# Surfactant removal and biomass production in a microalgal-bacterial process: effect of feeding regime

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## ABSTRACT

The influence of the feeding regime on surfactant and nutrient removal and biomass production was evaluated in three high rate algal ponds for primary domestic wastewater treatment. Feeding times of 24, 12 and 0.1 h $d^{-1}$ were studied in each reactor at a similar hydraulic retention time of 7.0 days and organic load of 2.3 mg $m^{-2}$ $d^{-1}$. Semi-continuous feeding at 12 and 0.1 h $d^{-1}$ showed better microalgal biomass production (0.21–0.23 g $L^{-1}$) and nutrient removal, including nitrogen (74–76%) and phosphorus (80–86%), when compared to biomass production (0.13 g $L^{-1}$) and nitrogen (69%) and phosphorus (46%) removals obtained at continuous feeding (24 h $d^{-1}$). Additionally, the removal efficiency of surfactant in the three reactors ranged between 90 and 97%, where the best result was obtained at 0.1 h $d^{-1}$, resulting in surfactant concentrations in the treated effluent (0.3 mg $L^{-1}$) below the maximum freshwater discharge limits.


**Key words** | anionic surfactant, biodegradation rate, domestic wastewater, *Scenedesmus* sp.

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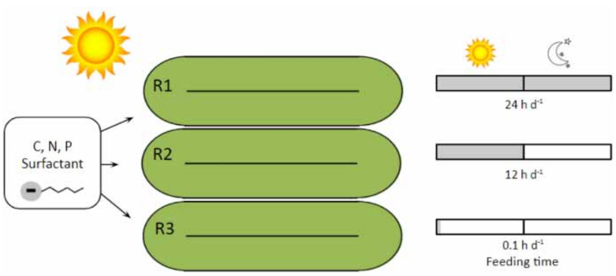
## HIGHLIGHTS

- The surfactant removal efficiency in the three reactors ranged between 90 and 97%.
- The best surfactant removal was obtained at 0.1 h $d^{-1}$ semi-continuous feeding.
- Surfactant concentration of 0.3 mg $L^{-1}$ was obtained in the treated effluent.
- Semi-continuous operation in daylight period showed better performance than continuous operation.
- Feeding time at 12 and 0.1 h $d^{-1}$ promoted higher biomass production and nutrients removal.

## GRAPHICAL ABSTRACT

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## INTRODUCTION

According to the World Health Organization (WHO), there are still many countries that treat less than half of the wastewater generated. In Brazil, only 56% of domestic wastewater was treated at secondary level until 2017 (UNICEF & WHO 2019). Furthermore, conventional treatment processes do not completely remove nutrients and emerging contaminants, including surfactants, which as a result are discharged in water bodies continuously, increasing the damage done to aquatic ecosystems. Surfactants comprise a vast number of chemical compounds and are divided into the classes of anionic, cationic, nonionic, and amphoteric surfactants, where anionic surfactants are traditionally the most used surfactants (around 60%) due to their detersive properties and lower costs (Pirsaheb *et al.* 2014; Palmer & Hatley 2018; Siyal *et al.* 2020). On the other hand, cationic surfactants account for about 30% of surfactant use (Siyal *et al.* 2020). The largest volumes of surfactant-containing products come from the cleaning products (detergents and soaps), petroleum and personal care products industries (Nitschke & Pastore 2006). Hence, these compounds are released into the water bodies from wastewater treatment plants (WWTPs) or on agricultural lands from sludge (Scott & Jones 2000), where the potential for environmental risk depends on the concentration and type of surfactant (Lechuga *et al.* 2016). Siyal *et al.* (2020) emphasized that when the length of the alkyl groups increases, the toxicity of the surfactant also increases. The main environmental damage resulting from the discharge of these surfactants in water bodies includes: reduced surface tension of the water, reduced breeding ability of aquatic organisms, and reduced oxygen levels in water bodies as a result of their degradation, among others (Palmer & Hatley 2018). According to Market Wired (2017), about 24.2 million tons of surfactant are expected to be produced in 2022.

Traditionally, technologies based on physical–chemical methods, such as chemical coagulation, electrochemical oxidation and photocatalytic degradation, have been used to remove the surfactants from water (Aboulhassan *et al.* 2006; Palmer & Hatley 2018). However, these technologies have drawbacks, like high operational costs and in some cases the production of hazardous by-products (Palmer & Hatley 2018). On the other hand, biological treatment of surfactants by aerobic microorganisms requires a lot of energy for aeration, while degradation by anaerobic microorganisms has only a limited removal efficiency, of around 40–85%, depending on the type of surfactant (Palmer & Hatley 2018). In contrast, microalgal-bacterial processes in high rate algal pond (HRAP) systems may represent a less energy intensive and more environmentally friendly alternative for an efficient removal of these contaminants. Contextually, this process is based on the cooperative interactions between microalgae and bacteria, with $CO_2$ and $O_2$ exchanges resulting from oxidation of organic matter by bacteria concomitantly with assimilation of $CO_2$ and nutrients by microalgae (Muñoz & Guieysse 2006). Thus, in HRAP systems, mechanical aeration may not be required, while nutrients such as nitrogen and phosphate, responsible for eutrophication, can be concomitantly taken up by the microalgae and removed. Other pollutants can be removed in this process as well, including surfactants. The microorganisms may use the surfactants as an energy or as a nutrient source, by (co-)metabolization (Palmer & Hatley 2018). In this context, surfactant removal by microalgae-based technologies can occur via abiotic (sorption, volatilization or photodegradation) and/or biotic (biodegradation, microalgae uptake or metabolization) mechanisms (Matamoros *et al.* 2015).

Hena *et al.* (2015) show high growth rates of *Scenedesmus* sp., *Chlamydomonas* sp., *Chlorococcum humicola*, *Botryococcus braunii* and *Chlorella* sp. in batch experiments using municipal wastewater with a high anionic surfactant content (51 mg $L^{-1}$), reaching removal efficiencies of above 97.9% in 10 days. More recently, Katam & Bhattacharyya (2018) compared the anionic surfactant removal in a microalgal reactor with that in an activated sludge process: removal efficiencies reached up to 80 and 95%, respectively. In spite of these promising results, the effectiveness of HRAPs for anionic surfactant removal is not yet considered proven technology in the literature. Furthermore, despite the promising results with wastewater treatment obtained in HRAPs with continuous and semi-continuous feeding (de-Bashan *et al.* 2002; Kim *et al.* 2014; Posadas *et al.* 2014, 2015; Beydes & Kapdan 2018; Ruas *et al.* 2018; Salgueiro *et al.* 2018), varying feeding regimes affect pollutant removal and biomass productivity in HRAPs, but the exact effects of different feeding regimes are still barely known, while this knowledge is crucial to understand and optimize the performance of microalgal-bacterial systems.

The objective of this work was thus to evaluate the influence of different feeding regimes (continuous versus semi-continuous) on the removal of anionic surfactants and nutrients, as well as on the biomass production in three identical HRAPs treating primary domestic wastewater (PDW), at identical organic loading rates.

## MATERIALS AND METHODS

### Inoculum

The HRAPs were inoculated with a consortium formed mainly of *Scenedesmus* sp. (≈98%) previously cultivated in outdoor reactors treating domestic wastewater, with a total suspended solids (TSS) concentration of 1.4 g $L^{-1}$. Activated sludge was also inoculated in the HRAPs, and collected from the WWTP in Campo Grande-MS (Brazil), with 4.2 gTSS $L^{-1}$.

### Primary domestic wastewater

PDW was collected from a primary treatment tank of a WWTP located in Campo Grande-MS, Brazil, and stored in a 300 L agitation cooling tank (Implemis, Brazil) at 4 °C prior to feeding into the HRAPs. Influent soluble concentrations of surfactant, chemical oxygen demand (COD), total organic carbon (TOC), inorganic carbon (IC), total organic nitrogen (TN), ammonium ion ($N\text{-}NH_4^+$) and total phosphorus as $P\text{-}PO_4^{3-}$ (TP) in the PDW are summarized in Table 1. All parameters were analysed according to *Standard Methods for the Examination of Water and Wastewater* (APHA *et al.* 2012). Nitrite ($N\text{-}NO_2^-$) and nitrate ($N\text{-}NO_3^-$) concentrations were below detection limits using ion chromatography (see section 'Analytical procedures').

**Table 1** | Physical–chemical characteristics of the primary domestic wastewater during the experiment

| Parameter | Unit | Concentration |
|---|---|---|
| Surfactant | mg $L^{-1}$ | 9.9 ± 0.7 |
| COD | mg $L^{-1}$ | 127 ± 11 |
| TOC | mg $L^{-1}$ | 119 ± 9 |
| IC | mg $L^{-1}$ | 53 ± 11 |
| TN | mg $L^{-1}$ | 66 ± 15 |
| $N\text{-}NH_4^+$ | mg $L^{-1}$ | 20 ± 5 |
| TP | mg $L^{-1}$ | 6.1 ± 0.4 |
| pH | – | 8.0 ± 0.1 |

### Experimental setup

The experimental setup consisted of three polypropylene 21 L HRAPs (R1, R2 and R3), with an illuminated surface of ≈0.13 $m^2$ and 16 cm cultivation broth depths (Figure 1), installed outdoors. One submerged pump with a nominal flow rate of 540 L $h^{-1}$ (Sarlo Better B500, Brazil) was located at the bottom of each reactor, maintaining a liquid recirculation velocity of 20 ± 2 cm $s^{-1}$ in order to promote complete agitation (Ruas *et al.* 2018). Each HRAP was followed by a 1 L sedimentation tank (S1, S2 and S3), with a hydraulic retention time (HRT) of ≈8 ± 0 h.

### Operational conditions

The HRAPs were operated at similar organic loading rate and HRT, of 2.3 ± 0.4 mgCOD $m^{-2}$ $d^{-1}$ and 7.0 ± 0.2 days, respectively, but with different feeding regimes, in order to evaluate the influence of feeding regime on PDW treatment. R1 was fed continuously (24 h $d^{-1}$), whilst R2 and R3 were fed semi-continuously, for 12 and 0.1 h $d^{-1}$, respectively. The feeding of R2 and R3 both started at 9:00 a.m but finished at 9:00 p.m. and 9:06 a.m., respectively. The experiment was conducted at the Effluents Laboratory of the Federal University of Mato Grosso do Sul (Campo Grande-MS, Brazil) for 36 days at a temperature of ≈29 °C.

### Sampling

Two samplings were performed three times a week to elucidate the performance of the HRAPs: in the morning (T1) and afternoon (T2). The first sampling, T1, occurred at 9:00 a.m. in the cultivation broth of R1, R2 and R3, before starting feeding of R2 and R3; and also at 9:06 a.m. in the effluent (E1, E2 and E3) of the settlers, after stopping feeding of R3. The second sampling, T2, occurred at 4:00 p.m., only in the cultivation broths of R1, R2 and R3, after several hours of exposure to sunlight. At T1, samples of 200 mL were taken to determine TSS and soluble concentrations of COD, TOC, IC, TN, $N\text{-}NH_4^+$, $N\text{-}NO_2^-$, $N\text{-}NO_3^-$, TP and anionic surfactant. At T2, samples of 20 mL were drawn to determine the soluble anionic surfactant. Samples of dissolved compounds were obtained from the samples by filtering through 0.45 μm glass fibre filters prior to analysis. The temperature, pH and dissolved oxygen (DO) concentrations were monitored daily at T1 and T2. All parameters were analysed according to *Standard Methods for the Examination of Water and Wastewater* (APHA *et al.* 2012). Furthermore, the

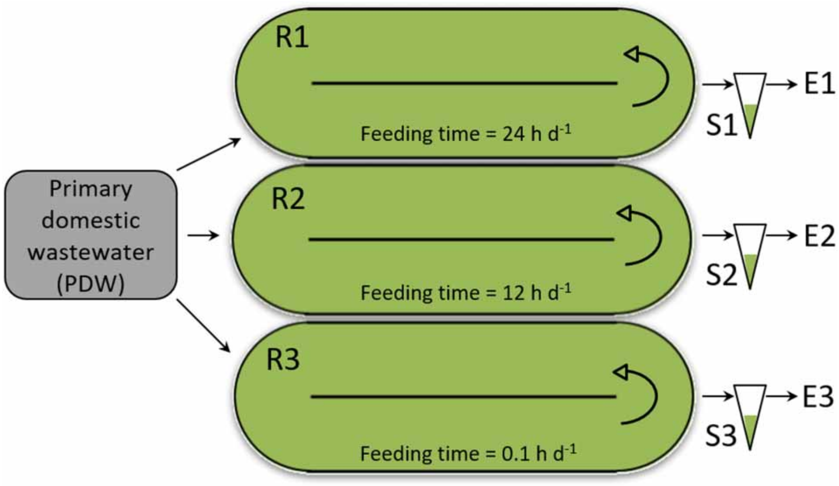


**Figure 1** | Experimental setup of the three 21 L HRAPs for primary domestic wastewater treatment.

daily evaporation rate was determined from the difference between the influent and effluent flow rates.

### Analytical procedures

The TOC, IC and TN were determined using a TOC analyser (Vario TOC Cube, Elementar, Germany). COD was analysed using the closed reflux dichromate and acid digestion method (APHA *et al.* 2012). N-$NH_4^+$ and pH were measured using Orion Dual Star (Thermo Scientific, The Netherlands) ammonia and pH electrodes, respectively, while N-$NO_2^-$, N-$NO_3^-$ and P-$PO_4^{3-}$ were analysed using a Dionex UltiMate ICS 1100 ion chromatography system with IonPac AG19/AS19 column (Thermo Scientific, USA). The anionic surfactant concentration was determined using methyl dodecylbenzene sulphonate reagent in MN Nanocolor® Tube Tests. Temperature and DO were measured using a Jenway 9500 DO2 oximeter (Jenway, UK). The light intensity (photosynthetically active radiation) was recorded with a Quantum meter MQ-200 (Apogee Instruments, USA). The microalgae identification was carried out by microscopic examination (Olympus BX41, USA) of samples fixed with 5% lugol acid and stored at 4 °C prior to analysis.

## RESULTS AND DISCUSSION

### Surfactant removal efficiency

The influent surfactant concentration in this study, of 9.9 ± 0.7 mg $L^{-1}$, was in the same range as in previous studies indicating 2–21 mg $L^{-1}$ (Pirsaheb *et al.* 2014). Surfactant concentrations comparable to the discharge limit in fresh water in Brazil of 0.5 mg $L^{-1}$ (Brasil 2005) were found in all samples from the cultivation broth of R1 and R2, at T1 and T2 (Figure 2(a)), and from the effluent of the settler E1 and E2 (Figure 2(b)). This removal corresponds to about 95% removal, suggesting continuous removal of surfactants from both reactors. Concentrations below the discharge limit were only recorded in R3 (0.3 ± 0.1 mg $L^{-1}$)

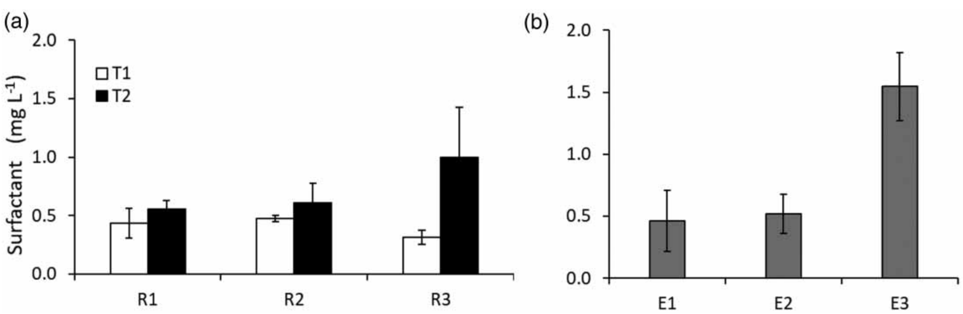


**Figure 2** | Surfactant concentration of (a) the cultivation broth of R1, R2 and R3 at T1 and T2, and (b) the effluent E1, E2 and E3 from the settler.

just before feeding at T1, corresponding to a 97 ± 1% removal. On the other hand, surfactant concentrations reached 1.5 ± 0.3 mg $L^{-1}$ just after feeding (E3) (Figure 2(b)), and reduced to 1.0 ± 0.4 mg $L^{-1}$ after 7 hours of daylight at T2. This result shows that bacteria are responsible for the removal (biodegradation) as even in R1 (continuous feeding) the concentrations at the end of the day are slightly higher than results obtained in the morning. Considering that the treated effluent could be removed from the system before receiving new influent (batch operation), a short feeding time, such as 0.1 h $d^{-1}$, can be a great alternative to remove surfactants and other pollutants in HRAPs. In this sense, an economical viability analysis is crucial to verify the feasibility of this operation, considering for instance the energy used in pumping and the construction costs of treatment and storage units, suggesting a niche for future investigations.

High removal efficiencies of anionic surfactants from municipal wastewater by *Scenedesmus* sp. (97.5%), *Chlamydomonas* sp. (98.0%), *Chlorella* sp. (99.4%), *Chlorococcum humicola* (97.9%) and *Botryococcus braunii* (99.1%) were recorded by Hena *et al.* (2015) in 10 d batch experiments with an initial concentration of 50 mg $L^{-1}$. More recently, Katam and Bhattacharyya (2018) studied the effect of solid retention times (SRTs) (2–12 days) on anionic surfactant removal in a microalgal reactor and an aerobic bacterial reactor. Removal efficiencies reached up to 80 and 95%, respectively, at 10 days SRT. Anionic surfactants were also removed by 98.3% from domestic wastewater in a wetlands wastewater treatment plant (Kruszelnicka *et al.* 2019), while removal in continuous activated sludge systems ranged from 93.7 to 96.7% (initial concentration of ≈16 mg $L^{-1}$) (Pirsaheb *et al.* 2014). On the other hand, Matamoros *et al.* (2015) obtained removal efficiencies of a non-ionic surfactant in HRAPs varying between 59% (cold season, 4 days HRT) and 93% (warm season, 8 days HRT).

Finally, the surfactant concentration in R3 clearly shows first-order degradation kinetics (Figure 3), with a rate constant of 1.18 $d^{-1}$. This biodegradation rate constant is similar to that obtained by Andrade *et al.* (2017) of 0.91–1.30 $d^{-1}$, using activated sludge for linear alkyl benzene sulphonate removal; however, lower than the rate constant calculated from results of Hena *et al.* (2015) of 2.72–4.98 $d^{-1}$, using different microalgae for municipal wastewater treatment.

## Biomass productivity and settleability

The different feeding regimes in R1, R2 and R3 promoted mixed culture biomass concentrations of 0.13 ± 0.02,

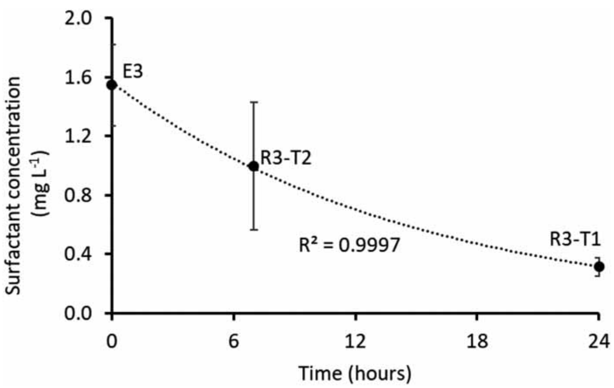


**Figure 3** | Time course of surfactant degradation of R3 in the effluent E3 (after feeding) and cultivation broth at T1 and T2.

0.23 ± 0.03 and 0.21 ± 0.01 g $L^{-1}$ (Table 2), corresponding to biomass productivity rates of 1.5, 3.6 and 3.3 g $m^{-2}$ $d^{-1}$, respectively. These rates were lower than those found by Ruas *et al.* (2018) and Posadas *et al.* (2015) of ≈4 and 5 g $m^{-2}$ $d^{-1}$ in continuous HRAPs treating domestic wastewater at 5 and 6 days HRT, respectively. The low carbon and nutrient loading rates applied to the HRAPs probably explain these lower recorded biomass productivities, as already reported by Posadas *et al.* (2014). On the other hand, the higher biomass production rate obtained with the semi-continuous feeding regime, when compared to the continuous feeding regime, may be directly related to two factors: (i) the operation mode, as according to Beydes & Kapdan (2018), an intermittent feeding mode provides higher biomass concentrations and easier control of environmental conditions, as well as a better resistance to toxic or inhibitory compound loadings; and (ii) the higher nutrient concentration available for microalgal growth during the period of exposure to sunlight. Kim *et al.* (2014) studied biomass growth in, and nutrient removal from raw municipal wastewater in a 60 L HRAP operated semi-continuously at HRTs of 2, 4, 6, and 8 days, and obtained a positive correlation between these parameters and increasing HRT, producing biomass concentrations of about 1.00, 1.26, 1.45 and 1.74 g $L^{-1}$, respectively. On the other hand, Ruas *et al.* (2018) found concentrations of 0.11–0.12 g $L^{-1}$ in continuously operating HRAPs treating domestic wastewater at a 5 days HRT, while 0.32–0.49 g $L^{-1}$ was recorded by Posadas *et al.* (2015) at 2.7–6.7 days HRT.

A good settleability of 81% was obtained in R2, followed by R1 (35%) and R3 (18%); however, settleability was not correlated with the microalgal population found in the reactors. In all three reactors, *Scenedesmus* sp. was the main species (>98%) found after 36 days of operation, in line with

**Table 2** | Environmental conditions, COD and nutrient removal efficiencies, and biomass concentration and settleability found in the three HRAPs at T1

| | Unit | R1 | R2 | R3 |
|---|---|---|---|---|
| Environmental conditions | | | | |
| pH | – | 8.6 ± 0.5 | 10.4 ± 0.1 | 10.1 ± 0.3 |
| DO | mg $L^{-1}$ | 7.6 ± 1.3 | 13.4 ± 1.3 | 11.8 ± 1.6 |
| Temperature | °C | 27.4 ± 1.9 | 27.3 ± 1.8 | 27.3 ± 1.8 |
| Evaporation losses | L $m^{-2}$ $d^{-1}$ | 0.9 ± 0.7 | 0.9 ± 0.7 | 1.0 ± 0.7 |
| Removal efficiencies | | | | |
| COD | % | 74 ± 8 | 73 ± 8 | 70 ± 13 |
| TOC | % | 41 ± 8 | 37 ± 10 | 42 ± 14 |
| IC | % | 67 ± 4 | 59 ± 5 | 58 ± 8 |
| TN | % | 65 ± 8 | 74 ± 6 | 76 ± 8 |
| N-$NH_4^+$ | % | 100 ± 0 | 100 ± 0 | 100 ± 0 |
| TP | % | 46 ± 5 | 86 ± 1 | 80 ± 7 |
| Microalgal biomass | | | | |
| TSS | mg $L^{-1}$ | 0.13 ± 0.02 | 0.23 ± 0.03 | 0.21 ± 0.01 |
| Settleability | % | 35 ± 10 | 81 ± 6 | 18 ± 6 |

literature: this species has been commonly reported in continuous photobioreactors treating domestic wastewater (Muñoz & Guieysse 2006; Posadas *et al.* 2015), but also in a semi-continuous HRAP treating raw municipal wastewater, together with *Chlorella* sp. and *Stigeoclonium* sp. (Kim *et al.* 2014). Settleability in this last case was very high, at 99%.

## Removal efficiency of COD and nutrients

Despite the elevated temperatures (≈27.2 °C) obtained in the reactors (Table 2), relatively low evaporation losses (≈0.9 L $m^{-2}$ $d^{-1}$) were recorded, when compared to those estimated by Guieysse *et al.* (2013) under outdoor conditions in tropical climates (1.3 L $m^{-2}$ $d^{-1}$). On the other hand, in the cultivation broth of R1 a lower pH and DO were recorded than in R2 and R3, which was directly related to microalgal-bacterial growth (Muñoz & Guieysse 2006).

Similar COD and TOC removals of 70–74 and 37–42%, respectively, were found in all reactors, due to similar bacterial activity in spite of different operational conditions. Kim *et al.* (2014) found slightly lower COD and TOC removal efficiencies, of 63 and 34%, respectively, in a semi-continuous HRAP treating raw municipal wastewater at 8 days HRT (initial COD and TOC of 110 and 60 mg $L^{-1}$, respectively). In continuous HRAPs, COD and TOC removals were in the range 66–86 and 54–70%, respectively, at 6.0–6.7 days HRT (Posadas *et al.* 2015). Apart from organic carbon, microalgal processes may reduce IC as well, as a result of photosynthesis. In R1, the removal of IC was higher (67 ± 4%) than observed in R2 (59 ± 5%) and in R3 (58 ± 8%). Based on the carbon content of around 50–53% for *Scenedesmus* sp. biomass cultivated in domestic wastewater, as found by Posadas *et al.* (2015), it can be inferred that in all reactors the main mechanism of carbon removal was assimilation into biomass. Stripping of carbon was also recorded, but only in R1 (11–16%), which can explain the higher removal of IC in R1.

The TN removal efficiency as obtained in R1 (69 ± 8%) was slightly lower than in R2 (74 ± 6%) and in R3 (76 ± 8%), which can be related to the higher biomass productivity recorded in R2 and R3. Ammonia (N-$NH_4^+$) was completely removed in all reactors, and nitrite (N-$NO_2$) and nitrate (N-$NO_3$) were not detected in the effluent. Phosphate removal in R1 (46 ± 5%) was also lower than in R2 and R3, with the removal from R2 (86 ± 1%) slightly higher than from R3 (80 ± 7%). Kim *et al.* (2014) found TN and TP removal efficiencies of 92–95 and 81–95%, respectively, in a semi-continuous HRAP treating raw municipal wastewater at 2–8 days HRT, with an initial TN of 44.8 mg $L^{-1}$ and an initial TP of 4.7 mg $L^{-1}$. Alternatively, Posadas *et al.* (2015) recorded TN and TP removals of 60–97 and 33–70%, respectively, in continuous HRAPs treating primary domestic wastewater during different seasons, at 2.8–6.7 days HRT and using a controlled pH (initial TN of 52–70 mg $L^{-1}$ and TP of 9–11 mg $L^{-1}$). Higher ammonium and phosphorus removal efficiencies from synthetic wastewater, using *Chlorella vulgaris* (UTEX 2714), were also found by de-Bashan *et al.* (2002) in semi-continuous cultures, when compared to the removal in continuous and batch cultures.

Based on the biomass N (8.4–9.0%) and P (0.8–1.3%) content of *Scenedesmus* sp. cultivated in domestic wastewater as obtained by Posadas *et al.* (2015), we can infer that the main mechanism of N removal from R1 was ammonia stripping (>73%), while in R2 and R3 it was ammonia stripping (54–57%) and assimilation into biomass (43–46%). Considering the pH of the reactors, a higher share of ammonium ion in the cultivation broth was evidenced in R1, while ammonia was recorded in R2 and R3. In contrast, assimilation into biomass accounts for the same range in the three reactors (34–58%); however, the high pH recorded may have promoted significant precipitation of P (Muñoz & Guieysse 2006).

## CONCLUSIONS

Semi-continuous feeding in HRAPs operated during the daylight period showed to be more advantageous than the usual continuous operation, with respect to both the microalgal biomass productivity and the nutrient removal efficiencies. Especially considering surfactant removal, a feeding for only 0.1 h $d^{-1}$ may give the best results when the treated effluent is withdrawn from the system before admitting new influent (batch operation). However, further research is still needed to increase the performance of microalgal-bacterial systems operated in batch or semi-continuously for domestic wastewater treatment, as well as an investigation into the removal of other groups of surfactants and an improvement of nutrient recovery into biomass, and also an analysis of economic viability.

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