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**1Performance of a microalgal photobioreactor treating toilet  
2wastewater: Pharmaceutically active compound removal and biomass  
3harvesting**

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18

**19Abstract**

20In this study, a 1200 L outdoor pilot scale microalgal photobioreactor (PBR) was used

21for toilet wastewater (WW) treatment and evaluate its ability to remove

22pharmaceutically active compounds (PhACs). The PBR was operated at two different

23hydraulic retention times (HRTs), which were 8 and 12 days, during Period I

24(September-October) and Period II (October-December), respectively. Algal biomass

25concentrations varied by operating period because of seasonal changes. Nutrients

26(ammonia, nitrogen and total phosphorous) and chemical oxygen demand (COD) were  
27monitored and efficiently removed in both periods (>80%), attaining the legislation  
28limits. At the theoretical hydraulic steady state in both periods, pharmaceutical removal  
29reached high levels (>48%). Two harvesting techniques were applied to the PBR  
30microalgae effluent. Gravity sedimentation was efficient for biomass removal (>99% in  
317 minutes) in Period I when large particles, flocs and aggregates were present. In  
32contrast, a longer sedimentation time was required when biomass was mainly composed  
33of single cells (88% clarification in a 24 h in Period II). The second harvesting  
34technique investigated was the co-pelletization of algal biomass with the ligninolytic  
35fungus *Trametes versicolor*, attaining >98% clarification for Period II biomass once  
36pellets were formed. The novel technology of co-pelletization enabled the complete  
37harvesting of single algae cells from the liquid medium in a sustainable way, which  
38benefits the subsequent use of both biomass and the clarified effluent.

39

## 401 **Introduction**

41Recently, the application of microalgae systems to wastewater (WW) treatment has been  
42investigated because of their capacity for nutrient and organic matter removal in  
43symbiosis with heterotrophic bacteria. Microalgae grow using sunlight as an energy  
44source, as well as the CO<sub>2</sub> released from bacterial respiration and the atmospheric, along  
45with inorganic nutrients (nitrogen, phosphorous, etc.); they release O<sub>2</sub> that is used by  
46bacteria. This symbiosis reduces the nutrients, metals and micropollutant concentrations  
47in WW (Ficara et al., 2014; Hoh et al., 2016; Hultberg et al., 2016; Mallick, 2002;  
48Muñoz and Guieysse, 2006; Prajapati et al., 2014, 2013; Ramanan et al., 2016; Sturm  
49and Lamer, 2011).

50The presence of emerging contaminants (ECs) in WW has attracted great interest  
51because of the possible undesirable effects many of these pollutants can have on the  
52environment and living organisms (Arnold et al., 2013). ECs include pharmaceuticals,  
53personal care products, and pesticides, among others (Kümmerer, 2008; Petrovic et al.,  
542003). Some technologies have been proposed to remove ECs, such as physico-  
55chemical and biological treatments (Ávila et al., 2014; Baccar et al., 2012; Cruz-Morató  
56et al., 2013a; Dorival-García et al., 2013; Fagan et al., 2016; Gimeno et al., 2016;  
57Nguyen et al., 2014; Secondes et al., 2014). EC removal by pure microalgae strains has  
58been proven effective (Della Greca et al., 2008; Hom-Diaz et al., 2015; Xiong et al.,  
592016). However, EC removal by microalgal-based technologies has not been widely  
60studied, and scarce literature is available (Cuellar-Bermudez et al., 2016). The first work  
61investigating EC removal in microalgal ponds was published by de Godos et al. (2012),  
62in which the antibiotic tetracycline was removed from a pilot scale high rate algal pond  
63(HRAP) treating synthetic WW, and the mechanisms implied were mainly  
64photodegradation and biosorption. Recently, Matamoros et al. (2015) studied the effect  
65of hydraulic retention time (HRT) and ambient temperature/sunlight irradiation  
66(seasonality) on the removal efficiency of 26 ECs in two HRAP pilot plants fed with  
67real urban wastewater. It was reported that HRT had a great impact on EC removal  
68depending on the season in which HRAPs were operated. It has been demonstrated that  
69microalgal-bacterial systems are good candidates for EC removal.

70Microalgal biomass has ample post-application potential; it could be a source of high-  
71value products for use as biofuels and bioproducts (Mennaa et al., 2015; Molinuevo-  
72Salces et al., 2016; Passos et al., 2015; Zhang et al., 2016).

73One of the main challenges in microalgae biotechnology and WW treatment is the  
74efficient and reliable separation of microalgae from the effluent after treatment. The

75small size of microalgae, typically in the range of 2–20  $\mu\text{m}$ , the low density difference  
76between algae and the growth medium, and the diluted concentrations of algal cultures  
77make the harvesting processes a key challenge, especially at the industrial scale (Li et  
78al., 2008; Mennaa et al., 2015). Depending on the species, cell density, and culture  
79conditions, harvesting algal biomass has been estimated to contribute 20-30% of the  
80production costs (Christenson and Sims, 2011; Molina Grima et al., 2003).  
81Consequently, the harvesting strategy must be based on a low energy method to  
82overcome these problems and make algae production economically feasible and the  
83system commercially viable (e.g., to produce biodiesel with algae technology).

84The selection of separation techniques depends on the value of the target products, the  
85species, the biomass concentration, the size of microalgae cells of interest, and the  
86desired final product (Brennan and Owende, 2010; Li et al., 2008; Olaizola, 2003).  
87Gravity or natural sedimentation is the process of solid-liquid separation that separates a  
88feed suspension into a slurry of higher solids concentration and a substantially clear  
89liquid effluent. This process depends on the characteristics of the solid and liquid, which  
90determine if the sedimentation rate is fast or slow (Olaizola, 2003; Sukenik and Shelef,  
911984). Because of the large volumes of WW treated and the low value of the biomass  
92generated, sedimentation is the most common technique for harvesting algal biomass in  
93WW treatment (Nurdogan and Oswald, 1996). However, the method is only suitable for  
94large microalgae (ca.  $>70 \mu\text{m}$ ) (Brennan and Owende, 2010). Co-pelletization is a novel  
95harvesting technique; the use of filamentous fungi is an attractive bioflocculating  
96technique because of the self-pelletization of the fungi, which can efficiently trap the  
97microalgal biomass. Fungal self-pelletization has been observed for numerous  
98filamentous strains, leading to the development of aggregates/pellets, and several

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

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

106

## 1072 **Materials and methods**

### 1082.1 *Wastewater characteristics*

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

116

117 Table 1. Influent average composition for Period I and II photobioreactor operation.  
118 (Total suspended solids (TSS), chemical oxygen demand (COD), ammonia nitrogen (N-  
119  $\text{NH}_4^+$ ), total phosphorous (TP), hydraulic retention time (HRT)).

<b>Parameter</b>	<b>Period I (HRT 8 d)</b>	<b>Period II (HRT 12 d)</b>
TSS (mg/L)	36±11	36±9
COD (mg/L)	462±53	398±115
N- $\text{NH}_4^+$ (mg/L)	79±42	121±22

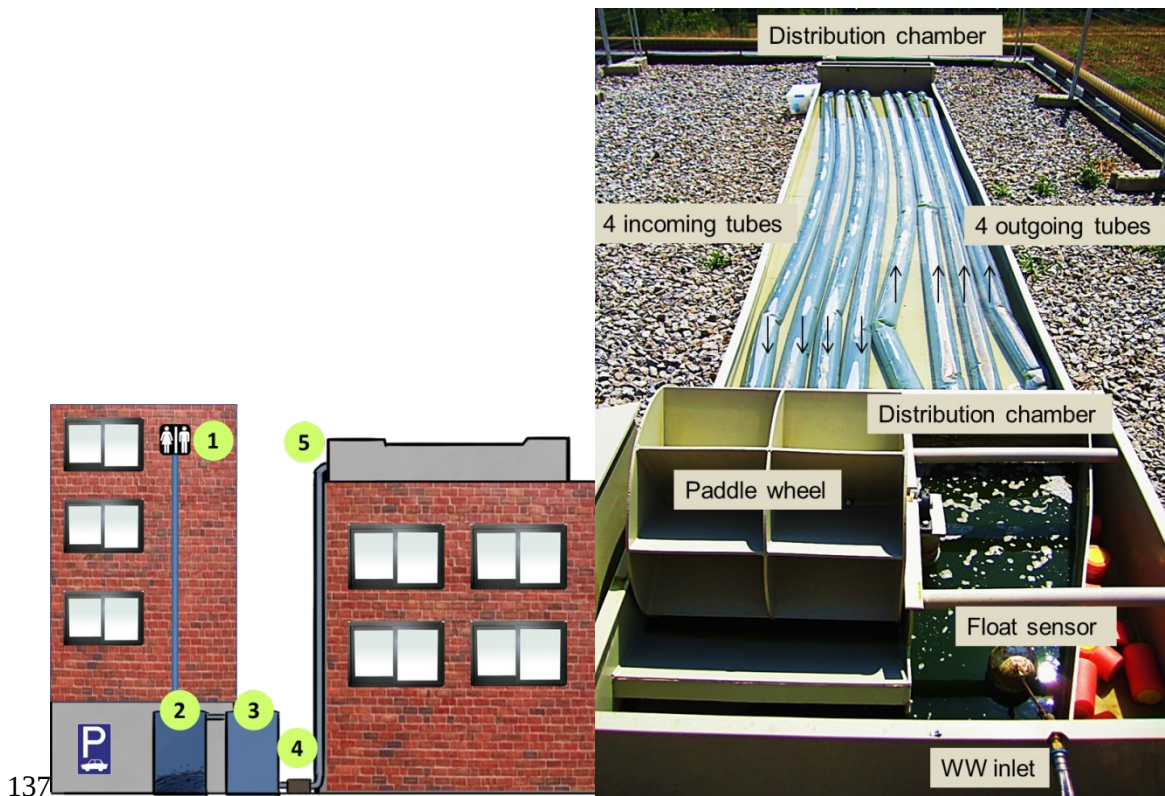
TP (mg/L)	15±4	7±2
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### 1222.2 *Microalgal photobioreactor*

123The experimental setup was located on the roof of the Department, consisting of an  
124enclosed 1200 L multitubular PBR (Fig. 1). Two distribution chambers were placed at  
125each end of the tubes to transfer and distribute the culture evenly between the tubes. The  
126tubes are made of low density polyethylene (PE); they are soft and moldable, whereas  
127the distribution chambers are made of propylene (PP), giving them a robustness. The  
128tubes are placed on a PP cuvette filled with tap water to avoid abrupt daily temperature  
129changes. A PP paddle wheel was placed in one of the distribution chambers, which  
130provides movement and aeration to the microalgal PBR by drawing the culture in from  
131the 4 incoming tubes and raising the culture into the distribution chamber and the 4  
132gravity-fed outgoing tubes. The WW inlet was placed in the same distribution chamber  
133as the paddle wheel together with a ball float level sensor to avoid a liquid overflow.  
134The distribution chambers have a total working volume of 0.14 m<sup>3</sup>, with a liquid level of  
13515 cm. The tubes have a working volume of 0.24 m<sup>3</sup> (230 mm internal diameter x 1 mm  
136thickness x 7.0 m long). The paddle wheel provides a constant velocity of 0.13 m/s.



137  
 138Figure 1. Microalgal photobioreactor (PBR). Left: Schematic PBR located on the roof  
 139of the Chemical, Biological and Environmental Engineering Department from  
 140Universitat Autònoma de Barcelona, Spain: (1) toilet sewage; (2) 1<sup>st</sup> settler; (3) 2<sup>nd</sup>  
 141settler; (4) WW pump; (5) PBR. Right: PBR in operation.

142

143The inoculation of the system is described in the Supplementary Material, SM1. Briefly,  
 144WW was pumped, via peristaltic pump, from the second settler and entered the  
 145distribution box immediately following the paddle wheel. The PBR treated 150 L/day,  
 146corresponding to an HRT of 8 days, in Period I and 100 L/day, corresponding to an HRT  
 147of 12 days, in Period II. The PBR performance was monitored by taking samples from  
 148September 14, 2015 to October 16, 2015 during Period I and from October 20, 2015 to  
 149December 22, 2015 during Period II. Three samples were taken on alternate days at the  
 150theoretical hydraulic steady state for the detection of pharmaceutical compounds at the  
 151end of Periods I and II. Samples were collected from the inlet WW and the PBR



152effluent; detailed information of sampling has been included in the Supplementary  
153Material, SM2.

#### 1542.3 *Analytical methods*

155Water temperature, pH and dissolved oxygen (DO) were measured in situ with a PCE-  
156PHD 1 multimeter (PCE Instruments, Albacete, Spain). The following parameters were  
157determined from the influent and effluent of the PBR. Total suspended solids (TSS) and  
158soluble chemical oxygen demand (COD) were measured according to Standard Methods  
159(APHA et al., 1999). Nitrogen ammonia (N-NH<sub>4</sub><sup>+</sup>) and total phosphorus (TP) were  
160measured using an Analyzer Y15 (Biosystems, Barcelona, Spain). Total, organic and  
161inorganic carbon was measured using an OI Analytical TOC Analyzer (Model 1020A).  
162Nitrite (NO<sub>2</sub><sup>-</sup>), sulfate (SO<sub>4</sub><sup>2-</sup>) and nitrate (NO<sub>3</sub><sup>-</sup>) concentrations were determined by ion  
163chromatography with conductivity detection using a Dionex ICS-2000. Analyses were  
164performed in triplicate, and the results are given as the mean values.

165Eighty-one pharmaceutical residues were measured following the analytical  
166methodology previously described by Gros et al. (2012) (complete results are presented  
167in the Supplementary Material, SM3). Briefly, samples were filtered through a 1 µm  
168glass fiber filter followed by a 0.45 µm PVDF membrane filter (Millipore; Billerica,  
169MA, USA). After filtration, Na<sub>2</sub>EDTA was added to a final concentration of 0.1% (g  
170solute/g solution), and an appropriate volume of each sample (25 and 50 mL for WW  
171and treated WW, respectively) was loaded into the Solid Phase Extraction (SPE)  
172cartridges Oasis HLB cartridges (60 mg, 3 mL) (Waters Corp. Mildford, MA, USA)  
173were conditioned with 5 mL of methanol followed by 5 mL of high performance liquid  
174chromatography (HPLC) grade water. After sample loading, cartridges were rinsed with  
1756 mL of HPLC grade water and further dried with air for 5 minutes to remove the  
176remaining water. Finally, analytes were eluted from the cartridges using 6 mL of pure

177methanol. The extracts reconstituted in methanol/water (10:90, v/v) were analyzed using  
178an ultra-performance liquid chromatography (UPLC) system (Waters Corp. Mildford,  
179MA, USA) equipped with a turbo Ion Spray source. Chromatographic separations were  
180performed in an Acquity HSS T<sub>3</sub> column (50 mm × 2.1 mm inner diameter, 1.8 μm  
181particle size; Waters Corp. Mildford, MA, USA) under positive ionization (PI) mode  
182and in an Acquity BEH C<sub>18</sub> column (50 mm × 2.1 mm inner diameter, 1.7 μm particle  
183size; Waters Corp. Mildford, MA, USA) under negative ionization (NI) mode. The  
184UPLC system was coupled to a quadrupole-linear hybrid ion trap mass spectrometer  
1855500 QTRAP (Applied Biosystems, Foster City, CA, USA). All data were recorded by  
186using Scheduled MRM™ algorithm monitoring two SRM transitions for each  
187compound; the first transition was for quantification, and the second transition was for  
188confirmation of the compounds. Concentrations were calculated by internal calibration  
189with the corresponding isotopically labelled standards using Analyst 1.5.1 software.

#### 1902.4 *Harvesting techniques*

191Natural sedimentation was conducted following the guidelines proposed by Nollet  
192(2000). A 1 L transparent glass measuring cylinder ([height/diameter] ratio≈5.7) was  
193filled with 1 L of microalgal suspension. It was kept vibration free, and disturbance of  
194the settled matter was avoided. Height decreases from the solid-liquid interphase and  
195time values were recorded to obtain the sedimentation curve and the sedimentation  
196velocity. After sedimentation, the cell concentration of the supernatant was determined  
197by absorbance at 683 nm in a spectrophotometer, and TSS were measured.

198The co-pelletization harvesting technique was conducted using the fungus *Trametes*  
199*versicolor* (American Type Culture Collection #42530). Mycelia were obtained as  
200previously described in Font et al. (2003). A total co-cultivation volume of 120 mL was  
201introduced into 500 mL Erlenmeyer flasks, containing 0.5 mL of *T. versicolor* mycelia,

202algal biomass and fungal defined media. Three different volumetric ratios of algal  
203biomass-to-fungal defined media were used (1:5, 1:2, and 1:1 (v/v)). The mixtures were  
204then cultivated for 3 days under constant agitation (130 rpm) and maintained at  $25\pm 1^\circ\text{C}$ .  
205The absorbance and the TSS concentration were measured using the supernatant after  
206agitation was stopped and pellets had settled to the bottom of the Erlenmeyer flask.  
207The *T. versicolor* growth culture medium was 8 g/L glucose, 3.3 g/L ammonium tartrate  
208dibasic, 3.3 g/L 2,2-dimethylsuccinic acid, 10 mL/L macronutrient solution and 1 mL/L  
209micronutrient solution (Blázquez et al., 2004).

#### 2102.5 *Statistical analysis*

211The standard deviation and means were analysed for significance. One-way ANOVA  
212test was used for the statistical analysis between Period I and II  $\text{N-NH}_4^+$ , total P and  
213COD removal percentages, as well as for each PhACs removal percentages.

214

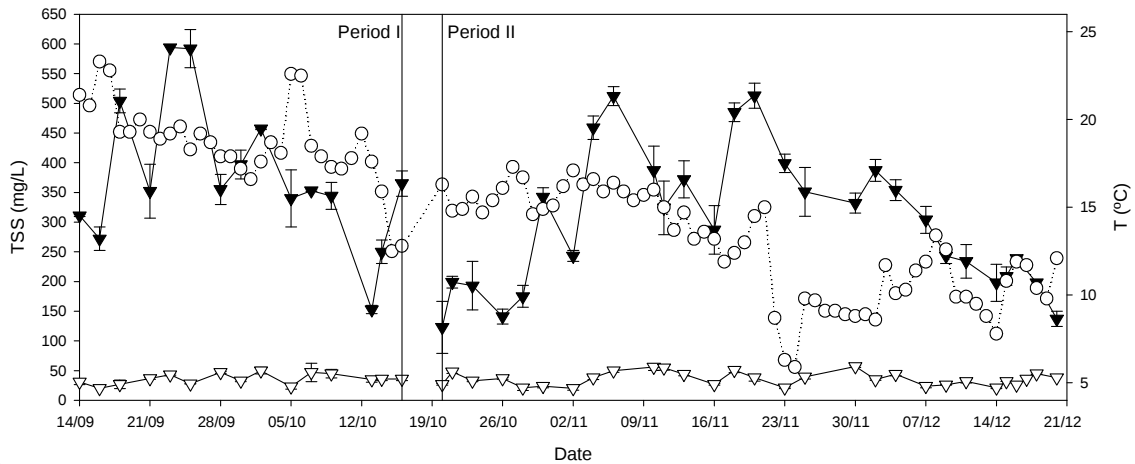
### 2153 **Results and discussion**

#### 2163.1 *Performance of the microalgal photobioreactor*

217PBR inoculation was conducted by introducing 100 L of water from a nearby lake  
218located in Sant Cugat del Vallès (Barcelona, Spain) (detailed information is described in  
219Supplementary Material, SM1), and the remaining volume was filled with wastewater  
220coming from the settler previously described. Inlet WW characteristics are presented in  
221Table 1. Influent TSS variations were negligible and could be considered constant  
222across both operating periods and were lower than typical raw wastewater because of  
223previous settling. Microalgal concentrations were measured as total suspended solids  
224(TSS) from the PBR effluent (Figure 2). Higher biomass concentrations were detected  
225during Period I (HRT=8 d) compared to during Period II (HRT=12 d),  $303\pm 83$  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).

230



231

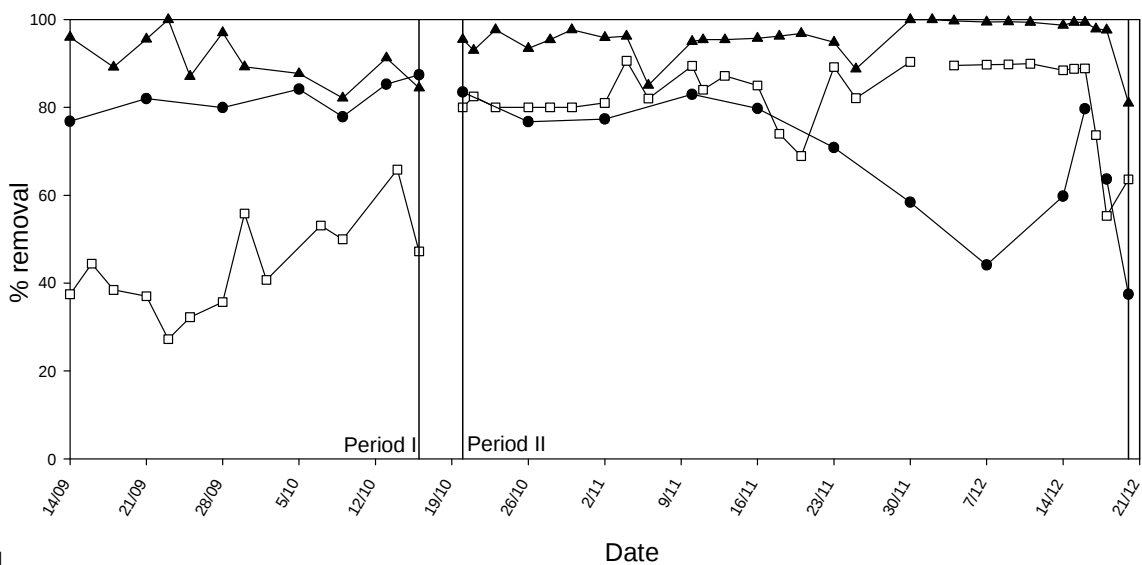
232Figure 2. Performance of PBR treating toilet wastewater. TSS from the influent (▽) and  
 233effluent (◻) during Period I (HRT 8 d) and Period II (HRT 12 d) and daily average  
 234outdoor temperature (○).

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<sub>4</sub><sup>+</sup> 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<sub>4</sub><sup>+</sup>  
 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).

253



254

255Figure 3. Nutrient removal percentages for Period I (HRT 8 d) and Period II (HRT 12  
 256d). (●) COD; (▲) N-NH<sub>4</sub><sup>+</sup>; (□) TP.

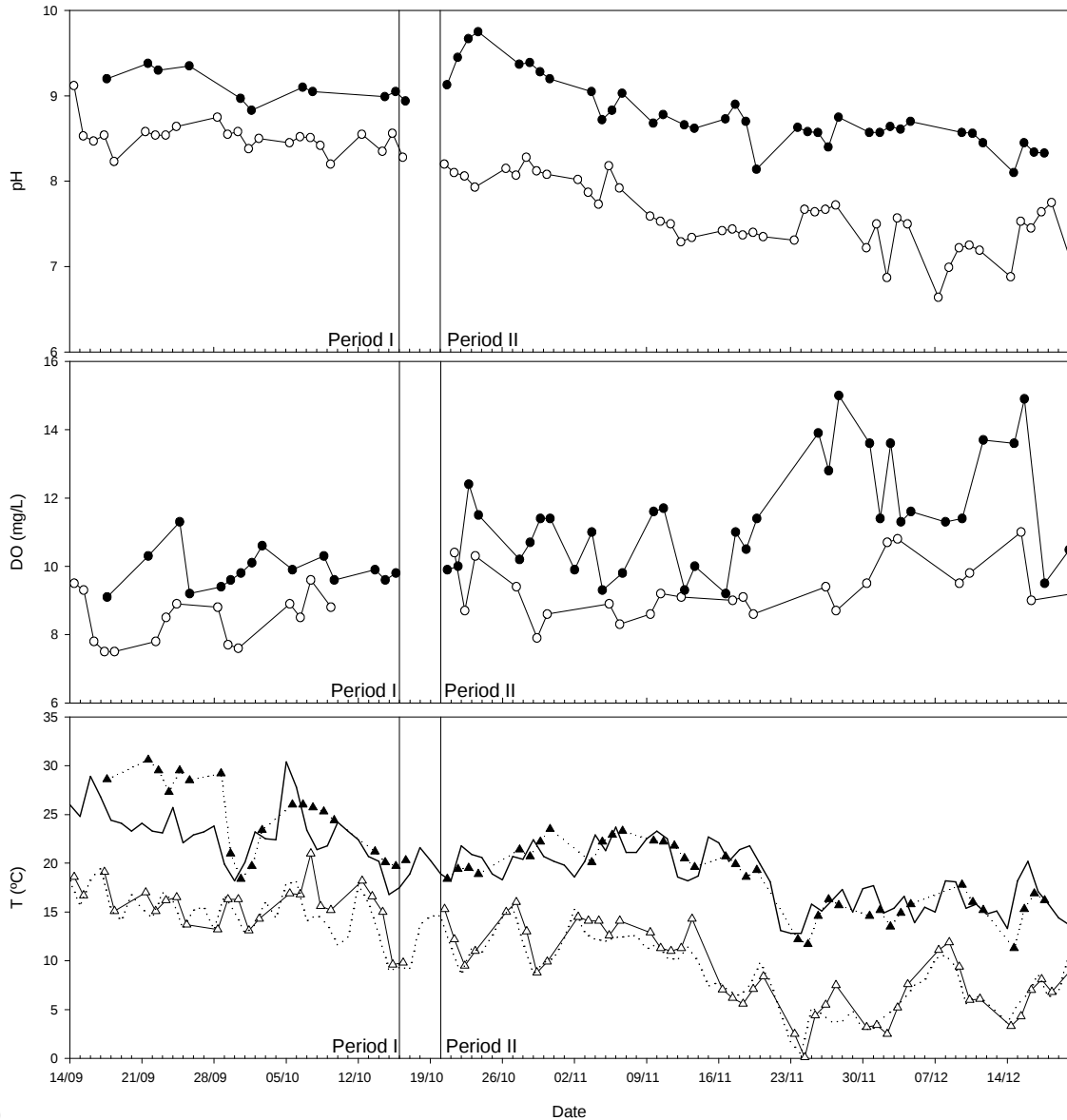
257

### 2583.2 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.

268



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

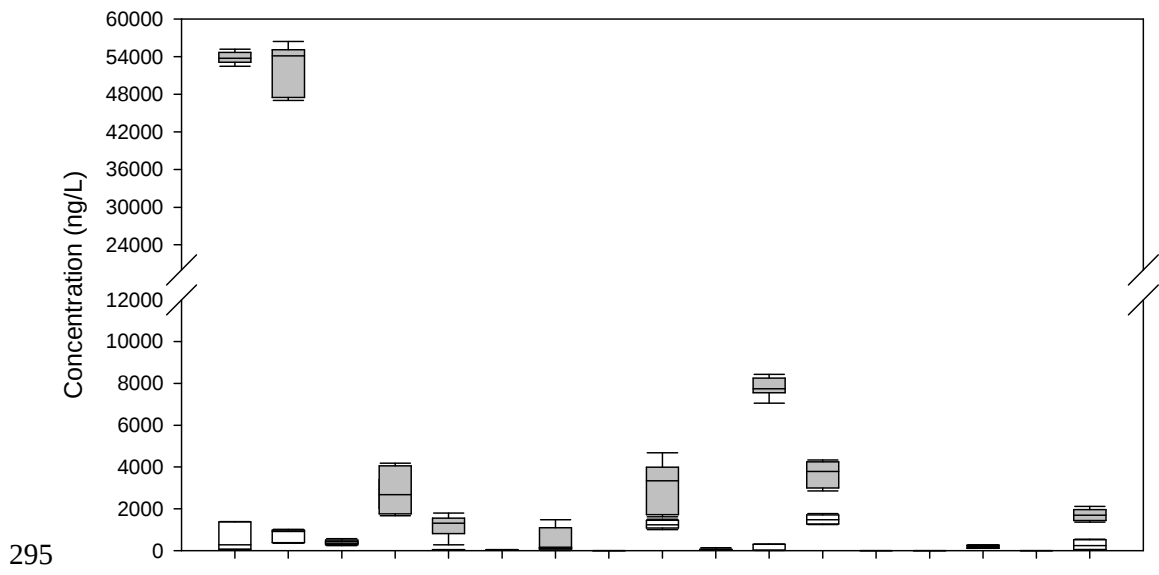
276 During the night, the lack of algal photosynthetic activity in conjunction with the  
277 continuous respiration of algae and other microorganisms resulted in low pH and DO  
278 values after the dark cycle. Algal photosynthetic activity increased after sunrise,  
279 producing higher pH and DO values.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

284

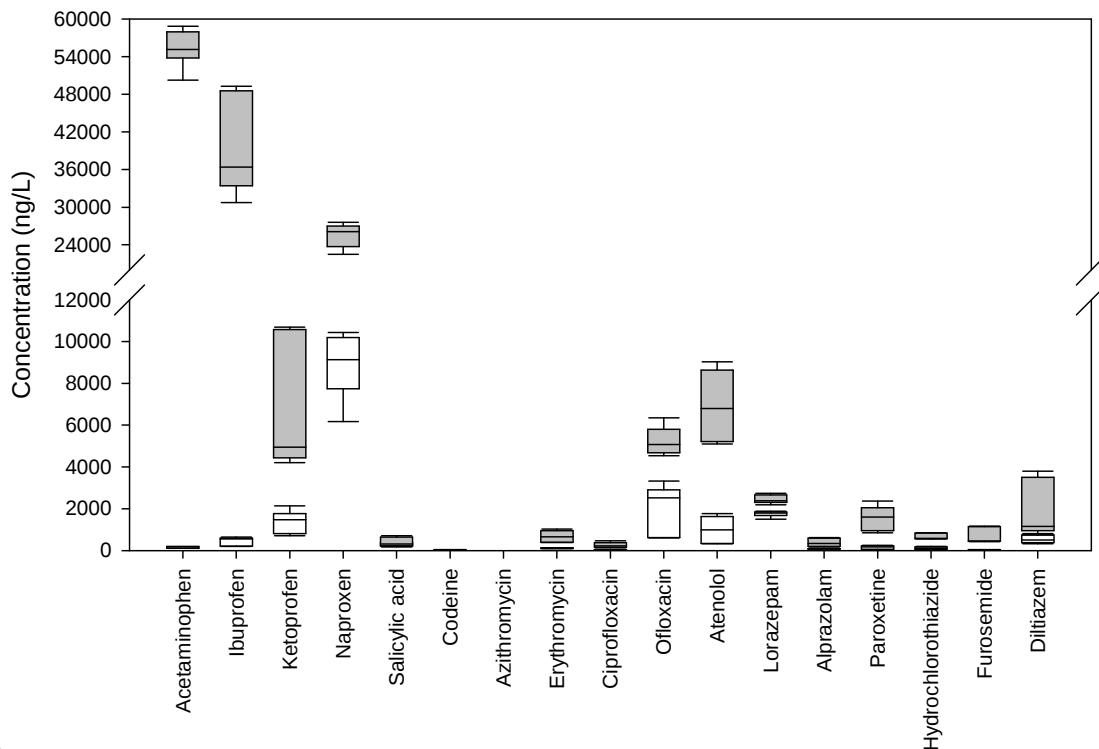
### 285 3.3 *Pharmaceutical compound removal*

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

294







296

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 25<sup>th</sup> and 75<sup>th</sup> 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

312highest concentrations in both sampling periods, with values ranging from 50.2 to 58.7  
313 $\mu\text{g/L}$  (Fig. 5). Removal percentages obtained for acetaminophen in the microalgal PBR  
314were higher than 99% (Table SM1) during both periods, in accordance with other  
315authors who also reported very good removal percentages using other microorganisms,  
316such as fungi or microalgal systems (Cruz-Morató et al., 2013b; Escapa et al., 2016;  
317Matamoros et al., 2015). Direct photolysis has been described as an important  
318mechanism for acetaminophen removal in freshwater systems (Laurentiis et al., 2014).  
319Other authors have described acetaminophen as a readily biodegradable compound and  
320have shown its significant biodegradability and removal via bio-sorption during WW  
321treatment (Joss et al., 2006; Kasprzyk-Hordern et al., 2009; Radjenovic et al., 2009).

322Ibuprofen, the second most abundant compound detected in the influent WW during  
323both operating periods (39.0-52.8  $\mu\text{g/L}$ , Fig. 5), also exhibited high removal percentages  
324in the PBR (>98%, Table SM1). Santos et al. (2009) detected ibuprofen as the most  
325abundant compound in four Spanish WWTPs, where concentration levels ranged from  
3263.73 to 603  $\mu\text{g/L}$ . Several studies reported that the dominant ibuprofen removal  
327mechanism in biological systems (membrane bioreactors (Abegglen et al., 2009) and  
328immobilized cell processes (Yu et al., 2011)) is biodegradation. Other authors also  
329attribute the high removal of ibuprofen to aerobic biodegradation processes during WW  
330treatment rather than to sorption processes. Ibuprofen has a low octanol-water partition  
331coefficient (high polarity), so it is not expected to be sorbed onto organic matter (Ávila  
332et al., 2010; Kasprzyk-Hordern et al., 2009). Another possible pathway for ibuprofen  
333removal is the presence of photosensitizers, such as dissolved organic matter (Yu-Chen  
334Lin and Reinhard, 2005).

335Ketoprofen inlet concentrations increased by one order of magnitude between Period I  
336(472 $\pm$ 52 ng/L) and Period II (6729 $\pm$ 413 ng/L) (Fig. 5). Its removal percentage during

337Period I (36%, Table SM1) is similar to the ones reported after activated sludge  
338processes in conventional WWTPs (Jiang et al., 2013). However, it was lower than  
339removals reported in a pilot scale HRAP by Matamoros et al. (2015) (50-95%), although  
340both algal systems were treating influents with similar initial concentrations of  
341ketoprofen. The removal percentage of this compound was statistically different  
342between periods ( $p=4.35 \cdot 10^{-6}$ ).

343Naproxen exhibited the lowest removal percentage among all the anti-inflammatories  
344detected during Period I (10%) but increased during Period II (69%) (Table SM1), being  
345this percentage statistically significant ( $p=8.14 \cdot 10^{-10}$ ). Values from Period II are in  
346accordance with those reported by Matamoros et al. (2015) in a pilot scale HRAP  
347treating urban WW (48-89%). Naproxen removal in a WWTP is mainly attributed to its  
348biodegradability, whereas sorption processes are not considered because of the low  
349octanol-water partition coefficient of naproxen (Kasprzyk-Hordern et al., 2009). Several  
350authors also studied the occurrence and the removal percentage of this compound,  
351obtaining variable removal efficiency depending on the system (Verlicchi et al., 2012).

352Salicylic acid is an active metabolite of the highly consumed acetylsalicylic acid, but it  
353is also a common derivate of phenol. Therefore, it is a typical pollutant in both urban  
354and industrial wastewaters, and its removal from aqueous solutions has received a great  
355deal of attention in recent years because of its high toxicity and accumulation in the  
356environment (Combarros et al., 2014; Evgenidou et al., 2015). Salicylic acid inlet  
357concentrations during Periods I and II were  $1349 \pm 738$  and  $368 \pm 12$  ng/L, respectively  
358(Fig. 5). Total removal was achieved with the PBR in the Period I, whereas only 33% of  
359the salicylic acid was removed during Period II (Table SM1). Escapa et al. (2015)  
360observed high removal percentages (93%) of salicylic acid using the algae *C.*  
361*sorokiniana* in a semi-continuous system.

362The analgesic codeine was only detected during Period I ( $33\pm 1$  ng/L, Fig. 5). This  
363concentration was similar to the urban wastewater treated by Cruz-Morató et al. (2013a)  
364and lower than the WWTP influent reported by Kasprzyk-Hordern et al. (2009). The  
365effluent concentration was below the limit of detection (LOD 4.17 ng/L, LOQ 13.91  
366ng/L).

367As in the case of anti-inflammatories and analgesics, antibiotic levels varied between  
368the two studied periods (Fig. 5). Ciprofloxacin and ofloxacin were detected at  
369concentrations of 2629 ng/L and 65 ng/L, respectively, during Period I and 294 ng/L and  
3705662 ng/L, respectively, during Period II. Azithromycin was only detected in Period I  
371(385 ng/L), and erythromycin was only detected in Period II (661 ng/L). The removal  
372percentages obtained ranged from 48% to complete removal, depending on the  
373antibiotic considered (Table SM1). Ofloxacin removal percentage between periods was  
374not significantly different ( $p=0.313$ ). Antibiotic removals between 35% and 76% in  
375conventional activated sludge processes and between 25% to 95% in membrane  
376bioreactors have been previously described (Radjenovic et al., 2009).

377The  $\beta$ -blocker atenolol was detected at high concentrations in Period I, 7.8  $\mu\text{g/L}$ , and  
378Period II, 6.9  $\mu\text{g/L}$  (Fig. 5); removal percentages were above 80% (Table SM1),  
379although the differences between Period I and II were significantly different ( $p=1.90$   
380 $\cdot 10^{-5}$ ). The inlet concentration is in accordance with values reported by previous authors  
381in WWTP influents (Verlicchi et al., 2012). Escolà Casas et al. (2015b) obtained 40%  
382removal in a continuously moving bed biofilm reactor, whereas almost complete  
383removal was found in a hybrid biofilm and activated sludge system (Escolà Casas et al.,  
3842015a). Biodegradation of atenolol has been linked to the activity of ammonia-oxidizing  
385bacteria 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 ( $p=6.58 \cdot 10^{-7}$ ). 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( $p=1.39 \cdot 10^{-10}$ )) (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 ( $p=1.08 \cdot 10^{-3}$ ). Very diverse diltiazem removal percentages have

411been described in the literature. High removal percentages (88%-99%) were reported by  
412Du et al. (2014) for different WWTPs (i.e., municipal treatment plant, aerobic treatment  
413plant and septic treatment system coupled with a subsurface constructed wetland),  
414whereas removals between 30 and 88% were observed in several Swedish free water  
415surface wetlands (Breitholtz et al., 2012). Only 13% removal was achieved in a  
416conventional activated sludge wastewater treatment process (Blair et al., 2015).

417Clear differences appear in the removal efficiencies of the two study periods (Table  
418SM1). For some compounds, such as naproxen, salicylic acid, ketoprofen,  
419hydrochlorothiazide and lorazepam, a lower influent concentration corresponded to  
420lower removal efficiency. There is a lack of information about EC removal by  
421microalgae. Biodegradation may require a threshold concentration before microbial  
422degradation can be triggered, suggesting an adaptation of the biomass is necessary for  
423the degradation of these compounds (Spain and Van Veld, 1983). This adaptation is  
424enhanced at high concentrations rather than at low concentrations. The removal  
425efficiency of other pollutants (i.e., codeine, ofloxacin, ibuprofen and acetaminophen)  
426was independent of the initial concentration.

427 In general, higher removal efficiencies were obtained during Period II, despite the  
428lower temperature and light irradiation. This could be a consequence of the HRT  
429increase from 8 to 12 days. Some authors reported that biological wastewater treatment  
430technologies for removing ECs are highly dependent on HRT because it enhances  
431biodegradation, photodegradation and sorption removal processes (Matamoros et al.,  
4322015). HRT is indeed a key design parameter for the removal efficiency of  
433microcontaminants in microalgal-based treatment systems; the higher the HRT, the  
434greater the EC removal efficiency (Garcia-Rodríguez et al., 2014; Víctor and Rodríguez,  
4352016).

436The biodegradation of organic compounds in microalgal-based treatment systems is the  
437result of facultative chemoautotrophy; therefore, the organic compounds are directly  
438biodegraded (Hom-Diaz et al., 2015; Priya et al., 2014; Semple et al., 1999). Little is  
439known about PhAC removal mechanisms because of the complexity of the microbial  
440variations in these systems. Microbial communities continuously change in open  
441systems because they need to adapt to the conditions (environmental and/or  
442operational). This fact poses an inconvenience in PhAC removal because not all species  
443are able to remove the same compounds. Cell walls of microalgae contain  
444polysaccharides, proteins and lipids, which in turn contain functional groups, including  
445amino, hydroxyl, carboxyl, and sulfate, that act as binding sites and are used to  
446sequester many different pollutants through adsorption or an ion-exchange process  
447(Priya et al., 2014; Yu et al., 1999). Moreover, microalgae produce peptides, which can  
448also bind to micropollutants.

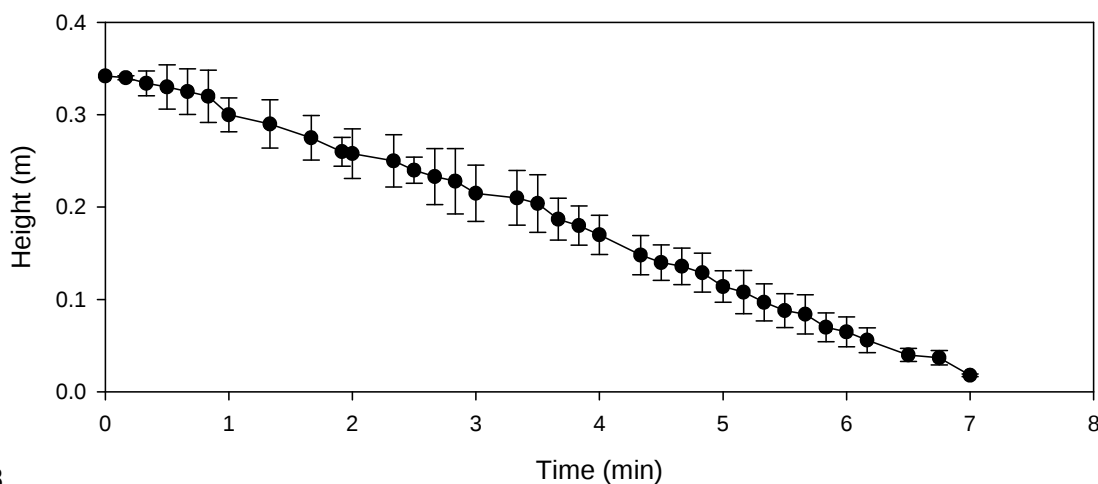
449The EC removal efficiencies significantly vary and can range from negligible removal  
450to 99% removal depending on the physico-chemical characteristics of the compound  
451and the cultivation and operation parameters, such as HRT and environmental  
452temperature (Cuellar-Bermudez et al., 2016).

#### 4533.4 *Microalgae harvesting*

454The gravity sedimentation profile from the PBR effluent at the theoretical hydraulic  
455steady state of Period I is shown in Fig. 6; it follows a linear tendency. The initial and  
456final parameters and the percentage of solid removal are presented in Table 2. The  
457calculated sedimentation velocity was  $0.049\pm 0.005$  m/min, and 99% of TSS were  
458removed within 7 minutes. Biomass from Period I had a good settling capacity, in  
459contrast to the microalgal biomass effluent from Period II operation, where no  
460interphase was observed, and as a consequence, the sedimentation curve could not be

461plotted. The settling velocity of Period II effluent decreased to  $2.29 \cdot 10^{-4}$  m/min within  
 46224 h, achieving a final TSS removal from the supernatant of 88% (Table 2). The  
 463differences between the two periods are attributed to biomass composition. During  
 464Period I, several filamentous species (*Phormidium*, a self-aggregating cyanobacteria  
 465used by previous authors in WW treatment (Olguín, 2003), has the ability to auto-  
 466flocculate and self-aggregate, immobilizing the smaller microalgae cells) were  
 467microscopically observed, while during Period II, a decrease of filamentous species and  
 468an increase of unicellular microalgae decreased the sedimentation rate of the effluent.  
 469Outdoor systems are in a constant state of change because of environmental conditions  
 470affecting biomass composition and harvesting efficiency. Algal harvesting depends on  
 471cell size, microalgae composition and other parameters (Brennan and Owende, 2010; Li  
 472et al., 2008; Olaizola, 2003). Park et al. (2013) stated that the harvesting efficiency was  
 473highly dependent on the dominant algae for a high rate algal pond (HRAP). Removals  
 474between 75 and 85% were achieved when *Pediastrum* sp. was dominant. Although there  
 475is scarce literature available on this subject, the values are similar to the ones reported in  
 476this study (Table 2).

477



478

479Figure 6. PBR Period I effluent sedimentation curve.



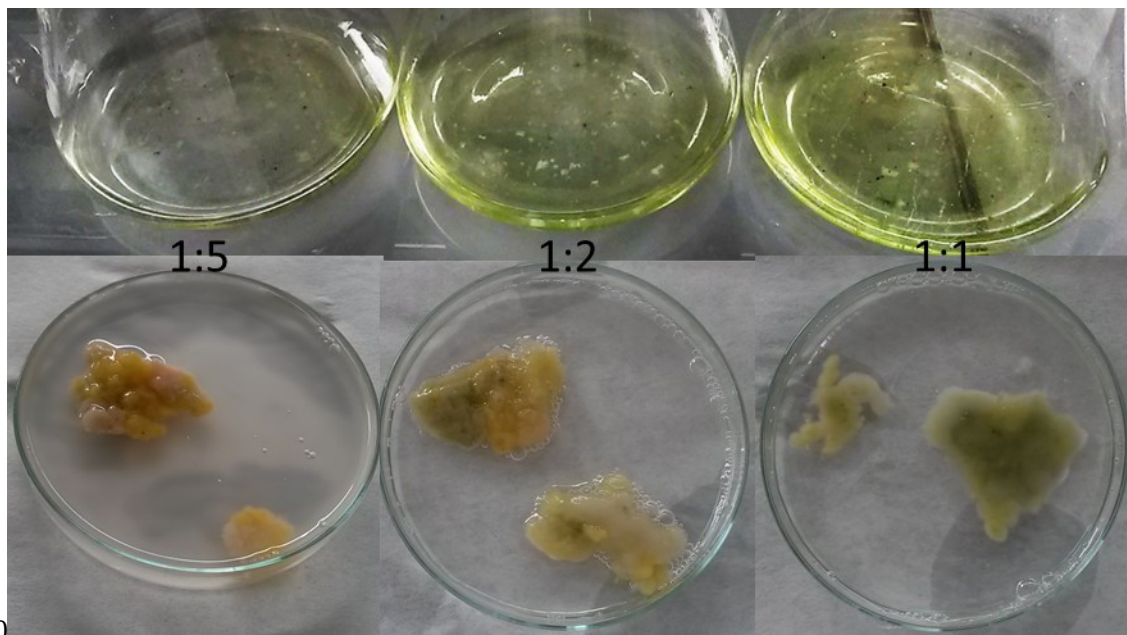
481Table 2. TSS removal percentages after the application of the harvesting technique,  
 482sedimentation and/or co-pelletization for PBR biomass in Period I and II. In brackets: \*  
 483biomass-to-fungal defined media ratio, \*\*sedimentation time.

<b>Harvesting technique</b>	<b>Period (ratio)*</b>	<b>TSS Initial (mg/L)</b>	<b>TSS Final supernatant (mg/L) (time)**</b>	<b>% removal</b>
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

484

485

486To improve the biomass harvesting from the Period II effluent, the novel technique of  
 487co-pelletization was applied using the fungus *Trametes versicolor*. The co-cultivated  
 488algal biomass and fungus can form pellets. The size of the pellets will allow their simple  
 489harvest by mesh sieve filtration or sedimentation. The co-pelletization was achieved at  
 490different algal biomass-to-fungal defined media co-cultivation ratios. Three ratios (1:1,  
 4911:2 and 1:5) were used to study co-pelletization. The results highlight the largely similar  
 492effects of the different algal biomass-to-fungal defined media ratios used in co-  
 493cultivation (Table 2). Co-cultivation can attain nearly complete removal of microalgal  
 494cells from the liquid medium, obtaining a clear, transparent supernatant (Figure 7). At  
 495the three ratios of algal biomass-to-fungal defined media, microalgal biomass was  
 496completely removed from the liquid medium after three days of co-cultivation and  
 497entrapped into the fungal pellets. Fungal pellets became more green (Figure 7) as the  
 498algal concentration increased and as more algal biomass was entrapped by the fungus.



500

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

503

504 The co-pelletization harvesting technique has scarcely been reported for a real  
505 microalgal effluent (Bhattacharya et al., 2017; Muradov et al., 2015; Wrede et al.,  
506 2014). Previous works have focused on pure microalgae cultures (i.e., *Chlorella*)  
507 (Wrede et al., 2014; Xie et al., 2013; Zhang and Hu, 2012; Zhou et al., 2013), and the  
508 promising results obtained encourage further study and the implementation of this  
509 technique in different real microalgal effluents. Harvesting microalgae from effluents  
510 using co-pelletization could be an efficient method to obtain a clarified supernatant and  
511 recover biomass for further valorization.

512

#### 5134 Conclusions

514  $\text{N-NH}_4^+$ , total phosphorous and COD removal percentages of higher than 80% were  
515 obtained by treating WW for four months in a microalgal photobioreactor operating at  
516 two different hydraulic retention times (8 days during Period I and 12 days during

517Period II). PBR performance is highly impacted by temperature and solar irradiation.  
518Low temperatures and few light hours decrease the TSS concentration (Period II),  
519which is directly related to productivity as well as nutrient removal.

520PhAC removal was also evaluated. Overall high removals (98%) were achieved for anti-  
521inflammatory drugs (ibuprofen, acetaminophen, salicylic acid, and codeine) and some  
522compounds, such as the diuretics hydrochlorothiazide (84%) and furosemide (total  
523removal). Lower removals (>48%) were obtained for antibiotics (azithromycin,  
524ciprofloxacin, ofloxacin and erythromycin) and the psychiatric drug lorazepam (30-  
52557%). These results demonstrate that algal systems are a good option for the biological  
526treatment of toilet WW.

527Microalgal photobioreactor effluent harvesting depends on the biomass characteristics.  
528In Period I, flocs were easily formed, allowing good clarification at high sedimentation  
529velocities (0.049 m/min and 7 min) using the natural sedimentation technique.  
530Sedimentation velocity decreased during Period II ( $2.29 \cdot 10^{-4}$  m/min), and the  
531clarification percentage also decreased (88%). The novel harvesting technology, co-  
532pelletization using *T. versicolor*, provided a solution to problems associated with current  
533energy-intensive and costly algae harvesting processes. Despite the good results attained  
534in this study (>98% microalgae entrapment), further research is still needed to study the  
535detailed pelletization conditions for large scale industrial applications.

536

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547

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