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Comparative evaluation of piggery wastewater treatment in algal-bacterial photobioreactors under indoor and outdoor conditions

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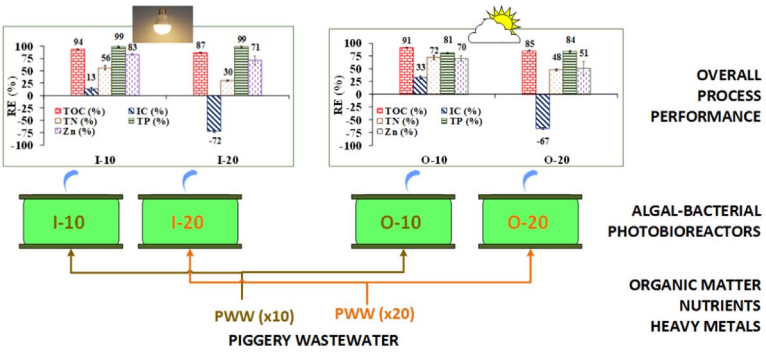
GRAPHICAL ABSTRACT



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ABSTRACT

This work evaluated the performance of four open algal-bacterial photobioreactors operated at ≈26 days of hydraulic retention time during the treatment of 10 (×10) and 20 (×20) times diluted piggery wastewater (PWW) under indoor (I) and outdoor (O) conditions for four months. The removal efficiencies (REs) of organic matter, nutrients and zinc from PWW, along with the dynamics of biomass concentration and structure of algal-bacterial population were assessed. The highest TOC-RE, TP-RE and Zn-RE (94 ± 1%, 100% and 83 ± 2%, respectively) were achieved indoors in ×10 PWW, while the highest TN-RE (72 ± 8%) was recorded outdoors in ×10 PWW. *Chlorella vulgaris* was the dominant species regardless of the ambient conditions and PWW dilution. Finally, DGGE-sequencing of the bacterial community revealed the occurrence of four phyla, *Proteobacteria* being the dominant phylum with 15 out of the 23 most intense bands.

1. Introduction

Europe, with an annual production of 23.5 million tn of pork meat, was the second largest pig producer in the world in 2015 (Statista, 2016). Europe's pig production accounted for 149 million heads, which represented approx. 44.3 % of the total European livestock in 2015 (EU, 2015; MAGRAMA, 2015). However, this relevant economic sector annually generates 217–434 million m^3 of piggery wastewater (PWW)

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(4–8 L/d·pig) containing high concentrations of organic matter, nutrients, solids and heavy metals (De Godos et al., 2009; Franchino et al., 2016). The treatment of such high strength wastewaters represents both a technical challenge and a severe economic burden for the livestock sector. In this context, next generation PWW treatment technologies should allow complying with European wastewater regulations (1999/31/EC) (Council Directive, 1999) while producing added-value bioproducts out of the organic matter and nutrients present in PWW (2008/98/EC) (European Commisssion, 2008).

Algal-bacterial symbiosis has emerged as a promising platform for resource recovery and recycling from PWW in rural areas (where space is often not limiting). Algal-bacterial symbiosis has been successfully applied in photobioreactors for the treatment of domestic wastewater (García et al., 2017a; Oswald et al., 1957), digestates (Anbalagan et al., 2016; Wang et al., 2013), livestock effluents (Tigini et al., 2016), parboiled rice wastewater (Bastos et al., 2009), olive oil mill wastewater and wastewater from the pulp and paper industry (Muñoz and Guieysse, 2006). The use of microalgae during PWW treatment can support a cost-effective removal of organic matter, nutrients, heavy metals, pathogens and emerging pollutants as a result of their dual autotrophic and heterotrophic metabolisms, photosynthetic O_2 release and ability to increase the pH of the cultivation broth (García et al., 2017a; Muñoz and Guieysse, 2006). The ability of microalgae to grow on both wastewater alkalinity and the carbon dioxide (CO_2) released during organic matter oxidation entails 2–3 folds larger productivities (compared to activated sludge systems) of a biomass that can be used as a feedstock for the production of biofertilizers or bioenergy. In addition, the lower energy demand of microalgae-based wastewater treatment, along with the CO_2 fixation ability of microalgae, significantly increase the environmental sustainability of this technology (Cheah et al., 2016; Dassey and Theegala, 2013). Despite the merits of algal-bacterial processes for PWW treatment and the intensive research conducted in this field in the past 10 years, very few studies have been carried out outdoors under the periodically fluctuating and high solar irradiations and temperatures (De Godos et al., 2009; García et al., 2017b; Posadas et al., 2017). In this context, the absence of comparative studies systematically assessing the representativeness of the results obtained indoors (under artificial irradiation and temperature controlled environments) compared to those supported by outdoors photobioreactors severely limits the use of most data available in literature for the design and operation of full-scale microalgae-based systems.

This work aimed at systematically evaluating the potential of open algal-bacterial photobioreactors for the treatment of PWW under indoor and outdoor conditions. The removal of carbon, nitrogen, phosphorus and heavy metals was assessed at two PWW dilutions under solar and artificial illumination. Finally, the influence of both PWW dilution and environmental conditions on the structure of the microalgae and bacteria communities was investigated.

2. Materials and Methods

2.1. Algal-bacterial inoculum and piggery wastewater

An acclimated *Chlorella vulgaris* culture, obtained from an indoor open algal-bacterial photobioreactor treating 15% diluted PWW at the Department of Chemical Engineering and Environmental Technology at Valladolid University (Spain), was used as inoculum. Fresh PWW was collected from a nearby farm at Cantalejo (Spain) and stored at 4 °C. The PWW was centrifuged for 10 min at 10,000 rpm before dilution to reduce the concentration of suspended solids. The average composition of the 10 and 20 folds diluted PWW is shown in Table 1.

2.2. Experimental system

The indoors experimental set-up consisted of two 3 L open photobioreactors (15.8 cm depth, 15.5 cm internal diameter) illuminated at 1417 ± 82 μmol/m^2·s for 12 h a day (08h00–20h00) by LED lamps arranged in a horizontal configuration 60 cm above the photobioreactor surface under indoor conditions (Fig. 1, Table 1). Likewise, two similar open photobioreactors were located outdoors at the Department of Chemical Engineering and Environmental Technology at Valladolid University (Spain). The average photosynthetic active radiation (PAR) in these systems at 11h00 was 1394 ± 171 μmol/m^2·s (Fig. 1, Table 1). This value was comparable to the daily average PARs provided by the official AEMET meteorological station located at the University of Valladolid during the experimental period (1210 ± 126 μmol/m^2·s). The temperature of the indoor and outdoor photobioreactors was partially controlled using a water bath to prevent the high temperatures induced by both LEDs and solar irradiation. The algal-bacterial cultivation broth in the photobioreactors was gently mixed via water immersion pumps. The indoors and outdoors photobioreactors were fed with both 10 and 20 times diluted PWW using an auto control 205U7CA multi-channel cassette pump (Watson-Marlow, UK). PWW dilutions were selected based on previous investigations carried out with this kind of wastewater and aiming to avoid microbial inhibition as a consequence of PWW toxicity (De Godos et al., 2009; García et al., 2017b; González et al., 2008). Pure CO_2 was added to the cultivation broth of the phobioreactors to automatically maintain the pH at 8.0 using a Crison multimeter M44 control unit (Crison Instruments, Spain).

2.3. Experimental design and sampling procedure

The indoors photobioreactors fed with 10 and 20 times diluted PWW (namely I-10 and I-20, respectively) and the outdoors photobioreactors fed with 10 and 20 times diluted PWW (namely O-10 and O-20, respectively) were inoculated with a fresh *Chlorella vulgaris* culture at an initial TSS concentration of ≈680 mg/L (corresponding to an initial microalgae cell concentration of ≈$1.06{\cdot}10^9$ cells/L, respectively). The photobioreactors, which were initially filled with tap water, were operated at an average hydraulic retention time (HRT) of ≈26 days for 120 days (from May-2016 to Sept-2016). A higher HRT than in conventional HRAPs (3–10 days) was chosen in this research to guarantee an effective carbon and nutrients removal, and to prevent toxicity effects on microbial population due the high loads of organic matter and nutrients of the PWW treated in this study (Aguirre et al., 2011; De Godos et al., 2009). The effluent from the photobioreactors overflowed separately as a function of the evaporation rates. Liquid samples from the influent PWWs and effluents of the photobioreactors were taken weekly to determine the concentration of total organic carbon (TOC), inorganic carbon (IC), total nitrogen (TN), nitrate (NO_3^-), nitrite (NO_2^-), total phosphorus (TP), zinc (Zn) and total suspended solid (TSS). Likewise, the structure of the microalgae population in the photobioreactors was periodically assessed from biomass samples preserved with lugol acid at 5% and formaldehyde at 10%, and stored at 4 °C prior to analysis. A cultivation broth sample from the photobioreactors was also collected under steady state (day 120) and immediately stored at −20 °C to evaluate the richness and composition of the bacterial communities (Alcántara et al., 2015). Dissolved oxygen (DO) concentration and temperature in the photobioreactors were measured twice per day (11h00 and 17h00), while the influents and effluents flowrates were daily recorded to monitor water evaporation losses (Table 1). Finally, the C, N and P content of the algal-bacterial biomass present in the photobioreactors was measured under steady state.

The removal efficiencies of C, N, P and Zn were calculated according to Eq. (1):

$$RE\,(\%) = \frac{(C_{feed} \times Q_{feed}) - (C_{eff} \times Q_{eff})}{C_{feed} \times Q_{feed}} \times 100 \quad (1)$$

where C_{feed} and C_{eff} represent the dissolved concentrations of TOC, IC, TN, TP and Zn in the influent PWWs and photobioreactors effluents,

Table 1
Operational conditions and physical/chemical characterization of the piggery wastewater (PWW) and cultivation broth in the photobioreactors.

Parameters		PWW (×10)	PWW (×20)	I-10	I-20	O-10	O-20
Operation period (days)		*	*	120	120	120	120
HRT (days)		*	*	≈26	≈26	≈26	≈26
pH (units)		*	*	8.0	8.0	8.0	8.0
PAR (μmol/m^2.s)		*	*	1417 ± 82	1417 ± 82	1394 ± 171	1394 ± 171
Temperature (°C)	11h00	*	*	21 ± 4	21 ± 4	26 ± 5	26 ± 5
	17h00	*	*	24 ± 4	24 ± 4	35 ± 5	35 ± 5
Dissolved Oxygen (mg/L)	11h00	*	*	3.5 ± 2.2	6.1 ± 4.4	1.9 ± 0.8	3.9 + 1.7
	17h00	*	*	2.3 ± 1.7	5.8 ± 4.8	1.3 + 0.3	2.7 ± 1.2
Evaporation rates (%)		*	*	27	27	44	44
TOC (mg/L)		963 ± 71	497 ± 33	80 ± 5	91 ± 5	150 ± 11	133 ± 8
IC (mg/L)		160 ± 15	82 ± 2	188 ± 3	191 ± 5	191 ± 9	241 ± 6
TN (mg/L)		341 ± 27	170 ± 3	201 ± 6	162 ± 5	168 ± 6	158 ± 7
Nitrate (mg/L)		< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5
Nitrite (mg/L)		< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5
TP (mg/L)		4.9 ± 0.2	2.5 ± 0.1	0.07 ± 0.01	0.04 ± 0.01	1.67 ± 0.08	0.70 ± 0.07
Zinc (mg/L)		0.66 ± 0.04	0.37 ± 0.02	0.16 ± 0.02	0.15 ± 0.05	0.35 ± 0.03	0.32 ± 0.08
TSS (mg/L)		291 ± 3	156 ± 3	1284 ± 71	720 ± 16	1328 ± 28	655 ± 13

*Not applicable.

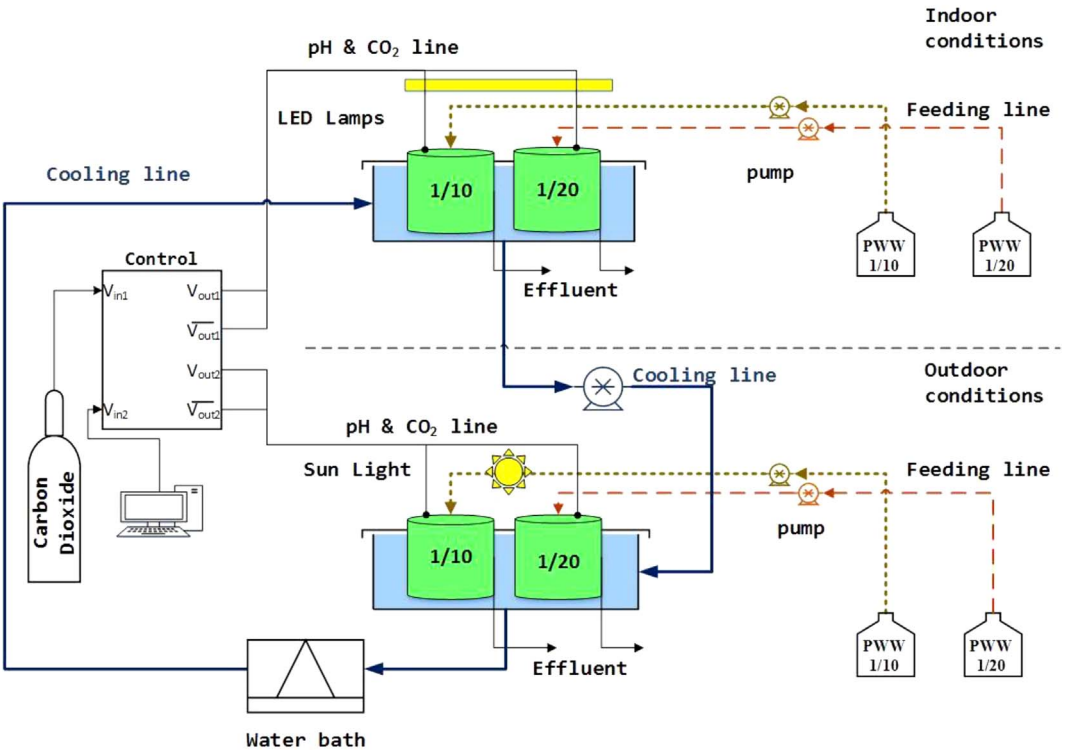

Fig. 1. Schematic diagram of the algal-bacterial photobioreactor set-up equipped with carbon dioxide supplementation for pH control under indoor and outdoor conditions.

respectively, while Q_{feed} and Q_{eff} represent the PWWs and effluents flow rate, respectively. The mass flow rate of C–CO_2 injected to control the pH was negligible compared to the input mass flow rate of C in the influent PWW (data not shown). The process was considered under steady state when the TSS concentrations in the photobioreactors remained stable for at least four consecutive samplings (~1 month). The results obtained were here provided as the average ± standard deviation from duplicate measurements along the one-month steady state period (days 91–120).

2.4. Analytical procedures

pH was in-situ measured using a Crison M44 multimeter and a Crison PH 28 meter. An OXI 330*i* oximeter was used to measure the DO and temperature (WTW, Germany). A LI-250A light meter (LI-COR Biosciences, Germany) was used to measure the light intensity as PAR. TOC, IC and TN concentrations were determined using a TOC-V CSH analyzer equipped with a TNM-1 module (Shimadzu, Japan). Nitrate and nitrite were analyzed by high performance liquid chromatography-ion conductivity (HPLC-IC) (Posadas et al., 2013). N-NH_4^+ was not analyzed based on the fact that no inhibition was expected at a pH of 8.0, where the ammonium share is greater than > 90% of the total nitrogen (Metcalf and Eddy, 2003). TP and TSS concentrations were determined according to Standard Methods (APHA, 2005). The analysis of the C, N and P biomass content in pre-dried and grinded algal-bacterial biomass was carried out using a LECO CHNS-932 elemental analyzer. Zinc was determined using a 725-ICP Optical Emission Spectrophotometer (Agilent, USA) at 213.62 nm. The concentrations of arsenic and copper were not determined based on the results obtained by (García et al., 2017b), who observed that the concentration of these

heavy metals in this PWW always remained below the detection limit (< 0.6 mg/L).

The identification and quantification of microalgae were conducted by microscopic examination (OLYMPUS IX70, USA) according to Sournia (1978). Molecular analysis of the bacterial populations was carried out according with Frutos et al. (2015). The genomic desoxyribonucleic acid (DNA) was extracted using the protocol described in the Fast® DNA Spin Kit for Soil (MP Biomedicals, LLC) handbook. The genes in the V6-V8 regions of the bacterial 16S ribosomal ribonucleic acid (rRNA) were amplified by Polymerase Chain Reaction (PCR) analysis using the universal bacterial primers 968-F-GC and 1401-R (Sigma-Aldrich, St. Louis, MO, USA) (Nübel et al., 1996). The denaturing gradient gel electrophoresis (DGGE) analysis of the amplicons was performed with a D-Code universal mutation system (Bio Rad Laboratories) using 8% (w/v) polyacrylamide gels with a urea/formamide denaturing gradient of 45–65%. DGGE running conditions were applied according to Roest et al. (2005). Sequences were deposited in GenBank Data Library under accession numbers MF380643 al MF380665. The Shannon-Wiener diversity index (H) was determined using the peak heights in the densitometric curves. This index, which reflects both the sample richness and evenness and ranges from 1.5 to 3.5 (low and high species evenness and richness, respectively), can be calculated according to Eq. (2) (MacDonald, 2003):

$$H = -\sum [P_i \ln(P_i)] \quad (2)$$

where H is diversity index and P_i is the importance probability of the bands in a lane ($P_i = n_i/n$, where n_i is the height of an individual peak and n is the sum of all peak heights in the densitometric curves). Similarity indices of the compared profiles were calculated from the densitometric curves of the scanned DGGE profiles by using the Pearson product–moment correlation coefficient (Häne et al., 1993). The taxonomic position of the sequenced DGGE bands was obtained using the RDP classifier tool (50% confidence level) (Wang et al., 2007). The closest cultured and uncultured relatives to each band were obtained using the BLAST search tool at the NCBI database (National Centre for Biotechnology Information) (McGinnis and Madden, 2004).

3. Results and discussion

3.1. Biodegradation of carbon, nitrogen and Phosphorous

The range of temperatures, DO and PAR along with pH control resulted in a successful PWW treatment regardless of the environmental and operational conditions imposed (Table 1, Fig. 2). The higher evaporation rates under outdoor conditions (44% of the influent PWW flowrate compared to 27% under indoor conditions) resulted in a significant deterioration of the quality of the treated effluent (Table 1).

The TOC-REs accounted for 94 ± 1, 87 ± 2, 91 ± 1 and 85 ± 1% in I-10, I-20, O-10 and O-20, respectively, which resulted in average TOC concentrations in the effluent at the end of the operational period of 80 ± 5, 91 ± 5, 150 ± 11 and 133 ± 8 mg/L, respectively (Table 1, Fig. 2). These high REs were supported by the DOs > 1 mg O_2/L in the cultivation broth of the four photobioreactors mediated by an intense photosynthetic activity (Table 1). The slightly higher TOC-REs during the treatment of 10 times diluted PWW regardless of the environmental conditions can be explained by the differences in microbial population structure and biomass concentration encountered in the photobioreactors under steady state. Thus, a higher share, diversity and concentration of bacteria compared to microalgae was present in the photobioreactors supplied with 10 times diluted PWW as revealed by the TSS measurements, DGGE analyses and microalgae population characterization. (Table 1). On the other hand, the higher DO concentrations recorded in the indoor photobioreactors regardless of the organic loading rate applied were likely caused by the lower temperatures (mediating a higher O_2 solubility and a lower bacterial activity) and by the constant PAR (resulting in a higher microalgal activity) (Posadas et al., 2015). The results here obtained were in agreement with the organic matter removal efficiencies reported by De Godos et al. (2009) under outdoor conditions in a 464 L HRAP operated at 10 days of HRT (COD-REs of 76 ± 11% during the treatment of 10 and 20 folds diluted PWW). Likewise, Aguirre et al. (2011) recorded COD-REs ≥ 90 % during the treatment of raw PWW in a 400 L HRAPs under outdoor environmental conditions at a HRT of 40 days. Finally, IC-REs of 13 ± 3, −72 ± 2, 33 ± 2 and −67 ± 13% were recorded under steady state in I-10, I-20, O-10 and O-20, respectively, which resulted in average IC concentrations in the effluent of 188 ± 3, 191 ± 5, 191 ± 9 and 241 ± 6 mg/L, respectively (Table 1, Fig. 2). The negative IC-REs recorded in I-20 and O-20 resulted from the accumulation of inorganic carbon mediated by the high TOC oxidation activity in the systems, which was in agreement with the results obtained by (Posadas et al., 2013). The higher IC-REs during the treatment of 10 times diluted PWW were likely supported by the higher biomass concentrations (≈1300 mg TSS/L in I-10 and O-10 compared to ≈700 mg TSS/L in I-20 and O-20). In this context, process operation at high biomass concentrations in photobioreactors under high PARs can prevent microalgae photoinhibition and thus induce high photosynthetic activities. Carbon removal by stripping (prior mineralization of the organic carbon to CO_2) was the main mechanism accounting for carbon removal in I-10 and O-10, since only 49 and 37 % of the total carbon removed was recovered in the form of harvested biomass, respectively.

The TN-REs accounted for 56 ± 4, 30 ± 14, 72 ± 8 and 48 ± 9% in I-10, I-20, O-10 and O-20, respectively, which resulted in average TN concentrations in the effluent under steady state conditions of 201 ± 6, 162 ± 5, 168 ± 6 and 157 ± 7 mg/L, respectively (Table 1, Fig. 2). The higher temperatures prevailing outdoors likely lowered NH_3 solubility and therefore increased N removal by stripping (Fig. 2) (Metcalf and Eddy, 2003). In addition, the systems supporting higher biomass concentrations (I-10 and O-10) mediated higher N

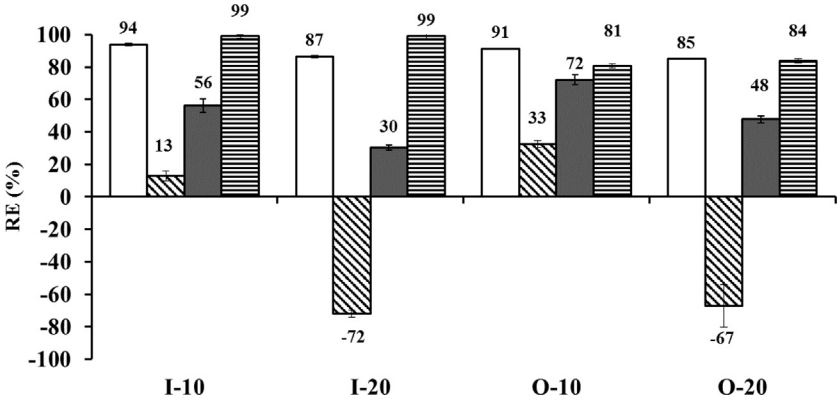

Fig. 2. Average removal efficiencies of TOC (□), IC (▨), TN (■) and TP (▤) under steady state. Bold numbers indicate the steady state removal efficiencies, while vertical bars represent the standard deviation from replicate measurements during steady state operation.

removals compared to process operation with 20 folds diluted PWW (Fig. 2). The TN-REs here achieved were similar to those obtained by Aguirre et al. (2011) who reported TN-REs ranging from 65 to 85% during PWW treatment in 400 L HRAPs operated at HRTs of 40–80 days, but lower than those reported by García et al. (2017b) (82–85%) during the treatment of 15% diluted PWW in open photobioreactors at a HRT of ≈27 days operated indoors. The nitrogen mass balances conducted revealed that stripping was the main N removal mechanism in I-10, O-10 and O-20, with nitrogen assimilation into biomass accounting for only 44, 25 and 37% of the total nitrogen removed, respectively. However, nitrogen assimilation was the main N removal mechanism in I-20 (85% of TN removed) likely due to the lower temperatures and TN concentrations prevailing in the cultivation both.

The TP-REs accounted for 99 ± 3, 99 ± 5, 81 ± 8 and 84 ± 13% in I-10, I-20, O-10 and O-20, respectively, which resulted in average TP concentrations in the effluent of 0.07 ± 0.07, 0.04 ± 0.08, 1.67 ± 0.08 and 0.70 ± 0.07 mg/L, respectively, under steady state (Table 1, Fig. 2). The fact that higher TP-REs were recorded indoors regardless of the PWW dilution and biomass concentration at a constant pH requires further investigation. The TP-REs herein obtained were similar to those reported by García et al. (2017b) during the treatment of 15% diluted PWW in indoors open photobioreactors (90–92%). Phosphorous assimilation into algal-bacterial biomass was likely the main removal mechanism based on the moderate pH values prevailing in the photobioreactors during the entire experiment (pH = 8.0), which did not support a significant phosphate precipitation (García et al., 2017a). Thus, a phosphorus mass balance revealed that 100, 99, 100 and 100% of the total removed phosphorus was recovered in the harvested biomass in I-10, I-20, O-10 and O-20 respectively.

3.2. Heavy metal removal

The overall steady state Zn-REs in I-10, I-20, O-10 and O-20 accounted for 83 ± 2, 71 ± 9, 70 ± 5 and 51 ± 13%, respectively, which resulted in average Zn concentrations at the end of the operational period of 0.16 ± 0.02, 0.15 ± 0.05, 0.35 ± 0.03 and 0.32 ± 0.08 mg/L, respectively (Table 1). In this context, the higher abundance of microalgae and cyanobacteria induced by laboratory conditions likely supported the higher Zn-REs recorded indoors, while the higher biomass concentrations in the HRAPs treating 10 folds diluted PWW explained the superior Zn-REs in I-10 and O-10 compared to I-20 and O-20, respectively. The latter suggests that biosorption was the main mechanism governing Zn removal (Javanbakht et al., 2014; Kaplan et al., 1987). The Zn-REs herein obtained were higher than those reported by García et al. (2017b) during PWW treatment in 3 L indoors HRAPs operated at a HRT of ≈27 days (26 to 49%).

3.3. Concentration, productivity and elemental composition of the algal-bacterial biomass

The algal-bacterial biomass concentration in I-10, I-20 and O-20 initially decreased from 680 mg TSS/L to 127, 177 and 170 mg TSS/L, respectively, during the first 28 days of operation, while biomass concentration slightly increased from 680 to 800 mg TSS/L in O-10 during process start-up (Fig. 3). The previous acclimation of microalgae to the pollutants loading rate and environmental conditions imposed to I-20 explain this increase in biomass concentration. Biomass concentration increased exponentially afterwards in I-10 and O-10 up to steady state values of 1284 ± 71 mg TSS/L and 1328 ± 28 mg TSS/L, respectively. Similarly, biomass concentration in I-20 and O-20 increased up to steady state values of 720 ± 16 and 620 ± 79 mg TSS/L, respectively (Fig. 3). Indeed, the steady state biomass concentrations in systems supplied with 10 folds diluted PWW were ~2 times higher than those recorded in the photobioreactors fed with 20 folds diluted PWW (Table 1). On the other hand, the higher water evaporation rates in the outdoor photobioreactors resulted in slightly lower biomass productivities: 5.6, 3.1, 4.8 and 2.4 g/m^2·d in I-10, I-20, O-10 and O-20, respectively. The lower biomass productivities recorded outdoors could be also explained by the pernicious effects on microbial metabolism caused by the high and fluctuating temperatures and irradiations. These biomass productivities were comparable to those reported by García et al. (2017b) under indoor conditions during the treatment of 15% diluted PWW in 3 L HRAPs operated at ≈27 days of HRT (5.8 to 7.8 g/m^2·d).

The C, N and P content of the biomass cultivated indoors averaged 49.3 ± 0.6, 8.6 ± 0.4 and 0.52 ± 0.06% (which entailed a C/N/P of 100/17/1), respectively, and 46.0 ± 0.02, 8.2 ± 0.02 and 0.58 ± 0.02% (C/N/P of 100/17/1), respectively, when cultivated outdoors. These elemental compositions were in agreement with those reported by Cabanelas et al. (2013), who observed a C, N and P content in the harvested biomass of ≈44, 7.5 and 0.5%, respectively, in a photobioreactor inoculated with *Chlorella vulgaris* and supplemented with CO_2 during the treatment of settled domestic wastewater.

3.4. Time course of the microalgae population structure

Chlorella vulgaris, which achieved a maximum cell concentration of $1.74 \cdot 10^9$ cells/L by day 92, represented the dominant photosynthetic species in I-10 throughout the entire experimental period. *Pseudanabaena* sp. was also identified in I-10 from day 92 onwards at concentrations of $\approx 0.30 \cdot 10^9$ cells/L (Fig. 4a). A similar microalgae population dynamics was recorded in I-20, with *C. vulgaris* representing the dominant species with maximum cell concentrations of $2.95 \cdot 10^9$ by days 42 and 70. However, *Pseudanabaena* sp. became dominant by day 120 with a concentration of $0.46 \cdot 10^9$ cells/L as a result of the gradual decrease in *C. vulgaris* population from day 70 (Fig. 4b). *C. vulgaris* was also dominant in the photobioreactors operated outdoors regardless of the PWW dilution applied. However, the maximum concentration of *C. vulgaris* in O-10 was recorded in the inoculum ($0.52 \cdot 10^9$ cells/L), with a gradual decrease afterwards. *Pseudanabaena* sp. was identified by days 105 and 120 at concentrations of $0.15 \cdot 10^9$ and $0.08 \cdot 10^9$ cells/L, respectively, in O-10. In addition, *Aphanothece* sp. was also detected from day 70 to 120 in O-10, but at negligible concentrations (Fig. 4c). Finally, the maximum cell concentration of *C. vulgaris* in O-20 was $1.73 \cdot 10^9$ cells/L by day 25, with *Acutodesmus obliquus* (identified by day 25) and *Aphanothece* sp. (identified by days 56 and 72) detected at negligible concentrations, and *Pseudanabaena* sp. identified by day 92 at a concentration of $0.59 \cdot 10^9$ cells/L (Fig. 4d). The high tolerance of *C. vulgaris* to organic and heavy metals pollution likely supported the observed dominance of this microalga regardless of the operational and environmental conditions. Thus, *C. vulgaris* ranked 11/80 in the ranking of pollution-tolerant microalgae species published by Palmer (1969), while the *Chlorella* ranked 5/60 at a genus level. Process inoculation with *C. vulgaris* at a high concentration, along with the high tolerance of this microalga to organic pollution, guaranteed its long-term dominance and an effective PWW treatment. *Pseudanabaena* sp., which belongs to the order of *Oscillatoriales,* was also identified at relevant concentrations under steady state (Acinas et al., 2009). The tolerance of *Pseudanabaena* sp to organic pollution herein recorded was in agreement with the observations of García et al. (2017a), who identified *Pseudanabaena* sp. during the treatment of domestic wastewater in an enclosed photobioreactor at a HRT of 2 day, and by Serejo et al. (2015) during the treatment of digested vinasse in a 180 L HRAP. This study also suggested that the high and fluctuating temperatures and irradiations prevailing under outdoors operation resulted in both a reduced population of microalgae and cyanobacteria compared to indoors cultures, and in lower biomass productivities (Fig. 4a,b). Finally, the highest microalgae concentration recorded during the treatment of 20 times diluted PWW regardless of the environmental conditions was likely caused by the lower toxicity at increasing PWW dilutions.

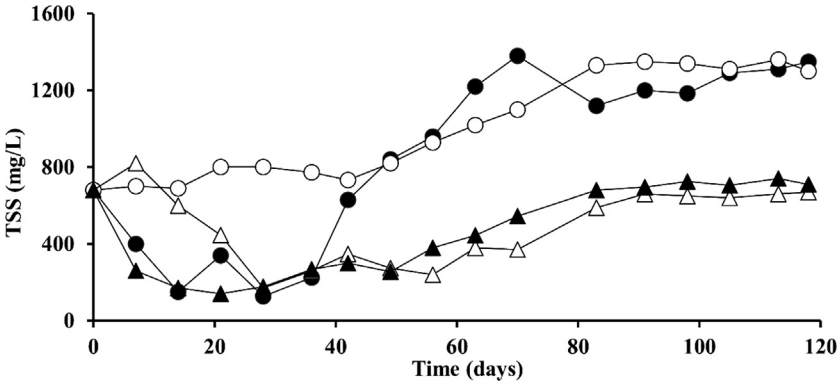

Fig. 3. Time course of TSS concentration in I-10 (●), O-10 (○), I-20 (▲) and O-20 (Δ).

3.5. Bacteria population structure

The DGGE analysis of the microbial communities present in the open photobioreactors revealed the occurrence of 4 phyla and 23 bands (Fig. 5). *Proteobacteria*, which is ubiquitous in the environment, was the dominant phylum (15 out of 23 bands sequenced) in the inoculum and in all photobioreactors (bands 1–15) (Fig. 5) (Shin et al., 2015). Despite not present in the inoculum, the phylum *Bacteroidetes* was identified under steady state in all photobioreactors (bands 16–20). The phylum *Firmicutes* was identified in the inoculum (bands 21 and 22) and in O-20, while the phylum *Cyanobacteria/Chloroplast* corresponded to band 23 in the inoculum and in I-10, I-20 and O-10 (Fig. 5). In this context, the open nature of the photobioreactors, along with the different environmental conditions and characteristics of the PWW fed, likely induced the enrichment of photobioreactor-specific bacterial populations different from the inoculum. Bacteria from the phyla *Proteobacteria, Bacteriodetes* and *Firmicutes* were likely the responsible for the biodegradation of organic matter in the photobioreactors. Thus, bacteria from the phylum *Proteobacteria* belonging to the genus *Psychrobacter* (I-10, I-20, O-10 and O-20), the class *Betaproteobacteria* (I-10, I-20, O-10 and O-20) and the genus *Thauera* (I-10, I-20 and O-10) have been identified in synthetic wastewater, swine effluents, anaerobic digesters treating feedstock from cheese manufacturing, wastewater from dye industry and anoxic biotrickling filters treating BTEX, which confirmed the capacity of these microorganisms to biodegrade the organic pollutants present in PWW (Akmirza et al., 2017; Lucas et al., 2013). Similarly, bacteria from the phylum *Bacteriodetes* have been identified during the anoxic removal of BTEX, Laboratory-scale partial nitrifying-ANAMMOX reactor and municipal wastewater treatment (Fig. 5) (Akmirza et al., 2017; Biswas and Turner, 2012). Finally, bacteria from the phylum *Firmicutes* (syntrophic microorganisms) were detected in a SBR reactor treating swine waste (Loureiro, 2008; Rivière et al., 2009).

The Shannon-Wiener diversity indexes (H) of the inoculum, I-10, I-20, O-10 and O-20 were 2.66, 2.69, 2.72, 2.63 and 2.17, respectively (Fig. 5). The photobioreactors operated in this study exhibited a relatively low-medium bacterial diversity (H ≈2.6) likely due to the extreme environmental conditions applied and to the high toxicity of the wastewater treated. The analysis of the similarity indexes (76.3% between I-10 and I-20 and 76.3% between O-10 and O-20) showed high similarities between the respective indoor and outdoor photobioreactors. On the other hand, low similarity indexes were recorded between I-10 and O-10 (14.2%) and I-20 and O-20 (41.6%). Thus, these results confirmed that temperature and irradiation under indoor and outdoor conditions can result in significantly different bacterial population structure. These results were in agreement with the findings reported by Ferrero et al. (2012), who observed that environmental parameters such as temperature or the impinging irradiation can play a more important role than organic matter and nutrients loading in the structure of the bacterial community.

4. Conclusions

This work demonstrated for the first time that neither pollutant removal nor the structure of microalgae and bacterial communities

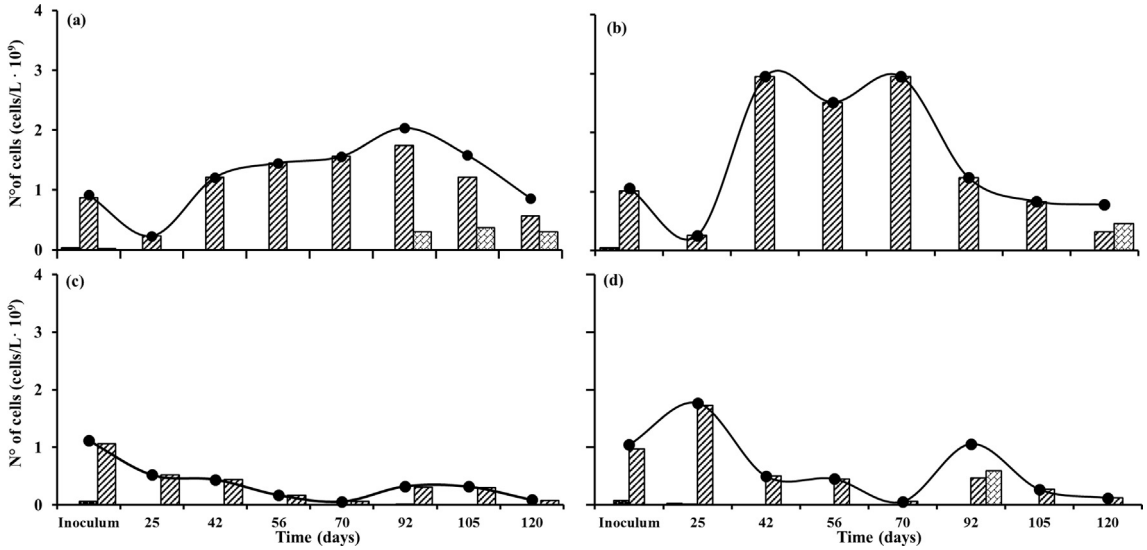

Fig. 4. Time course of the microalgae population structure in I-10 (a), I-20 (b), O-10 (c) and O-20 (d). *Acutodesmus obliquus* ▩, *Aphanothece* sp. ■, *Chlorella vulgaris* ▨, *Pseudanabaena* sp. ▨, and total numbers of cells (●).

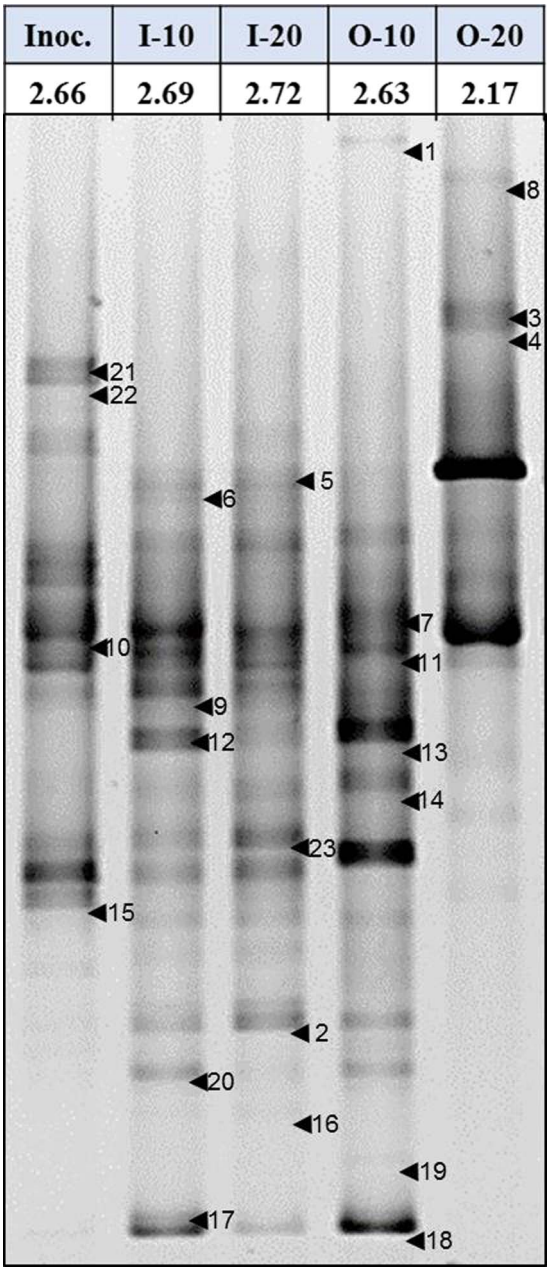

Fig. 5. Bacterial DGGE profile of the microbial communities in the inoculum (Inoc.) and in the open algal-bacterial photobioreactors I-10, I-20, O-10 and O-20. Horizontal arrows and numbers indicate the most abundant bacterial communities. The name of the samples and the Shannon-Wiener diversity indexes (H) are also shown in the upper part of the gel profiles.

under indoor conditions can be directly extrapolated to outdoors photobioreactors. Unexpectedly, the lowest PWW dilution always resulted in a superior PWW treatment performance. The dominance of *Chlorella vulgaris* in all photobioreactors regardless of the environmental conditions and PWW dilution confirmed the high pollution-tolerance of this species. The DGGE analysis revealed a high dominance of the *Proteobacteria* phylum in all photobioreactors, and the key influence of temperature and irradiation on the final bacterial population structure.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.biortech.2017.08.135.

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