0.01)	0.86 (0.07)	0.86 (0.07)
Lv-TOP [mg·L^{-1}·d^{-1}]	0.95 (0.24)	4.77 (1.18)	4.77 (1.18)

[a]Reactor operated at a coupled HRT and SRT of 10 d.
[b]Reactor operated at an uncoupled HRT of 2 days and SRT of 10 d.
[c]Reactor operated at an uncoupled HRT of 2 days and SRT of 5 d.

As it can be observed in Fig. 2, TN in the effluent (without the biomass) showed similar concentrations in the three reactors. However, when comparing the semi-continuous reactor with the sequencing batch it is noticeable that the best performance in terms of nutrients assimilation and removal was reached by the sequencing batch operation ($PSBR_{2\text{-}10}$ and $PSBR_{2\text{-}5}$). Indeed, considering the higher load applied to the sequencing batch reactors (Fig. 2b and 2c), these showed a higher removal rates of TN (>29 mg L^{-1} d^{-1}) than semi-continuous reactor (6.70 mg L^{-1} d^{-1}) (Fig. 2a). It is important to remark that Lv-TN was constituted by 63% of TON and 37% of TIN (Table 1). Since it is impossible for microalgae to uptake organic nitrogen, TON should have been mineralized to TAN before being consumed by microalgae (Pehlivanoglu and Sedlak, 2004). As observed in the three reactors, TON was almost totally transformed, whereas TAN presented a high variability during the experimental period, ranging from 0 to 13.45 mg L^{-1} (Fig. 2). This suggests that high concentrations of TAN could be derived from the mineralization of TON. Regarding N-NO_3^-, it can be seen that the three reactors showed similar concentrations during the experiment (around 12 mg L^{-1}) (Table 3). In this case, similar concentrations in N-NO_3^- were indicative of a higher removal. On the contrary, N-NO_2^- showed higher values in the reactors than in the influent, (3.84±3.33 mg L^{-1} in $SC_{10\text{-}10}$, 6.08±4.52 in $PSBR_{2\text{-}10}$ and

6.63±4.28 mg L^{-1} in $PSBR_{2-5}$), suggesting the inhibition of the nitrification process in the three reactors (Pollice et al., 2002).

Within the wastewater treatment context, due to the similar TN concentrations in the three reactors (Table 3), similar removal percentages were obtained (Table 4). Furthermore, higher removals were observed in TAN (>80%) and TON (99%), while $N\text{-}NO_2^-$ and $N\text{-}NO_3^-$ were not removed in any reactor. Despite such similarities in the general performance, the removal rates achieved in the two PSBRs for TN, TAN and TON were more than 4 times higher than those of the SC reactor (Table 4).

Table 3. Average (standard deviation) of the main nutrients concentrations of the supernatant of $SC_{10\text{-}10}$, $PSBR_{2\text{-}10}$ and $PSBR_{2\text{-}5}$ during the experiment (n=9-15).

Parameter	$SC_{10\text{-}10}$[a]	$PSBR_{2\text{-}10}$[b]	$PSBR_{2\text{-}5}$[c]
	Average	Average	Average
IC [mg·L^{-1}]	28.61 (23.69)	39.60 (18.99)	47.47 (20.77)
OC [mg·L^{-1}]	47.41 (9.80)	54.50 (23.46)	49.84 (10.76)
TN [mg·L^{-1}]	21.66 (7.12)	23.59 (6.89)	21.92 (4.96)
TAN [mg·L^{-1}]	4.10 (5.08)	3.71 (4.19)	2.82 (3.25)
$N\text{-}NO_2^-$[mg·L^{-1}]	3.85 (3.33)	6.08 (4.52)	6.63 (4.28)
$N\text{-}NO_3^-$[mg·L^{-1}]	13.53 (4.78)	12.33 (3.43)	12.12 (4.48)
TIN [mg·L^{-1}]	21.47 (7.20)	22.12 (8.07)	21.57 (5.62)
TON [mg·L^{-1}]	0.19 (0.63)	1.47 (2.93)	0.035 (1.17)
TP [mg·L^{-1}]	10.88 (2.89)	14.63 (5.71)	9.33 (6.69)
IP[mg·L^{-1}]	1.37 (1.05)	1.13 (1.41)	2.90 (2.90)
TOP [mg·L^{-1}]	6.89 (8.48)	13.5 (4.30)	6.43 (6.61)

[a]Reactor operated at a coupled HRT and SRT of 10 d.
[b]Reactor operated at an uncoupled HRT of 2 days and SRT of 10 d.
[c]Reactor operated at an uncoupled HRT of 2 days and SRT of 5 d.

Table 4. Nutrients removal performances and removal rate of the effluent of the three reactors during the experiment (n=9-15).

	$SC_{10\text{-}10}$[a]		$PSBR_{2\text{-}10}$[b]		$PSBR_{2\text{-}5}$[c]	
Parameter	Removal percentage [%]	Removal rate [mg·L^{-1}·d^{-1}]	Removal percentage [%]	Removal rate [mg·L^{-1}·d^{-1}]	Removal percentage [%]	Removal rate [mg·L^{-1}·d^{-1}]
TOC	86	29.32	84	128.78	85	130.81
TIC	53	3.91	40	13.13	35	11.63
TN	64	6.70	63	29.82	63	29.74

TAN	80	1.63	82	7.51	86	7.91
$N\text{-}NO_3^-$	-	-	-	-	-	-
$N\text{-}NO_2^-$	-	-	-	-	-	-
TIN	32	0.98	30	4.10	29	4.02
TON	99	5.29	99	23.58	99	23.58
TP	27	0.31	-	-	17	0.96
IP	20	0.03	34	0.29	-	-
TOP	29	0.27	-	-	33	1.56

[a]Reactor operated at a coupled HRT and SRT of 10 d.
[b]Reactor operated at an uncoupled HRT of 2 days and SRT of 10 d.
[c]Reactor operated at an uncoupled HRT of 2 days and SRT of 5 d.

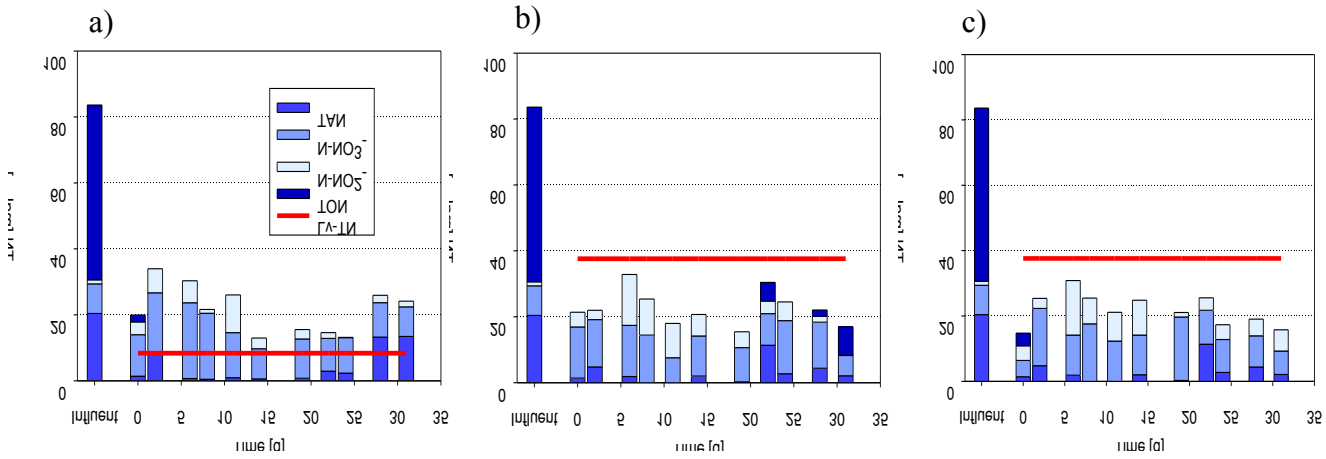

Fig. 2. Average influent and effluent TN concentrations during the experiment in a) $SC_{10\text{-}10}$, b) $PSBR_{2\text{-}10}$ and c) $PSBR_{2\text{-}5}$. The average Lv-TN is presented in mg L^{-1} d^{-1}.

On the other hand, TP in the effluent showed different patterns than those observed for TN. In general, the best performance was obtained in the semi-continuous reactor ($SC_{10\text{-}10}$) where the Lv-TP was very low (1.13±0.16 mg L^{-1} d^{-1}) and was removed at a rate of 0.30 mg L^{-1} d^{-1}. TP concentration in $PSBR_{2\text{-}10}$ showed an increasing pattern along the experimental time and values up to 15 mg L^{-1} were reached in the last week of operation (Fig. 3b). In the case of $PSBR_{2\text{-}5}$, concentrations of TP remained higher than 10 mg L^{-1} and then decreased to 6 mg L^{-1} in the two last weeks of operation (Fig. 3c).

These patterns in the three reactors depended on the mineralization of TOP in all the reactors (Fig. 3). As for TON, microalgae are also unable to uptake organic phosphorus, then it was necessary that a mineralization process took place to transform it to inorganic phosphorus species (Donald et al., 2017; Rodríguez and Fraga, 1999). High IP concentrations observed in the last two weeks in $SC_{10\text{-}10}$ and in the $PSBR_{2\text{-}5}$ indicated that TOP transformed to IP was not consumed. A better mineralization of TOP was observed in $PSBR_{2\text{-}5}$ even though both PSBRs received the same Lv-TP. This result could be related to the SRT of the reactors, since the best mineralization of TOP was obtained in the reactor operating at 5 days. It is known that the mineralization process is microorganism dependent (Rodríguez and Fraga, 1999), meaning that microalgae and bacteria growing under lower SRT conditions were able to consume more P than microorganisms growing in a SRT of 10 days.

Regarding wastewater treatment, the best TP removal efficiencies were achieved in $SC_{10\text{-}10}$ and $PSBR_{2\text{-}5}$ (Table 4). $PSBR_{2\text{-}5}$ showed a removal rate of TP of 1.56 mg L^{-1} d^{-1}, which is six times higher the removal rate of $SC_{10\text{-}10}$. Due to the increase in TOP concentration in $PSBR_{2\text{-}10}$, no net removal was observed.

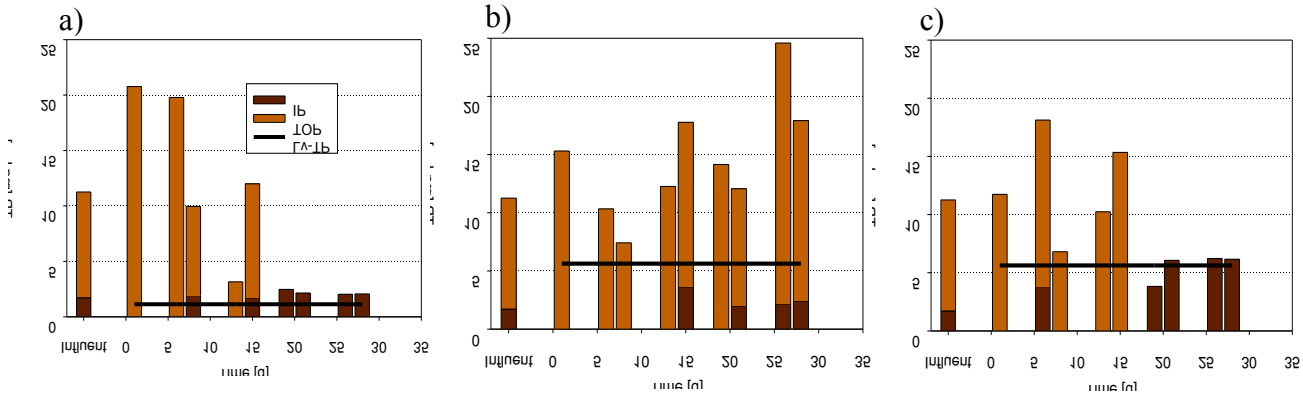

Fig. 3. Average influent and effluent TP concentration during the experiment in a) $SC_{10\text{-}10}$, b) $PSBR_{2\text{-}10}$ and c) $PSBR_{2\text{-}5}$. The average Lv-TP is presented in mg L^{-1} d^{-1}.

Regarding the uptake of the different carbon species, although the effluent in three reactors averaged similar concentrations along the experimental time (Table 3), they showed differences in the removals regarding the Lv-TOC (Fig. 4). Due to the similar concentrations of TOC in the three reactors, removal efficiencies were similar (85±1%) regarding the influent wastewater total content. However, removal rates in both PSBRs (128.78 mg L^{-1} d^{-1} for $PSBR_{2\text{-}10}$ and 130.81 mg L^{-1} d^{-1} for $PSBR_{2\text{-}5}$) were 4 times higher than that of the semi-continuous reactor (29.32 mg L^{-1} d^{-1}) (Table 4). Similarly, $PSBR_{2\text{-}10}$ and $PSBR_{2\text{-}5}$ also reached up to three times higher removal rates of TIC (Table 4).

Given the results obtained, it is clear that the treatment efficiency of both $PSBR_{2\text{-}10}$ and $PSBR_{2\text{-}10}$ is high enough to become a feasible alternative to treat uncentrifugued digestate diluted with secondary effluent in microalgal wastewater treatment systems. According to the removal rates obtained, both PSBRs achieved the highest removals of TN, TOC and TIC, and TP (with the exception of TP in $PSBR_{2\text{-}10}$). According to the higher transformation of TOP to IP in $SC_{10\text{-}10}$, the increase of the HRT in the PSBRs could be a good strategy to achieve better removal efficiencies. In the case of other nutrients and organic matter removal, it was demonstrated that the PSBRs showed a better performance in relation to the load applied. Moreover, such systems have the advantage that higher wastewater volumes can be treated per day.

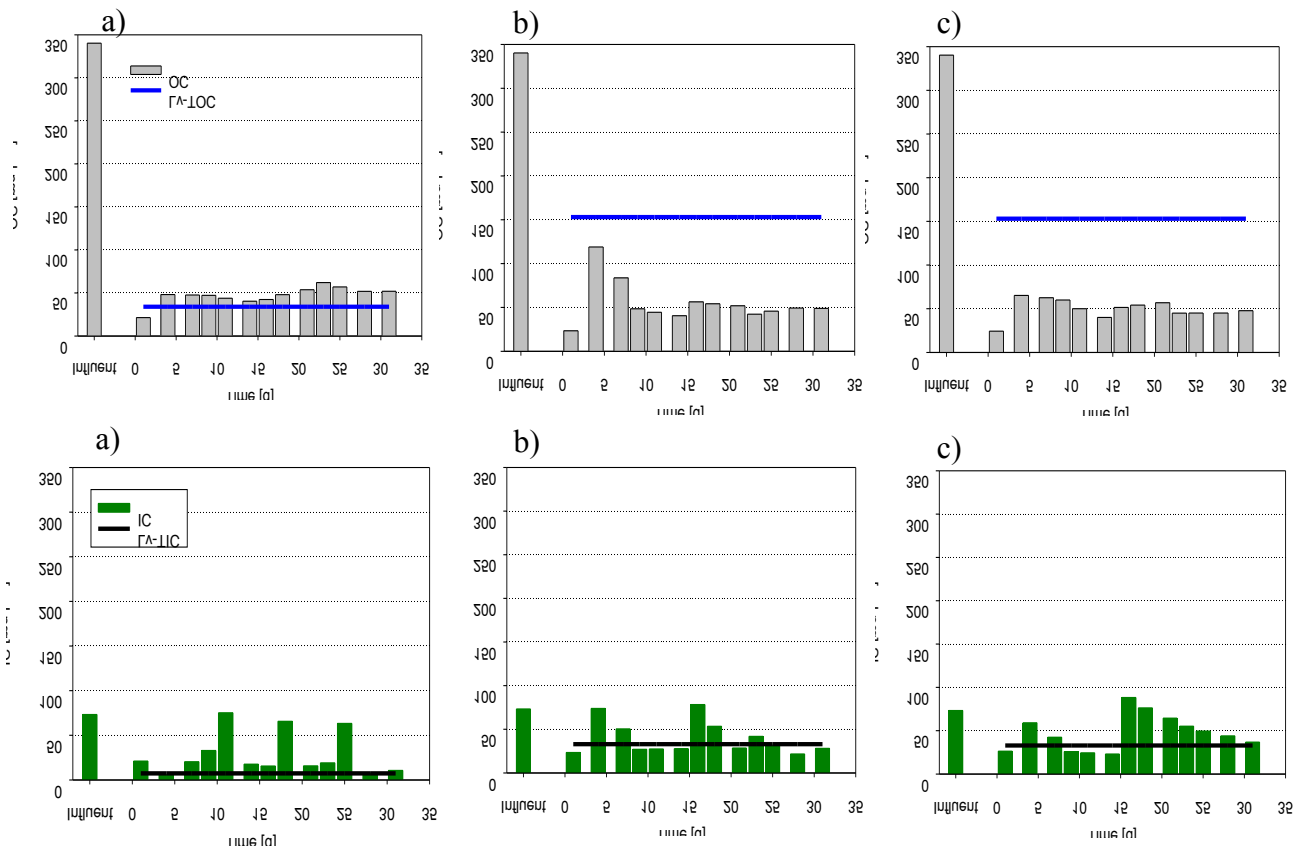

Fig. 4. Average TOC and TIC influent and effluent OC and IC concentration during the experiment in a) SC_{10-10}, b) $PSBR_{2-10}$ and c) $PSBR_{2-5}$. The average Lv-TOC/TIC is presented as mg L^{-1} d^{-1}.

3.2 Biomass production

Regarding the concentration of biomass, all the reactors showed an exponential increase during the first two weeks of operation. In the SC_{10-10} reactor, it increased from an initial concentration of 0.207±0.081 mg L^{-1} to 0.451 mg L^{-1} in day 15, and after that a constant biomass concentration of approximately 0.420 mg L^{-1} was maintained. $PSBR_{2-10}$ showed an increasing pattern until day 27, achieving then the highest concentration of 0.910 mg L^{-1}. In $PSBR_{2-5}$, the concentration increased from 0.207 mg L^{-1} to 0.652 mg L^{-1} on day 13 and then it decreased and oscillated between 0.434 and 0.586 mg L^{-1} during the rest of the experiment. Regarding the chlorophyll *a* content, it remained constant in SC_{10-10} and $PSBR_{2-5}$

during the experiment (0.597±0.091 and 0.829±0.279 mg L^{-1}, respectively) (Fig. 5), while $PSBR_{2\text{-}10}$ showed and increase from the initial concentration of 0.633 mg L^{-1} to 2.82 mg L^{-1} on the day 30.

In spite of the clear increase patterns registered in the biomass concentration, the highest biomass production was achieved in $PSBR_{2\text{-}5}$ (Fig. 5), due to the highest volume withdrawn. Thus, the biomass production reached by this reactor was 0.135 mg L^{-1} d^{-1} in day 15, and similarly to biomass concentration, it decreased during the following days, maintaining a quite constant production of approximately 0.11 g VSS $L^{-1} \cdot d^{-1}$. Despite that a lower mixed liquor volume was extracted in $PSBR_{2\text{-}10}$, the biomass production achieved was similar to that reached in $PSBR_{2\text{-}5}$ on day 27. On the contrary, the biomass production in $SC_{10\text{-}10}$ only increased from 0.021 to 0.04 g L^{-1} d^{-1} on day 10, maintaining similar values from that point on.

Fig. 5. Time course of biomass production and chlorophyll *a* content.

According to the microscopic monitoring, the microbial composition in $SC_{10\text{-}10}$ was similar during the whole experiment (Fig. 6). The biomass was composed mostly by microalgal mixed flocs containing diatoms, unicellular cyanobacteria cf. *Aphanocapsa* sp., green algae

species as *Chlorella* sp. and dispersed *Scenedesmus* sp., and rotifers protozoa. Bacterial colonies were also observed mostly during the last ten days of operation.

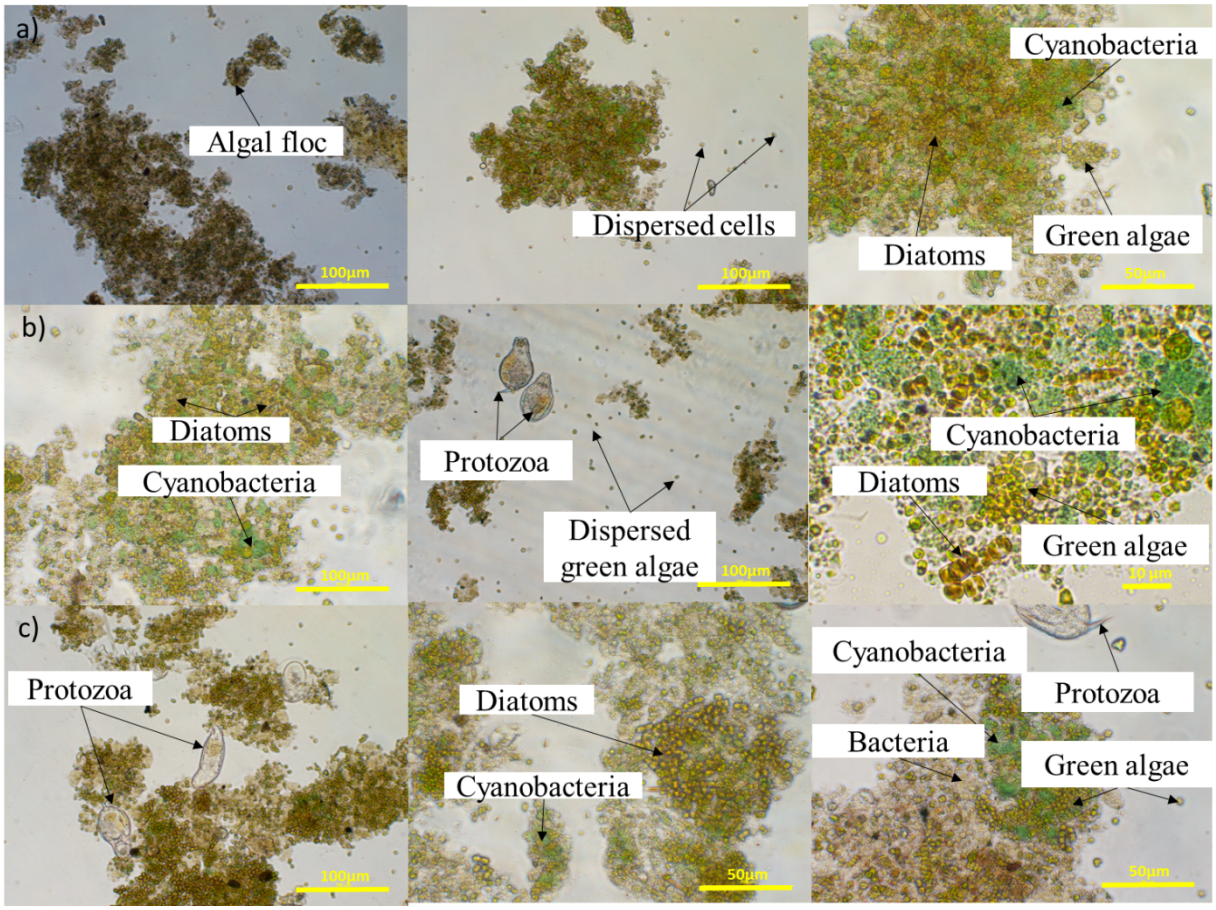

Fig. 6. Microscopic images illustrating microbial composition in $SC_{10\text{-}10}$ during the periods; a) days 1-10, b) days 11-20 and c) days 21-30.

In $PSBR_{2\text{-}10}$, a culture with the same composition observed in $SC_{10\text{-}10}$ was observed, with mixed flocs composed by green algae, some cyanobacteria and the presence of diatoms. However, microbial composition in the following days showed an increasing presence of bacterial colonies (Fig. 7). Contrary to the $SC_{10\text{-}10}$ reactor, green algae *Chlorella* sp increased in $PSBR_{2\text{-}10}$, whereas dispersed cells of *Scenedesmus* sp. were not observed. Protozoa species as *Vorticella* sp. were frequently visualized.

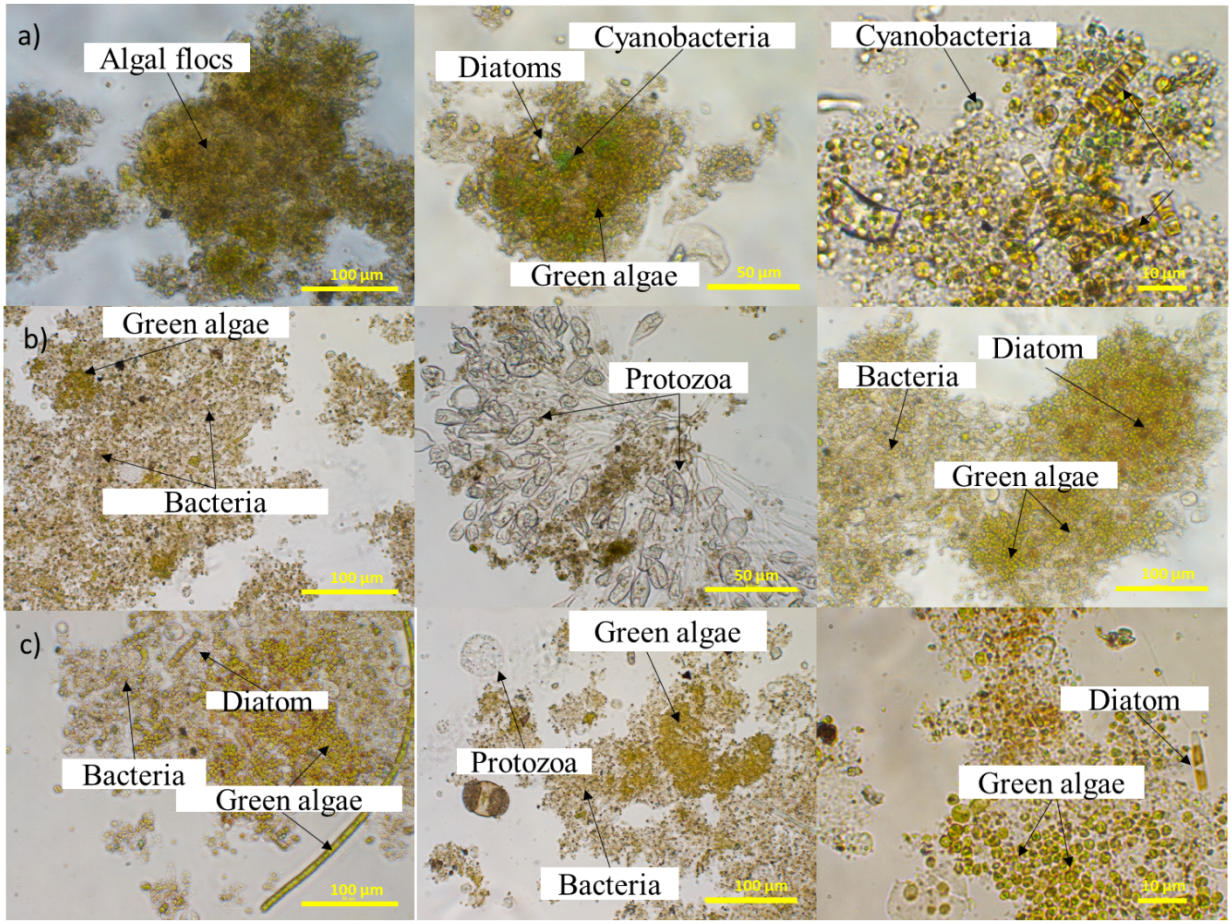

Fig. 7. Microscopic images illustrating microbial composition in $PSBR_{2\text{-}10}$ during the periods; a) 1-10 days, b) 11-20 days and c) 21-30 days.

On the other hand, $PSBR_{2\text{-}5}$ showed a different microbial evolution compared to the other reactors. As observed in Fig. 8, algal flocs were rarely observed, whereas bacterial flocs were observed from the first days of operation onwards. In this reactor, the green algae present in the culture belonged to species of *Chlorella* sp. and *Stigeoclonium* sp. Other species of protozoa, cyanobacteria and diatoms were rarely observed in the culture.

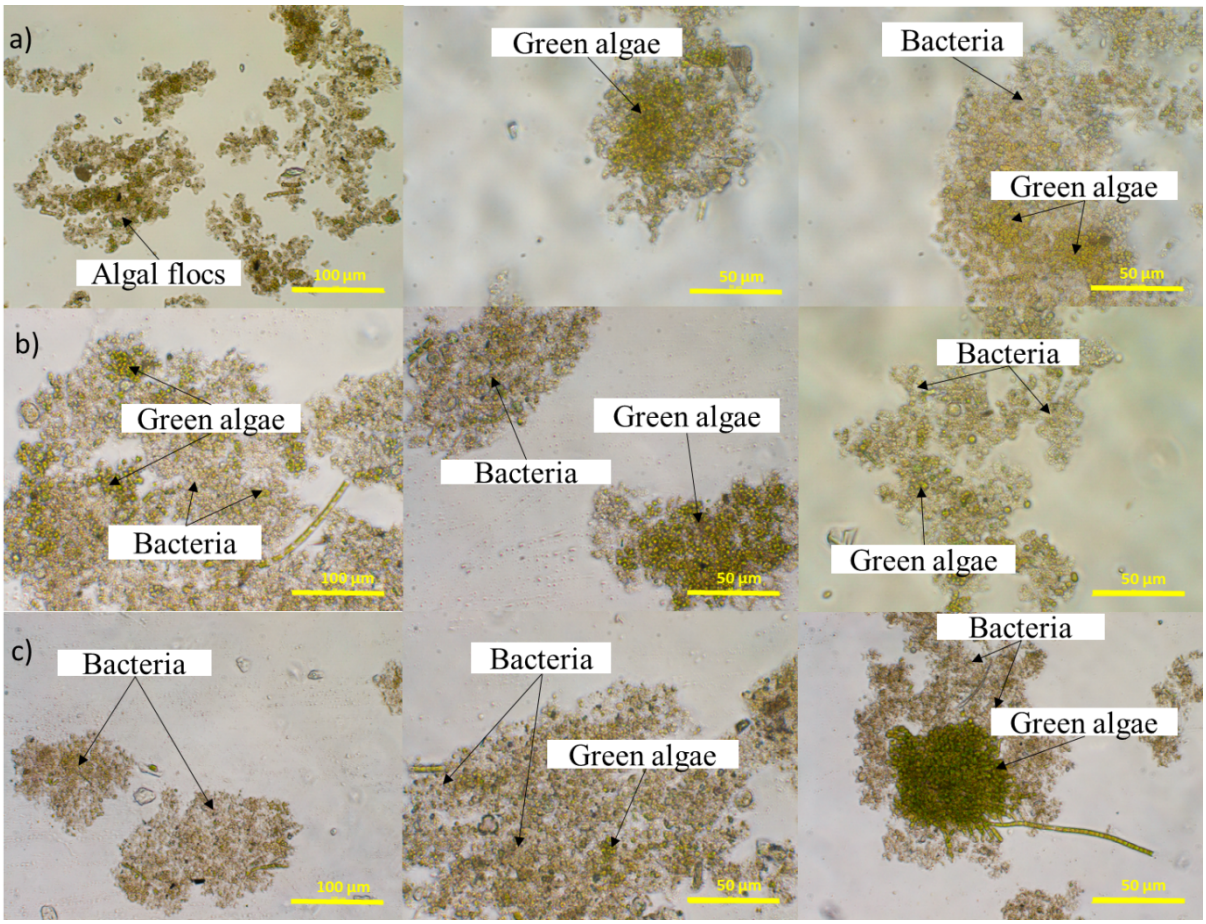

Fig. 8. Microscopic images illustrating microbial composition in $PSBR_{2\text{-}5}$ during the periods; a) days 1-10, b) days 11-20 and c) days 21-30.

In addition to the lack of dispersed cells observed by microscopy in $PSBR_{2\text{-}10}$ and $PSBR_{2\text{-}5}$, the concentration of SSV in the supernatant was 0.075±0.021 mg L^{-1} and 0.072±0.003 mg L^{-1}, respectively, from the first days of operation and remained quite constant during the experimental time. Such values implied a settleability of 86 to 92%. When comparing the biomass composition of the three reactors, it is clear that the strategy of operating $PSBR_{2\text{-}10}$ and $PSBR_{2\text{-}5}$ applying uncoupled SRT and HRT improved the capacity of the microorganisms to form flocs and perform a fast settling process, which are good results regarding the achievement of a feasible the harvesting process.

Considering these systems for biomass production, this study demonstrated that microbial composition could be controlled by nutrients loads and, at the same time, influenced by the SRT. For instance, it was shown that protozoa and diatoms can survive in a wide range of nutrients loads since their presence was observed in either low loads ($SC_{10\text{-}10}$) or high loads ($PSBR_{2\text{-}10}$). However, their presence was conditioned by long SRT (10 days), as it is usually observed in this type of systems (Shariati et al., 2011). On the other hand, the fact that the presence of cyanobacteria occurred in $SC_{10\text{-}10}$ (low loads) but not in $PSBR_{2\text{-}10}$ (high loads), even if they operated at the same SRT, showed that nutritional conditions highly affect cyanobacteria presence. It would be important to improve the competition capacity of these species in microalgae-based wastewater treatments, since they are potential PHB and carbohydrates producers.

Another important result to consider is that bacterial presence increased more in $PSBR_{2\text{-}10}$ and $PSBR_{2\text{-}5}$ than $SC_{10\text{-}10}$. This suggests that the introduction of high loads of nutrients, especially TOC in the PSBRs, promoted the growth of heterotrophic bacteria. Another important fact to consider is that the influent used in this experiment (secondary effluent and digestate) contained high TOC:TIC ratio (4.64). In a previous study of Van Den Hende et al., (2014), it was shown that TOC:TIC ratios higher than 2.39 improved heterotrophic bacteria domination in PSBRs operated at 2 days of HRT. The semi-continuous reactor (using the same influent but a lower nutrient load) showed a dominance of microalgae, confirming that the load applied to the reactors played an important role in the microbial community composition.

It is important to highlight that although microalgae based wastewater treatments similar to the ones described in this study, have been successfully used for the treatment of digestate

from different sources, the majority of the studies until now have employed batch or semi-continuous operation (Cañizares-Villanueva et al., 1994; Pouliot et al., 1989; Ruiz-Marin et al., 2010; Sepúlveda et al., 2015; Uggetti et al., 2014; Viruela et al., 2016). However, such operations presented limitation in nutrients removal rates, biomass production and the possibility to produce an easily settling culture. The strategy of using sequencing batch operation of photobioreactors for digestate removal is still limited to only a few studies. In the study of Van Den Hende et al., (2014), a 4 L PSBR operated at an HRT of 2 d to treat manure digestate was used. The authors obtained removal rates of TN and TP of 4.5 mg L^{-1} d^{-1} and 0.11 mg L^{-1} d^{-1}, respectively, producing 0.068 g L^{-1} d^{-1} of biomass. Remarkably, the results of $PSBR_{2\text{-}5}$ in this study reached higher removals rates (29.82 mg L^{-1} d^{-1} and 1.05 mg L^{-1} d^{-1} of TN and TP were removed, respectively) and, at the same time, a higher biomass production was achieved (0.11 g L^{-1} d^{-1}). On the other hand, the removal rate of TN of the present study was lower than that obtained by Wang et al., (2015), who used a 8 L PSBR operated at an HRT of 4 d to remove of diluted digestate. These authors obtained a removal rate of 71 mg L^{-1} d^{-1} of TN, applying nitrification and denitrification strategies in the PSBR, and at the same time producing 0.15 g L^{-1} d^{-1} of biomass.

3.3 Biopolymers accumulation

As mentioned in Section 3.1, none of the reactors presented nutrients limitation along the experiment, which influenced the low biopolymers accumulation observed in the cultures. Regarding carbohydrates content, only a low content was achieved. Hence, $SC_{10\text{-}10}$ reached 11.18±1.76 % VS^{-1}, while $PSBR_{2\text{-}10}$ and $PSBR_{2\text{-}5}$ achieved 11.47±2.78 and 9.90±2.60 % VS^{-1}, respectively. Despite the fact that the three reactors showed similar percentages, different concentrations were achieved considering the biomass concentrations: the highest

concentration (128.60±13.69 mg L^{-1}) was obtained in $PSBR_{2-10}$, while $PSBR_{2-10}$ and $PSBR_{2-5}$ maintained a constant concentration of 53.11±10.04 mg L^{-1} (Fig. 9). The accumulation of carbohydrates reached in this study was lower than that obtained by Arcila and Buitrón (2016) in a HRAP of 50 L operated at coupled HRT and SRT of 2d, 6d and 10d (14%, 16% and 22%, respectively). It is important to remark that this study and the study of Arcila and Buitrón (2016), although performed at different scales, were conducted in absence of nutrients limitation, which is an important factor limiting the accumulation of carbohydrates (De Philippis et al., 1992; Markou et al., 2013). Regarding PHB accumulation, it was <0.5% PHB VS^{-1} in all the reactors during the experimental time. The fact that PHB was not accumulated was caused by the lack of cyanobacteria in the cultures. As already explained in Section 3.2, reactors were mostly composed by green algae, which do not accumulate PHB. Thus, the low concentration values of this polymer were expected. Furthermore, the accumulation of this polymer is favoured under starvation conditions of nitrogen or phosphorus (Arias et al., 2018b; Samantaray et al., 2011), which did not take place during the experiments presented. These two facts strongly influenced the poor accumulation of this polymer in this study.

Fig. 9. Time course of carbohydrates concentration.

4. Conclusions

In this study, nutrients removal and biomass growth were analyzed in photosynthetic sequencing batch reactors (PSBR) treating digestate diluted with secondary effluent. Two PSBRs were operated at hydraulic retention time (HRT) of 2 days and solids retention time (SRT) of 10 and 5 days, comparing the results obtained with those of a semi-continuous (SC) reactor operating at HRT and SRT of 10 days. PSBR showed removals rates of 30 mg L^{-1} d^{-1} of total nitrogen and up to 1 mg L^{-1} d^{-1} of total phosphorus. Concerning inorganic carbon and organic carbon uptake, PSBRs achieved removal rates of 128-130 mg TOC L^{-1} d^{-1} and 12-13 mg TIC L^{-1} d^{-1}. These results were in general 1-5 times higher than the removal rates obtained in the semi-continuous reactor. PSBRs were able to produce biomass up to 0.09 g L^{-1} d^{-1}, more than two fold the biomass produced by SC, obtaining also a biomass settleability of 86-92%. Furthermore, this study demonstrated that microbial composition could be controlled by nutrients loads, since the three reactors were dominated by different species depending on the nutrients concentrations. Concerning biopolymers

accumulation, carbohydrates achieved similar values in the three reactors (11%), whereas, in contrast, <0.5 % of polyhydrohybutyrates (PHB) was produced. These low values in biopolymers production could be related to the lack of cyanobacteria in the cultures, as they are the microorganism accumulating carbohydrates/PHB. Future studies will focus on the determination of nutrients load strategies to select appropriated microorganisms and at the same time enhance biopolymers accumulation.

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