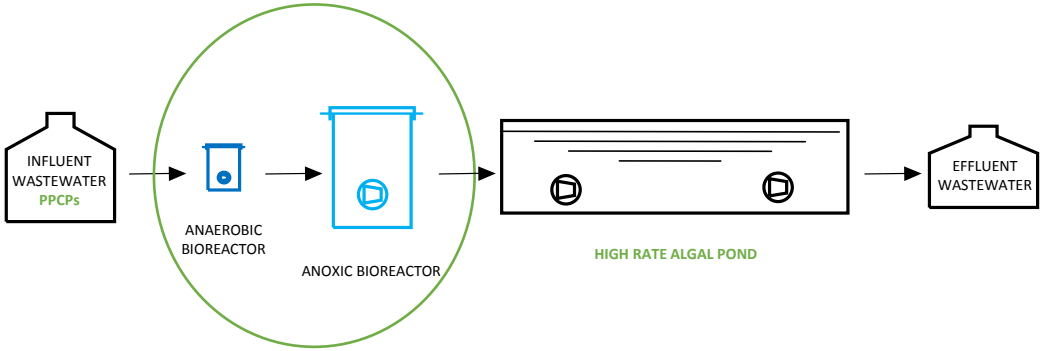
***Graphical Abstract**



***Highlights (for review : 3 to 5 bullet points (maximum 85 characters including spaces per bullet point)**

Research highlights

- This work evaluated the removal of ECs in novel AX-HRAP and ANA-AX-HRAP PBRs
- Triclosan and propylparaben showed total elimination during ANA-AX-HRAP set-up
- REs > 90% were obtained for ibuprofen and salicylic acid in the ANA-AX-HRAP PBR
- Naproxen was the most recalcitrant with eliminations rates below 52% in all cases
- Biodegradation and sorption were hypothesized as main removal mechanisms

Removal of contaminants of emerging concern from urban wastewater in novel algal-bacterial photobioreactors

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ABSTRACT

This work evaluates the removal of five pharmaceuticals and personal care products, i.e., ibuprofen, naproxen, salicylic acid, triclosan and propylparaben, from urban wastewater under two novel algal-bacterial photobioreactor settings. The first configuration (phase A) consisted of an anoxic-aerobic photobioreactor operating at a hydraulic retention time (HRT) of 2 d at different concentrations of total organic carbon (TOC) (90 mg L^{-1} – 200 mg L^{-1}). In the second configuration (phase B) an anaerobic step was introduced before the anoxic tank to set a photosynthetic A_2O process. In this phase, the HRT varied between 3

and 4 d and the TOC was kept constant at 200 mg L^{-1}. In addition, the impact of external aeration in the aerobic photobioreactor was assessed. The maximum removals for ibuprofen, naproxen, salicylic acid, triclosan and propylparaben (94±1%, 52±43%, 98±2%, 100±0%, 100±0%, respectively) were recorded during phase B. In phase A, low TOC concentrations triggered higher ibuprofen and naproxen removals likely due to the high contribution of biological oxidation on their removal. In phase B, total or very high removal efficiencies were observed for ibuprofen, propylparaben and triclosan independently on the operating conditions. In contrast, the removal efficiency of naproxen and salicylic acid decreased when the HRT dropped from 4 to 3 d in the absence of external aeration, which suggests that biodegradation played a key role in their removal. In addition, sorption might have contributed to the elimination of triclosan and propylparaben from the wastewater.

Keywords:

Algal-bacterial processes; emerging pollutants; microalgae; microcontaminants; micropollutants; PPCPs; wastewater treatment.

1. Introduction

Multiple organic compounds initially present in pharmaceuticals, personal care products, plasticizers, surfactants and pesticides are daily released into the environment via wastewater discharge from conventional wastewater treatment plants (WWTPs), which were not originally designed for the removal of such recalcitrant compounds (Matamoros et al., 2015; Rivera-Utrilla et al., 2013). These pollutants are referred to as contaminants of emerging concern (CECs), and their pernicious effects on the environment and human health are still unknown (although reproductive disruption in fish and other aquatic organisms such as alligators and frogs has been reported (Orlando and Ellestad, 2014)) (Alan et al., 2008). In this context, and despite the lack of regulatory limits of discharge of CECs into natural water bodies, the recent Directive (2013/39/EU) (European Commission, 2013) has updated the previous list of 41 priority substances to 45, which has boosted research on cost-effective methods for CEC removal. Conventional physical-chemical technologies such as adsorption on activated carbon and advanced oxidation processes (i.e. ozonation, photooxidation, radiolysis and electrochemical oxidation) have been tested both at lab and industrial scale to remove CECs from wastewaters. However, the tentative toxicity of the resulting transformation products and the lack of consistent analytical data together with the high investment and operating costs of these technologies has limited their application in WWTPs (Rivera-Utrilla et al., 2013). In this context, algal-bacterial processes have recently emerged as a cost-effective and

environmentally friendly alternative to remove CECs from wastewaters (Norvill et al., 2016). Microalgae-based wastewater treatment is based on the solar-driven conversion of carbon and nutrients from wastewater into algal-bacterial biomass (de Godos et al., 2012; Hom-Diaz et al., 2017a; Matamoros et al., 2015; Norvill et al., 2016). Although this green technology was not originally engineered to remove CECs from wastewaters, process operation at high sludge retention time (4-20 d), the enhanced penetration of UV light as a result of their high surface area to volume ratios, and the high daily variations of pH (7-11), dissolved oxygen concentration (2-25 mg O_2 L^{-1}) and temperature (depending on the climatic zone) render algal-bacterial photobioreactors a promising platform for the removal of CECs (Hom-Diaz et al., 2017a; Matamoros et al., 2015; Norvill et al., 2016; Norvill et al., 2017). Thus, (Matamoros et al., 2015) evaluated the effect of the hydraulic retention time (HRT) on the removal of 26 CECs from urban wastewater in two outdoor high rate algal ponds (HRAPs) of 470 L and concluded that up to 90% of the contaminant risk was removed during microalgae-based treatment. More recently, (Hom-Diaz et al., 2017a) recorded removal efficiencies above 48% in 17 pharmaceuticals detected in toilet wastewater and treated by a pilot-scale HRAP working at a HRT of 8 and 12 d. Similarly, (Norvill et al., 2017) and (de Godos et al., 2012) reported tetracycline removal efficiencies (REs) of 93% and 69±1% in a 180-L outdoor HRAP and in a 14-L lab-scale HRAP, respectively. Finally, (Hom-Diaz et al., 2017b) also reported a successful removal of ciprofloxacin in a 1000-L outdoor HRAP during domestic wastewater treatment.

Despite these previous studies have consistently shown the potential of HRAPs for CEC removal, the fate of these pollutants in innovative algal-bacterial photobioreactor

81 configurations has not been yet investigated. Hence, anoxic-aerobic algal-bacterial
82 photobioreactors have emerged as new configurations capable of treating low
83 carbon/nitrogen (C/N) wastewaters with a superior carbon and nutrient removal than
84 conventional HRAPs (Alcántara et al., 2015; de Godos et al., 2014; García et al., 2017). In
85 addition, anaerobic-anoxic-aerobic configurations based on photosynthetic oxygenation
86 have been proposed to foster phosphorous removal during wastewater treatments
87 (Metcalf et al., 2003).

88 In this context, the present work assessed for the first time the CEC removal performance
89 of two novel configurations consisting of anoxic-aerobic (phase A) and anaerobic-anoxic-
90 aerobic photobioreactors (phase B). The influence of parameters such as organic load,
91 HRT and external aeration on the removal of 5 pharmaceuticals and personal care
92 products (PPCPs) typically found in urban wastewaters (i.e., ibuprofen, naproxen, salicylic
93 acid, triclosan and propylparaben) was studied.

94

95 **2. Materials and methods**

96 **2.1 Experimental set-ups**

97 The experimentation was carried out in two phases. The first one, phase A, consisted of a
98 3.75-L enclosed anoxic bioreactor (AX) (15 cm long, 15 cm wide, 17 cm deep), an 11.25-L
99 open photobioreactor (HRAP) (30 cm long, 15 cm wide, 25 cm deep) and a 1-L conical
100 settler (Fig. 1). The 0.25/0.75 volume ratio of the AX and HRAP, and the depth of the

HRAP, were selected according to (Mosquera Corral, 2013) and (Sutherland et al., 2014), respectively. Culture mixing in the anoxic tank and HRAP was provided by Eheim compact 300 immersion pumps (Spain) (one pump in the AX and two pumps in the HRAP). The HRAP was exposed to a 12:12 h:h light:dark illumination regime at an average photosynthetically active radiation (PAR) over the photobioreactor surface of 1314±12 µmol m^{-2} s^{-1} via high-intensity LED PCBs (Phillips SA, Spain). An internal liquid recirculation (IR) from the HRAP to the AX supported the denitrification process and an external liquid recirculation (ER) from the bottom of the settler to the AX supported algal-bacterial biomass retention. The wastage of algal-bacterial biomass was conducted from the bottom of the settler, to maintain a constant sludge retention time (SRT) (Fig. 1). Both the SWW and treated effluent were stored in 20 L glass containers to avoid CECs adsorption.

In the second configuration, phase B, the set-up from phase A was modified by implementing a 0.65-L enclosed anaerobic bioreactor (ANA) (8.7 cm long, 8.7 cm wide, 8.7 cm deep) before the AX. The ANA unit was directly fed with synthetic wastewater and the ER from the settler, and discharged into AX by gravity. ANA was also mixed by an Eheim compact 300 immersion pump (Spain) (Fig. 2).

2.2 Algal-bacterial inoculum

The anoxic-aerobic photobioreactor (phase A) was inoculated with a microalgal consortium, collected from an outdoor pilot-scale HRAP treating centrate at the Department of Chemical Engineering and Environmental Technology (University of

Valladolid, Spain) and with secondary activated sludge from Valladolid WWTP (operated with a denitrification-nitrification configuration), in order to attain an initial total suspended solids (TSS) concentration of 0.2 and 0.6 g TSS L^{-1} of microalgae and activated sludge, respectively.

On the other hand, the anaerobic-anoxic-aerobic photobioreactor (phase B) was inoculated with a microalgae consortium collected from the anoxic-aerobic configuration and 1 L of aerobic-anoxic sludge from Valladolid WWTP.

2.3 Synthetic domestic wastewater

Synthetic domestic wastewater (SWW) was prepared according to (Frutos et al., 2016) in order to simulate a typical urban wastewater composition (Metcalf et al., 2003). The SWW was composed of (g L^{-1}): casein peptone 0.16, meat extract 0.11, NH_2COH_2 0.03, NaCl 0.007, $CaCl_2 \cdot 2H_2O$ 0.004, $MgSO_4 \cdot 7H_2O$ 0.002, $CuCl_2 \cdot 2H_2O$ 5·10-685, $K_2HPO_4 \cdot 3\ H_2O$ 0.112, $C_6H_{12}O_6$ 0.25 and $NaHCO_3$ 1.10. This resulted in concentrations of chemical oxygen demand (COD) of 632±45 mg L^{-1} , total organic carbon (TOC) of 196±9 mg L^{-1}, inorganic carbon (IC) of 195±12 mg L^{-1} , total nitrogen (TN) of 43±3 mg L^{-1}, $N\text{-}NH_4^+$ of 24±3 mg L^{-1}, $P\text{-}PO_4^{-3}$ of 13.1±0.8 mg L^{-1} and in a pH of 7.7±0.2. All reagents were purchased from PANREAC (Barcelona, Spain) with a purity >98%. Ibuprofen, naproxen, salicylic acid, triclosan and propylparaben were selected as model CECs based on their relevant concentration levels and ubiquity in aquatic environments as well as their variable degradability in conventional WWTPs (Hughes et al., 2013). An aliquot of a stock solution

143 containing the five CECs in methanol was daily added to the SWW to achieve a final
144 concentration of 8955±959 ng L^{-1} of ibuprofen, 4177±128 ng L^{-1} of naproxen, 62137±1449
145 ng L^{-1} of salicylic acid, 537±11 ng L^{-1} of triclosan and 408±31 ng L^{-1} of propylparaben. This
146 represented a realistic scenario according to the typical values determined in different
147 wastewater treatment plants in Spain (Ortiz de García et al., 2013; Reyes-Contreras et al.,
148 2012; Reyes-Contreras et al., 2011). Additionally, these concentrations are not expected
149 to exert any biomass inhibitory effect. In fact, previous studies by the authors showed no
150 significant effect on HRAP performance at 2 mg L^{-1} of tetracycline, which represents more
151 than 20 times higher concentration of antibiotic than the combined concentration of all
152 the PPCPs assessed in the present study (de Godos et al., 2012). The CECs standards were
153 purchased from Sigma-Aldrich with a purity >99% and methanol was purchased from
154 PANREAC (purity >99%).

155

156 **2.4 Operational stages and sampling procedure**

157 In phase A, the bioreactors were initially filled with SWW, inoculated and operated at a
158 hydraulic retention time (HRT) of 4 d using a Watson Marlow 120 S pump (United
159 Kingdom) in order to acclimate the algal-bacterial consortium to the SWW. CECs were not
160 supplemented to the SWW during the acclimation period. The flow rates of the IR and ER
161 pumps (Watson Marlow 120 S 124 pump, United Kingdom, and Masterflex 7021-24,
162 United States, respectively) corresponded to 200% and 50% of the SWW flow rate,
163 respectively. The SRT of the system was fixed at 10 d. The initial 47 d acclimation period

was followed by a decrease in the HRT to 2 d and the supplementation of the SWW with CECs. The flow rates of IR and ER were adjusted to the new SWW flow rate, while the SRT was maintained at 10 d. Under these operational conditions, the influence of the COD concentration (669±6 mg L^{-1} during stage A-I, 493±11 mg L^{-1} during stage A-II and 434±11 mg L^{-1} during stage A-III) on process performance was evaluated. Each operational stage was maintained for 40 d (≈4 times the SRT) to achieve representative steady states, which were kept for at least 10 days (thus allowing 4 replicate measurements). The system was considered under steady state when the TOC, IC and TN removals, as well as the TSS concentrations, varied less than 10% compared to the average values. The pH, dissolved oxygen concentration (DO) and temperature were daily measured in the AX and HRAP. The influent and effluent flow rates were also daily measured in order to determine the evaporation rate, while the PAR was weekly monitored. Liquid samples of 50 mL were drawn twice per week from the AX and HRAP to monitor the concentrations of TSS. The influent and effluent concentrations of COD and CECs were measured at steady state. The concentration of the H_2O_2 produced from photosynthetic microalgae activity in the HRAP was also measured under steady state to determine its effect on CECs removal.

No acclimation period was necessary in phase B since the inoculum in the anaerobic-anoxic-aerobic photobioreactor was obtained from phase A. The system was initiated at an HRT of 4 d (Stage B-I). The flow rates of the IR and ER pumps corresponded to 200% and 50% of the SWW flow rate, respectively. The SRT of the system was fixed at 10 d. The SWW was supplemented with CECs. During stage B-II, the HRT was decreased to 3 d under similar operating conditions to stage B-I (the flow rates of IR and ER were adjusted to the

186 new SWW flow rate, while the SRT was maintained at 10 d). Finally, 30 mL min^{-1} of air
187 were bubbled into the HRAP during the night period in stage B-III in order to keep the
188 HRAP DO levels above 1.5 mg L^{-1}. Each operational stage was maintained for ≈4 times the
189 SRT in order to achieve representative steady states. The pH, dissolved oxygen
190 concentration (DO) and temperature were daily measured in the ANA, AX and HRAP. The
191 influent and effluent flow rates were also daily measured in order to determine the
192 evaporation rate, while the PAR was weekly monitored. Liquid samples of 50 mL were
193 drawn twice per week from the ANA, AX and HRAP to monitor the concentrations of TSS.
194 The influent and effluent concentrations of COD and CECs were measured under steady
195 state.

196

197 **2.5 Analytical methods**

198 The DO concentration and temperature were monitored with an OXI 330i oximeter (WTW,
199 Germany), while a pH meter Eutech Cyberscan pH 510 (Eutech instruments, The
200 Netherlands) was used for pH determination. The PAR was recorded with a LI- 250A light
201 meter (LI-COR Biosciences, Germany). The determination of TSS and COD concentration
202 was carried out according to standard methods (Eaton et al., 2005). The quantification,
203 identification and biometry measurements of microalgae population structure were
204 performed by microscopic examination (OLYMPUS IX70, USA). H_2O_2 was measured with a
205 Pierce Quantitative Peroxide Assay Kit (Thermo Scientific, USA).

206 CEs analyses were carried out according to (López-Serna et al., 2018). In brief, influent and
207 effluent wastewater samples (100 mL, in duplicate) were saturated with NaCl (400 g L^{-1})
208 and pH adjusted to 3. Seventeen milliliters of the resulting solution were spiked with 100
209 ng of five isotopically labelled internal standards (i.e., ibuprofen-d3, propylparaben-d7,
210 salicylic acid-d4, naproxen-d3, triclosan-d3) and subsequently automatically analyzed by
211 online direct immersion solid phase microextraction (DI-SPME) on-fiber derivatization
212 followed by gas chromatography (GC) on a capillary HP-5MS column (30 m length,
213 0.25 mm i.d., 0.25 µm film thickness) coupled to mass spectrometry (MS) in selected ion
214 monitoring (SIM). Quantification based on peak areas was concurrently performed by
215 both matrix-matched and internal standard approaches. Method accuracy parameters are
216 shown as Supplementary data.

217

218 **3. Results and discussion**

219 The evaporation rate in the HRAP remained approx. constant at 15 L m^{-2} d^{-1} regardless of
220 the operational stage in phase A (Table 1). During phase B, evaporation rates accounted
221 for 13-16 L m^{-2} d^{-1} in stages B-I and B-II, respectively, and increased to 20 L m^{-2} d^{-1} in stage
222 B-III (Table 2). These high evaporation rates compared to the water losses in industrial
223 scale HRAP (≈3-8 L m^{-2} d^{-1}) were caused by the high turbulence induced by an
224 overdimensioned immersion pump in this lab scale HRAP (Guieysse et al., 2013). This
225 phenomenon was aggravated in stage B-III, where additional aeration was supplied during

226 the night period. In this context, water losses resulted in 7-16% higher CECs
227 concentrations in the effluent along the whole experimentation.

228 The organic nitrogen present in the SWW as protein (around 19 mg L^{-1}) was expected to
229 undergo ammonification in the anaerobic tank and subsequent nitrification in the HRAP
230 tank. In the absence of strong natural buffers, the pH was expected to drop as a result of
231 NH_4^+ oxidation to nitrate. However, photosynthetic activity increased the pH in the HRAP
232 compared to the anaerobic and anoxic tanks (Table 1 and Table 2). Surprisingly, lower
233 organic loads mediated lower pHs in the HRAP during phase A, which ultimately resulted
234 in similar pHs during stage A-III in both bioreactors (≈8.2 and 8.4 in AX and HRAP,
235 respectively). In phase B, the lowest pH values were observed in ANA (≈7.8), which was
236 attributed to the typical acidification of anaerobic processes. The highest values were
237 again observed in the HRAP (≈9.8), which were higher than in the HRAP during phase A
238 and considered a proxy of a more intense photosynthetic activity. In any case, pH values
239 remained within the optimum range to support microbial activity (Posadas et al., 2015).

240 The DO concentrations in AX remained lower than ≈ 0.3 mg L^{-1} (Table 1) during phase A
241 due to its enclosed nature and absence of photosynthetic activity. These DO levels were
242 suitable to support denitrification, which typically requires DO concentrations lower than
243 1 mg L^{-1} (Metcalf et al., 2003). DO concentration in the HRAP increased at decreasing COD
244 loads in phase A due to the reduced heterotrophic oxygen demand to oxidize the organic
245 matter present in the SWW. During phase B, DO concentrations remained at ≈ 0 mg O_2 L^{-1}
246 and below 1 mg O_2 L^{-1} in ANA and AX, respectively (Table 2). DO in the HRAP remained

247 around 13 mg O_2 L^{-1} in stage B-I, but dropped to 5 mg O_2 L^{-1} during stage B-II due to the
248 increase in the heterotrophic and nitrifying oxygen demand mediated by the decrease in
249 HRT. This severely limited the nitrification processes during stage B-II, which was
250 recovered in stages B-III by bubbling air during the night period.

251 The temperatures in the cultivation broth of the HRAP were usually the highest in both
252 phases as a result of the heat irradiated by the LEDs (Table 1 and Table2). In phase B, the
253 temperatures in ANA remained above those recorded in AX (Table 2). Regardless, the
254 observed temperatures in all the bioreactors (23-30 C) were suitable to support an
255 optimum biological wastewater treatment regardless of the stage. In this context,
256 Removal efficiencies (REs) of ibuprofen, naproxen and salicylic acid have been reported to
257 increase by ~ 10-20% when temperature increased from 12 C to 26 C (Hijosa-Valsero et al.,
258 2010). In our particular study, the influence of temperature on CECs removal was likely
259 negligible due to the fact that the highest difference in temperature per unit along each
260 phase was 5 C (Table 1 and Table 2).

261 The microalgae population (percentage of cells) during phase A was mainly composed of
262 *Chlorella vulgaris*, with abundances of 55% and 48% in A-I and A-II, respectively. However,
263 *Phormidium sp.*, which was absent during A-I and represented only 27% of microalgae
264 cells in A-II, increased up to 39% during A-III, representing the most popular species
265 followed by *Chlorella vulgaris*, which dropped to 28%. During phase B, *Chlorella vulgaris*
266 was also the most abundant species with abundances of 65 and 47% in B-I and B-II,
267 respectively, followed by *Scenedesmus acuminatus* (with abundances of 24 and 38% in

stage B-I and B-II, respectively). Interestingly, the addition of external aeration modified the microalgae population structure. Hence, *Pseudonabaena acicularis* and *Scenedesmus acutus* accounted for 67 and 16% of the total microalgae population during stage B-III, respectively, while *Chlorella vulgaris* dropped to levels of 8% during this stage. At this point it should be highlighted that the active internal and external recirculation (2.5 times the SWW flowrate) resulted in a complete mixing of the cultivation broth and therefore a similar microalgae population structure in the three bioreactor units.

These novel configurations and operational conditions supported effective COD removals of 84±0%, 89±1%, 86±1%, 90±1%, 94±1% and 94±0%, which resulted in COD effluent concentrations of 116±4 mg L^{-1}, 61±5 mg L^{-1}, 67±7 mg L^{-1}, 70±10 mg L^{-1}, 45±4 mg L^{-1} and 46±1 mg L^{-1} during stages A-I, A-II, A-III, B-I, B-II and B-III, respectively. In this regard, the treated effluent complied with the limits of COD concentration (≤125 mg L^{-1}) required by the EU Water Framework Directive for wastewater disposal into the environment (Directive 2000/60/EC) (European Commission, 2000) (European Commission, 2000).

The highest TSS concentrations were recorded in the HRAP (≈ 0.7-1.4 g L^{-1}) (Table 1 and Table 2), which was likely mediated by the superior carbon and nutrient removal mediated by algal activity in the photobioreactor (Posadas et al., 2013). In addition, effluent TSS concentrations were always within the discharge limit for wastewater during phases A and B regardless of the operational conditions.

Finally, H_2O_2, a byproduct from algal photosynthetic activity, was not detected along the whole experimentation, which ruled out a potential contribution of chemical oxidation to

289 CECs removal in the HRAP (Fukuzumi, 2016). Likewise, UV photodegradation was not
290 considered as a potential mechanism of CECs' removal in this research based on the fact
291 that the wavelength of the LEDS here used only comprised the range 400-700 nm.
292 Similarly, volatilization was not likely a significant mechanism of CEC removal based on the
293 fact that the vapor pressures of the 5 target CECs remained below 0.15 Pa at the range of
294 temperatures prevailing in the experimental set-up along the experiment.

295

296 **3.1 Ibuprofen removal**

297 In the anoxic-aerobic photobioreactor, Ibuprofen REs of 26±13%, 76±2% and 91±1% were
298 recorded in stages A-I, A-II and A-III, respectively (Fig. 3). The concentration of ibuprofen
299 in the effluent at the highest RE was ≈894 ng L^{-1}, which would correspond to ≈834 ng L^{-1} in
300 absence of water evaporation. The elimination of ibuprofen in stage A-III was similar to
301 that reported by (Hom-Diaz et al., 2017a; Matamoros et al., 2015), who always detected
302 ibuprofen removals >86% and concentrations < 900 ng L^{-1} in the effluent of two 470-L
303 outdoor single-stage HRAPs during the treatment of urban wastewater regardless of the
304 season and HRT. It should be noticed that in those studies, solar UV-mediated
305 photodegradation was expected to be an important elimination mechanism. In our
306 particular study, lower COD loads were correlated to higher ibuprofen REs, likely due to
307 the increase in DO in the cultivation broth of the HRAP, which triggered the removal of
308 this CEC by biological oxidation (Matamoros et al., 2016). An enhancement in the
309 nitrification activity was also observed in stages A-II and A-III mediated by the higher DO.

310 This could have also contributed to the improvement in the ibuprofen removal as
311 previously reported by (Fernandez-Fontaina et al., 2012) in activated sludge systems.

312 In the anaerobic-anoxic-aerobic photobioreactor, Ibuprofen was removed at higher
313 efficiencies than in phase A. Indeed, REs of 94±1%, 93±3% and 81±0% were recorded in
314 stages B-I, B-II and B-III, respectively (Fig. 3). Hence, no significant influence of HRT and
315 the DO was observed in the anaerobic-anoxic-aerobic photobioreactor. These results
316 confirmed that this novel configuration did not impact the good CEC removal performance
317 observed in single-stage HRAPs by (Hom-Diaz et al., 2017a; Matamoros et al., 2015),
318 where REs above 86% were recorded at a HRT of 4 d.

319 Ibuprofen removal by biomass sorption could be considered negligible in both phases at
320 the operational pH in ANA, AX and HRAP due to its negative charge (ibuprofen pKa≈4.41)
321 and the electrochemical negative charge of the microalgae cells walls (Matamoros et al.,
322 2015; Matamoros et al., 2016). Hence, biodegradation was likely the main mechanism
323 governing ibuprofen removal in this study. This was in agreement with the study
324 conducted by (Matamoros et al., 2016), who reported an increase of up to 40% in
325 ibuprofen removal due to the synergetic interaction between algae and bacteria.

326

327 **3.2 Naproxen removal**

328 In the anoxic-aerobic photobioreactor, naproxen removal was only effective during stage
329 A-III at 28±7%, which resulted in concentrations in the effluent of ≈3239 ng L^{-1} (≈3022 ng L^{-}

[1] without evaporation) (Fig. 4). In fact, naproxen was the CEC that exhibited the lowest RE of all studied ones. These naproxen REs were in accordance with the 33% reported in a waste stabilization pond during urban wastewater treatment (Hijosa-Valsero et al., 2010). Even lower elimination efficiencies (10%) were observed by (Hom-Diaz et al., 2017a) when toilet wastewater was treated in a single-stage pilot-scale HRAP operated at a HRT of 8 d. In contrast, (Matamoros et al., 2015) recorded naproxen REs of 60-90% during urban wastewater treatment in two 470-L outdoor single-stage HRAPs, where in contrast to our study, solar UV-mediated photodegradation was likely a key removal mechanism. In our particular study, the lower COD loads and higher DO concentration in stage A-III could have supported a significant naproxen removal by biological oxidation. In this sense, the enhanced nitrification activity occurring under these operational conditions could have also influenced naproxen removal (Fernandez-Fontaina et al., 2012). Additionally, microalgae inhibition at naproxen concentrations higher than 100 ppm has been reported (Fernandez-Fontaina et al., 2012). In this context, and despite the lower concentrations here tested ($\leq 4.2 \cdot 10^{-3}$ ppm), the absence of naproxen removal observed during the first two stages in phase A might have been due to microbial acclimation to this pollutant.

Overall, the anaerobic-anoxic-aerobic photobioreactor was more efficient at removing naproxen than the anoxic-aerobic photobioreactor. Hence, REs of 44±11%, 24±1% and 52±7% were recorded in stage B-I, B-II and B-III, respectively (Fig. 4). Thus, biodegradation could be pointed out as a major elimination mechanism again as a result of the enhanced photosynthetic activity and longer HRT during phase B. Those removal efficiencies were similar to the ones reported by (Matamoros et al., 2015) at a HRT of 4 d in a single-stage

352 HRAP during cold season (48±5%). Interestingly, a reduction in naproxen removal
353 efficiency was observed in stage B-II when DO levels in HRAP decreased below 1.5 mg L^{-1},
354 which suggests the key role of biological oxidation on the removal of this anti-
355 inflammatory drug. Finally, it should be highlighted that CEC sorption into biomass could
356 be discarded as a removal mechanism in the systems due to its low pKa (≈4.84) compared
357 to the pH in the bioreactors.

358

359 **3.3 Salicylic acid**

360 Steady state salicylic acid REs during the operation of the anoxic-aerobic photobioreactor
361 accounted for 63±1%, 83±5% and 74±8% in stages A-I, A-II and A-III, respectively (Fig. 5).
362 The minimum salicylic acid concentration in the effluent was ≈11691 ng L^{-1} during stage A-
363 II (≈10756 ng L^{-1} without water evaporation). The moderate increase in RE from stage A-I
364 to stages A-II and A-III showed the low influence of COD load on salicylic acid removal. The
365 REs herein recorded were lower than the reported values of 93% and 98% by (Escapa et
366 al., 2017) during the evaluation of salicylic acid removal at 25 mg L^{-1} and at 250 mg L^{-1},
367 respectively, by *Chlorella sorokiniana* in a 250 mL bubbling column photobioreactor
368 illuminated at 370 µmol m^{-2} s^{-1} at 12:12 h with 8 fluorescent lamps (58 W, 2150 lumen,
369 Philips, France). These authors concluded that *C. sorokiniana* was able to use salicylic acid
370 as an additional carbon source. Differences in results could be attributed to the high
371 concentrations tested there, in comparison to the realistic ppb levels assessed in our
372 study.

373 On the other hand, salicylic acid REs in the anaerobic-anoxic-aerobic photobioreactor
374 were generally similar to those recorded in the anoxic-aerobic system (except in stage B-
375 II). In particular, REs of 97±2%, 34±25% and 75±7% were achieved in stages B-I, B-II and B-
376 III, respectively (Fig. 5). These high elimination efficiencies were similar to the ones
377 reported by (Escapa et al., 2017; Hom-Diaz et al., 2017a) in the studies described above,
378 despite no UV photodegradation contribution occurred in the present research. The
379 occurrence of episodes at DO close to 0 were frequent during stage B-II and could explain
380 the poor removal of this CEC during that stage. This would lead to confirm biodegradation
381 as a major salicylic acid removal mechanism. Similarly to the rationale described for
382 naproxen and ibuprofen, sorption was not considered a relevant mechanism based on the
383 low pKa (≈3.01) of this CEC, which is far too low from the pH values observed in the
384 bioreactors along the whole experimentation.

385

386 **3.4 Triclosan**

387 Triclosan REs of 68±3%, 85±0% and 83±0% were recorded in the anoxic-aerobic
388 photobioreactor in stages A-I, A-II and A-III, respectively (Fig. 6). Thus, the minimum
389 concentration of triclosan in the effluent was ≈89 ng L^{-1} (≈82 ng L^{-1} without water losses).
390 Similarly to the pattern recorded for salicylic acid, comparable degradation efficiencies of
391 triclosan were recorded regardless of the COD load applied. These high REs agreed with
392 those reported by (Matamoros et al., 2015), who classified triclosan as a moderate-highly

393 degradable CEC (REs of 60-90%) during urban wastewater treatment in two 470-L outdoor
394 HRAPs.

395 The anaerobic-anoxic-aerobic photobioreactor supported higher efficiencies than those
396 achieved in the anoxic-aerobic photoreactor. In particular, triclosan REs of 73±0%, 100±0%
397 and 100±0% were recorded in stage B-I, B-II, B-III and B-IV, respectively (Fig. 6). This
398 showed that the enhanced denitrification processes achieved during phase B, as well as
399 the higher HRT, contributed positively to triclosan elimination. Thus, this novel
400 photobioreactor configuration turned out to be more advantageous than single-stage
401 HRAPs for the elimination of triclosan (Matamoros et al., 2015).

402 The high temperatures prevailing in our experimental set-ups in phase A and B likely
403 supported an effective triclosan removal by biodegradation (Hoque et al., 2014). In fact,
404 (Wang et al., 2018) recently concluded that cellular uptake and biotransformation are
405 major removal mechanisms of triclosan in microalgae cultures at a pH of 7.5, which
406 suggests the key role of enzymatic triclosan degradation in our photobioreactors.
407 However, triclosan removal by sorption into biomass should be also considered based on
408 its pKa (≈7.80), which was close to the above-mentioned range of pHs in the ANA and AX
409 units (Matamoros et al., 2015).

410

411 **3.5 Propylparaben**

412 Propylparaben REs of 87±0%, 81±17% and 85±15% were recorded in stages A-I, A-II and A-
413 III, respectively, in the anoxic-aerobic photobioreactor (Fig. 7). These high REs resulted in
414 propylparaben concentrations in the effluent of ≈ 55 ng L^{-1} (≈52 ng L^{-1} without water
415 losses). This research was the first time propylparaben was assessed under microalgae-
416 based water treatment. Nonetheless, these REs were similar to the eliminations reported
417 in conventional activated sludge WWTPs (>90%) (Haman et al., 2015). Interestingly,
418 propylparaben removal was not dependent on COD loads.

419 The anaerobic-anoxic-aerobic photobioreactor supported steady state propylparaben REs
420 of 73±0%, 100±0% and 100±0% during stage B-I, B-II and B-III, respectively) (Fig. 7).

421 The degradation profiles recorded in phase A and B were almost identical to the ones
422 observed for triclosan. Indeed, propylparaben exhibits a high pKa (≈8.23) too, similar to
423 the pH value of the cultivation in ANA, AX and HRAP during most of both experiments,
424 which likely supported a significant contribution of sorption into biomass to the removal
425 of propylparaben as well. Nevertheless, there is also consistent evidence in literature that
426 supports the high biodegradability of parabens (>90%), which suggests that
427 biodegradation might have played also a major role on propylparaben fate in the
428 photobioreactors (González-Mariño et al., 2011).

429

430 **4. Conclusions**

To the best of our knowledge, this research constitutes the first time the removal of CECs from domestic wastewater was evaluated in two novel anoxic-aerobic and anaerobic-anoxic-aerobic photobioreactor configurations. Overall, the set-up including the anaerobic process exhibited higher removals of the five PPCPs than the anoxic-aerobic photobioreactor. This superior performance could have been mediated by the higher HRT, and enhanced denitrification processes and photosynthetic oxygenation, which supported biodegradation as a major mechanism in the elimination of the five CECs. In contrast, volatilization and photodegradation phenomena were discarded due to the low vapor pressure of the contaminants and the use of visible LED lamps, respectively. On other hand, the high pKa of triclosan and propylparaben could have promoted sorption onto the biomass as an extra via of elimination for these two CECs, which could explain the higher REs observed for triclosan and propylparaben in comparison to the other more polar CECs. Furthermore, sorption is not expected to depend on the COD or DO levels and could have supported their efficient REs during stages A-I, A-II and B-II despite the low DO. Overall, despite these novel photobioreactor configurations have not been specifically designed to eliminate CECs but to enhance nitrogen removal, CEC-REs were improved with respect to single-stage HRAPs.

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Tables

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Table 1. Data of process performance during the removal of ECs from synthetic urban wastewater in the anoxic (AX) and aerobic (HRAP) bioreactors during phase A under steady state.

	STAGE A-I **HRT=2 d** **COD=669±6 mg O_2 L^{-1}**	**STAGE A-II** **HRT=2 d** **COD=493±11 mg O_2 L^{-1}**	**STAGE A-III** **HRT=2 d** **COD=434±11 mg O_2 L^{-1}**
Evaporation rate (L m^{-2} d^{-1})	14±1	15±2	15±2
pH $_{AX}$	8.2±0.2	8.1±0.1	8.2±0.2
pH $_{HRAP}$	9.0±0.1	8.7±0.1	8.4±0.3
DO $_{AX}$ (mg O_2 L^{-1})	0.1±0.1	0.2±0.1	0.3±0.2
DO $_{HRAP}$ (mg O_2 L^{-1})	0.4±0.1	3.4±2.6	4.7±3.6
T $_{AX}$ (ºC)	24±1	26±2	27±1
T $_{HRAP}$ (ºC)	26±0	28±2	29±1
TSS $_{AX}$ (g L^{-1})	0.7±0.1	0.5±0.1	0.6±0.1
TSS $_{HRAP}$ (g L^{-1})	1.0±0.1	0.7±0.0	1.0±0.1

Table 2. Data of process performance during the removal of ECs from synthetic urban wastewater in the anaerobic (ANA), anoxic (AX) and aerobic (HRAP) bioreactors during phase B under steady state.

	STAGE B-I **HRT=4 d** **COD=669±6 mg O_2 L^{-1}**	**STAGE B-II** **HRT=3 d** **COD=669±6 mg O_2 L^{-1}**	**STAGE B-III** **HRT=3 d** **COD=669±6 mg O_2 L^{-1}**
Evaporation rate (L m^{-2} d^{-1})	13±1	16±3	20 ±0
pH $_{ANA}$	8.6±0.1	7.4±0.2	7.7±0.2
pH $_{AX}$	9.0±0.2	8.1±0.1	8.1±0.1
pH $_{HRAP}$	9.8±0.1	8.8±0.3	8.7±0.2
DO $_{ANA}$ (mg O_2 L^{-1})	0.0±0.0	0.0±0.0	0.0±0.1
DO $_{AX}$ (mg O_2 L^{-1})	0.9±0.7	0.2±0.7	0.3±0.6
DO $_{HRAP}$ (mg O_2 L^{-1})	13.0±6.0	5.0±5.4	10.4±6.3
T $_{ANA}$ (C)	27	28	28
T $_{AX}$ (C)	25	27	29
T $_{HRAP}$ (C)	29	29	30
TSS $_{ANA}$ (g L^{-1})	0.7±0.2	1.2±0.1	1.5±0.1
TSS $_{AX}$ (g L^{-1})	0.6±0.2	0.7±0.1	1.2±0.2
TSS $_{HRAP}$ (g L^{-1})	1.0±0.2	1.2±0.0	1.4±0.4

Figure
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Figure 1. Schematic of the anoxic-aerobic photobioreactor.

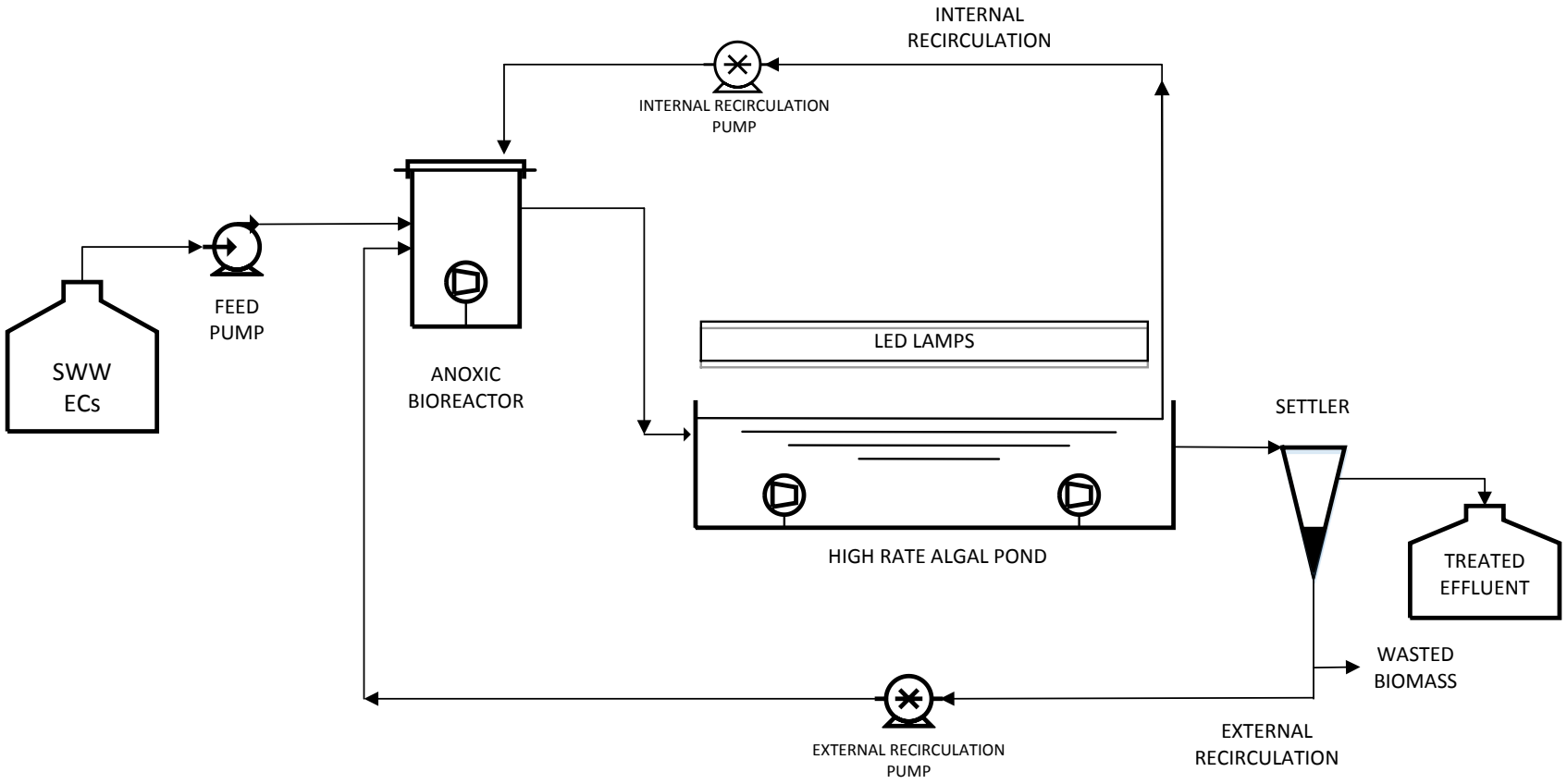


Figure 2. Schematic of the anaerobic-anoxic-aerobic photobioreactor.

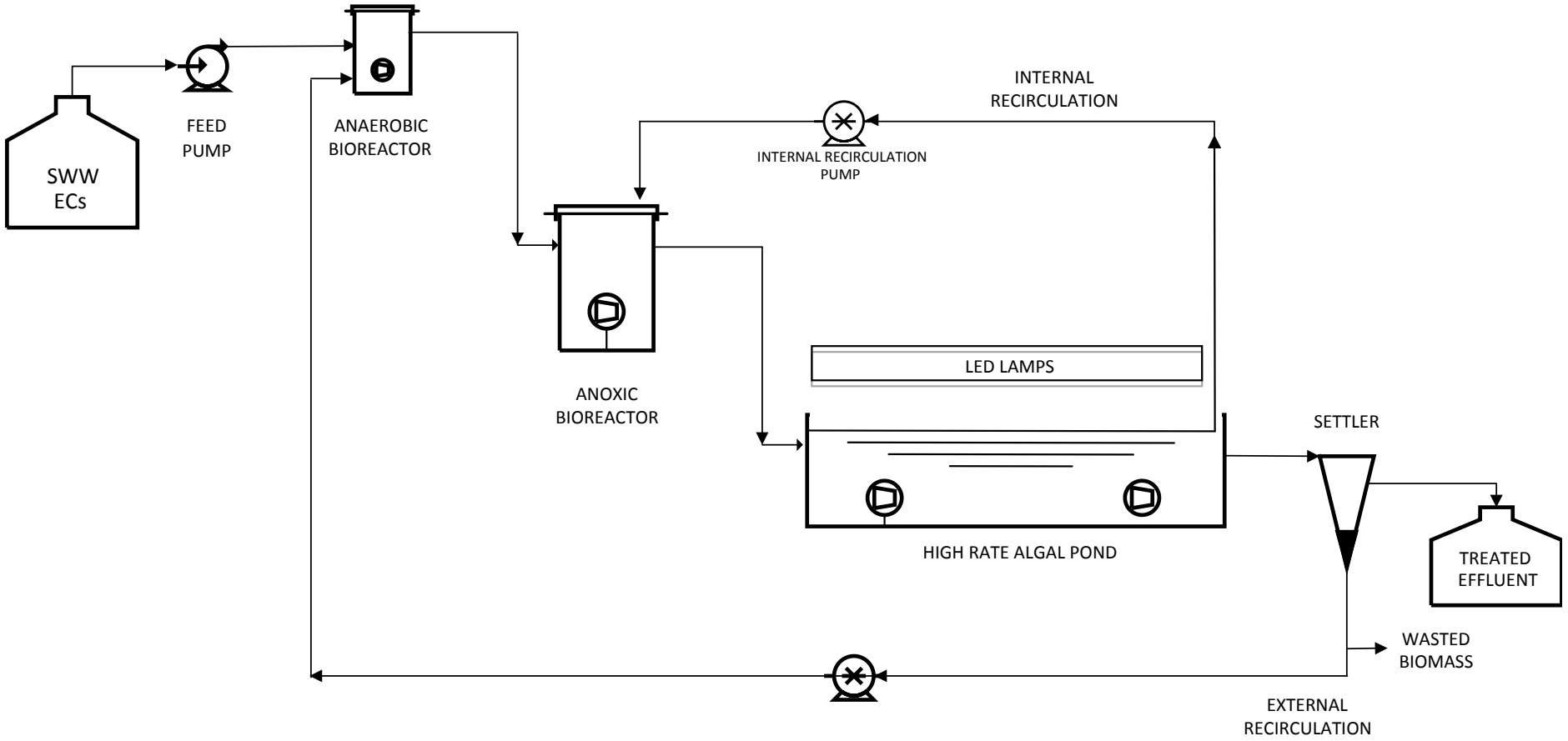
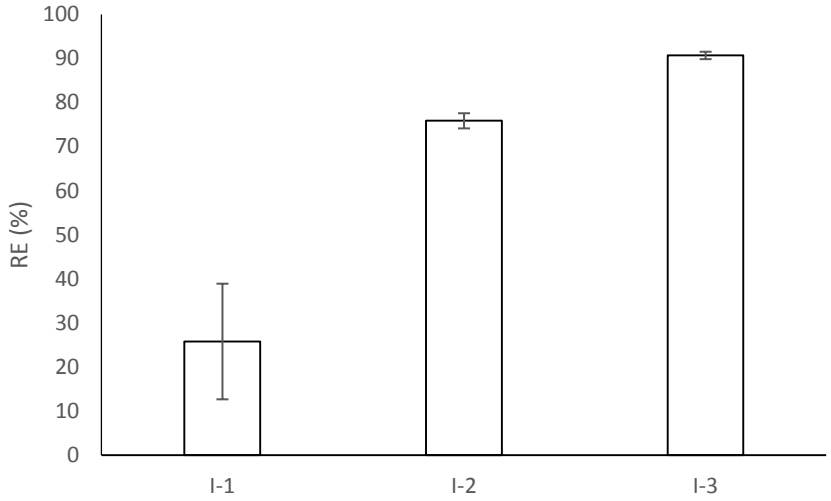


Figure 3. Steady state removal efficiencies of ibuprofen during phase A (a) and phase B (b). Vertical bar correspond to standard deviations from replicates.

a)



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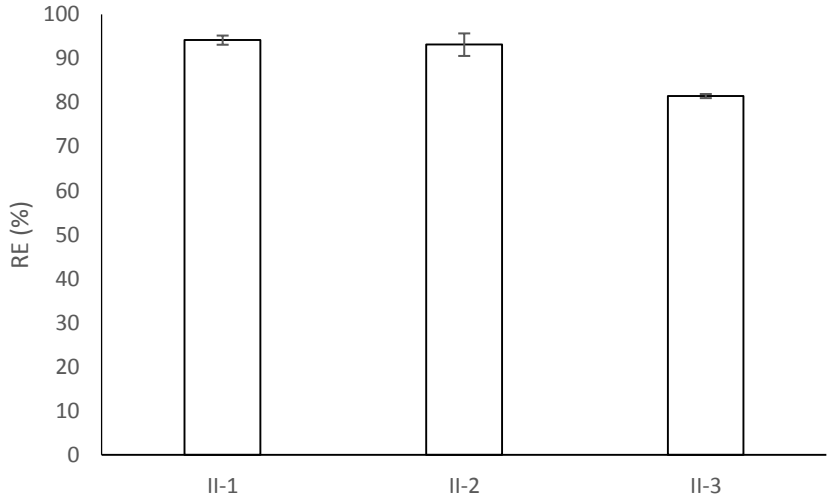
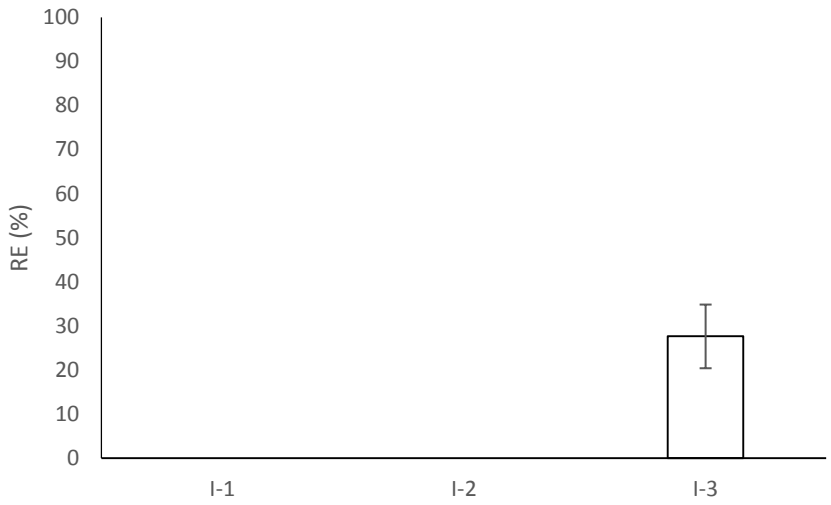


Figure 4. Steady state removal efficiencies of naproxen during phase A (a) and phase B (b). Vertical bar correspond to standard deviations from replicates.

a)



b)

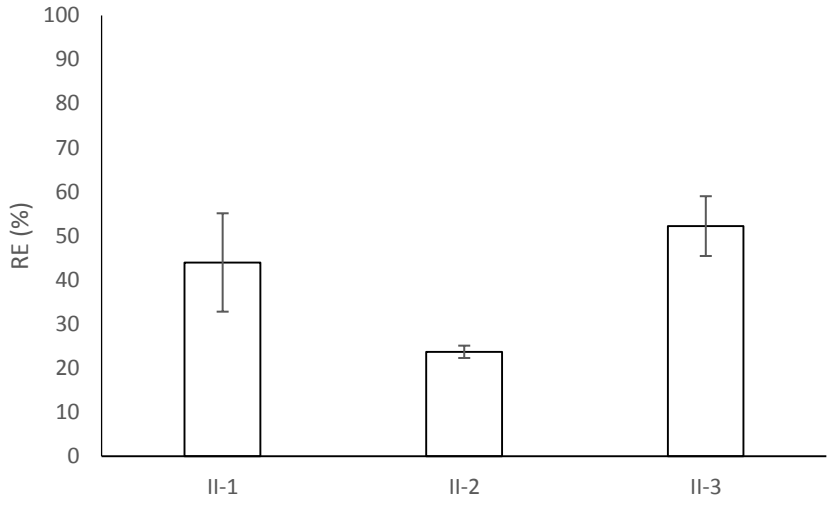
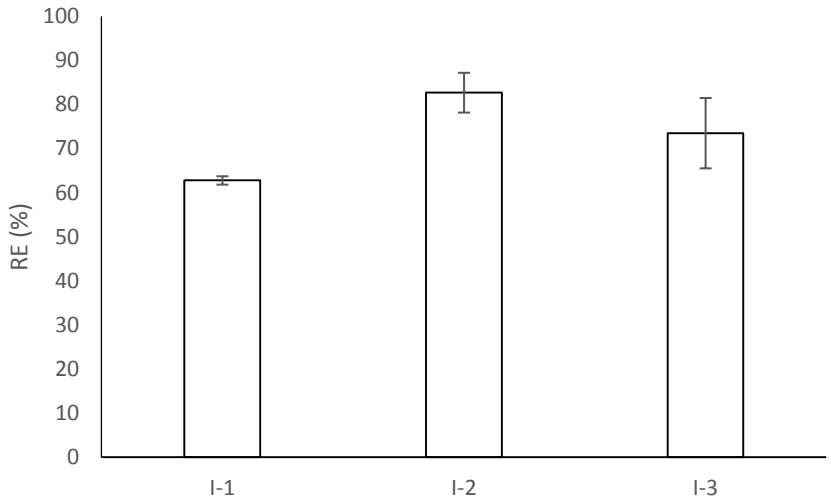


Figure 5. Steady state removal efficiencies of salicylic acid during phase A (a) and phase B (b). Vertical bar correspond to standard deviations from replicates.

a)



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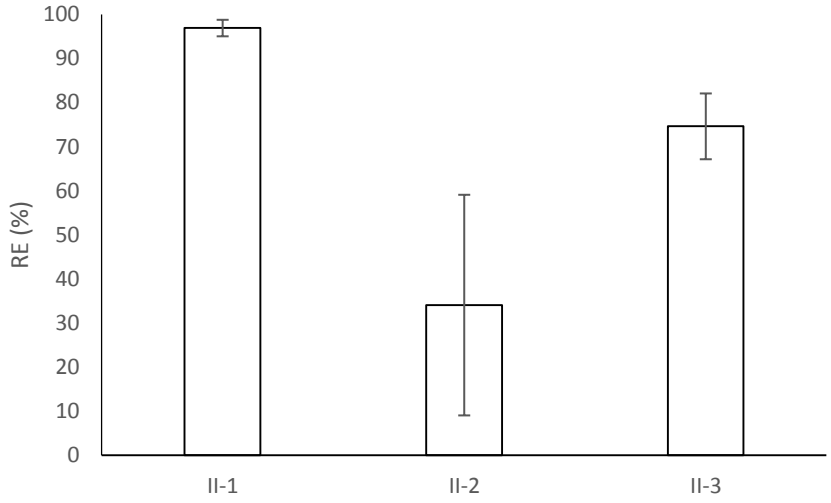
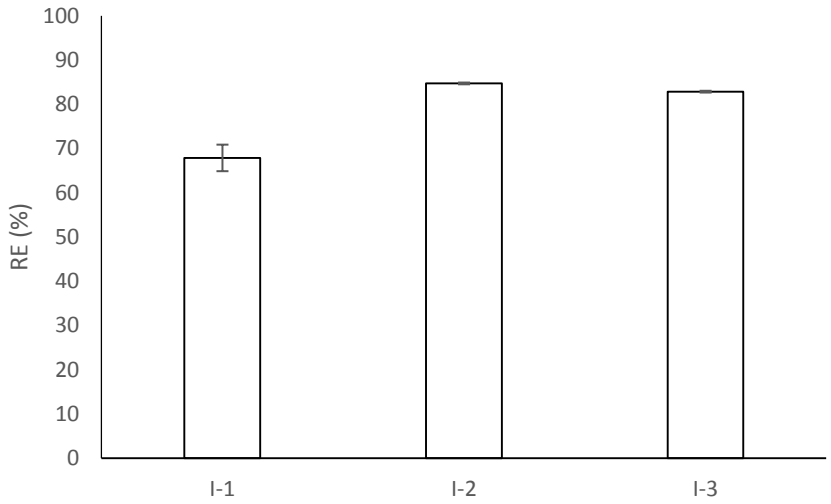


Figure 6. Steady state removal efficiencies of triclosan during phase A (a) and phase B (b). Vertical bar correspond to standard deviations from replicates.

a)



b)

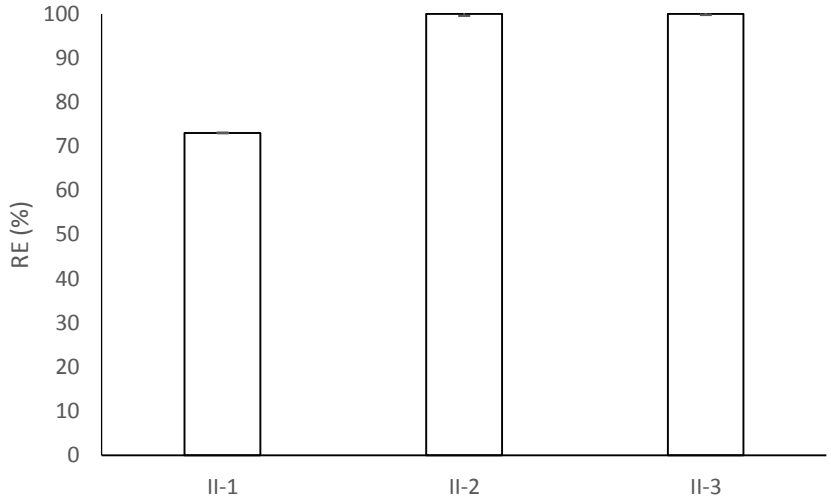
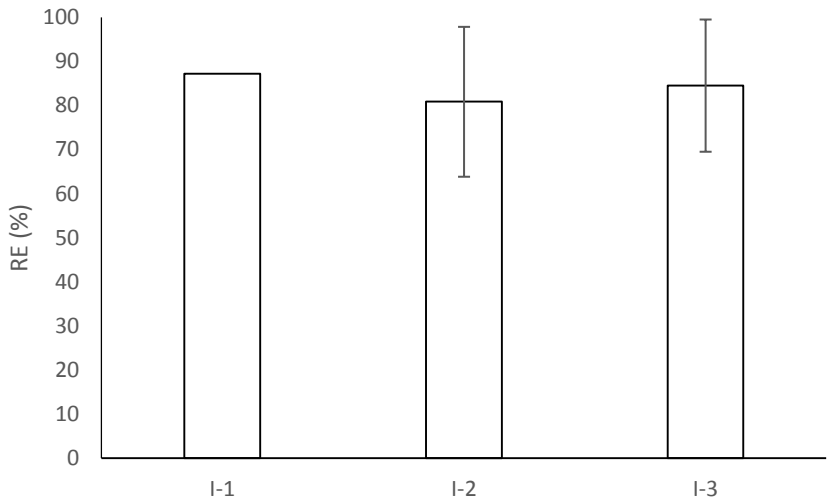


Figure 7. Steady state removal efficiencies of propylparaben during phase A (a) and phase B (b). Vertical bar correspond to standard deviations from replicates.

a)



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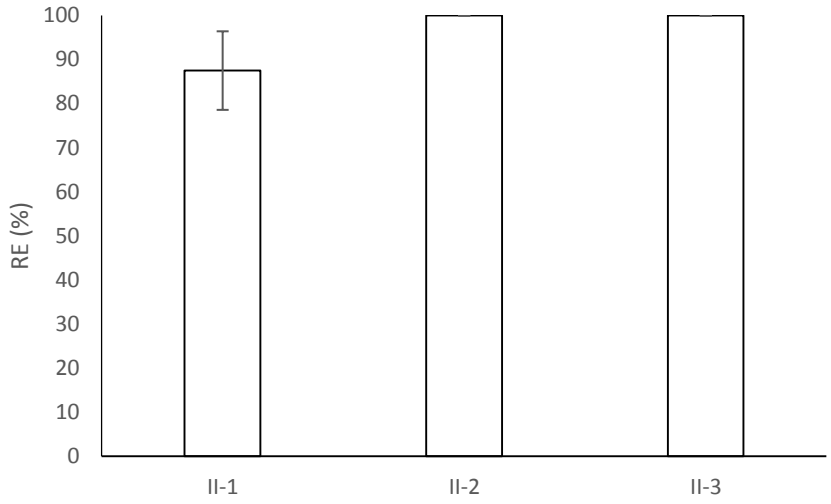

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