Journal of Environmental Management 354 (2024) 120340

Contents lists available at ScienceDirect

Journal of Environmental Management

journal homepage: www.elsevier.com/locate/jenvman

Research article

Removal of contaminants of emerging concern from pig manure in different operation stages of a thin-layer cascade photobioreactor. Relationship with concentrations in microalgae and manure

Rebeca López-Serna [a,b], Belén Franco [a,b], Silvia Bolado [b,c], Juan José Jiménez [a,b,*]

[a] Department of Analytical Chemistry, Faculty of Sciences, University of Valladolid, Campus Miguel Delibes, Paseo de Belén 7, 47011 Valladolid, Spain
[b] Institute of Sustainable Processes, Dr. Mergelina s/n, 47011 Valladolid, Spain
[c] Department of Chemical Engineering and Environmental Technology, School of Industrial Engineering, University of Valladolid, Dr. Mergelina, s/n, 47011 Valladolid, Spain

ARTICLE INFO

Handling Editor: Prof Raf Dewil

Keywords:
Thin-layer cascade photobioreactor
Micropollutants
Microalgae biomass
Routine removal
Pig manure

ABSTRACT

The performance of a pilot-scale thin-layer cascade photobioreactor, operated in semicontinuous mode, for the removal of veterinary drug residues and other contaminants of emerging concern (CECs) from pig manure has been assessed in six operation stages. *Chlorella* sp. (70–90%), *Scenedesmus* sp. (10–25%) and *Diatomea (<5%)* comprise the microalgae species present during the stages. The global performance to remove the total CEC content in the photobioreactor effluent varied from 62 to 86% on each stage, while an CEC mean amount close to 8% was accumulated in the photobioreactor biomass. A relation with weather conditions was not observed. Elimination ratio was not related to the concentration in the influent which reached up to 8000 ng L^{-1} for some CECs. As expected, the concentrations of veterinary drugs were higher than those of non-veterinary CECs. The concentrations accumulated in the grown biomass were relative low, lower than 10 ng per fresh g excepting for a few cases. However, statistical data suggested that the linkage of CECs to microalgae biomass boosted their removal from the influent. Furthermore, it was observed that the manure liquid phase contained higher amounts of CECs than the solid phase.

1. Introduction

The use of microalgae-based technology is being tested for the treatment of livestock and urban wastewaters, which contain great amounts of organic and inorganic matter. The bioremediation of wastewater by microorganisms such as microalgae and their bacteria consortium is an interesting purification tool in comparison to other technologies, which are basically advanced oxidation processes and membrane procedures, with high energy and chemical consumption requirements. Microalgae can easily grow in a medium rich in nutrients, consuming atmospheric carbon dioxide. Also, they can easily adapt to changes in temperature, light intensity, salinity, pH and nutrient availability in the medium they grow. This biological treatment is environmentally sustainable and enables a combination of CEC removal mechanisms that can hardly be achieved by other purification process. These mechanisms, which can act individually or jointly, are basically biodegradation, direct and indirect photodegradation, hydrolysis and adsorption to the biomass (Silva et al., 2019; Tolboom et al., 2019). Different configurations of photobioreactors have been developed over time, from outdoor culture systems with uncontrolled conditions to indoor culture systems with predictable conditions. At this respect, the open systems, such as the thin-layer reactors, entail a lower cost of investment and operation in addition to an easy and sustainable performance (Tolboom et al., 2019; Vo et al., 2019; Wang et al., 2017). On the other hand, a reduction of the operation economic costs associated with the use of photobioreactors could be possible thanks to the microalgae biomass harvested. The production of added value products such as carbohydrates, proteins and others from biomass is being optimized. (Dang et al., 2022; Lorenzo et al., 2019; Martín et al., 2019).

Weather conditions (radiation intensity and temperature) could affect the fate of the CECs in a photobioreactor. Thus, the biomass productivity is favored by high radiation values and relatively high

* Corresponding author. Department of Analytical Chemistry, Faculty of Sciences, University of Valladolid, Campus Miguel Delibes, Paseo de Belén 7, 47011 Valladolid, Spain.
E-mail address: jjjimenez@uva.es (J.J. Jiménez).

https://doi.org/10.1016/j.jenvman.2024.120340
Received 19 October 2023; Received in revised form 26 January 2024; Accepted 8 February 2024

temperatures and, consequently, nutrient and CEC removals by biodegradation should be enhanced under these weather conditions too. Furthermore, an increase of the solar radiation entails a higher absorption of radiation by the CECs increasing the breakdown capacity of its structure (direct photodegradation). Alternatively, the incident radiation on organic matter can yield oxygen radical species, which could react with the CECs (indirect photodegradation). This last pathway would not only be fostered by an increase in solar radiation but also by an increase in temperature. The rate of chemical reactions, including hydrolysis reactions, increases with temperature. A possible drawback of the high environmental temperature is the decrease of the CEC adsorption on the microalgae cell walls (Couto et al., 2022; Mojiri et al., 2022; Sánchez Zurano et al., 2020; Tian et al., 2019).

Microalgae have demonstrated to be a valid approach to decrease the CEC content in wastewaters. Table SM1 (in supplementary material) shows some removal percentages reported in the scientific literature for over fifty contaminants of emerging concern (CECs). Most of the gathered data has been obtained in laboratory studies working with wastewaters or basal media to promote the growth of microalgae biomass. CEC removal can widely vary, depending on the type of microalgae, photobioreactor, matrix and CEC concentration, in addition to the retention time.

The CEC removal data achieved with outdoor pilot-scale microalgae photobioreactors are scarce in the scientific literature, and they work under variable environmental conditions. As these photobioreactors are a promising alternative to purify wastewaters it is advisable to know their CEC removal capacity over time. Thus, in this work, the CEC removal performance of a thin-layer cascade photobioreactor fed with diluted wastewater from a piggery has been assessed in different operation stages over time. The thin-layer reactor operated in semi-continuous mode under constant controllable operational parameters and different weather conditions. Forty-six CECs have been monitored and the concentrations of twenty-six detected microcontaminants have been determined in the solid and liquid phases of the manure collected from the feeding lagoon, the influent (diluted manure) and effluent of the photobioreactor. Also, the CEC amounts accumulated in the biomass grown in the photobioreactor have been determined, which entails a remarkable novelty because data about the occurrence of CECs in the biomass compartments of the photobioreactors are mostly negligible.

2. Experimental

2.1. Laboratory material and reagents

Forty-six environmental microcontaminants with a purity higher than >95% were purchased from LGC Standards (Barcelona, Spain) and Sigma-Aldrich (Tres Cantos, Madrid, Spain). Twenty-four of these were drugs for veterinary use (Table SM2). Oasis HLB cartridges (60 mg) for solid-phase extraction (SPE) were acquired from Waters (Milford, MA, USA) and ultrapure water was in-house generated by a Milli-Q Advantage Ultrapure Water purification system (Billercia, MA, USA). Methanol and acetonitrile were supplied by Sigma-Aldrich and Scharlau (Barcelona, Spain). Primary secondary amine (PSA), alumina and PTFE disposable filter units were purchased from Scharlab (Barcelona, Spain). An N-Evap 11,250 evaporation system, a Univeba ultrasonic bath and a PK120 centrifuge were acquired from Organomation (Berlin, MA, USA), Selecta (Barcelona, Spain) and ALC (Winchester, VA, USA) respectively.

2.2. Operation of the photobioreactor, collection of samples

Six operation stages of a photobioreactor placed in the experimental station "Las Palmerillas" (University of Almeria, El Ejido, Almeria, Spain) were sampled in the years 2019 (May, June and November) and 2020 (February and March). An open 30 m^2 thin-layer cascade reactor with a channel width of 1 m was operated at a culture depth of 0.02 m. The reactor was built of glass fibre and was elevated approximately 1.5 m above the ground. It had a 680 L collector allowing a total working volume of 1.280 L. The culture was pumped from the collector to the beginning of the channel, lifting it 0.5 m, and flowed gravitationally downwards at 0.2 m s^{-1}. Fig. 1 shows a simple operation scheme and resumes the weather conditions. The reactor was feed with pig slurry diluted at 5%. Table SM3 shows the carbon, nitrogen and phosphorus contents, as well as the total solids in suspension, of the influent at each stage. Water loss was compensated for daily using freshwater (Ciardi et al., 2022). The photobioreactor was operated 24 h per day in semi-continuous mode, under recirculation (75%). The photobioreactor was considered in steady-state when the biomass concentration remained constant for at least three consecutive days. The pH value of the culture medium was continuously measured and adjusted to pH 8.0 by the on-demand injection of CO_2 in the collector of the reactor, in order to prevent large deviations from the pH considered optimal (Posadas et al., 2017). The hydraulic retention time was 12 days and the dilution rate was 0.3 day^{-1}. The biomass was harvested automatically using a GEA Wetfalia Separator OTC-3-02- 137 (Oelde, Germany) operating at 10.000 rpm. The most abundant microalgae in the stages, according to the cell percentage, were *Chlorella* sp. (70–90%), *Scenedesmus* sp. (10–25%) and *Diatomea* (<5%). The identification and quantification measurements of the microalgae species were performed by optical microscopy (OLYMPUS IX70, Hamburg, Germany) using at least three different samples and a counting chamber according to Sournia (1978). Biomass samples were fixed with acidic Lugol solution at 5%. Microalgae biomass was taken from other smaller photobioreactor fed with freshwater and commercial nutrients. In one stage (March 2020) the reactor was covered with a 200 μm thickness plastic film from Sotrafa (Almería, Spain; total transmission of visible light, 90%, EN 2155-5 standard; light diffusion, 55%, EN 2155-9 standard; thermal effect, 90%, EN 13206 standard) to limit the loss of water by evaporation.

Aliquots of the influent (diluted raw manure) of the photobioreactor were collected in each stage and frozen at -5° until analysis. Aliquots of the effluent were also taken, and fresh microalgae were separated from the liquid phase by centrifugation. Then, biomass was lyophilized (moisture 78–79%) and kept under −5 °C until use, as the effluent liquid phase.

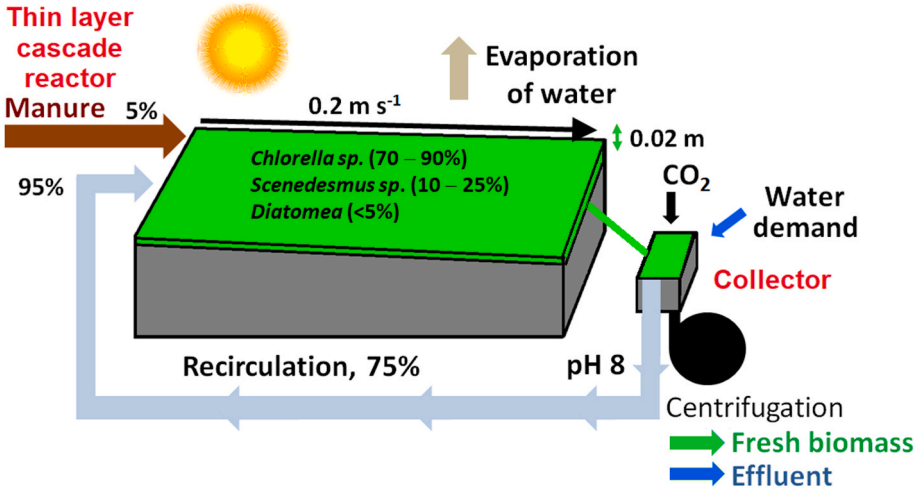
Raw swine manure samples were collected from the lagoon attached to the reactor before dilution. For each raw sample, the manure solid phase (MSP) and the manure liquid phase (MLP) were separated in the laboratory by centrifugation at 10,000 rpm for 10 min. The amount of solids in suspension, which constitute the MSP, was in the range 3.9–5.2% (w/w) and the density of MLP (range 94.8–96.1%, w/w) was 1.01–1.02 g mL^{-1}. MSP samples were lyophilized (moisture about 78%) and both phases were kept at -5 °C before their individual analysis. The concentration of each CEC in the raw manure (C_{RM}) was calculated from the CEC content measured in the MSP and MLP by Eq. (1), taking into account the percentage by weight of each phase ($\%_{wMSP/wRM}$ and $\%_{wMLP/wRM}$) in the raw manure, the MSP moisture to convert the concentration determined on lyophilized MSP (C_{MSP}) in a concentration per fresh g, and the inverse of the density of the MLP (V/w) to convert the measured MLP concentration (C_{MLP} in ng/L) in ng per g.

$$C_{RM} = C_{MSP} \times \left[1 - \frac{\%_{moisture}}{100}\right] \times \frac{\%_{w_{MSP}/w_{RM}}}{100} + C_{MLP} \times (V/w)_{MLP} \times \frac{\%_{w_{MLP}/w_{RM}}}{100} \quad (1)$$

2.3. Sample preparation

2.3.1. Influent and effluent of the photobioreactor

Samples were treated according to a procedure already described (López-Serna et al., 2019). Briefly, a volume of 100 mL filtered through 0.45 μm disks was mixed with 2 mL of EDTA at 5% (w/v) and subjected to SPE on Oasis HLB cartridges. Cartridges were eluted with 3 × 2 mL of acetonitrile and the extract was evaporated at room temperature under a



Stage	Solar radiation (MJ m^{-2} d^{-1}) Mean daily values	Temperature (ºC) Mean daily values	Lyophilized biomass amount (g L^{-1})
May 2019	26.6	19.5	1.5
June 2019	29.0	21.2	1.8
November 2019	11.7	16.8	1.2
February 2020	14.5	14.5	1.3
March 2020	16.3	15.2	1.3
March 2020 Covered	17.4	16.2	1.3

Fig. 1. Scheme of the photobioreactor and weather conditions of the stages.

N_2 stream. Finally, extracts were reconstituted in 1 mL of water/-methanol (95:5, v/v).

2.3.2. *Liquid and solid phase of the manure*

The MLP was filtered successively through 0.70 and 0.45 μm pore-size filters, and a volume of 5 mL of MLP was mixed with 2 mL of EDTA at 5% (w/v) and diluted to 100 mL with ultrapure water. Then, a SPE procedure similar to that described in section 2.3.1 was applied. CECs in the lyophilized MSP were determined by a previously described procedure (Argüeso et al., 2021). Briefly, an amount of 0.3 g of lyophilized MSP, mixed with activated alumina, was extracted twice with 15/10 mL of a 90:10 (v/v) water/methanol mixture by solid-liquid extraction assisted with ultrasounds. The combined extracts were diluted to 100 mL with water after adding EDTA to achieve a concentration of 0.1% (w/v) and, then, the SPE procedure and the extract reconstitution described in section 2.3.1 were carried out.

2.3.3. *Algae biomass*

The content of CECs in algae biomass was determined by an analytical method previously developed for veterinary drugs (López-Serna et al., 2022). In brief, lyophilized microalgae samples (0.3 g) were extracted twice with 15/10 mL of a 90:10 (v/v) water/methanol mixture by ultrasonic shaking in presence of PSA. The supernatants separated by centrifugation were combined, EDTA was added and the solution was diluted to 100 mL with water before carrying out the SPE, evaporation and reconstitution procedures described in section 2.3.1.

2.4. *Determination by HPLC-MS/MS*

An Exion LC AD liquid chromatograph coupled to a triple quadrupole 6500+ mass spectrometer, both from AB Sciex (Framingham, MA, USA), were used, following the chromatographic conditions previously described elsewhere (Argüeso et al., 2021). MS/MS data were acquired by an Analyst (AB Sciex) software with an electrospray ionization interface in positive mode, following two transitions on selected reaction monitoring (SRM) mode (Table SM4).

Twenty veterinary drugs, including the hormone progesterone, were monitored in the three stages operated along the year 2019. Additionally, forty-six CECs, including twenty-four veterinary drugs, were monitored in the other three stages carried out during the year 2020. A tailor-made standard addition calibration method was developed for each type of matrix. Peak areas of the most intense SRM transitions were used for quantification; they were measured in the chromatograms by using the OS software (AB Sciex).

Table SM5 shows the linearity ranges, limits of detection and quantitation, and coefficients of determination of the calibration graphs after a linear fitting. The repeatability of the analytical methods, expressed as relative standard deviation, was commonly lower than 20%, n = 5 (Argüeso et al., 2021; López-Serna et al., 2019, 2022). Table SM6 details the precision values for the compounds detected in the analyzed matrices.

3. Results and discussion

3.1. *Influent and manure concentrations*

Table 1 shows the concentrations of CECs determined in the three types of samples collected from the photobioreactor (influent, effluent, biomass) and the liquid and solid phases of the manure used to feed the photobioreactor after its dilution with the recirculated liquid. As regards to the influent results, danofloxacin, oxytetracycline, sulfadiazine, sulfadimidine and tylosin were detected in the influents of all six stages, and tiamulin, sulfamethoxazole, fenbendazole and progesterone were also frequently detected compounds. Concentrations up to 8000 ng L^{-1} (for oxytetracycline) were measured in them. Ciprofloxacin (684 ng L^{-1}), danofloxacin (376 ng L^{-1}), doxycycline (342 ng L^{-1}), enrofloxacin (449 ng L^{-1}), naproxen (337 ng L^{-1}), oxytetracycline (365 ng L^{-1}), progesterone (391 ng L^{-1}) and sulfadiazine (2480 ng L^{-1}) showed the highest concentrations according to their median values. These data, along with the ones from the samples collected in the manure lagoon, are charted in Fig. 2 and summarized in Table SM7). Enrofloxacin, oxytetracycline, sulfadiazine, sulfadimidine and tylosin were detected in

Table 1
Concentrations of veterinary drugs in the manure solid phase (SP, ng per g of lyophilized phase), manure liquid phase (LP, ng L^{-1}), influent of the photobioreactor (INF, ng L^{-1}), algae biomass grown in the photobioreactor (BIO, ng per g of lyophilized biomass) and effluent of the photobioreactor (EFF, ng L^{-1}).

	Use[a]	SP ng g^{-1}	LP ng L^{-1}	INF ng L^{-1}	BIO ng g^{-1}	EFF ng L^{-1}	SP ng g^{-1}	LP ng L^{-1}	INF ng L^{-1}	BIO ng g^{-1}	EFF ng L^{-1}	SP ng g^{-1}	LP ng L^{-1}	INF ng L^{-1}	BIO ng g^{-1}	EFF ng L^{-1}
Date:		**May 2019**					**June 2019**					**November 2019**				
Ciprofloxacin	VET	<37	3980	<147	7	108	97	1777	1778	25	104	–	–	–	–	–
Danofloxacin	VET	–	–	–	6	–	–	–	–	–	–	–	2880	7237	30	380
Doxycycline	VET	796	16,800	560		104	999	16,468	124	–	258	–	–	–	–	–
Enrofloxacin	VET	57	>40,000	1034	20	804	74	27,514	780	24	902	189		250	<17	–
Fenbendazole	VET	<31	–	111	29	25	<31	–	20	92	–	–	–	<9	–	–
Florphenicol	VET	–	26,530	–	–	–	–	15,538	–	–	–	–	–	–	–	–
Oxytetracycline	VET	1652	39,550	8154	17	2926	1709	38,736	2958	20	183	215	1634	202	16	179
Penicillin G	VET	–	–	–	–	–	–	–	–	–	–	–	<1706	–	–	–
Progesterone	HOR	<130	5910	732	<34	<45	<130	10,468	1526	<34	–	329	860	<45	<34	–
Sulfadimidine	VET	2	670	14	2	16	8	2738	22	3	12	50	368	12	3	23
Sulfadiazine	VET	808	27,068	402	23	7394	654	29,836	3214	30	5010	367	10,504	2317	1.4	1445
Sulfomethoxazole	VET	–	–	–	–	–	–	–	–	–	–	3	v	31	–	–
Sulfathiazole	VET	–	54	21	–	–	–	–	–	–	–	–	–	–	–	–
Tiamulin	VET	–	–	–	–	–	6	32	14	11		394	16	74	80	
Trimethoprim	VET	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Tetracycline	VET	–	–	–	–	–	<66	–	–	–	–	357	–	–	–	–
Tylosin	VET	>2911	>34,000	342		26	>2911	>34,000	277	10	–	78	5628	<9	9	133
	Use*	SP ng g^{-1}	LP ng L^{-1}	INF ng L^{-1}	BIO ng g^{-1}	EFF ng L^{-1}	SP ng g^{-1}	LP ng L^{-1}	INF ng L^{-1}	BIO ng g^{-1}	EFF ng L^{-1}		Use*	SP ng g^{-1}	LP ng L^{-1}	INF ng L^{-1}
Date:		**February 2020**					**March 2020**									
Ciprofloxacin	VET	177	–	–	16	–	–	–	–	–	–	87	–	684	–	–
Danofloxacin	VET	9	226	<16	–	–	42	257	743	53	444	90	446	<16	33	–
Doxycycline	VET	–	–	–	–	–	–	–	–	–	–	–	–	–	<34	<9
Enrofloxacin	VET	<165	388	71	57	<24	343	686	493	18	<24	920	333	404	48	<24
Fenbendazole	VET	–	11	–	37	<9	–	<12	<9	44	–	–	27	–	33	<9
Florphenicol	VET	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Oxytetracycline	VET	168	933	125	8	<18	63	2145	527	4		187	1154	128		
Penicillin G	VET	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Progesterone	HOR	<130	584	50	43	200	–	<42	–	–	–	–	–	–	–	–
Sulfadimidine	VET	2	27	100	–	5	20	82	105	3	12	22	132	97	–	78
Sulfadiazine	VET	1104	7584	2121	12	621	891	18,561	2643		682	331	25,083	2964	15	1191
Sulfomethoxazole	VET	2	–	16	13	<12	28	–	15	11	–	89	110	23	7	–
Sulfathiazole	VET	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Tiamulin	VET	116	213	28	14	8	212	687	45	10	36	138	295	38	11	23
Trimethoprim	VET	–	164	–	–	–	56	1600	45	21	–	–	83	–	–	–
Tetracycline	VET	72	–	–	–	–	–	–	–	–	–	128	–	–	–	–
Tylosin	VET	–	309	10	–	13	–	259	12	4	–	–	131	176	–	14
Acetaminophen	VET	–	<13	–	11	–	–	–	–	–	–	–	<13	24	3	–
Salicylic acid	VET	–	–	–	23		1050		277	26	434	–	–	–	22	
Diclofenac	VET	<16	1239	136	–	–	38	291	25	–	–	70	1013	66		28
Clofibric acid	NON	<2	256	11	12		31	81	14	7	–	53	128	23	7	–
Atrazine	NON	–	–	–	–	–	–	–	8	–	25	–	–	–	–	–
Atorvastatin	NON	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Caffeine	NON	53	190	32	10	50	11	73	17	5	58	13	253	13	5	16
Carbamazepine	NON		209	20	7	5	–	<5	4	3	8	–	230	119	0.3	6
DEET	NON	17	1077	35	25		19	382	114	20	117	40	593	239	14	107
Gemfibrozil	NON	–	–	–	–	–	–	–	–	–	–	<20	–	–	–	–
Methylparaben	NON	<33	–	<37	200	–	<33	–	–	212	–	135	–	<37	260	56
Naproxen	NON	22	1672	308	18		134	1617	401			120	2014	337	–	413
Ofloxacin	NON	<41	–	–	6	–	–	–	19	–	–	–	–	–	–	–
Propanolol	NON	<93	207	<6	–	<125	–	–	21	–	–	–	<61	<6	–	<125

–: not detected.
[a] Use: VET, veterinary drug; NON, non-veterinary drug; HOR, hormone.

all the manure samples, while ciprofloxacin, danofloxacin, fenbendazole, tiamulin, tetracycline, sulfamethoxazole and progesterone were usually present in the manure samples. All these compounds were detected in very variable amounts, reaching total concentrations up to 3500–5000 ng per fresh g. Total CEC concentrations in raw manure, calculated from the concentrations measured in each phase, are shown in Tables SM8 and SM9. Total concentration of each CEC in the manure and their respective concentrations in the photobioreactor influent were positively correlated ($r = 0.59$, $p < 0.0001$, $n = 81$) as expected. After removing two outliers the coefficient of correlation increased up to $r = 0.70$ (Fig. SM1). However, there was not a perfect correspondence in the composition of both media because some compounds detected in the influent were not detected in the manure sampled at the same time, which was attributed to a lack of homogeneity in the manure lagoon. The occurrence levels of the compounds, and their wide concentration ranges, were expected as they were previously observed in related studies published elsewhere (Argüeso et al., 2021; Spielmeyer, 2018; Wohde et al., 2016).

The total concentration in manure was positively correlated with the MLP concentration (in ng per g, $r = 0.52$, $p < 0.0001$, $n = 71$) and MSP concentration (in ng per fresh g, $r = 0.43$, $p = 0.0002$, $n = 70$). Obviously, the concentrations found in both phases were also correlated ($r =$

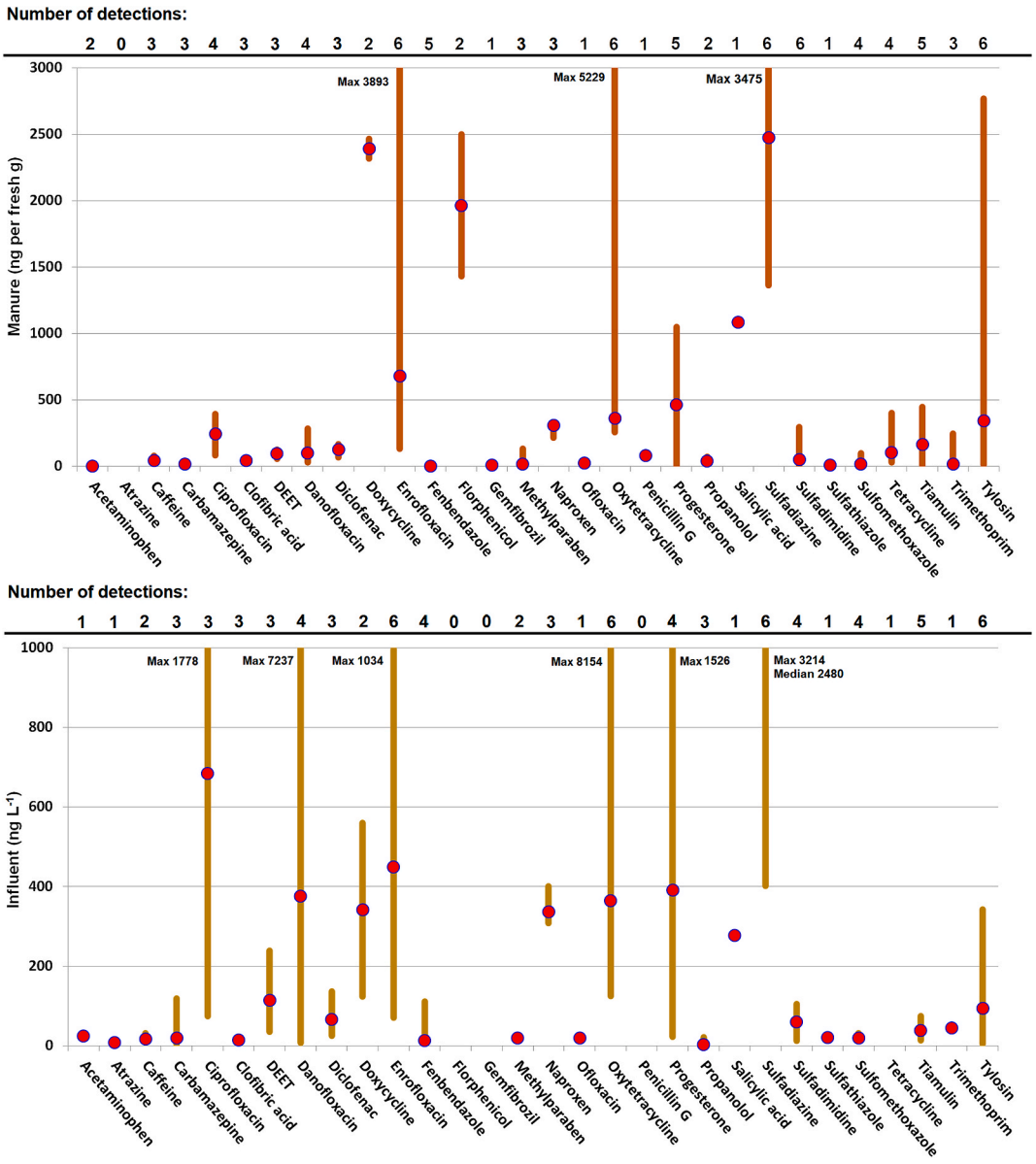


Fig. 2. Median, minimum and maximum concentration values for the compounds detected in manure and influent (left axis), and number of detections.

0.71, $p < 0.0001$, $n = 60$) but not all the compounds detected in the MLP were detected in its corresponding MSP.

Thus, a few CECs have been observed exclusively in the MLP, while for most of them the total amount was distributed between both phases. However, the affinity for the MLP seems to be higher because around 70% of the total amount was determined in the MLP (Fig. 3, Tables SM8, SM9 and SM10). This affinity can be explained based on the polar or moderately polar nature of the CECs (Ohoro et al., 2019; Xin et al., 2021). Only tiamulin and sulfomethoxazole had a higher affinity for the MSP. On the other hand, gemfibrozil, methylparaben, ofloxacin, salicylic acid and tetracycline were only detected in the solid phase. Thus, based on these findings, CEC concentration in MLP could be proposed as a suitable parameter to estimate their occurrence in pig manure, rather than concentrations in MSP.

In addition, the log P value was negatively correlated with the total concentration of each CEC in manure ($r = -0.23$, $p = 0.021$, $n = 98$), and the same was observed for the MLP ($r = -0.25$, $p = 0.023$, $n = 80$) and MSP ($r = -0.23$, $p = 0.041$, $n = 78$) concentrations. These results suggest that the raw manure from the piggeries is relatively richer in hydrophilic CECs which could be interpreted in two ways: a) the lipophilic compounds are more easily bioaccumulated in the animals and, then, they are less mobilized, b) the more polar compounds are easily solubilized and dragged when the facilities soiled with feces and other residues are washed with water, whereas the more lipophilic compounds are retained on particulates and surfaces.

It is remarkable the presence in the manure of CECs of non-veterinary use such as caffeine, clofibric acid, DEET, methylparaben and carbamazepine, which were detected in all 3 stages in which they were monitored. Their occurrence in manure must be ascribed to an environmental pollution phenomenon. However, it is unknown if they arose from the water applied during the water cleaning of the piggery, the feces of the animals, the rests of feed or straw bed, or several of these possibilities. As it could be expected, their concentrations were notably lower than those found for veterinary drugs, and they remained below 150 ng per fresh manure g (Fig. SM2). Progesterone, an animal hormone whose use in piggeries is not authorized in the European Union, was also detected in manure, as in previous works (HSo et al., 2014; Portela et al., 2022).

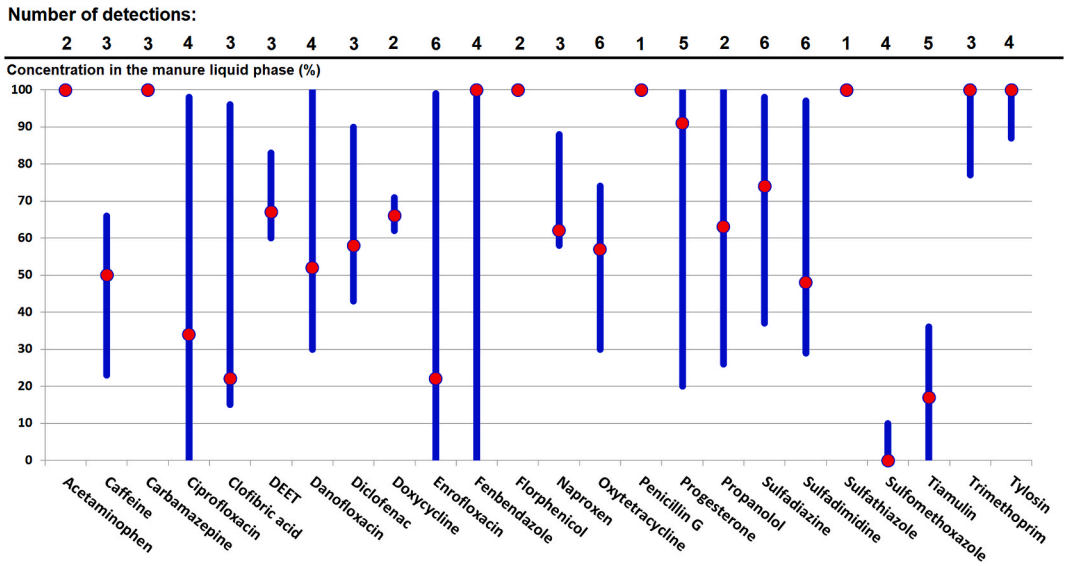


Fig. 3. Median, minimum and maximum concentration values (in percentage) in the liquid phase of the manure (MLP) for the compounds detected (left axis), and number of detections.

3.2. *Effluent concentrations and removal of CECs*

The number of compounds detected (56) in the effluents of the six stages was lower than the number of compounds found (85) in the influents that fed the reactor in the different stages. Sulfadimidine and sulfadiazine were monitored in all analyzed effluents while carbamazapine, doxycycline, fenbendazole, oxytetracycline and tiamulin were detected, at least, in three of them. In general, the concentrations in the effluents were below 200 ng L^{-1} although concentrations about 400 ng L^{-1} were observed for danofloxacin, naproxen and salicylic acid, and concentrations higher than 2000 ng L^{-1} were detected in some stages for oxytetracycline and sulfadiazine. The concentrations of CECs in the effluent were positively correlated with the manure concentrations ($r = 0.564$, $p < 0.0001$, $n = 54$) and those found in the influent ($r = 0.366$, $p = 0.0071$, $n = 53$).

Fig. 4 (Table SM11) resumes the concentrations in the effluent and the percentages of removal of CECs during the photobioreactor operation. Fig. 5 (Table SM12) shows the removal percentages for each detection in the assayed stages. It can be stated that the amount of fluoroquinolones, in general terms, is decreased in presence of microalgae. The removal of oxytetracycline and danofloxacin varied within the 40–100% range for the six stages, while ciprofloxacin and enrofloxacin were removed almost completely except for two stages. Doxycycline was detected in two stages. It resulted to be persistent in one of them but it was removed by 81% in the other one. The sulphonamides sulfomethoxazole and sulfathiazole (1 stage only) tended to be removed in percentages about 66–100%, whereas sulfadiazine and sulfadimidine were less removed in various stages and, even, their concentrations in the effluent increased (up to a 92%) in three cases. Tiamulin was removed in different proportions, between 20 and 100%, while the antibiotic tylosin, with high molecular weight, was removed about 92–100% in four stages but remained intact in other stage. Fenbendazole and diclofenac were removed in all stages in which they were detected, in ranges varying between 72–100% and 58–100%, respectively. Trimethoprim, acetaminophen and the non-veterinary antibiotic ofloxacin were completely removed in the only stage in which they were monitored. On the other hand, salicylic acid and the herbicide atrazine were not removed (1 stage), and caffeine demonstrated to be persistent after the operation in the three stages. In fact, the persistence of the CECs during the photobioreactor operation, or a low removal-rate, could entail in some cases an accumulation of the compounds in the photobioreactor and, subsequently, the concentration in the effluent could be higher than the concentration in the influent. As regards the discussion of the removal percentages it must be clarified that some of the most negative estimated percentages (−1000% or lowest, for example for propanolol) are not representative of the drug behavior because their calculation involved the comparison of concentrations below the corresponding LOQs. In these cases (indicated with the sign < in Table 1), the LOQ divided by two was considered for calculations.

The behavior of tetracyclines and fluoroquinolones observed in this work was similar to that described in the bibliography (Table SM1 and López-Serna et al., 2019), as they are partially removed. Sulfadiazine and sulfadimidine resulted to be rather persistent in the wastewater, which also agrees with the literature but sulfomethoxazole and sulfathiazole were removed somewhat when literature data for these CECs are variable (Table SM1; García-Galán et al., 2020; Gentili and Fick, 2017; Vassalle et al., 2020b). Carbamazepine and naproxen are CECs generally considered as persistent in photobioreactors with microalgae (García-Galán et al., 2020; Jiménez et al., 2020) although they have been removed, at least in part, in this work. In contrast, caffeine has not been removed in any of the stages in which it was detected despite bibliographic data indicate that the caffeine concentration is diminished (Lindberg et al., 2021). Trimethoprim was completely removed in its only detection, which was not expected according to bibliographic data (Table SM1, Gentili and Fick, 2017). Finally, acetaminophen, tiamulin, tylosin, diclofenac, clofibric acid, naproxen, progesterone, fenbendazole and methylparaben were similarly removed as compared to data previously reported, and salicylic acid was persistent as expected (Table SM1; García-Galán et al., 2020; Jiménez et al., 2020; López-Serna et al., 2019; Matamoros et al., 2015).

Regarding the performance of the photobioreactor the seasonality was expected to be a relevant factor, thus the variations found in the CEC removal could be ascribed to the change in temperature and solar radiation intensity. Also, temperature influences the adsorption of CECs on microalgae and other particles (Hena et al., 2021; Leng et al., 2020). However, the variations in the removal rates estimated in this work could not be related to the seasonality as happened in some works (Matamoros et al., 2015; Villar-Navarro et al., 2018). Similarly, the variation in the photobioreactor microalgae composition did not influence the CEC removal. Hence, a one-way Anova did not show significant differences ($p < 0.05$) in the removal rates based on the operation stage. On this matter, it must be noted that a relatively high or low CEC specific load in the influent of the photobioreactor could influence the estimated removal percentages too. Thus, for instance, sulfadimidine and tylosin

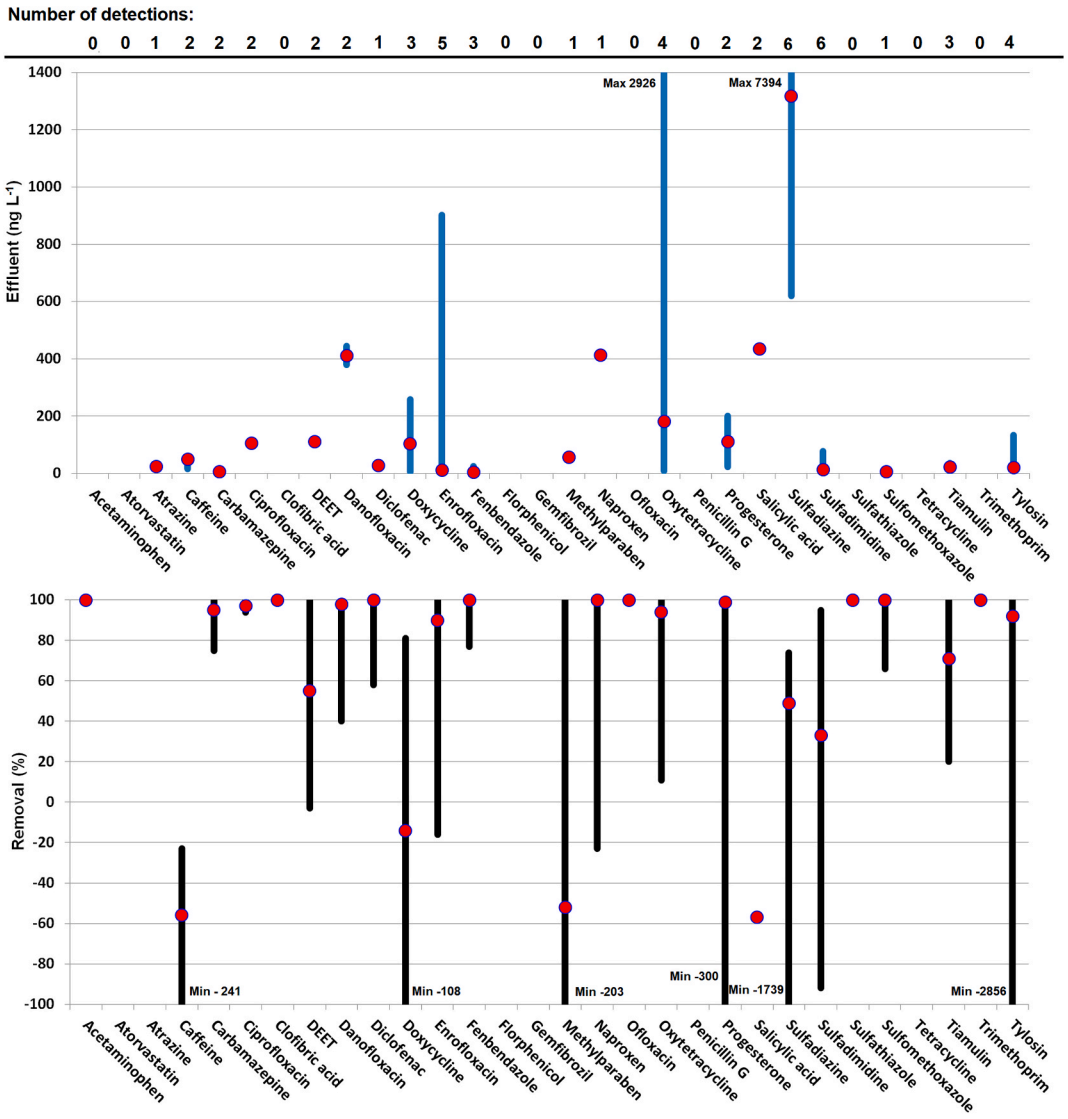


Fig. 4. Median, minimum (Min) and maximum (Max) values for the effluent concentrations and percentages of removal. Number of detections.

were accumulated in the photobioreactor in November-2019 (lower solar radiation and low temperature) while oxytetracycline was less removed as expected. However, the removal of enrofloxacin was higher in November-2019. In addition, the Anova result was relevant because the photobioreactor was covered with a plastic film in a stage carried out in March-2020 to decrease the loss of water by evaporation and the percentages of removal resulted to be basically similar to those achieved in other stages, particularly in the stage carried out in March-2020 (Fig. 5).

The removal of organic microcontaminants, such as CECs, by microalgae biodegradation involves one or more reactions catalyzed by enzymes, and the microcontaminant is often transformed in broken-down and polar derivatives. Biodegradation pathways entail patterns of metabolic degradation with specific enzymes which use the micro-contaminants are carbon and energy source, and patterns of co-metabolic degradation by non-specific enzymes which require an additional carbon and energy input (Ding et al., 2017b; Qiang et al., 2014; Xiong et al., 2017). Furthermore, the biodegradation outside the cell (intercellular) is possible by intracellular substances released into the medium (Naghdi et al., 2018), too. In addition, there is a cooperation between microalgae and their bacteria consortium that reinforces the biodegradation. In terms of bacteria population, the same microorganisms found in conventional activated sludge processes have been identified in photobioreactors, although the relative contribution to the metabolism is different in relation to the activated sludge systems due to the different design and operational conditions (Metcalf and Eddy, 2003).

The direct photodegradation is another removal mechanism. It is based in the direct absorption of solar radiation by the micro-contaminant and in this regard the UV sunlight radiation is more effective that the visible radiation of the sun to degrade pharmaceuticals (Jiménez et al., 2018b). The indirect photodegradation is clearly more successful to remove microcontaminants. In this case, the radiation is absorbed for one or more chemical species of the culture medium (the dissolved organic matter or algal components for instance) which would generate radical oxygen species, such as hydroxyl radicals, and favor the decomposition of the microcontaminants by radical reactions (Leng et al., 2020; Tian et al., 2019; Zhang et al., 2012).

Other two mechanisms whose relevance in the CEC removal is relative scarce are the volatilization and hydrolysis. Volatilization of CECs is almost negligible generally, according to their low Henry law constant values, while the hydrolysis reaction rates are rather slow at environmental temperature although they may be induced by enzymes (Jiménez et al., 2018a, 2018b; Hena et al., 2021; Prosenc et al., 2021). Irrespective of the removal mechanisms discussed above, side chain breakdown, ring cleavage, N-oxidations, hydroxylation, demethylation,

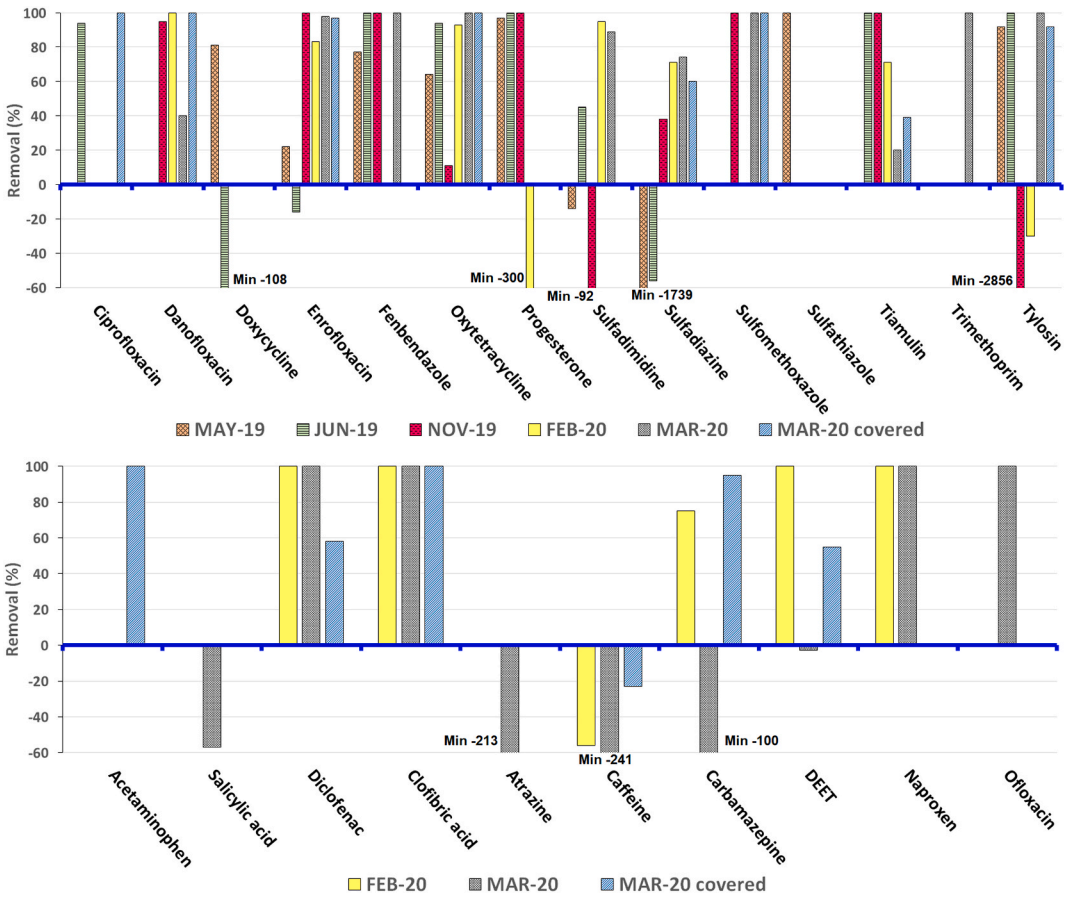


Fig. 5. Removal of veterinary drugs in the different stages. Letter "C": Covered with a plastic film.

decarboxylation, and dehydroxylation are usual CEC transformation pathways. Finally, the mechanism of adsorption of the CECs on the microalgae biomass is addressed in the next section.

Table 2 shows the global decrease in the CEC content from the piggery wastewater for the samples collected on each stage. The global concentration decrease varied between 0 and 79%. The values reflect a one-time situation and they are very different. The manure influent composition changes over time and modifies the effluent composition in that moment, too. In fact, if two effluent concentrations outliers are discarded the global efficiency is higher of 60% for the monitored stages. Thus, it can be stated that the thin-layer photobioreactors are a reliable option to decrease, but no to remove completely, the CEC total content in piggery wastewater.

Wastewaters are usually treated in open systems such as high-rate algae ponds (HRAPs) or thin-layer cascade photobioreactors. As regards the CEC removal, they differ basically in the culture depth so that the photodegradation and oxidation processes are favored in the thin-layer photobioreactor while HRAPs may contain some anaerobic bacteria in addition to aerobic bacteria. CEC removal data in thin-layer photobioreactors have not been found in the bibliography, therefore the global removal performance observed in this work (roughly 60%) has been compared with those published after assays in HRAPs. From examination of the data it can be inferred that the global removal rates are in essence similar in both types of photobioreactors (García-Galán et al., 2020; Gentili and Fick, 2017; Jiménez et al., 2020; Lindberg et al., 2021; Matamoros et al., 2015; Vassalle et al., 2020a; Vassalle et al., 2020b; Villar-Navarro et al., 2018). Here, it must be pointed out that the experimental conditions (such as the hydraulic retention time, predominant microalgae cells and their concentration, meteorological parameters), the wastewater treated in the photobioreactor, its design, and the monitored microcontaminants, were quite different in these assays. As a result of the CEC partial removal, the coupling of some other purification technology with a microalgae photobioreactor is a feasible option to improve the removal performance.

Table 2
Global efficacy in the removal of CECs, comparing their total concentration in effluent and influent.

Stage	Influent concentration (ng L^{-1})	Effluent concentration (ng L^{-1})	Global removal (%)
Mayo 2019[a]	11,444	11,426 (4032[a])	0 (64[a])
June 2019[a]	10,713	6469 (1459[a])	40 (86[a])
November 2019	10,154	2160	79
February 2020	3093	996	68
March 2020	5553	1828	67
March 2020C	5365	2016	62
All stages	46,322	17,501	62

[a] Excluding two outlier concentrations of sulfadiazine in the effluent.

3.3. Biomass concentrations

The concentrations of CECs measured in the biomass grown in the photobioreactor were rather low (Table 3 and SM10), generally lower than10 ng g^{-1} (per fresh g). This agrees with data obtained for biomass grown in urban wastewater (García-Galán et al., 2020). Methylparaben was detected in the three stages in which it was analyzed, at high concentrations ranging from 44 to 57 ng g^{-1}. Only fenbendazole, danofloxacin, enrofloxacin and tiamulin had concentrations slightly higher than 10 ng g^{-1} in some stages. These four compounds, in addition to oxytetracycline, sulfadiazine, progesterone, caffeine, clofibric acid and DEET were frequently detected in biomass. Tetracycline and gemfibrozil

Table 3
Mean, median, minimum (Min) and maximum (Max) values for the biomass concentrations, biomass accumulation factor (AcF, quotient between the biomass and effluent concentrations), and number of cases (n).

	Biomass (ng per fresh g)					Accumulation factor				
Compound	n	Mean	Median	Min	Max	n	Mean	Median	Min	Max
Acetaminophen	2	1.4	1.4	0.7	2	2	5	5	5	5
Atorvastatin	–	–	–	–	–	–	–	–	–	–
Atrazine	–	–	–	–	–	1	0	0	0	0
Caffeine	3	1.4	1.1	1.1	2	3	0.043	0.040	0.019	0.069
Carbamazepine	3	0.9	1	0.1	2	3	0.166	0.088	0.017	0.392
Ciprofloxacin	3	4	4	2	6	3	1.753	0.240	0.019	5
Clofibric acid	3	2	2	2	3	3	5	5	5	5
DEET	3	4	4	3	6	3	1.687	0.034	0.028	5
Danofloxacin	4	7	7	1.3	12	4	2.511	2.514	0.018	5
Diclofenac	–	–	–	–	–	1	0	0	0	0
Doxycycline	1	4	4	4	4	3	0.292	0.097	0	0.778
Enrofloxacin	6	7	5	2	13	6	1.228	0.625	0.005	5
Fenbendazole	5	10	8	6	20	5	2.715	1.778	0.240	5
Florphenicol	–	–	–	–	–	–	–	–	–	–
Gemfibrozil	–	–	–	–	–	–	–	–	–	–
Methylparaben	3	49	47	44	57	3	3.673	5	1.018	5
Naproxen	1	4	4	4	4	2	2.500	2.500	0	5
Ofloxacin	1	1.3	1	1.3	1.3	1	5	5	5	5
Oxytetracycline	5	3	4	0.9	4	5	1.076	0.137	0.001	5
Penicillin G	–	–	–	–	–	–	–	–	–	–
Progesterone	4	5	4	4	9	4	2.550	2.578	0.045	5
Propanolol	–	–	–	–	–	2	0	0	0	0
Salicylic acid	3	5	5	5	6	3	3.338	5	0.014	5
Sulfadiazine	5	4	3	0.3	7	6	0.002	0.002	0	0.005
Sulfadimidine	4	0.6	0.7	0.4	0.7	6	0.366	0.028	0	2.083
Sulfathiazole	–	–	–	–	–	0	–	–	–	–
Sulfomethoxazole	3	2	2	2	3	3	3.492	5	0.477	5
Tetracycline	–	–	–	–	–	–	–	–	–	–
Tiamulin	5	5	2	2	18	5	2.104	0.375	0.056	5
Trimethoprim	1	5	5	5	5	1	5	5	5	5
Tylosin	3	1.6	2	0.9	2	6	1.669	0.008	0	5

–: without data.

were only detected in biomass, not in the surrounding aqueous effluent, in two and one stages, respectively.

The relatively scarce content of CECs in microalgae biomass could be attributed to two non-exclusive reasons: a low adsorption-bioaccumulation capacity in the biomass and a relatively quick biodegradation of the CECs incorporated to the biomass (Ding et al., 2017; Hena et al., 2021; Qiang et al., 2014; Xiong et al., 2018; Zambrano et al., 2021). An adsorption process presumably takes place because there is a positive correlation between the log P values and the CEC concentrations in biomass ($r = 0.349$, $p = 0.0035$, $n = 68$, Fig. SM3), excluding the results for methylparaben, whose concentration values were extreme. Then, lipophilic compounds were incorporated in the biomass in a larger extent as already previously observed on a much smaller study (López-Serna et al., 2022). Consequently, it can be proposed that lipophilic compounds will be more easily removed from the manure and less abundant in the purified effluent. No relationship was found as regards the percentages of removal but it was obtained a negative correlation between the CEC concentrations in effluent and log P ($r = -0.286$, $p = 0.0327$, $n = 56$), which confirms that the incorporation of CECs to biomass notably helps to wastewater purification. Likewise, it can be inferred that the hydrophilic CECs tend to be less removed in the photobioreactor. On the other hand, Fig. SM4 shows the CEC concentration in biomass for the six stages. The concentration of CECs in biomass was seemingly a little higher in March-2020 when the solar radiation reached the photobioreactor through a plastic coverture, in comparison to those concentrations found in the uncovered stage carried out in the same period, but this difference was not statistically significant. According to a one-way Anova the content of CECs in biomass was similar along the different stages ($p > 0.05$) as well. The mean amount of CECs accumulated in the photobioreactor biomass varied from 1.6 to 4.0% for the first three stages but it increased to 10.8–22.3% in the last three assayed stages in which the number of compounds monitored was higher. The highest mean lipophilicity of the CECs determined in the last stages justify their accumulation rate (Table SM13).

The obtained results indicate that the adsorption mechanism on microalgae biomass is a noteworthy way to decrease the CEC content in piggery wastewater. The adsorption of CECs on the algae cell walls can be accomplished by several physico-chemical processes. Thus, the linkage can be established by hydrogen bonds, electrostatic attraction, pore-filling, hydrophobic effects, partitioning and π-π interactions (Leng et al., 2020). The adsorption phenomenon is more pronounced as the CEC hydrophobicity (related to the log P value) is high, as it shown in this work, and it is enhanced when the CEC carries on opposite electric charge to microalgae. The outer surface of microalgae cells often possesses a net negative charge at the culture pH values which favors the electrostatic interaction with positively charged microcontaminants. The Langmuir isotherm seems describe the adsorption process acceptably (Tan et al., 2015; Xiong et al., 2018; Zambrano et al., 2021).

Biosorption is an extracellular process, if the microcontaminants cross over the cell membrane they could undergo the above commented biodegradation mechanisms, or simply, they could be bioaccumulated (Mojiri et al., 2022). The bioaccumulation also plays a role in the removal of CECs (Bai and Acharya, 2016, 2017) but some accumulated compounds induce the production of reactive oxygen species resulting in severe damage (toxicity) to microalgae cells (Amin et al., 2013; Xin et al., 2021).

The biodegradation by microalgae and their bacteria consortium is likely the main removal pathway observed in this work as deduced from the experimental findings and authors' experience. The degradation of CECs by hydrolysis and oxidation chemical reactions in aqueous medium at pH-values close to neutrality is generally negligible after short periods of time, as in this case in which the hydraulic retention time was 12 days, even if the molecule has bonds that are susceptible of hydrolysis

(Jiménez et al., 2017, 2018a). Unlike chemical reactions, photochemical reactions perform a worthy role in the degradation of CECs but, in this work, their contribution to the CECs removal seemed lower in comparison to the biodegradation mechanism. This statement is supported by the lack of statistically significant differences among the removal rates of the six studied stages, and taking into account the high intensity of solar radiation in two of stages (May and June 2019) as opposed to the other four. Furthermore, non-significant differences were found in the removal rates between the two stages carried out in March 2020, after covering the reactor with a plastic film in one of them. The plastic cover decreased the transmission of visible radiation but also decreased the UV radiation intensity, which mostly promotes the photochemical reactions due to its higher energy. In relation to the removal of CECs by biosorption on microalgae it has been pointed out in laboratory assays on dead biomass that some CECs can be removed in high rates, about 32–100% (Zambrano et al., 2021; Xie et al., 2020). In fact, accumulation global rates on fresh biomass about 2–20% have been estimated in this work (Table SM13). These data include the biosorption percentages in addition to those of a possible bioaccumulation. In the absence of conclusive data we think that the removal performance of the photodegradation and biosorption mechanisms, in this work, would turn out similar in some cases, but the photodegradation seems a more important mechanism than biosorption in general terms. Finally, the relevance of the volatilization mechanism should be discarded on account of the nature of the studied compounds.

The incorporation of each CEC to the biomass can depend of its concentration in the surrounding aqueous medium. Thus, an accumulation factor (AcF) of CECs in biomass has been calculated as the quotient between the CEC biomass concentration and the CEC effluent concentration (Eq. (2)). The value of this factor depends on the distribution of the CEC between the biomass and the surrounding aqueous phase, and the density of biomass in the medium. This factor could be more suitable to assess the affinity of the CECs towards the biomass of the photobioreactor.

$$AcF = \frac{(ng\ CEC)_{biomass} / g\ biomass}{(ng\ CEC)_{effluent} / L\ effluent} = \frac{(ng\ CEC)_{biomass}}{(ng\ CEC)_{effluent}}\ x\ \frac{L\ effluent}{g\ biomass} \quad (2)$$

Table SM12 shows the calculated AcF values, taking into account that a AcF value of 5 was assigned when the CEC was only detected in the biomass phase. Table 3 resumes the data. High AcF values indicate a major linkage of the CEC to the biomass. Acetominophen, clofibric acid, danofloxacin, fenbendazole, methylparaben, naproxen, ofloxacin, progesterone, salicylic acid, sulfomethoxazole, tiamulin and trimethoprim showed high AcF values. These compounds, in general, presented relatively high concentrations in biomass. More interestingly is the positive correlation observed between the percentages of removal and the parameter AcF ($r = 0.439$, $p = 0.0001$, $n = 70$, after excluding those cases in which the calculation of removal percentages involved concentrations close to the LOQ), which suggests that the biomass plays a relevant role in the mechanism of removal of CECs contained in the manure, either for their adsorption or algae-favored degradation processes. Efforts should be conducted to increase the productivity of biomass in the photobioreactors to enhance the CEC removal. Moreover, it must be pointed out that the CEC concentration levels detected in the influent did not affect the microalgae biomass productivity, which was closely related to weather conditions (Sánchez Zurano et al., 2020).

It is accepted that the biosorption rate varies considerably depending on CEC and microalgae species due to their different hydrophobicity, functionality and involved chemical structures. However, a correlation between the biomass concentrations, or AcF values, and the microalgae species of each stage was not observed in this work, perhaps as a consequence of the relatively little variation in the microalgae composition (Table SM14). The microalgae composition was not correlated with the removal rates either. On the other hand, the chemical structures of the CECs have been summarized in certain characteristics such as elemental atom ratios (C/N, C/O, …) and presence of some functional groups (carboxylic acids, heterocyclic nitrogen, hydroxyalkyl groups, …) among other options, all of which are shown in Tables SM15 and SM16. Thus, remarkable correlations of experimental data with structural characteristics were now found. The accumulation factors of CECs in biomass (AcF) were negatively correlated with the presence of amine groups in the structure ($r = -0.28$, $p = 0.009$, $n = 83$) and with the elemental ratio N/O ($r = -0.28$, $p = 0.011$, $n = 79$). Similarly, the CEC biomass concentrations (expressed per fresh gram) were also negatively correlated with the presence of amine groups ($r = -0.25$, $p = 0.034$, $n = 71$) and the ratio N/O ($r = -0.28$, $p = 0.021$, $n = 68$). In addition, the biomass concentrations were negatively correlated with the percentage of nitrogen (%N, w/w) in the chemical structure ($r = -0.32$, $p = 0.007$, $n = 71$) and the elemental ratio N/C ($r = -0.30$, $p = 0.011$, $n = 71$). These biomass concentrations were positively correlated with the presence of hydroxylalkyl groups ($r = 0.26$, $p = 0.031$, $n = 71$) and hydroxyphenyl groups ($r = 0.36$, $p = 0.002$, $n = 71$) in the structure. The multivariant analysis of correlation was repeated after excluding the high biomass concentrations of methylparaben. The number of significant correlations was now drastically reduced but the relevance of the oxygen and nitrogen atoms, and amine groups, in the CEC accumulation on biomass was strengthened. Thus, in this last case, the ACF values were correlated negatively with the presence of amine groups ($r = -0.25$, $p = 0.023$, $n = 80$) and the ratio N/O ($r = -0.26$, $p = 0.024$, $n = 76$), and as a new finding, the AcF values also had a positive correlation on the limit of statistical significance with the elemental ratio Cl/H ($r = 0.22$, $p = 0.049$, $n = 80$). Experimental data clearly indicate that CECs with oxygen atoms are prone to accumulate on biomass, mainly those with a free hydroxyl group. On the contrary, the CECs with nitrogen atoms are less accumulated and this phenomenon seems to correspond with the presence of free amine groups rather than nitrogen-containing aromatic heterocycles. The electric charge of the CECs and their degree of unsaturation (number of cycles and double bonds) did not turn out a significant factor but the larger dipole moment of the bond C–Cl compared to that bond C–H, or perhaps the ability of the chlorine atom to share electrons pairs, did seem to favor the accumulation. The physico-chemical and biological interpretation of these experimental findings is not simple and it should not be forgotten that the concentrations measured in the microalgae biomass can have two origins because the superficial biosorption mechanism overlaps with the mechanism of bioaccumulation inside the microalgae when the CECs cross over the membrane cells.

4. Conclusions

CECs of non-veterinary use have been detected in pig manure in lower amounts than veterinary drugs. Their occurrence in manure is attributed to environmental pollution. The liquid phase of the manure contained, generally, higher CEC amounts than the solid phase. In this context, the determination of CECs in the manure liquid phase provides more useful information to estimate the occurrence of residues in the original manure.

The total content of CECs in piggery wastewater can be decreased in different rates during the routine operation of a microalgae photobioreactor over time. The mean removal of the total content in the effluent was about 60%. A combination with another purification treatment seems advisable to improve the removal. Caffeine and methylparaben seemed to be persistent. Sulfadiazine, sulfadimidine, and to a lesser degree enrofloxacin and ciprofloxacin, were less prone to be removed than other CECs.

The concentrations of CECs in biomass were small. The lipophilic CECs were found in higher amounts than the hydrophilic ones in the algae biomass. There is a linear relationship between the CEC concentration in biomass and log P. The linkage of the CEC to the biomass favors the removal, thus the microalgae growth should be enhanced as much as possible.

CRediT authorship contribution statement

Rebeca López-Serna: Validation, Resources, Methodology, Data curation. **Belén Franco:** Investigation, Data curation. **Silvia Bolado:** Project administration, Funding acquisition. **Juan José Jiménez:** Writing – review & editing, Supervision, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Silvia Bolado reports financial support was provided by Spain Ministry of Science and Innovation. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgements

This work was financed by the project PID2020-113544RB-I00/AEI/10.13039/501100011033 of the Spanish "Ministerio de Ciencia, Innovación y Universidades". The authors also acknowledge the support by the regional government of Castilla y León (UIC 338 and CL-EI-2021-07), the Spanish project PDC2021-121861-C22/AEI/10.13039/501100011033 and the European Union EU-FEDER and EU-Next Generation. It was partially carried out in the Laboratory of Instrumental Techniques from the University of Valladolid (UVa) to whom we thank the support provided.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jenvman.2024.120340.

Letter "C": covered with a plastic film.

References

Amin, A., Naik, A.T.R., Azhar, M., Nayak, H., 2013. Bioremediation of different waste waters-a review. Continent. J. Fish. Aquat. Sci. 7, 7–17. https://doi.org/10.5707/cjfas.2013.7.1.7.17.

Argüeso, M., Bolado, S., Jiménez, J.J., López-Serna, R., 2021. Determination of antibiotics and other veterinary drugs in the solid phase of pig manure. Chemosphere 275, 130039. https://doi.org/10.1016/j.chemosphere.2021.130039.

Bai, X., Acharya, K., 2016. Removal of trimethoprim, sulfamethoxazole, and triclosan by the green alga Nannochloris sp. J. Hazard Mater. 315, 70–75. https://doi.org/10.1016/j.jhazmat.2016.04.067.

Bai, X., Acharya, K., 2017. Algae-mediated removal of selected pharmaceutical and personal care products (PPCPs) from Lake Mead water. Sci. Total Environ. 581–582, 734–740. https://doi.org/10.1016/j.scitotenv.2016.12.192.

Ciardi, M., Gómez-Serrano, C., Lafarga, T., Acién, G., Llamas, B., Bolado, S., Fernández-Sevilla, J.M., 2022. Reduction in water consumption during the production of microalgae using diluted pig slurry in thin-layer cascade photobioreactors. J. Appl. Phycol. 34, 2905–2916. https://doi.org/10.1007/s10811-022-02826-8.

Couto, E., Assemany, P.P., Assis Carneiro, G.C., Ferreira Soares, D.C., 2022. The potential of algae and aquatic macrophytes in the pharmaceutical and personal care products (PPCPs) environmental removal: a review. Chemosphere 302, 134808. https://doi.org/10.1016/j.chemosphere.2022.134808.

Dang, B.T., Bui, X.T., Tran, D.P.H., Ngo, H.H., Nghiem, L.D., Hoang, T.K.D., Nguyen, P. T., Nguyen, H.H., Vo, T.K.Q., Lin, C., Lin, K.Y.A., Varjani, S., 2022. Current application of algae derivatives for bioplastic production: a review. Bioresour. Technol. 347, 126698 https://doi.org/10.1016/j.biortech.2022.126698.

Ding, T., Yang, M., Zhang, J., Yang, B., Lin, K., Li, J., Gan, J., 2017. Toxicity, degradation and metabolic fate of ibuprofen on freshwater diatom *Navicula sp.* J. Hazard Mater. 330, 127–134. https://doi.org/10.1016/j.jhazmat.2017.02.004.

García-Galán, M.J., Arashiro, L., Santos, L., Insab, S., Rodriguez-Mozaz, S., Barcelo, D., Ferrer, I., Garfi, M., 2020. Fate of priority pharmaceuticals and their main metabolites and transformation products in microalgae-based wastewater treatment systems. J. Hazard Mater. 390, 121771. https://doi.org/10.1016/j.jhazmat.2019.121771.

Gentili, F.G., Fick, J., 2017. Algal cultivation in urban wastewater: an efficient way to reduce pharmaceutical pollutants. J. Appl. Phycol. 29, 255–262. https://doi.org/10.1007/s10811-016-0950-0.

Hena, S., Gutierrez, L., Croué, J.P., 2021. Removal of pharmaceutical and personal care products (PPCPs) from wastewater using microalgae: a review. J. Hazard Mater. 403, 124041 https://doi.org/10.1016/j.jhazmat.2020.124041.

Hso, Y.B., Zakaria, M.P., Latif, P.A., Saari, N., 2014. Occurrence of veterinary antibiotics and progesterone in broiler manure and agricultural soil in Malaysia. Sci. Total Environ. 261–267. https://doi.org/10.1016/j.scitotenv.2014.04.109, 488–489.

Jiménez, E.M., Madera, C.A., Ortiz, A.C., Morales, P.A., Peña, E.J., Machuca, F., 2020. High-rate algal pond for removal of pharmaceutical compounds from urban domestic wastewater under tropical conditions. Case study: Santiago de Cali, Colombia. Water Sci. Technol. 82, 1031–1043. https://doi.org/10.2166/wst.2020.362.

Jiménez, J.J., Muñoz, B.E., Sánchez, M.I., Pardo, R., 2018a. Forced and long-term degradation assays of tenoxicam, piroxicam and meloxicam in river water. Degradation products and adsorption to sediment. Chemosphere 903–910. https://doi.org/10.1016/j.chemosphere.2017.10.056, 191C.

Jiménez, J.J., Pardo, R., Sánchez, M.I., Muñoz, B.E., 2018b. Photochemical, thermal, biological and long-term degradation of celecoxib in river water. Degradation products and adsorption to sediment. J. Hazard Mater. 342, 252–259. https://doi.org/10.1016/j.jhazmat.2017.08.037.

Jiménez, J.J., Sánchez, M.I., Pardo, R., Muñoz, B.E., 2017. Degradation of indomethacin in river water under stress and non-stress laboratory conditions: degradation products, long-term evolution and adsorption to sediment. J. Environ. Sci. 51, 13–20. https://doi.org/10.1016/j.jes.2016.08.021.

Leng, L., Wei, L., Xiong, Q., Xu, S., Li, W., Lv, S., Lu, Q., Wan, L., Wen, Z., Zhou, W., 2020. Use of microalgae based technology for the removal of antibiotics from wastewater: a review. Chemosphere 238, 124680. https://doi.org/10.1016/j.chemosphere.2019.124680.

Lindberg, R.H., Namazkar, S., Lage, S., Östman, M., Gojkovic, Z., Funk, C., Gentili, F.G., Tysklind, M., 2021. Fate of active pharmaceutical ingredients in a northern high-rate algal pond fed with municipal wastewater. Chemosphere 271, 129763. https://doi.org/10.1016/j.chemosphere.2021.129763.

López-Serna, R., Bolado, S., Irusta, R., Jiménez, J.J., 2022. Determination of veterinary drugs in microalgae biomass from photobioreactors fed with piggery wastewater. Chemosphere 287, 132076. https://doi.org/10.1016/j.chemosphere.2021.132076.

López-Serna, R., García, D., Bolado, S., Jiménez, J.J., Lai, F.Y., Golovko, O., Gago-Ferrero, P., Ahrens, L., Wiberg, K., Muñoz, R., 2019. Photobioreactors based on Microalgae-Bacteria and Purple Phototrophic Bacteria Consortia: a promising technology to reduce the load of veterinary drugs from piggery wastewater. Sci. Total Environ. 692, 259–266. https://doi.org/10.1016/j.scitotenv.2019.07.126, 2019.

Lorenzo, A., Ruiz, J., Vega, M., Bolado, S., 2019. Recovery of proteins from biomass grown in pig manure microalgae-based treatment plants by alkaline hydrolysis and acidic precipitation. Bioresour. Technol. 273, 599–607. https://doi.org/10.1016/j.biortech.2018.11.068.

Martín, J., Vega, M., Riol, E., Muñoz, R., Bolado, S., 2019. Optimisation of the production of fermentable monosaccharides from algal biomass grown in photobioreactors treating wastewater. Bioresour. Technol. 281, 239–249. https://doi.org/10.1016/j.biortech.2019.02.082.

Matamoros, V., Gutierrez, R., Ferrer, I., Garcia, J., Bayona, J.M., 2015. Capability of microalgae-based wastewater treatment systems to remove emerging organic contaminants: a pilot-scale study. J. Hazard Mater. 288, 34–42. https://doi.org/10.1016/j.jhazmat.2015.02.002.

Metcalf, I., Eddy, G., 2003. Wastewater Engineering and Reuse, fourth ed. McGraw Hill, New York. 9780071122504.

Mojiri, A., Zhou, J.L., Ratnaweera, H., Rezania, S., Nazari, Manosoureh, M.V., 2022. Pharmaceuticals and personal care products in aquatic environments and their removal by algae-based systems. Chemosphere 288, 132580. https://doi.org/10.1016/j.chemosphere.2021.132580.

Naghdi, M., Taheran, M., Brar, S.K., Kermanshahi-Pour, A., Verma, M., Surampalli, R.Y., 2018. Removal of pharmaceutical compounds in water and wastewater using fungal oxidoreductase enzymes. Environ. Pollut. 234, 190–213. https://doi.org/10.1016/j.envpol.2017.11.060.

Ohoro, C., Adeniji, A., Okoh, A., Okoh, O., 2019. Distribution and chemical analysis of pharmaceuticals and personal care products (PPCPs) in the environmental systems: a review. IJERPH 16, 3026. https://doi.org/10.3390/ijerph16173026.

Posadas, E., Alcántara, C., García-Encina, P.A., Gouveia, L., Guieysse, B., Norvill, Z., Gabriel Acién, F., Markou, G., Congestri, R., Koreivienė, J., Muñoz, R., 2017. In: Gonzalez, C., Muñoz, R. (Eds.), Microalgae Cultivation in Wastewater in: Microalgae-Based Biofuels and Bioproducts. Elsevier, pp. 67–92. https://doi.org/10.1016/B978-0-08-101023-5.00003-0 (Chapter 3).

Portela, C., Bolado, S., López-Serna, R., Jiménez, J., 2022. Determination of contaminants of emerging concern in raw pig manure as a whole: difference with the analysis of solid and liquid phases separately. Environ. Sci.: Process. Impacts 24, 2357. https://doi.org/10.1039/d2em00323f.

Prosenc, F., Piechocka, J., Skufca, D., Heath, E., Griessler Bulc, T., Istenic, D., Buttiglieri, G., 2021. Microalgae-based removal of contaminants of emerging concern: mechanisms in *Chlorella vulgaris* and mixed algal-bacterial cultures. J. Hazard Mater. 418, 126284 https://doi.org/10.1016/j.jhazmat.2021.126284.

Qiang, F., Ying, G.G., Yang, B., Liu, S., Lai, H.J., Liu, Y.S., Chen, Z.F., Zhou, G.J., 2014. Biotransformation of progesterone and norgestrel by two freshwater microalgae (Scenedesmus obliquus and Chlorella pyrenoidosa): transformation kinetics and

products identification. Chemosphere 95, 581–588. https://doi.org/10.1016/j.chemosphere.2013.10.013.
Sánchez Zurano, A., Garrido Cárdenas, J.A., Gómez Serrano, C., Morales Amarala, M., Acién-Fernández, F.G., Fernández Sevilla, J.M., Molina Grima, E., 2020. Year-long assessment of a pilot-scale thin-layer reactor for microalgae wastewater treatment. Variation in the microalgae-bacteria consortium and the impact of environmental conditions. Algal Res. 50, 101983 https://doi.org/10.1016/j.algal.2020.101983.
Silva, A., Delerue-Matos, C., Figuereido, S.A., Freitas, O.M., 2019. The Use of algae and fungi for removal of pharmaceuticals by bioremediation and biosorption processes: a review. Water 11, 1555. https://doi.org/10.3390/w11081555.
Sournia, A., 1978. Phytoplankton Manual. Unesco, United Kingdom, 9331015729.
Spielmeyer, A., 2018. Occurrence and fate of antibiotics in manure during manure treatments: a short review. Sustainable Chem. Pharm 9, 76–86. https://doi.org/10.1016/j.scp.2018.06.004.
Tan, X., Liu, Y., Zeng, G., Wang, X., Hu, X., Gu, Y., Yang, Z., 2015. Application of biochar for the removal of pollutants from aqueous solutions. Chemosphere 125, 70–85. https://doi.org/10.1016/j.chemosphere.2014.12.058.
Tian, Y., Wei, L., Yin, Z., Feng, L., Zhang, L., Liu, Y., Zhang, L., 2019. Photosensitization mechanism of algogenic extracellular organic matters (EOMs) in the phototransformation of chlortetracycline: role of chemical constituents and structure. Water Res. 164, 1149401–1149408. https://doi.org/10.1016/j.watres.2019.114940.
Tolboom, S.N., Carrillo-Nieves, D., de Jesús Rostro-Alanis, M., de la Cruz Quiroz, R., Barceló, D., Iqbal, H.M.N., Parra-Saldivar, R., 2019. Algal-based removal strategies for hazardous contaminants from the environment – a review. Sci. Total Environ. 665, 358–366. https://doi.org/10.1016/j.scitotenv.2019.02.129.
Vassalle, L., García-Galán, M.J., Aquino, S.F., de Cássia Franco Afonso, R.B., Ferrer, I., Passos, F., Rossas Mota Filho, C., 2020a. Can high rate algal ponds be used as post-treatment of UASB reactors to remove micropollutants? Chemosphere 248, 125969. https://doi.org/10.1016/j.chemosphere.2020.125969.
Vassalle, L., Sunyer-Caldú, A., Díaz-Cruz, M.S., Terumi Arashiro, L., Ferrer, I., Garfí, M., García-Galán, M.J., 2020b. Behavior of UV filters, UV blockers and pharmaceuticals in high rate algal ponds treating urban wastewater. Water 12, 2658. https://doi.org/10.3390/w12102658.
Villar-Navarro, E., Baena-Nogueras, R.M., Paniw, M., Perales, J.A., Lara-Martín, P.A., 2018. Removal of pharmaceuticals in urban wastewater: high rate algae pond (HRAP) based technologies as an alternative to activated sludge based processes. Water Res. 139, 19–29. https://doi.org/10.1016/j.watres.2018.03.072.
Vo, H.N.P., Ngo, H.H., Guo, W.S., Nguyen, T.M.H., Liu, Y., Liu, Y., Nguyen, D.D., Chang, S.W., 2019. A critical review on designs and applications of microalgae-based photobioreactors for pollutants treatment. Sci. Total Environ. 651, 1549–1568. https://doi.org/10.1016/j.scitotenv.2018.09.282.
Wang, Y., Liu, J., Kang, D., Wu, C., Wu, Y., 2017. Removal of pharmaceuticals and personal care products from wastewater using algae-based technologies: a review. Rev. Environ. Sci. Biotechnol. 16, 717–735. https://doi.org/10.1007/s11157-017-9446-x.
Wohde, M., Berkner, S., Junker, T., Konradi, S., Schwarz, L., Düring, R.A., 2016. Occurrence and transformation of veterinary pharmaceuticals and biocides in manure: a literature review. Environ. Sci. Eur. 28, 23. https://doi.org/10.1186/s12302-016-0091-8.
Xie, P., Chen, C., Zhang, C., Su, G., Ren, N., Ho, S.H., 2020. Revealing the role of adsorption in ciprofloxacin and sulfadiazine elimination routes in microalgae. Water Res. 172, 115475 https://doi.org/10.1016/j.watres.2020.115475.
Xin, X., Huang, G., Zhang, B., 2021. Review of aquatic toxicity of pharmaceuticals and personal care products to algae. J. Hazard Mater. 410, 124619 https://doi.org/10.1016/j.jhazmat.2020.124619.
Xiong, J.Q., Kurade, M.B., Jeon, B.H., 2017. Biodegradation of levofloxacin by an acclimated freshwater microalga, Chlorella vulgaris. Chem. Eng. J. 313, 1251–1257. https://doi.org/10.1016/j.cej.2016.11.017.
Xiong, J.Q., Kurade, M.B., Jeon, B.H., 2018. Can microalgae remove pharmaceutical contaminants from water? Trends Biotechnol. 36, 30–44. https://doi.org/10.1016/j.tibtech.2017.09.003.
Zambrano, J., García-Encina, P.A., Hernández, F., Botero-Coy, A.M., Jiménez, J.J., Irusta-Mata, R., 2021. Removal of a mixture of veterinary medicinal products by adsorption onto a *Scenedesmus almeriensis* microalgae-bacteria consortium. J. Water Process Eng. 43, 102226 https://doi.org/10.1016/j.jwpe.2021.102226.
Zhang, J., Fu, D., Wu, J., 2012. Photodegradation of Norfloxacin in aqueous solution containing algae. J. Environ. Sci. 24, 743–749. https://doi.org/10.1016/S1001-0742(11)60814-0.