Performance of a microalgal photobioreactor treating toilet wastewater: Pharmaceutically active compound removal and biomass harvesting

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Abstract

In this study, a 1200 L outdoor pilot scale microalgal photobioreactor (PBR) was used for toilet wastewater (WW) treatment and evaluate its ability to remove pharmaceutically active compounds (PhACs). The PBR was operated at two different hydraulic retention times (HRTs), which were 8 and 12 days, during Period I (September-October) and Period II (October-December), respectively. Algal biomass concentrations varied by operating period because of seasonal changes. Nutrients

(ammonia, nitrogen and total phosphorous) and chemical oxygen demand (COD) were monitored and efficiently removed in both periods (>80%), attaining the legislation limits. At the theoretical hydraulic steady state in both periods, pharmaceutical removal reached high levels (>48%). Two harvesting techniques were applied to the PBR microalgae effluent. Gravity sedimentation was efficient for biomass removal (>99% in 17 minutes) in Period I when large particles, flocs and aggregates were present. In contrast, a longer sedimentation time was required when biomass was mainly composed of single cells (88% clarification in a 24 h in Period II). The second harvesting technique investigated was the co-pelletization of algal biomass with the ligninolytic fungus *Trametes versicolor*, attaining >98% clarification for Period II biomass once pellets were formed. The novel technology of co-pelletization enabled the complete harvesting of single algae cells from the liquid medium in a sustainable way, which benefits the subsequent use of both biomass and the clarified effluent.

1 Introduction

Recently, the application of microalgae systems to wastewater (WW) treatment has been investigated because of their capacity for nutrient and organic matter removal in symbiosis with heterotrophic bacteria. Microalgae grow using sunlight as an energy source, as well as the CO_2 released from bacterial respiration and the atmospheric, along with inorganic nutrients (nitrogen, phosphorous, etc.); they release O_2 that is used by bacteria. This symbiosis reduces the nutrients, metals and micropollutant concentrations in WW (Ficara et al., 2014; Hoh et al., 2016; Hultberg et al., 2016; Mallick, 2002; Muñoz and Guieysse, 2006; Prajapati et al., 2014, 2013; Ramanan et al., 2016; Sturm and Lamer, 2011).

The presence of emerging contaminants (ECs) in WW has attracted great interest because of the possible undesirable effects many of these pollutants can have on the environment and living organisms (Arnold et al., 2013). ECs include pharmaceuticals, personal care products, and pesticides, among others (Kümmerer, 2008; Petrovic et al., 2003). Some technologies have been proposed to remove ECs, such as physico-chemical and biological treatments (Ávila et al., 2014; Baccar et al., 2012; Cruz-Morató et al., 2013a; Dorival-García et al., 2013; Fagan et al., 2016; Gimeno et al., 2016; Nguyen et al., 2014; Secondes et al., 2014). EC removal by pure microalgae strains has been proven effective (Della Greca et al., 2008; Hom-Diaz et al., 2015; Xiong et al., 2016). However, EC removal by microalgal-based technologies has not been widely studied, and scarce literature is available (Cuellar-Bermudez et al., 2016). The first work investigating EC removal in microalgal ponds was published by de Godos et al. (2012), in which the antibiotic tetracycline was removed from a pilot scale high rate algal pond (HRAP) treating synthetic WW, and the mechanisms implied were mainly photodegradation and biosorption. Recently, Matamoros et al. (2015) studied the effect of hydraulic retention time (HRT) and ambient temperature/sunlight irradiation (seasonality) on the removal efficiency of 26 ECs in two HRAP pilot plants fed with real urban wastewater. It was reported that HRT had a great impact on EC removal depending on the season in which HRAPs were operated. It has been demonstrated that microalgal-bacterial systems are good candidates for EC removal.

Microalgal biomass has ample post-application potential; it could be a source of high-value products for use as biofuels and bioproducts (Mennaa et al., 2015; Molinuevo-Salces et al., 2016; Passos et al., 2015; Zhang et al., 2016).

One of the main challenges in microalgae biotechnology and WW treatment is the efficient and reliable separation of microalgae from the effluent after treatment. The

small size of microalgae, typically in the range of 2–20 μm, the low density difference between algae and the growth medium, and the diluted concentrations of algal cultures make the harvesting processes a key challenge, especially at the industrial scale (Li et al., 2008; Mennaa et al., 2015). Depending on the species, cell density, and culture conditions, harvesting algal biomass has been estimated to contribute 20-30% of the production costs (Christenson and Sims, 2011; Molina Grima et al., 2003). Consequently, the harvesting strategy must be based on a low energy method to overcome these problems and make algae production economically feasible and the system commercially viable (e.g., to produce biodiesel with algae technology).

The selection of separation techniques depends on the value of the target products, the species, the biomass concentration, the size of microalgae cells of interest, and the desired final product (Brennan and Owende, 2010; Li et al., 2008; Olaizola, 2003). Gravity or natural sedimentation is the process of solid-liquid separation that separates a feed suspension into a slurry of higher solids concentration and a substantially clear liquid effluent. This process depends on the characteristics of the solid and liquid, which determine if the sedimentation rate is fast or slow (Olaizola, 2003; Sukenik and Shelef, 1984). Because of the large volumes of WW treated and the low value of the biomass generated, sedimentation is the most common technique for harvesting algal biomass in WW treatment (Nurdogan and Oswald, 1996). However, the method is only suitable for large microalgae (ca. >70 μm) (Brennan and Owende, 2010). Co-pelletization is a novel harvesting technique; the use of filamentous fungi is an attractive bioflocculating technique because of the self-pelletization of the fungi, which can efficiently trap the microalgal biomass. Fungal self-pelletization has been observed for numerous filamentous strains, leading to the development of aggregates/pellets, and several

authors have proposed this technique for biodiesel production (Gultom and Hu, 2013; Liu et al., 2008; Xia et al., 2014; Zhang and Hu, 2012).

The objective of this study was to evaluate the performance of a tubular microalgal reactor for wastewater treatment. The system was tested for its applicability in different seasonal periods and regimes for pharmaceutically active compounds (PhACs) degradation. Moreover, a preliminary study of two different harvesting techniques was also evaluated for biomass recovery and supernatant clarification.

2 Materials and methods

2.1 Wastewater characteristics

Toilet wastewater (WW) was collected from the toilet drainage of the Chemical, Biological and Environmental Engineering Department (Universitat Autònoma de Barcelona) and placed into a first settler. The supernatant was conducted to a second settler from which the WW was pumped into the microalgal photobioreactor (PBR) via peristaltic pump. The HRT of the setters was 48 hours, so they can be used as a homogenization tanks. The average characteristics of the toilet WW are presented in Table 1.

Table 1. Influent average composition for Period I and II photobioreactor operation. (Total suspended solids (TSS), chemical oxygen demand (COD), ammonia nitrogen (N-NH_4^+), total phosphorous (TP), hydraulic retention time (HRT)).

Parameter	Period I (HRT 8 d)	Period II (HRT 12 d)
TSS (mg/L)	36±11	36±9
COD (mg/L)	462±53	398±115
N-NH_4^+ (mg/L)	79±42	121±22

TP (mg/L)	15±4	7±2

2.2 *Microalgal photobioreactor*

The experimental setup was located on the roof of the Department, consisting of an enclosed 1200 L multitubular PBR (Fig. 1). Two distribution chambers were placed at each end of the tubes to transfer and distribute the culture evenly between the tubes. The tubes are made of low density polyethylene (PE); they are soft and moldable, whereas the distribution chambers are made of propylene (PP), giving them a robustness. The tubes are placed on a PP cuvette filled with tap water to avoid abrupt daily temperature changes. A PP paddle wheel was placed in one of the distribution chambers, which provides movement and aeration to the microalgal PBR by drawing the culture in from the 4 incoming tubes and raising the culture into the distribution chamber and the 4 gravity-fed outgoing tubes. The WW inlet was placed in the same distribution chamber as the paddle wheel together with a ball float level sensor to avoid a liquid overflow. The distribution chambers have a total working volume of 0.14 m^3, with a liquid level of 15 cm. The tubes have a working volume of 0.24 m^3 (230 mm internal diameter x 1 mm thickness x 7.0 m long). The paddle wheel provides a constant velocity of 0.13 m/s.

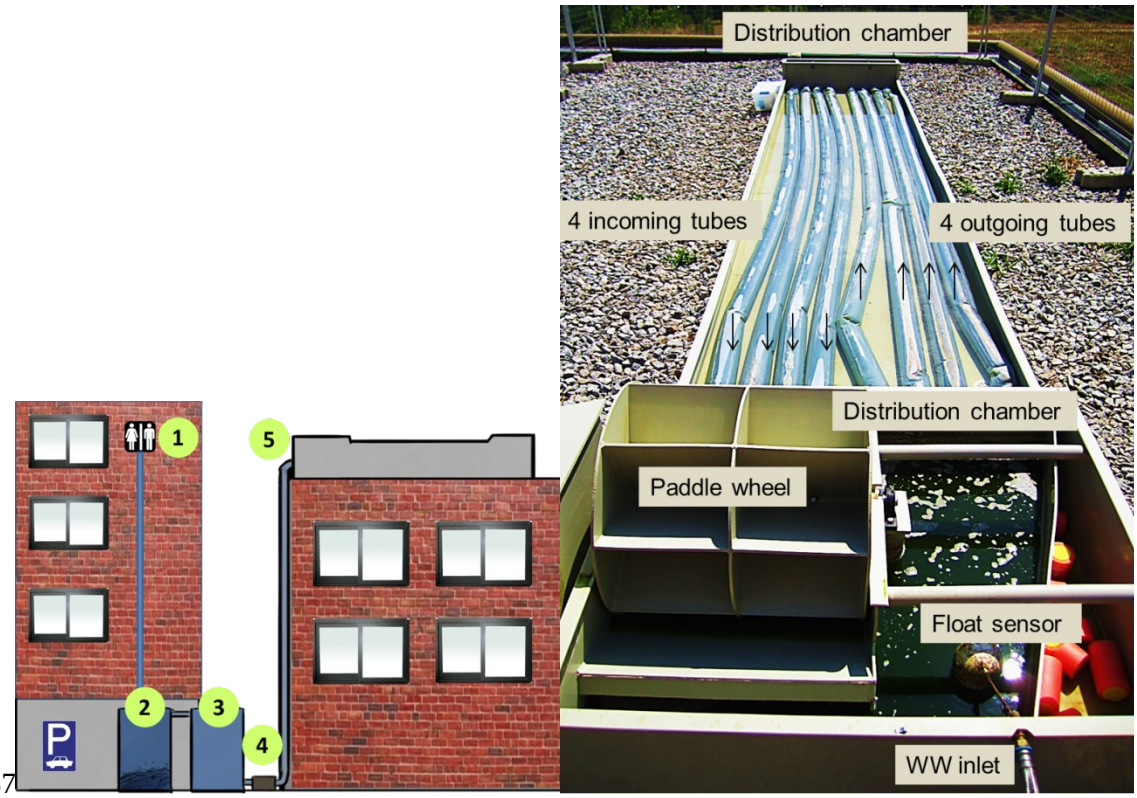

Figure 1. Microalgal photobioreactor (PBR). Left: Schematic PBR located on the roof of the Chemical, Biological and Environmental Engineering Department from Universitat Autònoma de Barcelona, Spain: (1) toilet sewage; (2) 1st settler: (3) 2nd settler; (4) WW pump; (5) PBR. Right: PBR in operation.

The inoculation of the system is described in the Supplementary Material, SM1. Briefly, WW was pumped, via peristaltic pump, from the second settler and entered the distribution box immediately following the paddle wheel. The PBR treated 150 L/day, corresponding to an HRT of 8 days, in Period I and 100 L/day, corresponding to an HRT of 12 days, in Period II. The PBR performance was monitored by taking samples from September 14, 2015 to October 16, 2015 during Period I and from October 20, 2015 to December 22, 2015 during Period II. Three samples were taken on alternate days at the theoretical hydraulic steady state for the detection of pharmaceutical compounds at the end of Periods I and II. Samples were collected from the inlet WW and the PBR

effluent; detailed information of sampling has been included in the Supplementary Material, SM2.

2.3 Analytical methods

Water temperature, pH and dissolved oxygen (DO) were measured in situ with a PCE-PHD 1 multimeter (PCE Instruments, Albacete, Spain). The following parameters were determined from the influent and effluent of the PBR. Total suspended solids (TSS) and soluble chemical oxygen demand (COD) were measured according to Standard Methods (APHA et al., 1999). Nitrogen ammonia ($N\text{-}NH_4^+$) and total phosphorus (TP) were measured using an Analyzer Y15 (Biosystems, Barcelona, Spain). Total, organic and inorganic carbon was measured using an OI Analytical TOC Analyzer (Model 1020A). Nitrite (NO_2^-), sulfate (SO_4^{2-}) and nitrate (NO_3^-) concentrations were determined by ion chromatography with conductivity detection using a Dionex ICS-2000. Analyses were performed in triplicate, and the results are given as the mean values.

Eighty-one pharmaceutical residues were measured following the analytical methodology previously described by Gros et al. (2012) (complete results are presented in the Supplementary Material, SM3). Briefly, samples were filtered through a 1 µm glass fiber filter followed by a 0.45 µm PVDF membrane filter (Millipore; Billerica, MA, USA). After filtration, Na_2EDTA was added to a final concentration of 0.1% (g solute/g solution), and an appropriate volume of each sample (25 and 50 mL for WW and treated WW, respectively) was loaded into the Solid Phase Extraction (SPE) cartridges Oasis HLB cartridges (60 mg, 3 mL) (Waters Corp. Mildford, MA, USA) were conditioned with 5 mL of methanol followed by 5 mL of high performance liquid chromatography (HPLC) grade water. After sample loading, cartridges were rinsed with 6 mL of HPLC grade water and further dried with air for 5 minutes to remove the remaining water. Finally, analytes were eluted from the cartridges using 6 mL of pure

methanol. The extracts reconstituted in methanol/water (10:90, v/v) were analyzed using an ultra-performance liquid chromatography (UPLC) system (Waters Corp. Mildford, MA, USA) equipped with a turbo Ion Spray source. Chromatographic separations were performed in an Acquity HSS T_3 column (50 mm × 2.1 mm inner diameter, 1.8 µm particle size; Waters Corp. Mildford, MA, USA) under positive ionization (PI) mode and in an Acquity BEH C_{18} column (50 mm × 2.1 mm inner diameter, 1.7 µm particle size; Waters Corp. Mildford, MA, USA) under negative ionization (NI) mode. The UPLC system was coupled to a quadrupole-linear hybrid ion trap mass spectrometer 5500 QTRAP (Applied Biosystems, Foster City, CA, USA). All data were recorded by using Scheduled MRM™ algorithm monitoring two SRM transitions for each compound; the first transition was for quantification, and the second transition was for confirmation of the compounds. Concentrations were calculated by internal calibration with the corresponding isotopically labelled standards using Analyst 1.5.1 software.

2.4 Harvesting techniques

Natural sedimentation was conducted following the guidelines proposed by Nollet (2000). A 1 L transparent glass measuring cylinder ([height/diameter] ratio≈5.7) was filled with 1 L of microalgal suspension. It was kept vibration free, and disturbance of the settled matter was avoided. Height decreases from the solid-liquid interphase and time values were recorded to obtain the sedimentation curve and the sedimentation velocity. After sedimentation, the cell concentration of the supernatant was determined by absorbance at 683 nm in a spectrophotometer, and TSS were measured.

The co-pelletization harvesting technique was conducted using the fungus *Trametes versicolor* (American Type Culture Collection #42530). Mycelia were obtained as previously described in Font et al. (2003). A total co-cultivation volume of 120 mL was introduced into 500 mL Erlenmeyer flasks, containing 0.5 mL of *T. versicolor* mycelia,

algal biomass and fungal defined media. Three different volumetric ratios of algal biomass-to-fungal defined media were used (1:5, 1:2, and 1:1 (v/v)). The mixtures were then cultivated for 3 days under constant agitation (130 rpm) and maintained at 25±1ºC. The absorbance and the TSS concentration were measured using the supernatant after agitation was stopped and pellets had settled to the bottom of the Erlenmeyer flask.

The *T. versicolor* growth culture medium was 8 g/L glucose, 3.3 g/L ammonium tartrate dibasic, 3.3 g/L 2,2-dimethylsuccinic acid, 10 mL/L macronutrient solution and 1 mL/L micronutrient solution (Blánquez et al., 2004).

2.5 Statistical analysis

The standard deviation and means were analysed for significance. One-way ANOVA test was used for the statistical analysis between Period I and II N-NH_4^+, total P and COD removal percentages, as well as for each PhACs removal percentages.

3 Results and discussion

3.1 Performance of the microalgal photobioreactor

PBR inoculation was conducted by introducing 100 L of water from a nearby lake located in Sant Cugat del Vallès (Barcelona, Spain) (detailed information is described in Supplementary Material, SM1), and the remaining volume was filled with wastewater coming from the settler previously described. Inlet WW characteristics are presented in Table 1. Influent TSS variations were negligible and could be considered constant across both operating periods and were lower than typical raw wastewater because of previous settling. Microalgal concentrations were measured as total suspended solids (TSS) from the PBR effluent (Figure 2). Higher biomass concentrations were detected during Period I (HRT=8 d) compared to during Period II (HRT=12 d), 303±83 vs.

226265±77 mg/L, respectively at the theoretical hydraulic steady state. The decrease in 227ambient temperature between periods affected microalgal biomass activity because low 228temperatures reduce the ability to use light, which may have caused photoinhibition, 229reducing microalgal growth (Davison, 1991).

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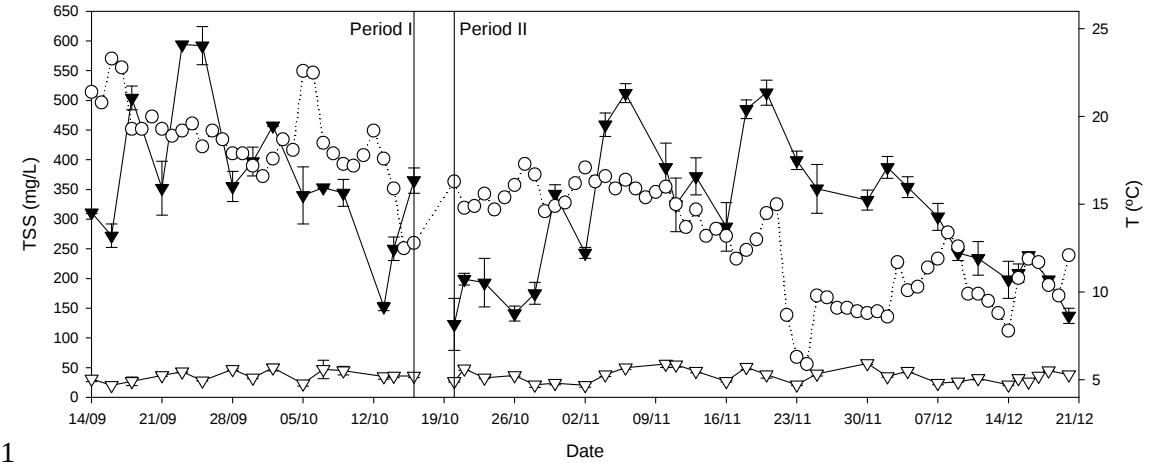

231

232Figure 2. Performance of PBR treating toilet wastewater. TSS from the influent (▽) and 233effluent (▯) during Period I (HRT 8 d) and Period II (HRT 12 d) and daily average 234outdoor temperature (○).

235

236Several studies have explored how consortia of microalgae and bacteria can be effective 237for nutrient removal in WW. Microalgae-bacteria consortia demonstrated high 238efficiency in COD and N-NH_4^+ removal and lower efficiencies in TP removal. Removal 239efficiency for COD was higher during Period I than during Period II, 84% vs. 60%, 240respectively (Figure 3). The decrease is attributed to a lower photosynthetic activity by 241microalgae as well as a decrease in microalgal biomass concentration as a consequence 242of light irradiance (detailed information in Supplementary Material SM4). The N-NH_4^+ 243removal percentages were similar for both periods, 86 vs. 98%, respectively, at the 244theoretical hydraulic steady state. Good removal percentages were obtained for total 245phosphorus during Period II, 89%, whereas during Period I, removal percentages were 246low, 50%. The N and P removal efficiencies depend on the microalgae species; the N/P

247ratio can vary from 8 to 45 g N/g P (Christenson and Sims, 2011; Cuellar-Bermudez et
248al., 2016). The presence of microalgal species able to uptake ‘luxury’ phosphorous has
249been previously reported in ponds treating WW (Powell et al., 2011). The system has a
250great capacity for nutrient removal from toilet wastewater as described by previous
251authors treating wastewater with microalgal-based systems (Cromar et al., 1996; García
252et al., 2006).

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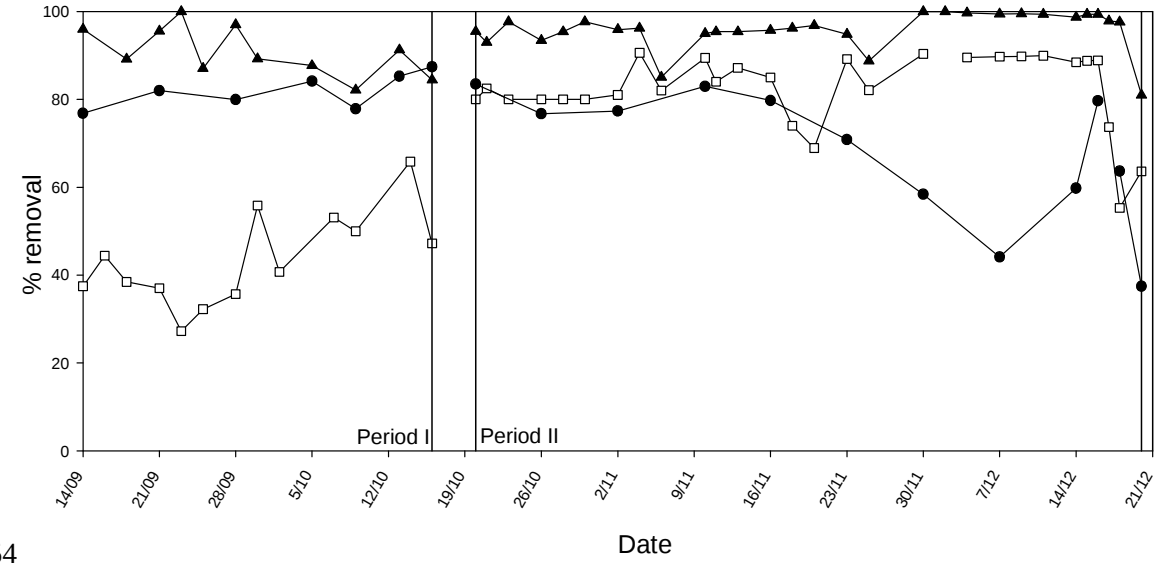

254

255Figure 3. Nutrient removal percentages for Period I (HRT 8 d) and Period II (HRT 12
256d). (●) COD; (▲) N-NH_4^+; (☐) TP.

257

258*3.2 Diurnal variations*

259The results of the present study indicate that the variables influenced by algal
260photosynthesis (DO and pH) have significant variations during the day in the mixed
261liquor of the photobioreactor (PBR) in relation to the diurnal solar radiation rhythm. The
262temperature inside the PBR is affected by the outside temperature. This trend has also
263been previously reported by some authors (El Ouarghi et al., 2000; García et al., 2006;
264Picot et al., 1993). Figure 4 illustrates the daily variations of pH and DO over the two
265periods, the data shown correspond to the dark cycle (morning) and the light cycle

266(afternoon), and the temperature inside the PBR as well as the ambient temperature after
267each cycle is also represented.

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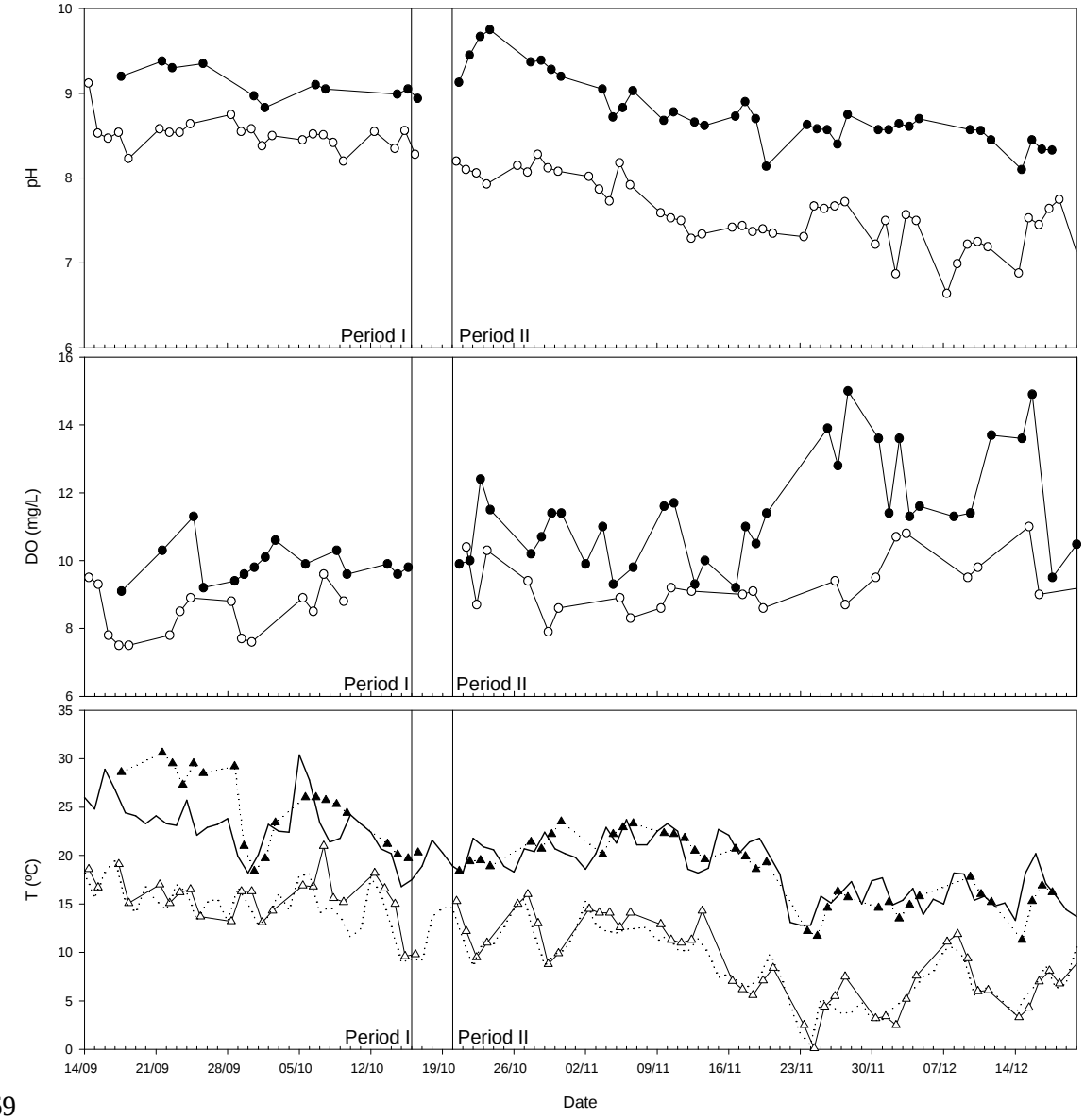


269

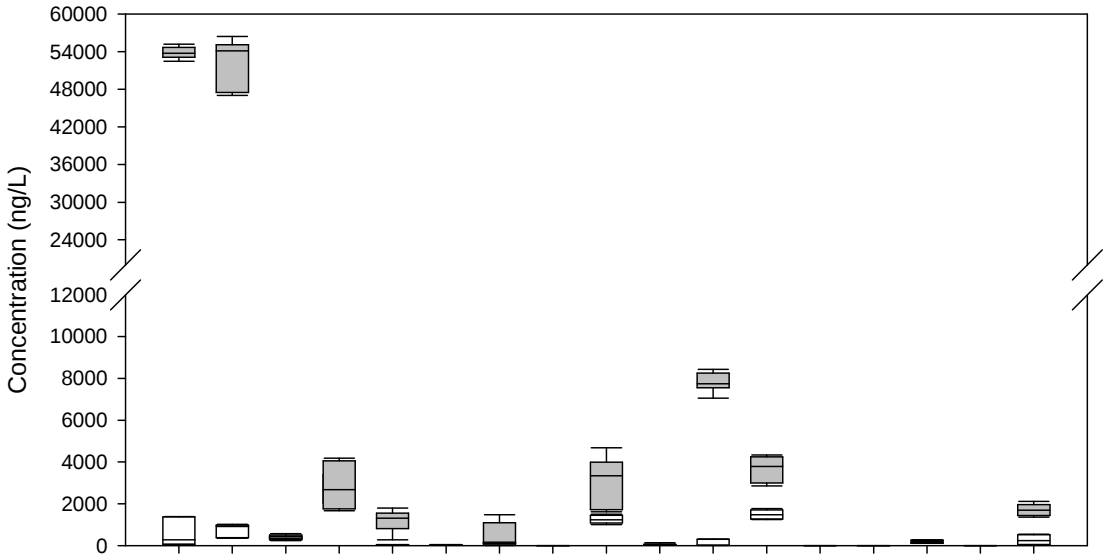
270Figure 4. Daily variations in the photobioreactor for Period I (HRT 8 d) and Period II
271(HRT 12 d). Top: pH daily monitoring; Middle: DO daily monitoring; Bottom:
272Temperature daily monitoring; (●) pH or DO after the light cycle; (○) pH or DO after
273the dark cycle; (▲) PBR temperature after the light cycle; (△) PBR temperature after
274the dark cycle; (—) maximum ambient temperature; (···) minimum ambient temperature.

275

During the night, the lack of algal photosynthetic activity in conjunction with the continuous respiration of algae and other microorganisms resulted in low pH and DO values after the dark cycle. Algal photosynthetic activity increased after sunrise, producing higher pH and DO values. The average differences in pH after the dark and light cycles were approximately 0.5 pH units during Period I and 1 pH unit during Period II. Differences were also observed between the two HRT periods. Higher pH values were measured during Period I than during Period II because of the higher microalgae photosynthetic activity.

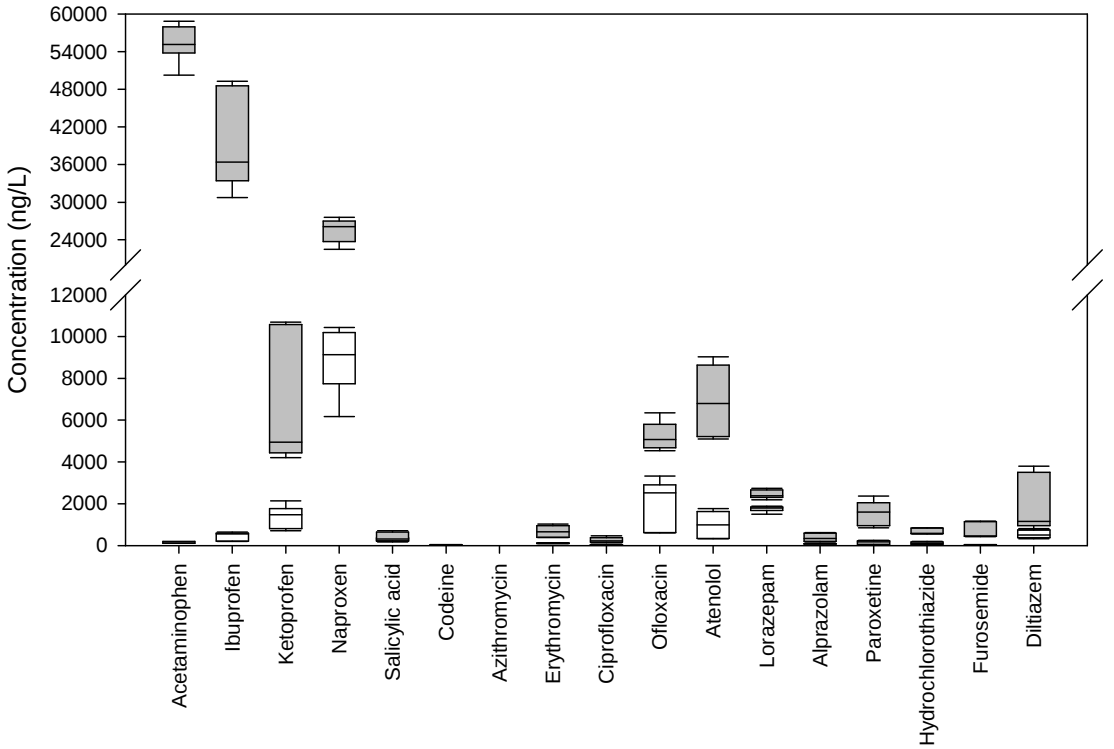
3.3 Pharmaceutical compound removal

In this study, PhACs were analyzed from inlet wastewater and effluent from the PBR. Three different samples were taken from the bioreactor influent and effluent during the theoretical hydraulic steady state. The steady state occurrence of PhACs in the PBR during Periods I and II is summarized in Figure 5. No large differences in PhAC levels in the influent water were observed between October and December (water collection dates). Seasonal fluctuations in PhAC levels have been reported by other authors and attributed to different climatic conditions and consumption patterns among different periods of the year (Kolpin et al., 2004).


Concentration (ng/L)
60000
54000
48000
42000
36000
30000
24000
12000
10000
8000
6000
4000
2000
0

295

1515



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297Figure 5. Box-plot of the occurrence of PhACs in the inlet wastewater (grey boxes) and
298PBR effluent (white boxes) from Period I (top) and Period II (bottom). The box-plots
299indicate the median, and the 25th and 75th percentile for each compound. (Note: For
300period I, ketoprofen, naproxen, and azithromycin grey boxes overlap white boxes)

301

302The reported concentrations of PhACs in the influent revealed differences among the
303pharmaceutical therapeutic groups, which were linked to population characteristics,
304local common diseases, drug metabolism (excretion rate), and environmental
305persistence among others. Compounds detected (with a high number of anti-
306inflammatory and antibiotic drugs) as well as their concentration in the toilet WW are in
307accordance with the concentrations reported by other authors (Cruz-Morató et al.,
3082013a; Jelic et al., 2011; Kasprzyk-Hordern et al., 2009; Matamoros et al., 2015;
309Radjenovic et al., 2009; Verlicchi et al., 2012).

310Five anti-inflammatory compounds (acetaminophen, ibuprofen, naproxen, salicylic acid
311and ketoprofen) were detected, and acetaminophen (paracetamol) was found at the

highest concentrations in both sampling periods, with values ranging from 50.2 to 58.7 µg/L (Fig. 5). Removal percentages obtained for acetaminophen in the microalgal PBR were higher than 99% (Table SM1) during both periods, in accordance with other authors who also reported very good removal percentages using other microorganisms, such as fungi or microalgal systems (Cruz-Morató et al., 2013b; Escapa et al., 2016; Matamoros et al., 2015). Direct photolysis has been described as an important mechanism for acetaminophen removal in freshwater systems (Laurentiis et al., 2014). Other authors have described acetaminophen as a readily biodegradable compound and have shown its significant biodegradability and removal via bio-sorption during WW treatment (Joss et al., 2006; Kasprzyk-Hordern et al., 2009; Radjenovic et al., 2009).

Ibuprofen, the second most abundant compound detected in the influent WW during both operating periods (39.0-52.8 µg/L, Fig. 5), also exhibited high removal percentages in the PBR (>98%, Table SM1). Santos et al. (2009) detected ibuprofen as the most abundant compound in four Spanish WWTPs, where concentration levels ranged from 3.73 to 603 µg/L. Several studies reported that the dominant ibuprofen removal mechanism in biological systems (membrane bioreactors (Abegglen et al., 2009) and immobilized cell processes (Yu et al., 2011)) is biodegradation. Other authors also attribute the high removal of ibuprofen to aerobic biodegradation processes during WW treatment rather than to sorption processes. Ibuprofen has a low octanol-water partition coefficient (high polarity), so it is not expected to be sorbed onto organic matter (Ávila et al., 2010; Kasprzyk-Hordern et al., 2009). Another possible pathway for ibuprofen removal is the presence of photosensitizers, such as dissolved organic matter (Yu-Chen Lin and Reinhard, 2005).

Ketoprofen inlet concentrations increased by one order of magnitude between Period I (472±52 ng/L) and Period II (6729±413 ng/L) (Fig. 5). Its removal percentage during

Period I (36%, Table SM1) is similar to the ones reported after activated sludge processes in conventional WWTPs (Jiang et al., 2013). However, it was lower than removals reported in a pilot scale HRAP by Matamoros et al. (2015) (50-95%), although both algal systems were treating influents with similar initial concentrations of ketoprofen. The removal percentage of this compound was statistically different between periods ($p=4.35\cdot10^{-6}$).

Naproxen exhibited the lowest removal percentage among all the anti-inflammatories detected during Period I (10%) but increased during Period II (69%) (Table SM1), being this percentage statistically significant ($p=8.14\cdot10^{-10}$). Values from Period II are in accordance with those reported by Matamoros et al. (2015) in a pilot scale HRAP treating urban WW (48-89%). Naproxen removal in a WWTP is mainly attributed to its biodegradability, whereas sorption processes are not considered because of the low octanol-water partition coefficient of naproxen (Kasprzyk-Hordern et al., 2009). Several authors also studied the occurrence and the removal percentage of this compound, obtaining variable removal efficiency depending on the system (Verlicchi et al., 2012).

Salicylic acid is an active metabolite of the highly consumed acetylsalicylic acid, but it is also a common derivate of phenol. Therefore, it is a typical pollutant in both urban and industrial wastewaters, and its removal from aqueous solutions has received a great deal of attention in recent years because of its high toxicity and accumulation in the environment (Combarros et al., 2014; Evgenidou et al., 2015). Salicylic acid inlet concentrations during Periods I and II were 1349±738 and 368±12 ng/L, respectively (Fig. 5). Total removal was achieved with the PBR in the Period I, whereas only 33% of the salicylic acid was removed during Period II (Table SM1). Escapa et al. (2015) observed high removal percentages (93%) of salicylic acid using the algae *C. sorokiniana* in a semi-continuous system.

The analgesic codeine was only detected during Period I (33±1 ng/L, Fig. 5). This concentration was similar to the urban wastewater treated by Cruz-Morató et al. (2013a) and lower than the WWTP influent reported by Kasprzyk-Hordern et al. (2009). The effluent concentration was below the limit of detection (LOD 4.17 ng/L, LOQ 13.91 ng/L).

As in the case of anti-inflammatories and analgesics, antibiotic levels varied between the two studied periods (Fig. 5). Ciprofloxacin and ofloxacin were detected at concentrations of 2629 ng/L and 65 ng/L, respectively, during Period I and 294 ng/L and 5662 ng/L, respectively, during Period II. Azithromycin was only detected in Period I (385 ng/L), and erythromycin was only detected in Period II (661 ng/L). The removal percentages obtained ranged from 48% to complete removal, depending on the antibiotic considered (Table SM1). Ofloxacin removal percentage between periods was not significantly different ($p=0.313$). Antibiotic removals between 35% and 76% in conventional activated sludge processes and between 25% to 95% in membrane bioreactors have been previously described (Radjenovic et al., 2009).

The β-blocker atenolol was detected at high concentrations in Period I, 7.8 μg/L, and Period II, 6.9 μg/L (Fig. 5); removal percentages were above 80% (Table SM1), although the differences between Period I and II were significantly different ($p=1.90 \cdot 10^{-5}$). The inlet concentration is in accordance with values reported by previous authors in WWTP influents (Verlicchi et al., 2012). Escolà Casas et al. (2015b) obtained 40% removal in a continuously moving bed biofilm reactor, whereas almost complete removal was found in a hybrid biofilm and activated sludge system (Escolà Casas et al., 2015a). Biodegradation of atenolol has been linked to the activity of ammonia-oxidizing bacteria and heterotrophs (Sathyamoorthy et al., 2013).

386The psychiatric drug lorazepam, present in both periods (Period I: 3.7 µg/L and Period
387II: 2.4 µg/L), achieved removals between 30 and 57% using the PBR (Table SM1),
388values significantly different in both periods ($p=6.58\cdot10^{-7}$). Jelic et al. (2011) found that
389lorazepam was biologically degraded only 30% during WW treatment, and sorption
390onto sludge was less than 5%. The psychiatric drugs alprazolam and paroxetine were
391only detected in Period II (389 and 1652 ng/L, respectively) and were efficiently
392removed during algal treatment, with 87% and 93% removal, respectively (Table SM1).
393Similar removal for paroxetine has been observed by other authors in activated sludge
394systems and membrane bioreactors (Sipma et al., 2010), whereas the conventional
395WWTP removal of paroxetine was worse (c.a. 77%). Biodegradation and/or chemical
396transformation are postulated to be the dominant removal mechanisms for these
397compounds in biological treatment systems (Radjenovic et al., 2009; Subedi and
398Kannan, 2015).
399The diuretic hydrochlorothiazide (Period I: 228 ng/L and Period II: 686 ng/L, Fig. 5)
400was partially removed in the PBR (44% during Period I and 84% during Period II,
401($p=1.39\cdot10^{-10}$)) (Table SM1). Its persistent behavior has been acknowledged by some
402authors (Bertelkamp et al., 2014; Radjenovic et al., 2009), where low or insignificant
403degradation was reported for hybrid biofilm-activated sludge processes, membrane
404bioreactors, etc (Falas et al., 2013; Kovalova et al., 2012). In contrast, for furosemide,
405another diuretic detected in the inlet WW during Period II (669 ng/L, Fig. 5), high
406removal efficiencies were reported for different WWTPs (Collado et al., 2014;
407Papageorgiou et al., 2016), as well as in the present study.
408The calcium channel blocker diltiazem was detected in the inlet WW, ranging from
4091600 to 1900 ng/L, and its removal ranged between 73 and 77% (Table SM1), values
410significantly different ($p=1.08\cdot10^{-3}$). Very diverse diltiazem removal percentages have

been described in the literature. High removal percentages (88%-99%) were reported by Du et al. (2014) for different WWTPs (i.e., municipal treatment plant, aerobic treatment plant and septic treatment system coupled with a subsurface constructed wetland), whereas removals between 30 and 88% were observed in several Swedish free water surface wetlands (Breitholtz et al., 2012). Only 13% removal was achieved in a conventional activated sludge wastewater treatment process (Blair et al., 2015).

Clear differences appear in the removal efficiencies of the two study periods (Table SM1). For some compounds, such as naproxen, salicylic acid, ketoprofen, hydrochlorothiazide and lorazepam, a lower influent concentration corresponded to lower removal efficiency. There is a lack of information about EC removal by microalgae. Biodegradation may require a threshold concentration before microbial degradation can be triggered, suggesting an adaptation of the biomass is necessary for the degradation of these compounds (Spain and Van Veld, 1983). This adaptation is enhanced at high concentrations rather than at low concentrations. The removal efficiency of other pollutants (i.e., codeine, ofloxacin, ibuprofen and acetaminophen) was independent of the initial concentration.

In general, higher removal efficiencies were obtained during Period II, despite the lower temperature and light irradiation. This could be a consequence of the HRT increase from 8 to 12 days. Some authors reported that biological wastewater treatment technologies for removing ECs are highly dependent on HRT because it enhances biodegradation, photodegradation and sorption removal processes (Matamoros et al., 2015). HRT is indeed a key design parameter for the removal efficiency of microcontaminants in microalgal-based treatment systems; the higher the HRT, the greater the EC removal efficiency (Garcia-Rodríguez et al., 2014; Víctor and Rodríguez, 2016).

The biodegradation of organic compounds in microalgal-based treatment systems is the result of facultative chemoautotrophy; therefore, the organic compounds are directly biodegraded (Hom-Diaz et al., 2015; Priya et al., 2014; Semple et al., 1999). Little is known about PhAC removal mechanisms because of the complexity of the microbial variations in these systems. Microbial communities continuously change in open systems because they need to adapt to the conditions (environmental and/or operational). This fact poses an inconvenience in PhAC removal because not all species are able to remove the same compounds. Cell walls of microalgae contain polysaccharides, proteins and lipids, which in turn contain functional groups, including amino, hydroxyl, carboxyl, and sulfate, that act as binding sites and are used to sequester many different pollutants through adsorption or an ion-exchange process (Priya et al., 2014; Yu et al., 1999). Moreover, microalgae produce peptides, which can also bind to micropollutants.

The EC removal efficiencies significantly vary and can range from negligible removal to 99% removal depending on the physico-chemical characteristics of the compound and the cultivation and operation parameters, such as HRT and environmental temperature (Cuellar-Bermudez et al., 2016).

3.4 Microalgae harvesting

The gravity sedimentation profile from the PBR effluent at the theoretical hydraulic steady state of Period I is shown in Fig. 6; it follows a linear tendency. The initial and final parameters and the percentage of solid removal are presented in Table 2. The calculated sedimentation velocity was 0.049±0.005 m/min, and 99% of TSS were removed within 7 minutes. Biomass from Period I had a good settling capacity, in contrast to the microalgal biomass effluent from Period II operation, where no interphase was observed, and as a consequence, the sedimentation curve could not be

plotted. The settling velocity of Period II effluent decreased to $2.29 \cdot 10^{-4}$ m/min within 24 h, achieving a final TSS removal from the supernatant of 88% (Table 2). The differences between the two periods are attributed to biomass composition. During Period I, several filamentous species (*Phormidium*, a self-aggregating cyanobacteria used by previous authors in WW treatment (Olguín, 2003), has the ability to auto-flocculate and self-aggregate, immobilizing the smaller microalgae cells) were microscopically observed, while during Period II, a decrease of filamentous species and an increase of unicellular microalgae decreased the sedimentation rate of the effluent. Outdoor systems are in a constant state of change because of environmental conditions affecting biomass composition and harvesting efficiency. Algal harvesting depends on cell size, microalgae composition and other parameters (Brennan and Owende, 2010; Li et al., 2008; Olaizola, 2003). Park et al. (2013) stated that the harvesting efficiency was highly dependent on the dominant algae for a high rate algal pond (HRAP). Removals between 75 and 85% were achieved when *Pediastrum* sp. was dominant. Although there is scarce literature available on this subject, the values are similar to the ones reported in this study (Table 2).

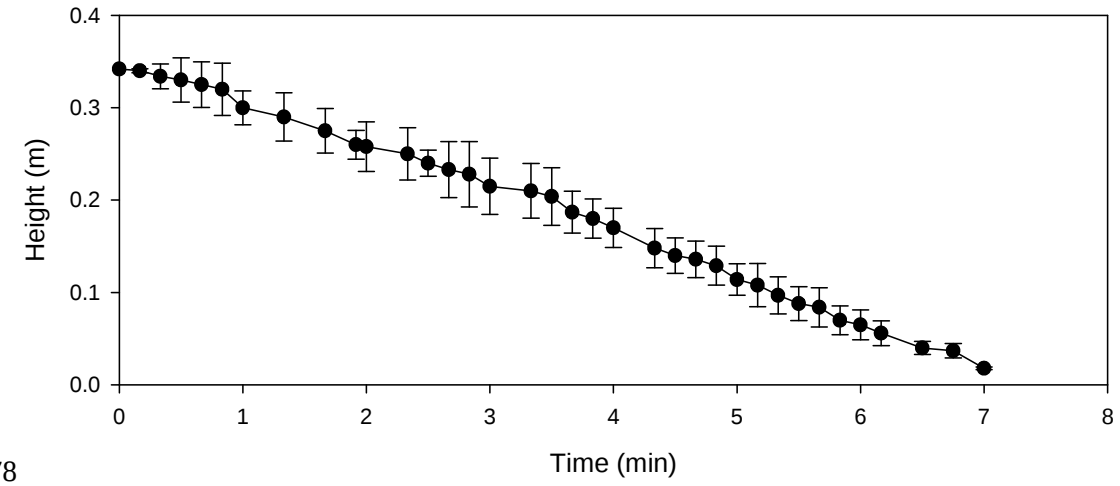

Figure 6. PBR Period I effluent sedimentation curve.

Table 2. TSS removal percentages after the application of the harvesting technique, sedimentation and/or co-pelletization for PBR biomass in Period I and II. In brackets: * biomass-to-fungal defined media ratio, **sedimentation time.

Harvesting technique	Period (ratio)*	TSS Initial (mg/L)	TSS Final supernatant (mg/L) (time)**	% removal
Sedimentation	Period I	454	4 (7 min)	99
	Period II	162	20 (24 h)	88
Co-pelletization	Period II (1:5)	27	0	100
	Period II (1:2)	54	1	98
	Period II (1:1)	81	2	98

To improve the biomass harvesting from the Period II effluent, the novel technique of co-pelletization was applied using the fungus *Trametes versicolor*. The co-cultivated algal biomass and fungus can form pellets. The size of the pellets will allow their simple harvest by mesh sieve filtration or sedimentation. The co-pelletization was achieved at different algal biomass-to-fungal defined media co-cultivation ratios. Three ratios (1:1, 1:2 and 1:5) were used to study co-pelletization. The results highlight the largely similar effects of the different algal biomass-to-fungal defined media ratios used in co-cultivation (Table 2). Co-cultivation can attain nearly complete removal of microalgal cells from the liquid medium, obtaining a clear, transparent supernatant (Figure 7). At the three ratios of algal biomass-to-fungal defined media, microalgal biomass was completely removed from the liquid medium after three days of co-cultivation and entrapped into the fungal pellets. Fungal pellets became more green (Figure 7) as the algal concentration increased and as more algal biomass was entrapped by the fungus.

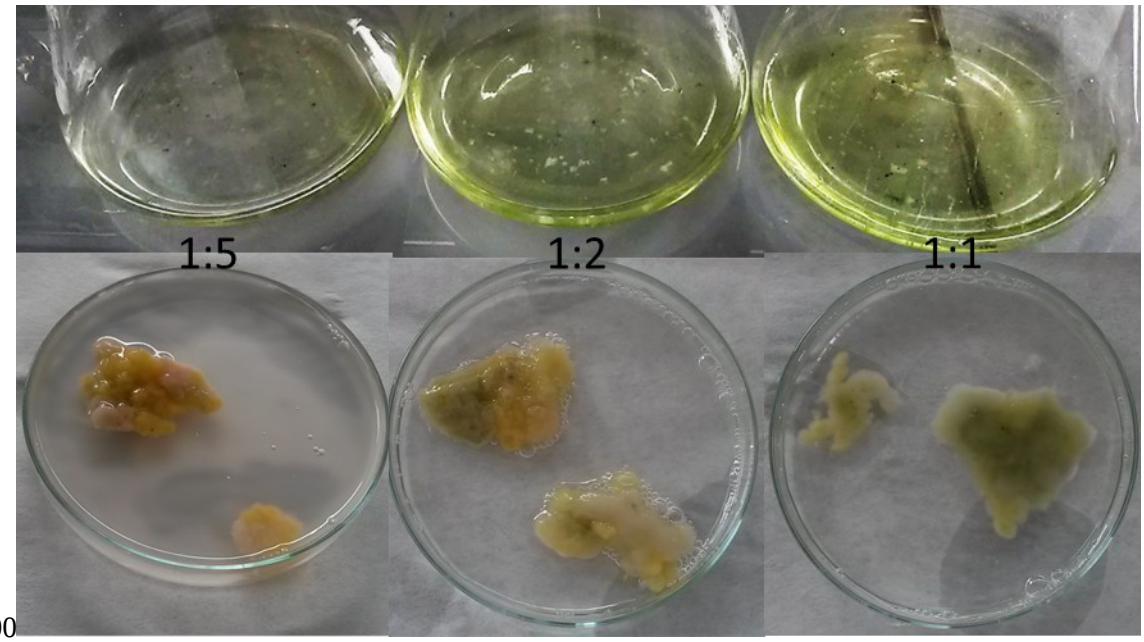

Figure 7. Co-pelletization assay using Period II PBR biomass at different algal biomass-to-fungal defined media ratios. Top: initial; bottom: final, 3 days.

The co-pelletization harvesting technique has scarcely been reported for a real microalgal effluent (Bhattacharya et al., 2017; Muradov et al., 2015; Wrede et al., 2014). Previous works have focused on pure microalgae cultures (i.e., *Chlorella*) (Wrede et al., 2014; Xie et al., 2013; Zhang and Hu, 2012; Zhou et al., 2013), and the promising results obtained encourage further study and the implementation of this technique in different real microalgae effluents. Harvesting microalgae from effluents using co-pelletization could be an efficient method to obtain a clarified supernatant and recover biomass for further valorization.

4 Conclusions

$N\text{-}NH_4^+$, total phosphorous and COD removal percentages of higher than 80% were obtained by treating WW for four months in a microalgal photobioreactor operating at two different hydraulic retention times (8 days during Period I and 12 days during

Period II). PBR performance is highly impacted by temperature and solar irradiation. Low temperatures and few light hours decrease the TSS concentration (Period II), which is directly related to productivity as well as nutrient removal.

PhAC removal was also evaluated. Overall high removals (98%) were achieved for anti-inflammatory drugs (ibuprofen, acetaminophen, salicylic acid, and codeine) and some compounds, such as the diuretics hydrochlorothiazide (84%) and furosemide (total removal). Lower removals (>48%) were obtained for antibiotics (azithromycin, ciprofloxacin, ofloxacin and erythromycin) and the psychiatric drug lorazepam (30-57%). These results demonstrate that algal systems are a good option for the biological treatment of toilet WW.

Microalgal photobioreactor effluent harvesting depends on the biomass characteristics. In Period I, flocs were easily formed, allowing good clarification at high sedimentation velocities (0.049 m/min and 7 min) using the natural sedimentation technique. Sedimentation velocity decreased during Period II ($2.29 \cdot 10^{-4}$ m/min), and the clarification percentage also decreased (88%). The novel harvesting technology, co-pelletization using *T. versicolor*, provided a solution to problems associated with current energy-intensive and costly algae harvesting processes. Despite the good results attained in this study (>98% microalgae entrapment), further research is still needed to study the detailed pelletization conditions for large scale industrial applications.

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