Contamination of N-poor wastewater with emerging pollutants does not affect the performance of purple phototrophic bacteria and the subsequent resource recovery potential.

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Abstract

Propagation of emerging pollutants (EPs) in wastewater treatment plants has become a warning sign, especially for novel resource-recovery concepts. The fate of EPs on purple phototrophic bacteria (PPB)-based systems has not yet been determined. This work analyzes the performance of a photo-anaerobic membrane bioreactor treating a low-N wastewater contaminated with a heterogeneous mixture of 25 EPs. The chemical oxygen demand (COD), N and P removal efficiencies were stable (76±8, 62±15 and 36±8%, respectively) for EPs loading rate ranging from 50 to 200 ng L^{-1} d^{-1}. The PPB community adapted to changes in both the EPs concentration and the organic loading rate (OLR) and maintained dominance with >85% of total 16S gene copies. Indeed, an increment of the OLR caused an increase of the biomass growth and activity concomitantly with a higher EPs removal efficiency (30±13 vs 54±11% removal for OLR of 307±4 and 590±8 mgCOD L^{-1} d^{-1}, respectively). Biodegradation is the main mechanism of EPs removal due to low EPs accumulation on the biomass, the membrane or the reactor walls. Low EPs adsorption avoided biomass contamination, resulting in no effect on its biological methane potential. These results support the use of PPB technologies for resource recovery with low EPs contamination of the products.

Keywords: Emerging pollutants; partition-release-recover; resource recovery; circular economy; purple phototrophic bacteria.

1. INTRODUCTION

A significant number of emerging pollutants (EPs) resulting from point and diffuse pollution is present in the aquatic environment [1]. These are in extremely low concentrations (ng/L-µg/L) within municipal sewage and different industrial sectors as food industry, pharmaceutical production plants, among others. The continuous discharge, accumulation and synergistic combination is becoming of growing concern because they can cause adverse effects to the environment and/or to humanity [2]. Typical examples of EPs are pesticides, endocrine disrupting compounds, pharmaceutical organic contaminants, personal care products, disinfectants and industrial additives. Nowadays, the capacity of new concept of sustainable wastewater facilities for the removal of EPs with environmental potential risk (CID (EU) 2018/840) is of paramount importance.

Numerous studies in the last decade have proved that EPs are not completely removed by conventional wastewater treatment processes [3]. Technically feasible solutions are chemical technologies as advanced oxidation processes [4]. However, biological treatment technologies are by far the most widely studied, including bacterial, fungal and phototrophic biomass due to the low costs of the process [5]. This fits well with the emerging vision for transforming wastewater facilities into sustainable biorefineries based on the water-energy-food nexus [6]. A trending topic in these novel wastewater treatment plants (WWTPs) is the use of photosynthetic biological processes, as phototrophic bacteria and algae photoreactors [7, 8].

The potential of purple phototrophic bacteria (PPB) has been described in the partition-release-recovery (PRR) concept which reduces the WWTP structure to three main stages, where the focus is on energy neutrality and the maximization

of resource recovery [9]. The partition stage is based on the accumulative and assimilative (not oxidative) capacity of PPB using infrared light as sole energy source which enables to maximize the carbon allocation and considerably limit carbon and nutrients loses [10, 11]. A key mechanism of PPB entails the N_2 fixation from the atmosphere, so these bacteria can be potentially use for assimilation of organics and nutrients from N-poor wastewater. This extends the applicability of the C recovery in the PRR concept to several industrial wastewater sources from sugar refineries, some dairies, paper mills, vegetables factories, breweries, wineries and other distilleries, among others [12]. The release stage is due to anaerobic digestion of the concentrated biomass, which provides energy recovery as biogas and concomitant release of nutrients to the liquid phase of the digestate for final recovery stage [13]. In this resource recovery-oriented strategy, the accumulation and release of EPs from the biomass is also a critical point to that must be evaluated [14]. This matter has not received much attention in the literature despite the proliferation of works focused on this concept for wastewater. The interaction between the microorganisms and EPs is therefore a key question in these processes.

Interestingly, the PPB can degrade some structures typically found in EPs: aromatic ring in benzene and toluene derivatives [15], aniline [16], n-containing heterocyclic aromatic compounds [17], butachlor [18], azo dyes [19] or complex organic polymers [20]. As such, the potential of PPB for the treatment of wastewater containing some of these pollutants in high concentrations has been demonstrated [21, 22]. However, despite the great potential to deal with these compounds, and the problematic arising from the management of EPs-contaminated wastewater, the efficiency of PPB systems on the removal of

complex mixtures of EPs at extremely low concentrations from wastewater has never been reported so far.

This work deals with the application of PPB as versatile organisms to address the treatment of a low nitrogen loading simulated wastewater contaminated with EPs. The removal of EPs is studied in a photo-anaerobic membrane bioreactor (PAnMBR) operating under anaerobic photoheterotrophic conditions at two different organic loading rates (OLRs). The effect of EPs on the performance of the PAnMBR, the development of the microbial communities and the release of energy by anaerobic digestion of the resultant PPB biomass is also addressed.

2. MATERIALS AND METHODS

2.1 Source of wastewater

A simulated wastewater (SWW) was used for a better control of the composition of the inlet stream and to limit hidden operational factors. The wastewater is characterized as N-deficient and medium load effluent, which is typical in some industrial streams as food washers, oil refineries, and mixed effluents from distilleries, sugar refineries and paper mills, among others. Detailed composition is included in Supporting Information. Resulting total chemical oxygen demand (TCOD) and soluble COD (SCOD) averaged values (n=19) were 349 (121) and 261 (134) mgCOD/L, respectively, with 100*N/COD and 100*P/COD ratios averaging 2.2 (1.4) mg N/100mgCOD and 1.3 (0.6) mgP/100mgCOD, respectively.

The SWW was supplemented with several pharmaceutical compounds of different therapeutic families, which are usually found in WWTPs [23], such as analgesic (4-acetominoantipyrine), lipid regulators (gemfibrozil), diuretic

(hydrochlorothiazide), antibiotics (metronidazole and sulfamethoxazole), stimulant (caffeine), psychiatric drug (carbamazepine), hormones (estrone), X-ray contrast (iohexol) and cytostatic agent (cyclophosphamide). Additionally, some biocides typically found in food wastewater [3, 23], such as herbicides (Atrazine, Clofibric acid, Isoproturon, Metamitron, Simazine, Terbutryn), fungicides (Azoxystrobin, Triclosan) and insecticides (Buprofezin, Dimethoate, Imidacloprid, Thiamethoxam) were also added, as well as plasticizers (tris-chloroethyl-phosphate (TCEP)) and life-style compounds (acesulfame K, N,N-Diethyl-meta-toluamide (DEET)). EPs were prepared in a ~50 µg/L concentrated mixture and added directly to the SWW at different concentrations as described below.

2.2 The PAnMBR system

An automatized PAnMBR reactor equipped with a submerged flat sheet membrane with 0.45 µm pore size and 0.12 m^2 surface area (Kubota, Osaka, Japan) was designed based on a previous work [11]. The reaction vessel was built on a rectangular structure connected to a mobile support for the artificial light system. The smart construction allowed to an optimized light distribution on the two big sides of the reactor by modifying the distance and the illumination angle, and therefore the irradiation was homogeneously distributed. Eight 850 nm LED lamps (4 by each side) were used as illumination modules, giving an average irradiance of 28.5 ± 1.7 W/m^2 (see irradiation maps on SI). The reactor was covered with a UV/VIS filtering foil that filters around 90% of the irradiation below 750 nm (ROLL-299 ND FILTER GEL, Transformation Tubes, Banstead, UK). This avoids the growth of photosynthetic microorganisms as microalgae and cyanobacteria. Temperature was not controlled and varied between 33 and 36ºC.

Anoxygenic conditions were ensured by a continuous flux of nitrogen, which also provides an extra source of N for the biomass. The hydraulic retention time (HRT) and the reactor working volume was fixed by using two peristaltic pumps connected to a PLC system controlling the wastewater inlet flow, the water column, the headspace and the transmembrane pressures (PID Eng&Tech, Madrid, Spain). A 3D scheme of the PAnMBR is shown in Figure 1. The solid retention time was fixed at 4 d by pumping out biomass using a peristaltic pump connected to the bottom of the reactor body.

2.3 PAnMBR operation

Initially, the PAnMBR was fed with a domestic wastewater (DWW) collected from the WWTP of the Mostoles Campus of the University Rey Juan Carlos (enrichment stage). This DWW was used as inoculum to enrich PPB for period of 8 days using a HRT and solid retention time (SRT) of 1 d and 4 d, respectively. Average composition (standard deviation in brackets) of DWW (n=5 samples) was as follows: 312 (193) mgTCOD/L, 123 (35) mgSCOD/L, 82 (18) mg TSS/L, 69 (12) mgVSS/L, 39 (4) mgNH_4^+-N/L and 4.2 (0.4) mgPO_4^{3-}-P/L.

After the enrichment stage, the DWW is replaced by the SWW used in this work. Firstly, the SWW, in absence of EPs, was fed for 31 d until the reactor performance (substrate and nutrients removal and biomass concentration) stabilized (acclimation stage). Afterwards, the inlet SWW was spiked with EPs (treatment stages, TS). Initially, the HRT was maintained at 1 d for 14 d, where EPs were spiked to 50 ng/L (TSI), and then, it was reduced to 0.5 d for further 23 days by doubling the SWW flowrate, where the EPs concentration was doubled, which increased considerably the EPs loading rate (TSII). Table 1 summarizes the operation conditions of the different stages. Operational parameters of the

PAnMBR (OLR, HRT) were calculated from single measurements and average values were calculated. For statistical purposes, the reactor performance was compared between stages by calculating the average of single measurements and their standard deviations. Where calculations were needed for obtaining operational parameters, 95% confidence intervals were obtained and used for statistical comparisons.

2.4 Specific Phototrophic Activity tests

The activity of the phototrophic biomass of the PAnMBR reactor was determined at days 35 and 48. To that purpose, standard Specific Phototrophic Activity (SPA) tests were performed following [24]. The organic substrate was acetate at 500 mgCOD/L. The experiments were inoculated with 100 mgCOD/L of active biomass extracted from the PAnMBR. The experiments were performed in 100 mL anaerobic serum bottles in a temperature-controlled incubator (at 30 ºC) illuminated with IR lamps at around 50 W/m^2 and covered with the UV/VIS filtering foil. Batch tests of SPAs were performed in triplicate. SPAs were estimated dynamically by using Aquasim 2.1d as specified in [24], so that they correspond with the k_{M_ac} parameter (mgCOD-acetate/mgCOD-biomass d). Confidence intervals (at 95%) were calculated based on two-tailed t-tests from the parameter standard error and used for statistical representative comparisons.

2.5 Biochemical methane potential tests

Biochemical methane potential (BMP) tests were performed following the recommendations of [25]. Anaerobic sludge from a full-scale anaerobic digestion of a WWTP located at Mostoles, Madrid, Spain, was used as inoculum. In order to have a representative analysis of the different operational stages of the

149 PAnMBR, four different samples were generated as an equal-mass mixture of
150 several PPB biomass samples extracted from the PAnMBR reactor during the
151 acclimation (days 27, 28, 29, 30) and treatment periods (TSI: days 42, 47, 49;
152 early TSII: days 57, 61; and late TSII: days 69, 75, 77). The active biomass
153 samples extracted from the PAnMBR were centrifuged and maintained at 4 ºC
154 before making the mixtures prior to the BMP tests. The four mixtures were then
155 fully characterized and used as substrate using an inoculum to substrate (I/S)
156 ratio of 2:1 (as VS) and a concentration of 10 gVS/L. An inoculum control was
157 used for subtracting the methane yield produced by the inoculum due to
158 endogenous digestion. BMP tests were performed by triplicate, yielding mL
159 CH_4/gVS. Kinetic parameters of methane production in BMP assays were
160 obtained by fitting first order models to the data as per [26], thereby calculating
161 the kinetic first order hydrolysis constant k_H (d^{-1}) and the BMP (B_0, in mL CH4/g
162 VS). Parameter uncertainty was determined using two-tailed t-tests calculated
163 from the standard error in the parameter value, obtained from the Fisher
164 information matrix. Parameter uncertainty surface (with k_H, B_0, $J = J_{crit}$, 5%
165 significance threshold) has also been assessed as described in [27]. Confidence
166 intervals (at 95%) were also calculated based on two-tailed t-tests from the
167 parameter standard error, as above, and used for statistical representative
168 comparisons. All the statistical analyses of BMP tests were performed by using
169 Aquasim 2.1d.

170 2.6 Analytical methods for water and sludge characterization.

171 COD, total and volatile solids (TS/VS), total and volatile suspended solids
172 (TSS/VSS) and total Kjeldalh nitrogen (TKN) were measured following standard
173 procedures [28]. NH_4^+, PO_4^{3-} and TP were measured by Merck® kits (Merck,

174 Darmstadt, Germany). Gas pressure from BMP tests was measured using a
175 Boyle-Mariotte apparatus, and CH_4 and CO_2 from gas samples were quantified
176 by GC/TCD following the method described in [26]. Irradiance was measured with
177 a spectroradiometer STN BlueWave VIS-NIR (StellarNet, Tampa, FL, USA).

178 In order to analyze the PPB enrichment, 400-950 nm absorbance spectra were
179 performed periodically with the biomass extracted from the PAnMBR, thereby
180 checking for the bacteriochlorophyll relative content (which have maximum peaks
181 at 590, 805 and 865 nm for most of PPB cultures, [29]). Biomass spectra was
182 analyzed by VIS-NIR spectrometry (V-630, Jasco, Madrid, Spain).

183 2.7 Analytical method for quantification of EPs

184 Figure 1 summarizes the method developed for EPs analysis. The method allows
185 to analyze the EPs in the influent (influent sample, S1) and to study the fate of
186 these compounds within the reaction system, e.g: completely mobilized in the
187 permeate (permeate sample, S2), mobilized in the reactor (reactor soluble
188 sample, S3) or immobilized onto/into the biomass (reactor particulate sample,
189 S4). The description of the analytical protocol is shown in Supplementary
190 Material. Representative samples from the influent, the PAnMBR and the effluent
191 (100 mL each) were extracted at the end of the TSI (day 49) and TSII (day 78)
192 an analyzed for EPs quantification. The extraction efficiency was included in the
193 calibration curve for each EP. The matrix effects have been studied by including
194 random internal standards and internal calibration in the samples list and has
195 been considered as negligible. This has been also stated in a previous work for
196 low organic load wastewater following the same extraction protocol [REF].

197 2.8 Analysis of microbial community

198 Representative samples from the PAnMBR were extracted at days 1 (initial), 9
199 (enrichment), 39 (acclimation), 49 (TSI), and 67 (TSII) for the analysis of the
200 development of the photosynthetic bacterial community by Illumina MiSeq.
201 Details of the method are given in Supplementary Material.

202 3 RESULTS AND DISCUSSION

203 3.1. Effect of the EPs on the performance of the PAnMBR

204 The performance of the PAnMBR was assessed in terms of the phototrophic
205 biomass development and COD, NH_4^+-N and P-PO_4^{3-}-P removal efficiencies.
206 Figure 2 summarized the results of C, N and P removal efficiencies of treated
207 wastewater (a) and VSS concentration and N and P contents of particulate matter
208 (b) for the different stages of the operation treatment.

209 a) Phototrophic biomass development

210 During the 8 days of the enrichment stage with a real DWW, the biomass was
211 quickly developed in the reactor, achieving a concentration around 300 mgVSS/L.
212 The biomass was then growing at the beginning of the acclimation stage up to
213 achieving a value close to 1000 mgVSS/L. Thereafter, the concentration
214 continuously decreased until achieving an equilibrium at around 200 mgVSS/L.
215 This is attributed to biofilm formation onto the photo-irradiated reactor walls that
216 is affecting negatively the reactor performance, reducing the VSS concentration
217 inside the reactor (Figure 2b) and the COD consumption efficiency from day 20
218 to 40 at the acclimation stage (Figure 2a). As the biofilm formation evidenced a
219 negative impact on the reactor performance, this biofilm was weekly detached by
220 internal superficial brushing. The cleaning periods began on TSI, causing an
221 immediate increment of the biomass concentration inside the reactor.

The effect of the cleaning periods is shown in Figure 3, where the proportion of the biomass between reactor, membrane and walls is depicted before and after the cleaning periods of stages I and II of treatment of SWW with EPs. The biomass turned from growing predominantly as biofilms (around 70% of VSS attached onto the reactor walls) to growth as suspended culture (above 70% VSS within the suspended liquor). This caused the SPA values to significantly increase from 0.59 to 0.97 mgCOD/mgCOD d while maintaining a similar growth yield (around 0.91 mgCOD/mgCOD), which essentially means a faster assimilative substrate usage by the biomass. This has implications on the downstream potential of the PPB biomass, which will be furtherly explored through BMP tests, and on the potential development of phototrophic bacterial biomass.

The development of the phototrophic bacteria was confirmed by analysis of the ratio of particulates concentration (expressed as TCOD) to absorbance at 805 or 865 nm (corresponding to the maximum peaks of absorption of Bacteriochlorophyll *a* [30]) and the ratio of particulates absorbance (generally given at 660 nm) to Bacteriochlorophyll *a* absorbance (at 805 or 865 nm). In both cases, the lowest the ratio is, the best phototrophic enrichment. This analysis was performed for biomass samples of the PAnMBR over the whole operation period, as is shown in Figure 4. According to this analysis, the phototrophic biomass development in the PAnMBR was very fast. The first 7 d of enrichment were enough for having a high proportion of Bacteriochlorophyll *a* as indicated by a sharp decrease of both the TCOD/$Abs_{805,865}$ and the $Abs_{660}/Abs_{805,865}$ ratios. These ratios continued decreasing for the acclimation step, increasing only slightly for the stages I and II of treatment of SWW with EPs. However, it is noteworthy that the increase of the OLR from TSI to II did not have an important

effect on studied ratios. This fact suggests that the PPB biomass can resist a chemical stress period (where the OLR and the EPs concentration were doubled) without losing the phototrophic ability.

b) Carbon, nitrogen and phosphorous removal efficiencies

During the acclimation stage, the biomass needed around 23 d to achieve stable COD, NH_4^+-N and PO_4^{3-}-P removal efficiencies of around 90, 60 and 70%, respectively. The addition of the EPs at TSI (day 37), working with an OLR of 310 ± 40 mgCOD/Ld, did not cause destabilization of the system, yielding COD, NH_4^+-N and PO_4^{3-}-P removal efficiencies of 86 ± 3, 78 ± 10 and 49 ± 9%, respectively. However, the increase of the OLR to 590 ± 84 mgCOD/Ld during the TSII (day 54), which doubled the EPs concentration into the reactor, caused destabilization of the system during ca. 10 d. The COD, N and P removal efficiencies dropped to around 40, 20 and 5%, respectively during this period of operation. Afterwards, the system performance enhanced continuously until the steady-state, yielding COD, N and P removal efficiencies of 88 ± 4, 97 ± 2 and 38 ± 5%, respectively, during the last week of operation.

The reactor performance is comparable with previous works where photo-MBR processes have been used. Low-strength wastewater has been treated achieving stable COD, N and P removal to discharge limits using a similar reactor that treated domestic sewage [11], even at low (10 ºC) temperature [31]. OLR changes did not affected the PAnMBR performance in those works, and only the removal of the organic co-substrate (ethanol or acetate) substantially damaged the reactor performance. Likewise, high-strength wastewater treatment (up to 9750 mgCOD/L) by PPB-based PAnMBR was also accomplished by applying high HRTs (around 10 d) for treating food-processing wastewater [32]. Thereby,

we suggest that the deterioration of the reactor performance at the beginning of the TSII is due to the increase of the EPs loading rate rather than to the increase of the OLR. In any case, the exploration of an N-poor wastewater treatment contaminated with EPs by phototrophic mixed culture is novel, and the nutrients assimilation reported here required further analyses.

In order to assess the effect of the EPs loading on the assimilation and removal of N and P nutrients, the COD/N/P ratios were analyzed. Average assimilation and removal of N and P are shown in Table 2. During the acclimation stage (see Figure 2), the N and P assimilation ratios of the biomass increased over time until achieving COD/N/P values around 100/7.5/1.7 during the TSI with EPs, like those reported in the literature for wastewater treatment by PPB [24, 29]. These values are maintained during the TSII despite the increase of the OLR and the EPs concentration in the effluent. The similarity of these values is likely due to the uniformity of the biomass composition, as will be furtherly discussed in section 3.3.

However, the COD/N/P removal ratios differed from the biomass assimilation ratios. The samples were extracted upon the cleaning periods, so the effect of the biomass growth on walls and membrane on these differences is discarded. The N removal ratio was higher than the organic N assimilation ratio in both TSI (100/10.1 vs 100/7.5) and TSII (100/12.4 vs 100/7.8), whereas the P removal ratio was lower than the organic P assimilation ratios in also both TSI (100/1.0 vs 100/1.7) and TSII (100/0.7 vs 100/1.5). These facts suggest the appearing of other processes not linked to growth, as in essence, all the carbon and nutrients go to biomass (assimilation or accumulation) during photoheterotrophic metabolism, and so, the removal and assimilation ratios should be similar. This

297 behavior is independent of the presence of EPs, as it is similar for stages I and II
298 working with different EPs loading rate, and even for the acclimation stage.

299 The occurrence of heterotrophic denitrification from the NO_3^- contained in the
300 SWW is a possible explanation for the higher N removal ratio, as some species
301 of PPB are able to denitrify [33]. Dissimilatory reduction of nitrate to ammonium
302 is discarded, as the product of this reaction (NH_4^+) can be used by PPB for
303 growing, and therefore it would not affect the COD/N ratios. In contrast, P cannot
304 be dissipated because of the microbial activity, so a loss of COD due to a side
305 reaction is the only explanation for the lower P removal ratio, likely due to
306 hydrogen production (by photofermentation, [34]). The lack of ammonium in the
307 wastewater composition, which is a strong inhibitor of the nitrogenase activity
308 [35], support this suggestion. Other chemoorganotrophic processes might have
309 a small effect as these processes are one order of magnitude slower than
310 photoheterotrophic mechanisms in PPB-based systems [24].

311 3.2. Removal of EPs by phototrophic biomass at PAnMBR

312 A comprehensive analysis of the removal and fate of EPs within the PAnMBR
313 system has been conducted. Figure 5 shows the main results from this analysis.
314 The concentration of EPs in the liquid phase (influent, reactor and permeated
315 effluent, ng/L) are shown in Figure 5a (day 49, TSI) and 5b (day 78, TSII),
316 whereas the concentration of EPs recovered from the biomass in sludge samples
317 of both Stages (µg EPs/gVSS) are shown in Figure 5c. In general, the
318 concentration of the EPs in the liquid phase inside the reactor and in the
319 permeated outlet effluent are quite similar in all cases. This indicates that the EPs
320 passed through the membrane without the inference of separation or abiotic
321 sorption processes. Previous works reported insignificant removal of EPs by flat

sheet micro-membranes in MBR reactors, and main removal mechanisms were identified as biodegradation/biotransformation and abiotic absorption onto the biomass [36, 37]. Generally, high removal of EPs is observed in the TSII as compared to the TSI. This is an early indication that the increase of OLR during TSII improved considerably the removal of EPs by PPB biomass.

Low accumulation of the EPs in the biomass (Figure 5c) suggests high conversion of the compounds by biological processes. In general, the accumulation is not related to the OLR, as the concentrations of the EPs in the biomass extracted during Stages I and II are similar. Accumulated compounds as Azoxystroblin, Buprofezin, Carbamazepine, Triclosan or TCEP have a high water/octanol partition coefficient (logD) that explain the high affinity for biomass adsorption due to high hydrophobicity (Table 3). The only exception is caffeine, which can be actively assimilated by PPB as a C source [38]. These results suggest that accumulative removal mechanisms are residuary and only relevant for highly hydrophobic EPs.

Figure 6 shows the removal efficiencies of the EPs compounds at the end of both stages I and II. Data has been sorted in descendent order according to the EPs removal efficiencies found in TSII for the sake of comparison. Data from TSII was organized as high removal (>50%), moderate removal (25-50%) and low removal (<25%). High removal efficiencies were achieved during the operation of the PAnMBR reactor in the TSII. Up to four EPs were completely removed (>99%) in the TSII (metronidazole, caffeine, sulfamethoxazole and isoproturon), whereas 14 out of 25 compounds were highly removed (efficiency >50%). In general, the results show that the increase of the OLR enhanced the removal efficiency of the EPs by PPB biomass. This is true for 22 out of the 25 EPs analyzed. The only

exceptions were Azoxystrobin, Thiamethoxam, and Dimethoate that, in any case, were highly removed during the TSII. As a conclusion, a higher biomass activity found in TSII, due to the higher OLR and similar COD removal efficiencies, promoted the removal of EPs by PPB biomass. The effect of extra organic source on the biomass activity can promote fortuitous co-metabolic processes that might have a role on the increment of the EPs removal efficiency. Indeed, it has been recently suggested that this is one of the main mechanisms of organics removal at very low concentrations under anaerobic conditions [39].

EPs removal efficiencies are independent of their hydrophobicity either in the TSI or in the TSII, as confirmed by the inexistence of linear correlation between the logD vs the EPs removal efficiencies (see Supplementary Material). Even the hydrophobicity does not completely relate to the accumulation of the EPs in the biomass, as some EPs with high logD values were not accumulated in the biomass and were poorly removed (as is the case of Acesulfame K and Simazine). This is in contrast with results reported by [37], which reported that hydrophobic (logD > 3.2) and biodegradable trace organics were well removed by an aerobic MBR with a VSS concentration between 4600-6700 mg/L and an infinite SRT imposed due to lack of biomass withdrawal. Under such circumstances, specific biomass activity is poor due to high biomass decay. Therefore, biosorption mechanisms are predominant, which are directly related to the hydrophobic affinity of the compound to the cell surface (given by the logD value). The removal of the EPs in the PAnMBR was instead related to their specific chemical structure and its affinity with PPB metabolism. Some highly removed compounds have an N-containing heterocyclic aromatic structure (e.g. Metronidazole, Isoproturon, Caffeine or Terbutryn) that fits well with PPB

372 metabolism [17], a benzene ring as the central structure (as Sulfamethoxazole)
373 that is biodegradable by PPB [15], or both (as Azoxystrobin, Buprofezin). Also,
374 the PPB seem to need an acclimation period for the removal of the EPs as
375 supported by the difference between the removal efficiencies shown in Stages I
376 and II (despite the higher EPs concentration added in TSII). During this
377 acclimation period, the specific activity of the biomass is increasing due to the
378 higher OLR imposed. These results taken as a whole strongly suggest that
379 biodegradation/biotransformation is the main mechanism of EPs removal by
380 PPB, which is reinforced by the low accumulation of EPs found in the PPB
381 biomass.

382 3.3. Microbial community analysis

383 The analysis of the microbial community evolution during the PAnMBR operation
384 has been analyzed by Illumina MiSeq sequencing. A summary of the results is
385 shown in Figure 7, where the identified operational taxonomic units (OTUs) have
386 been classified based on their phylum (Figure 7a), and those belonging to the
387 phylum *Proteobacteria* have been classified based on their closest species
388 affiliation (Figure 7b). The inoculum source (domestic wastewater) contained a
389 somehow highly diverse microbial community, where 96.5% of the OTUs were
390 classified as *Bacteria*, whereas only 3.5% where classified as *Archaea*. Most of
391 the bacterial phyla are represented with *Firmicutes*, *Proteobacteria* and
392 *Actinobacteria* being as dominant phyla. The PPB presence in the inoculum was
393 somehow residual, representing only 8% of the total OTUs belonging to the
394 phylum *Proteobacteria* (which corresponds to around 2.5% of the total copies of
395 *Bacteria*). This is a typical microbial community in domestic wastewater sources,
396 with high microbial diversity and lack of dominance of a specific community over

the others [40]. However, the structure of the microbial community changed drastically with the beginning of the photoreactor operation.

After the enrichment stage, the community structure changed dramatically. Most of the bacteria belonged to the phylum *Proteobacteria*, which includes all the PPB (Figure 7a). The super-dominance of *Proteobacteria* remained invariable along most of the operative time (>98% of the total detected OTUs). However, it slightly decreased during the TSII down to 94% dominance, probably due to the development of side communities because of changing operating conditions.

The dominance of *Proteobacteria* was intimately related to the super-dominance of anoxygenic phototrophic organisms. Despite the residual presence of PPB in the inoculum, these bacteria developed very quickly and super-dominate the microbial consortia even during the enrichment period (Figure 7b). Following the enrichment, >97% of the OTUs belonging to the phylum *Proteobacteria* were classified as PPB. This dominance remained during the rest of the experimental period. This is one of the most extreme case of phototrophic dominance reported ever in a photobioreactor treating wastewater with a mixed culture. Previous studies working with PPB showed a dominance of PPB as high as 80% of the total OTUs [11, 44]. The use of an external factor as a metabolic advantage to push for the dominance of PPB has been previously reported, e.g. PPB has been over-enriched in wastewater treatment by using low temperature [31]. Also, the low concentration of ammonium is an impediment for the development of non-nitrogen fixing heterotrophic bacteria, which create an ecological niche for the proliferation of nitrogen-fixing organisms, mostly related to *Proteobacteria*. Most of PPB detected are, indeed, nitrogen-fixing organisms. The dominance shown

421 here was generalized but not homogeneous, as there were changes in the
422 proportion of the dominant PPB OTUs along the reactor operation.

423 The enrichment period led to a dominance of two species: *Rhodopseudomonas*
424 *palustris* and *Blastochloris sulfoviridis*. At the end of the acclimation period, only
425 *R. palustris* remained as a dominant species, likely due to the switch from the
426 DWW to SWW. As suggested, this was probably due to a lack of ammonium as
427 an N source. *R. palustris* is a strong nitrogen-fixing organism as described in a
428 high number of studies [45], so nitrogen fixation could be the main environmental
429 factor to drive the over-dominance of this species over the rest of PPB. The
430 dominance of this species went on during the TSI, indicating that the EPs
431 introduction did not cause an effect in the community structure. However, during
432 the TSII the overall dominance of PPB within the *Proteobacteria* decreased down
433 to 89% of the total OTUs identified within this phylum. In addition, *R. palustris* lost
434 its dominance and other PPBs emerged in high proportion, especially two species
435 belonged to the *Rhodospirillaceae* family. In parallel, some other non-
436 phototrophic bacteria belonging to the *Proteobacteria* phylum appeared in high
437 proportion, including two predatory bacteria of the *Bdellovibrio* sp. genus and one
438 bacteria of the *Acinetobacter* sp. genus. *Bdellovibrio* sp. can develop in changing
439 communities, where some organisms are decaying and being replaced by others.
440 The motile capacity can provide an efficient depredation capacity to *Bdellovibrio*
441 sp. cells, to survive and thrive under these enrichment conditions dominated by
442 PPB strains [46]. *Acinetobacter* sp. can conduct aromatics degradation and may
443 be associated to the degradation of some EPs [47]. As a general observation, the
444 structure of the phototrophic community changed and adapted to new conditions,
445 with the appearing of flanking microbial communities. However, differently to

other biological systems, a super-dominance of a specific metabolic group (in this case, PPB) does not imply a decrease of the process stability [48]. This is explained by the high metabolic versatility of the PPB communities, which can occupy several ecological niches by modifying their functional activity [20, 49].

3.4. Effect of emerging pollutants on the biochemical potential of phototrophic biomass.

The analysis of the downstream potential of the PPB biomass was performed by checking their BMP. The BMP of the PPB biomass was analyzed in batch by using PPB biomass harvested from the PAnMBR reactor. Main results are shown in Figure 8, where the time course of the methane potential is shown in Figure 8a and the 95% confidence regions for the first-order kinetic parameters are depicted in Figure 8b. The increase of the OLR entailed an improvement of the BMP of the PPB biomass. The BMP values varied from 136 ± 5 mL CH_4/gVS at the beginning of the acclimation stage in absence of EPs (average OLR of 310 mgCOD/L d) to 227 ± 6 mL CH4/gVS at the end of the TSII with EPs in the inlet influent (after achieving the steady-state working under an OLR of 590 mgCOD/L d). Kinetics of the AD process were also considerably enhanced, (k_H increased from 0.22 ± 0.02 d^{-1} at the beginning of the acclimation stage to 0.29 ± 0.03 d^{-1} at the end of the TSII). Increasing the OLR may promote active accumulative processes in PPB bacteria like the production of polyhydroxyalkanoates or glycogen [50], which are easier to be anaerobically digested and converted into biogas [51]. The effect of the EPs on the modification of the community dynamics may also affect the digestibility of PPB biomass, as the community replacement influenced the biomass decay, as confirmed by the appearing of predatory bacteria like *Bdellovibrio* sp. Thereby, the promotion of the PPB growth by increasing the OLR

while maintaining constant SRT considerably improved the digestibility and the BMP of the PPB biomass. Importantly, the accumulation of EPs on the PPB biomass (Figure 5a) is irrelevant for the anaerobic digestion process, likely due to low EPs concentration. This essentially means that the biodegradation capability of the PPB community allows harvesting biomass with low EPs contamination, which can be downstream processed in a secure way. The emerging pollutants does not affect the quality of the biomass. This has important implications on the use of PPB for resource recovery from wastewater.

This work reports for the first time BMP tests of PPB biomass extracted from a continuous reactor. BMP values reported here are a preliminary indication that PPB biomass has a high energy potential and might drive a resource recovery-oriented wastewater platform energetically positive, which is a prerequisite for a successful process as discussed previously [9, 13]. More in depth analyses are encouraged to determine the extent of the dependency of the AD process in the PRR concept.

4. CONCLUSIONS

This work analyzes, for the first time, the performance of a PPB-based photobioreactor treating wastewater contaminated with EPs. Main conclusions are as follows:

- The EPs concentration barely affected the PAnMBR system performance, which was able to achieve remarkable removals of COD, N and P.
- The increase of OLR improved the EPs removal efficiency, which is explained by a higher biomass activity. This supports the ability of the phototrophic bacteria to stand with the chemical stress caused by the EPs.

This ability is reflected in the very high (>90%) dominance of PPB on the phototrophic system despite the changes in the OLR and the EPs concentration in the wastewater.

- Main mechanism of EPs removal by the phototrophic systems is through biodegradation, as bioaccumulation of the EPs is only evident with some highly hydrophobic organics, being the rest removed/transformed.
- The BMP of the PPB biomass extracted during the treating of a synthetic wastewater with EPs is enhanced when the biomass is more active (treating higher OLR values). Low EPs adsorbed into the PPB biomass seems to be irrelevant for the performance of the anaerobic digestion process.

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6. REFERENCES

[1] T. Deblonde, C. Cossu-Leguille, P. Hartemann, Emerging pollutants in wastewater: A review of the literature, International Journal of Hygiene and Environmental Health, 214 (2011) 442-448.

[2] Y. Luo, W. Guo, H.H. Ngo, L.D. Nghiem, F.I. Hai, J. Zhang, S. Liang, X.C. Wang, A review on the occurrence of micropollutants in the aquatic environment and their fate and removal during wastewater treatment, Science of the Total Environment, 473-474 (2014) 619-641.

[3] J. Wilkinson, P.S. Hooda, J. Barker, S. Barton, J. Swinden, Occurrence, fate and transformation of emerging contaminants in water: An overarching review of the field, Environmental Pollution, 231 (2017) 954-970.

[4] M.O. Barbosa, N.F.F. Moreira, A.R. Ribeiro, M.F.R. Pereira, A.M.T. Silva, Occurrence and removal of organic micropollutants: An overview of the watch list of EU Decision 2015/495, Water Research, 94 (2016) 257-279.

[5] A. Garcia-Rodríguez, V. Matamoros, C. Fontàs, V. Salvadó, The ability of biologically based wastewater treatment systems to remove emerging organic

532 contaminants—a review, Environmental Science and Pollution Research, 21
533 (2014) 11708-11728.
534 [6] D.J. Garcia, F. You, The water-energy-food nexus and process systems
535 engineering: A new focus, Computers and Chemical Engineering, 91 (2016) 49-
536 67.
537 [7] S. Abinandan, S. Shanthakumar, Challenges and opportunities in application
538 of microalgae (Chlorophyta) for wastewater treatment: A review, Renewable and
539 Sustainable Energy Reviews, 52 (2015) 123-132.
540 [8] A. Idi, M.H. Md Nor, M.F. Abdul Wahab, Z. Ibrahim, Photosynthetic bacteria:
541 an eco-friendly and cheap tool for bioremediation, Reviews in Environmental
542 Science and Biotechnology, 14 (2015) 271-285.
543 [9] D. Batstone, T. Hülsen, C. Mehta, J. Keller, Platforms for energy and nutrient
544 recovery from domestic wastewater: A review, Chemosphere, 140 (2015) 2-11.
545 [10] T. Cai, S.Y. Park, Y. Li, Nutrient recovery from wastewater streams by
546 microalgae: Status and prospects, Renewable and Sustainable Energy Reviews,
547 19 (2013) 360-369.
548 [11] T. Hülsen, E.M. Barry, Y. Lu, D. Puyol, J. Keller, D.J. Batstone, Domestic
549 wastewater treatment with purple phototrophic bacteria using a novel continuous
550 photo anaerobic membrane bioreactor, Water research, 100 (2016) 486-495.
551 [12] V. Singh Yadav, R. Vinoth, D. Yadav, Bio-hydrogen production from waste
552 materials: A review, in: MATEC Web of Conferences, 2018.
553 [13] D.J. Batstone, B. Virdis, The role of anaerobic digestion in the emerging
554 energy economy, Current Opinion in Biotechnology, 27 (2014) 142-149.

[14] V.K. Tyagi, S.-L. Lo, Sludge: A waste or renewable source for energy and resources recovery?, Renewable and Sustainable Energy Reviews, 25 (2013) 708-728.

[15] J. Gibson, C.S. Harwood, Degradation of aromatic compounds by nonsulfur purple bacteria, in: Anoxygenic photosynthetic bacteria, Springer, 1995, pp. 991-1003.

[16] V. Shanker, S.M. Rayabandla, R.N. Kumavath, S. Chintalapati, R. Chintalapati, Light-dependent transformation of aniline to indole esters by the purple bacterium Rhodobacter sphaeroides OU5, Current microbiology, 52 (2006) 413-417.

[17] N. Rajasekhar, C. Sasikala, C.V. Ramana, Toxicity of N-containing heterocyclic aromatic compounds and their utilization for growth by a few purple non-sulfur bacteria, Bulletin of environmental contamination and toxicology, 65 (2000) 375-382.

[18] K.M. Lee, H.-S. Lee, Effects of butachlor on the growth of purplenon sulfur bacteria, Misaengmul Hakhoechi, 29 (1991) 130-135.

[19] S. Kuroki, M. Yoshiyama, K. Furukawa, Biodegradation of acid azo dyes by newly isolated purple nonsulfur bacteria, Japanese Journal of Water Treatment Biology, 37 (2001) 69-75.

[20] C. Sasikala, C.V. Ramana, Biodegradation and metabolism of unusual carbon compounds by anoxygenic phototrophic bacteria, in: Advances in Microbial Physiology, 1998, pp. 339-377.

[21] X. Wang, X. Cheng, D. Sun, Y. Ren, G. Xu, Simultaneous nutrient and carbon removal from azo dye wastewater using a photorotating biological contactor

579 reactor, Journal of Chemical Technology and Biotechnology, 89 (2014) 1545-
580 1552.
581 [22] E. Madukasi, X. Dai, C. He, J. Zhou, Potentials of phototrophic bacteria in
582 treating pharmaceutical wastewater, International Journal of Environmental
583 Science & Technology, 7 (2010) 165-174.
584 [23] N.H. Tran, M. Reinhard, K.Y.-H. Gin, Occurrence and fate of emerging
585 contaminants in municipal wastewater treatment plants from different
586 geographical regions-a review, Water Research, (2017).
587 [24] D. Puyol, E.M. Barry, T. Hï¿½lsen, D.J. Batstone, A mechanistic model for
588 anaerobic phototrophs in domestic wastewater applications: Photo-anaerobic
589 model (PAnM), Water Research, 116 (2017) 241-253.
590 [25] I. Angelidaki, M. Alves, D. Bolzonella, L. Borzacconi, J. Campos, A. Guwy,
591 S. Kalyuzhnyi, P. Jenicek, J. Van Lier, Defining the biomethane potential (BMP)
592 of solid organic wastes and energy crops: a proposed protocol for batch assays,
593 Water science and technology, 59 (2009) 927-934.
594 [26] Y. Segura, D. Puyol, L. Ballesteros, F. Martínez, J. Melero, Wastewater
595 sludges pretreated by different oxidation systems at mild conditions to promote
596 the biogas formation in anaerobic processes, Environmental Science and
597 Pollution Research, 23 (2016) 24393-24401.
598 [27] D.J. Batstone, P.F. Pind, I. Angelidaki, Kinetics of thermophilic, anaerobic
599 oxidation of straight and branched chain butyrate and valerate, Biotechnology
600 and Bioengineering, 84 (2003) 195-204.
601 [28] W. APHA, Standard methods for the examination of water and wastewater,
602 American Public Health Association (APHA), Washington, DC, USA, 2005.

603 [29] T. Hülsen, D.J. Batstone, J. Keller, Phototrophic bacteria for nutrient recovery
604 from domestic wastewater, Water Research, 50 (2014) 18-26.
605 [30] A.G. McEwan, Photosynthetic electron transport and anaerobic metabolism
606 in purple non-sulfur phototrophic bacteria, Antonie van Leeuwenhoek, 66 (1994)
607 151-164.
608 [31] T. Hülsen, E.M. Barry, Y. Lu, D. Puyol, D.J. Batstone, Low temperature
609 treatment of domestic wastewater by purple phototrophic bacteria: Performance,
610 activity, and community, Water Research, 100 (2016) 537-545.
611 [32] S. Chitapornpan, C. Chiemchaisri, W. Chiemchaisri, R. Honda, K.
612 Yamamoto, Photosynthetic bacteria production from food processing wastewater
613 in sequencing batch and membrane photo-bioreactors, Water Science and
614 Technology, 65 (2012) 504-512.
615 [33] T. Satoh, Y. Hoshino, H. Kitamura, Rhodopseudomonas sphaeroides forma
616 sp. denitrificans, a denitrifying strain as a subspecies of Rhodopseudomonas
617 sphaeroides, Archives of microbiology, 108 (1976) 265-269.
618 [34] T.Y. Wu, J.X.W. Hay, L.B. Kong, J.C. Juan, J.M. Jahim, Recent advances in
619 reuse of waste material as substrate to produce biohydrogen by purple non-sulfur
620 (PNS) bacteria, Renewable and Sustainable Energy Reviews, 16 (2012) 3117-
621 3122.
622 [35] B. Masepohl, T. Drepper, A. Paschen, S. Groß, A. Pawlowski, K. Raabe,
623 K.U. Riedel, W. Klipp, Regulation of nitrogen fixation in the phototrophic purple
624 bacterium Rhodobacter capsulatus, Journal of Molecular Microbiology and
625 Biotechnology, 4 (2002) 243-248.
626 [36] D. Dolar, M. Gros, S. Rodriguez-Mozaz, J. Moreno, J. Comas, I. Rodriguez-
627 Roda, D. Barceló, Removal of emerging contaminants from municipal wastewater

628 with an integrated membrane system, MBR–RO, Journal of Hazardous Materials,
629 239-240 (2012) 64-69.
630 [37] L.N. Nguyen, F.I. Hai, J. Kang, W.E. Price, L.D. Nghiem, Removal of trace
631 organic contaminants by a membrane bioreactor–granular activated carbon
632 (MBR–GAC) system, Bioresource Technology, 113 (2012) 169-173.
633 [38] H. Kaspari, W. Busse, Oxidative degradation of purines by the faculative
634 phototrophic bacterium Rhodopseudomonas capsulata, Archives of
635 microbiology, 144 (1986) 67-70.
636 [39] T. Alvarino, S. Suarez, J. Lema, F. Omil, Understanding the sorption and
637 biotransformation of organic micropollutants in innovative biological wastewater
638 treatment technologies, Science of The Total Environment, 615 (2018) 297-306.
639 [40] Ł. Jałowiecki, J.M. Chojniak, E. Dorgeloh, B. Hegedusova, H. Ejhed, J.
640 Magnér, G.A. Płaza, Microbial Community Profiles in Wastewaters from Onsite
641 Wastewater Treatment Systems Technology, PLoS ONE, 11 (2016) e0147725.
642 [41] P.J. Rey, J.M. Alcántara, A.J. Manzaneda, A.M. Sánchez-Lafuente,
643 Facilitation contributes to Mediterranean woody plant diversity but does not
644 shape the diversity–productivity relationship along aridity gradients, New
645 Phytologist, 211 (2016) 464-476.
646 [42] R.M. Callaway, Positive interactions and interdependence in plant
647 communities, Springer, 2007.
648 [43] M. Holmgren, M. Scheffer, Strong facilitation in mild environments: the stress
649 gradient hypothesis revisited, Journal of Ecology, 98 (2010) 1269-1275.
650 [44] T. Hülsen, K. Hsieh, Y. Lu, S. Tait, D.J. Batstone, Simultaneous treatment
651 and single cell protein production from agri-industrial wastewaters using purple

652 phototrophic bacteria or microalgae – A comparison, Bioresource Technology,
653 254 (2018) 214-223.
654 [45] C. Kneip, P. Lockhart, C. Voß, U.-G. Maier, Nitrogen fixation in eukaryotes –
655 New models for symbiosis, BMC Evolutionary Biology, 7 (2007) 55.
656 [46] M.E. Hibbing, C. Fuqua, M.R. Parsek, S.B. Peterson, Bacterial competition:
657 surviving and thriving in the microbial jungle, Nature Reviews Microbiology, 8
658 (2010) 15.
659 [47] R. Mazzoli, E. Pessione, M.G. Giuffrida, P. Fattori, C. Barello, C. Giunta, N.D.
660 Lindley, Degradation of aromatic compounds by Acinetobacter radioresistens
661 S13: growth characteristics on single substrates and mixtures, Archives of
662 Microbiology, 188 (2007) 55-68.
663 [48] A. Cydzik-Kwiatkowska, M. Zielińska, Bacterial communities in full-scale
664 wastewater treatment systems, World Journal of Microbiology & Biotechnology,
665 32 (2016) 66.
666 [49] C.N. Hunter, F. Daldal, M.C. Thurnauer, J.T. Beatty, The purple phototrophic
667 bacteria, Springer Science & Business Media, 2008.
668 [50] P. Guerra-Blanco, O. Cortes, T. Poznyak, I. Chairez, E.I. García-Peña,
669 Polyhydroxyalkanoates (PHA) production by photoheterotrophic microbial
670 consortia: Effect of culture conditions over microbial population and biopolymer
671 yield and composition, European Polymer Journal, 98 (2018) 94-104.
672 [51] Q. Wang, J. Sun, C. Zhang, G.-J. Xie, X. Zhou, J. Qian, G. Yang, G. Zeng,
673 Y. Liu, D. Wang, Polyhydroxyalkanoates in waste activated sludge enhances
674 anaerobic methane production through improving biochemical methane potential
675 instead of hydrolysis rate, Scientific reports, 6 (2016) 19713.

676