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Removal of veterinary antibiotics in swine manure wastewater using microalgae–bacteria consortia in a pilot scale photobioreactor

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ABSTRACT

Scenedesmus almeriensis microalgae–bacteria consortia were evaluated for the removal of a mixture of tetracycline (TET), ciprofloxacin (CIP), and sulfadiazine (SDZ) from the real liquid fraction of pig slurry in a pilot scale photobioreactor. After 15 days of operation, the reactor was spiked with a mixture of 100 μg/L of each antibiotic. The experiment ran for 20 additional days. From the liquid phase, antibiotic removal were 77 ± 5 %, 90 ± 14 %, and 60 ± 27 % for TET, CIP, and SDZ, respectively. The antibiotics found in the solid phase were 979 ± 382 ng/g for TET and 192 ± 69 ng/g for SDZ; CIP was not detected in the biomass. The parameters analyzed before and after antibiotic addition showed that the antibiotics did not have a negative effect on the reactor biomass. The removal efficiencies of the analyzed parameters were 64.6 ± 0.6 % for TOC, 56.9 ± 0.6 % for IC, 63.9 ± 0.6 % for TN, 88.6 ± 0.9 % for N-NH_4^+, 64.9 ± 0.6 % for N-NO_3^-, and 30.1 ± 0.3 % for P-PO_4^{3-}. This study demonstrated the good performance of microalgae-based technology for swine manure wastewater treatment, not only in terms of organic matter and nutrient removal, but also regarding the removal of antibiotics. The mass balance analysis of the entire process is presented. Additionally, the present study is a validation of previous laboratory scale batch studies operating in a quasi-continuous mode on veterinary antibiotics (VA) removal efficiencies and kinetics.

1. Introduction

Pork meat is the most consumed meat worldwide, with an annual consumption of 110 million tons per year (Cheng et al., 2020). The increasing demand for pork meat has developed industrialized confined animal-feeding operations, where the proximity of the large number of animals increases the potential for the rapid spread of diseases, making the use of pharmaceuticals compulsory (Sarmah et al., 2006). The use of antibiotics as animal feed additives is often indiscriminate and there are no standards for the regulation of their concentrations in the environment. The most used antibiotics in

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swine production are tetracycline and sulfonamide. The annual global consumption of antimicrobials per kilogram of pigs is 172 mg/kg (Cheng et al., 2020). Following the administration of antibiotics to animals, significant amounts of these substances are excreted in their urine and feces, eventually ending up in aquatic environments. Due to their slow or negligible degradation, antibiotics can persist in wastewater for extended periods of time. In addition, pig manure is commonly used as fertilizer, and runoffs from these agricultural soils is used for crop irrigation, so plants are continuously exposed to these antibiotics. Moreover, the accumulation of antibiotics in wastewater can cause the development of antibiotic resistant organisms and disrupt the ecological balance (Huang et al., 2021; Rocha et al., 2021; Tasho and Cho, 2016) .

Wastewater treatment plants (WWTP) are not intended to remove antibiotics. However, adsorption, biodegradation, disinfection, and membrane separation has shown to remove antibiotics to some extent. Physical (Ben et al., 2014; Saucier et al., 2015; Taheran et al., 2016), chemical (de Souza Santos et al., 2015; Iakovides et al., 2019; L. Opez-Ortiz et al., 2018), and biological treatment (A et al., 2021; Feng et al., 2017; Lucas et al., 2016) technologies, as well as advanced oxidation processes (AOPs) (Fiorentino et al., 2019; Kanakaraju et al., 2015; Ormeno-Cano and Radjenovic, 2022), have been widely studied for antibiotic removal. Some examples can be cited. Tian et al. (2019) prepared biochar via pyrolysis from livestock feces in order to remove antibiotics. Tian et al. (2019) obtained the complete removal of TET and SDZ in a pilot scale using a temperature of 600 °C. Even though pyrolysis showed to completely remove VA, pyrolysis is a complex process which requires high operational and investment costs. A et al. (2021) used an hybrid constructed wetland and a layered biological filter to treat sewage treatment plant effluent for VA removal. A et al. (2021) obtained removals of 59%–67% for tetracyclines, 28%–64% for quinolones, and no removal for sulfonamides. This kind of technique presents some disadvantages — constructed wetlands require high land areas; biological filters are prone to clogging and they need a preliminary treatment before the wastewater can be treated. Regarding AOPs, Zambrano et al. (2022) obtained 83% removal for SDZ and complete removal for TET and CIP by using photocatalysis with TiO_2. However, this process has some technical limitations such as the inefficient utilization of visible light and the post-recovery of the catalyst. In this respect, the use of microalgae for wastewater treatment has emerged as an ecofriendly technology with low costs, providing natural disinfection, greenhouse gasses emission reduction, and better nutrient removal than conventional WWTP.

An additional benefit to microalgae-based technology is the option to obtain algae-derived products such as biofuel, biofertilizer, proteins, carbohydrates, pigments, and vitamins (Casagli et al., 2022; Li et al., 2023; Xiong et al., 2021). In this regard, it is essential to determine the elimination of antibiotics not only from the liquid phase but also from the algal biomass from the antibiotic-contaminated wastewater treatment process. The objective of this study was to determine the antibiotics, nutrients, and organic matter removal efficiency of a thin layer reactor fed with a diluted liquid fraction of swine manure and spiked with a mixture of antibiotics. The determination of the antibiotic removal in the aqueous and solid phase and the mass balance analysis of the entire process is also presented.

2. Materials and methods

2.1. Chemicals

High-purity grade (>95%) standards of TET, CIP, and SDZ were acquired from Sigma-Aldrich (Madrid, Spain). Individual stock solutions (1 g/L) were prepared in methanol (CH_3OH), except for CIP which was prepared in water/methanol (1:1) containing 0.2% (v/v) hydrochloric acid (HCl). CH_3OH of high analytical grade and HCl (37%) were purchased from Sigma-Aldrich (Madrid, Spain). Ultrapure water was acquired from Milli-Q (MQ) Advantage Ultrapure Water purification system from Merck Millipore (Billercia, USA).

2.2. Batch experiments

Four different configurations were used to distinguish between hydrolysis, biosorption, photolysis, and the global process for antibiotic removal using microalgae bacteria consortia. Amber glass beakers were used to study the removal of antibiotics using only biosorption (containing 1 g/L lyophilized microalgae bacteria consortia, dead biomass, without light exposure) or only hydrolysis (containing neither biomass nor light exposure). Transparent glass beaker were used to study the removal of antibiotics using only photolysis (exposed to light but without biomass) or to study the whole process for antibiotic removal using the microalgae bacteria consortia (1 g/L active biomass in 1 L BG11 medium Stanier et al., 1971).

The reactors were spiked with a mixture of 20, 100, 500, and 1000 μg/L of TET, CIP, and SDZ. The temperature was maintained at 25 °C and LED lights with a light intensity of 12.6 mW/cm^2 were used for illumination at 12 h light/12 h dark cycles. Samples were collected at different time intervals up to 5 days, filtered through 0.22 mm nylon syringe filters (Fisherbrand), and stored at 4 °C until the antibiotic analysis was performed. All experiments were performed in triplicate. The data of the batch experiments were previously reported separately in a study by Zambrano et al. (2023).

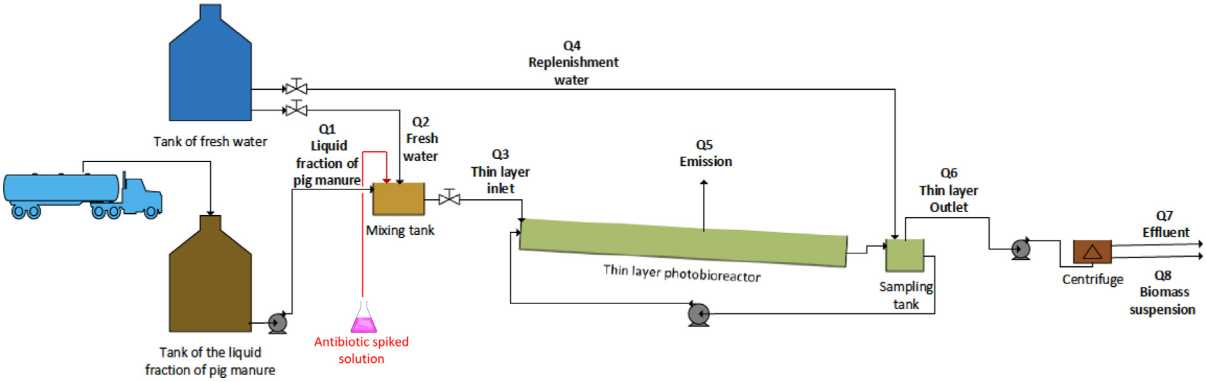

Fig. 1. Process flow diagram of microalgal treatment of the diluted liquid fraction of swine manure (SM) in a pilot scale thin layer photobioreactor.

2.3. Quasi-continuous experiments

Quasi-Continuous mode tests were conducted in a thin-layer cascade reactor with two-channels (Fig. 1) located at the Institute for Agricultural and Fisheries Research and Training (IFAPA) in Almería, Spain. The fiberglass thin layer photobioreactor had an area of 30 m^2 and a depth of 0.04 m. The reactor was inoculated using a microalgae–bacteria consortia, mainly composed of *Scenedesmus almeriensis*, with a concentration of 1.0 g/L.

The reactor was operated in quasi-continuous mode at 5 days HRT. It was fed twice a day with 240 L/d of a diluted liquid fraction of pig slurry at 5%. After 15 days, the diluted liquid fraction of pig slurry was spiked with a mixture of TET, CIP, and SDZ to a final concentration of 100 μg/L for each antibiotic, which was fed to the reactor for 20 days more. Temperature, evaporation, and quantum yield were registered during the experiment (Fig. 2). Freshwater was also used to compensate for water evaporation every day.

Liquid samples were taken on different days during the 35 days of operation from both the influent and the effluent. One fifth of the total reactor volume was harvested in the morning after homogenizing the reactor. This volume was transferred to a separate tank and centrifuged using an industrial SSD 6-06-007 centrifuge (GEA Westfalia Separator, Oelde, Germany). The harvested biomass was frozen and lyophilized, and the liquid phase was frozen at −20 °C until further analysis. From the liquid phase, 1.5 L were used to determine the TOC, IC, TN, N-NH_4^+, N-NO_3^- and P-PO_4^{3-}, and 500 mL was taken to determine dissolved VA concentrations. From the lyophilized biomass, 1 g was taken to analyze VA concentration in the solid phase. All experiments were performed in triplicate.

2.4. Analytical methods

TOC, IC and TN concentrations were measured using a TOC-V CSH analyzer equipped with a TNM-1 module (Shimadzu, Japan). N-NH_4^+ analyses were carried out with an ammonium specific electrode Orion Dual Star (Thermo Scientific, The Netherlands). N-NO_3^- and P-PO_4^{3-} concentrations were determined by high performance liquid chromatography (Waters 515 HPLC pump) coupled with a detector based on ion [conductivity] (Waters 432 IC) (HPLC-IC). The biomass concentration was quantified according to standard methods as described elsewhere (APHA, 2005). The chlorophyll fluorescence ratio (F_v/F_m) is a robust indicator of the maximum [quantum yield] which was determined using an AquaPen AP 100 fluorometer (Photon System Instruments, The Czech Republic).

The quantitative analyses of the veterinary antibiotics were adapted based on the work of López-Serna et al. (2019) for the liquid phase and Argüeso-Mata et al. (2021) for the solid phase. In brief, the liquid samples (100 mL) were vacuum filtered through 0.45 μm and spiked with a 5% EDTA solution and 0.5 ppm internal standard. After that, a solid phase extraction (SPE) was performed using Oasis® HLB cartridges (60 mg, 3 cc; Waters Chromatography, Barcelona, Spain). Then, the cartridges were eluted with 6 mL of acetonitrile, and the resulting organic solutions were subsequently evaporated in a nitrogen evaporator (Organomation N-Evap 11250) and reconstituted in 1 mL of 0.1% formic acid in a mixture of water/methanol (95:5, v/v). Finally, the samples were filtered through 0.22 μm PTFE and analyzed by ultra-high performance liquid chromatography (UHPLC) – tandem mass spectrometry (MS/MS) according to Zambrano et al. (2022).

For the solid samples, 0.3 g of lyophilized biomass was spiked with 0.3 g of activated PSA (primary secondary amino) and 2 ppm internal standard. After that, two extraction steps were performed. For the first extraction, 10 mL of a methanol/water mixture (10:90, v/v) was added to the solution which was vortexed and subjected to ultrasound. The second extraction followed the same steps using 15 ml of the methanol/water mixture. The extracts from both extraction cycles were combined, and 2 mL of 5% EDTA solution were added and the solution was diluted up to 100 mL with water before the solid phase extraction (SPE) with Oasis® HLB cartridges. Then, cartridges were eluted with 6 mL of acetonitrile,

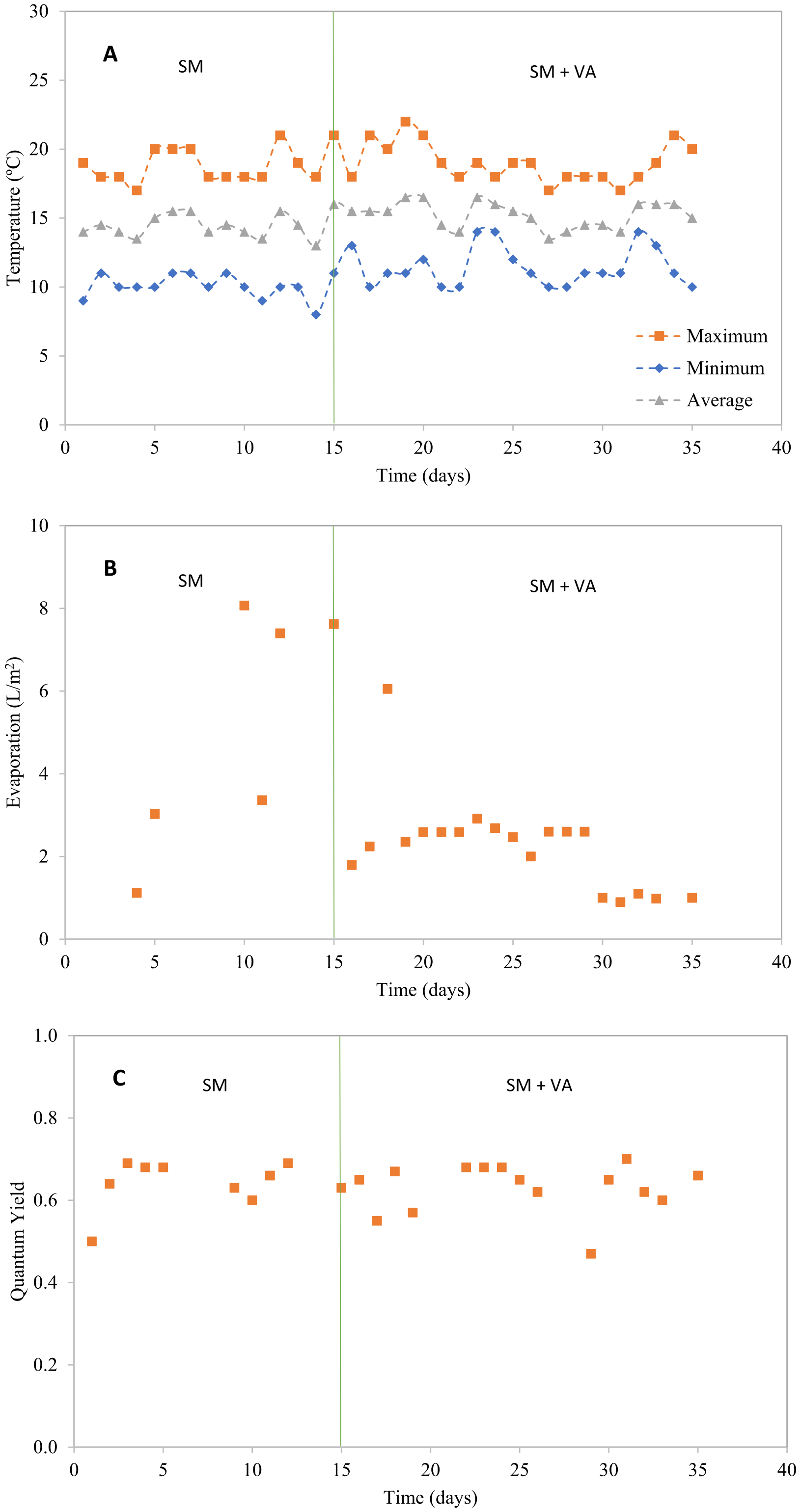

Fig. 2. (A) Temperature, (B) evaporation, and (C) quantum yield during the thin layer photobioreactor operation before VA addition (SM) and after VA addition (SM + VA).

Table 1
Photobioreactor performance.

Parameters	Concentration (μg/L)			
	Before doping		After doping	
	Influent Q_3	Effluent Q_6	Influent Q_3	Effluent Q_6
TOC	1491 ± 124	662 ± 82	1404 ± 39	497 ± 31
IC	376 ± 25	137 ± 1	508 ± 44	219 ± 22
TN	365 ± 14	123 ± 4	377 ± 10	136 ± 2
$N\text{-}NH_4^+$	35 ± 2	6 ± 1	24 ± 4	3 ± 2
$N\text{-}NO_3^-$	66 ± 5	16 ± 3	76 ± 10	27 ± 4
$P\text{-}PO_4^{3-}$	8 ± 1	6 ± 1	7 ± 1	5 ± 1

and the resulting organic solutions were subsequently evaporated and reconstituted in 1 mL of 0.1% formic acid in a mixture of methanol/water (95:5, v/v). Finally, the samples were filtered through 0.22 μm PTFE and analyzed by ultra-high performance liquid chromatography (UHPLC) – tandem mass spectrometry (MS/MS) according to Zambrano et al. (2022).

3. Results and discussions

3.1. Bioremediation performance of the photobioreactor

TOC, IC, and TN were measured from the dilution of the liquid fraction of pig slurry fed to the reactor before and after its treatment by the photobioreactor (Table 1). The removal efficiency of TOC before VA addition averaged 56 ± 1%, while after VA addition it increased to 65 ± 1%. IC removal efficiency before VA addition averaged 64 ± 2%, while after VA addition it slightly decreased to 57 ± 4%. TN presented a similar behavior. Before VA addition, the removal efficiency of TN averaged 66 ± 2%, which was similar to the removal efficiency of 64 ± 1% after VA addition.

Similar results can be found in the literature. López-Serna et al. (2019) studied the removal of VA from pig slurry in a 3 L open photobioreactor inoculated with microalgae bacteria consortia mainly composed of *Chlorella vulgaris.* With a 4 day HRT, 66 ± 3% of TOC and 47 ± 1% of TN removals were obtained. Meanwhile, Posadas et al. (2013) studied the carbon and nutrient removal from pre-treated domestic wastewater (by screening and primary sedimentation) using a 31 L algal–bacterial biofilm bioreactor. With an HRT of 10.4 d, they achieved 90 ± 3% TOC, 91 ± 6% IC, and 70 ± 8% TN removals. The higher TOC and IC removal rates were the consequence of the higher HRT used by Posadas et al. (2013) who doubled the HRT used in the present study.

$N\text{-}NH_4^+$, $N\text{-}NO_3^-$, and $P\text{-}PO_4^{3-}$ were measured in the inlet and outlet of the thin layer reactor (Table 1). The removal efficiency of $N\text{-}NH_4^+$ before VA addition was 82 ± 1% and it increased to 89 ± 3% after VA addition. For $N\text{-}NO_3^-$, the removal efficiency was 77 ± 5% before VA addition, and it decreased to 65 ± 5% after VA addition. In the case of $P\text{-}PO_4^{3-}$, the removal efficiency was similar both before and after VA addition, 28 ± 2 and 30 ± 1%, respectively.

Similar results were reported in the literature. Ciardi et al. (2022) studied the annual production of *S. almeriensis* in a thin layer cascade reactor by feeding the reactor with pig slurry. The $N\text{-}NH_4^+$, $N\text{-}NO_3^-$, and $P\text{-}PO_4^{3-}$ removal percentages were 99.1, 87.2, and 34.5%, respectively. Meanwhile, Hena et al. (2018) used an HRAP to treat dairy cattle wastewater with *Arthrospia platensis*, obtaining removals of 100% $N\text{-}NH_4^+$, 99.6% $N\text{-}NO_3^-$, and 98.8% $P\text{-}PO_4^{3-}$. The higher removals obtained by Hena et al. (2018) might be due to the low concentrations of $N\text{-}NH_4^+$, $N\text{-}NO_3^-$, and $P\text{-}PO_4^{3-}$ in dairy farm wastewater; 0.75 – 5.56, 1.45 – 5.44, and 4.33 – 7.01 mg/L, respectively.

The significance of the removal efficiencies for TOC, IC, TN, $N\text{-}NH_4^+$, $N\text{-}NO_3^-$, and $P\text{-}PO_4^{3-}$, before and after VA addition, was determined using analysis of variance (ANOVA), as shown in Table S1. Based on the statistical analysis, no significant changes were observed in the removal efficiency after VA addition to the photobioreactor, except for TOC, where the removal efficiency increased significantly. Moreover, the quantum yield (Fig. 2C) did not show variations before and after VA addition. A decrease in the quantum yield suggests that there are stress factors influencing the performance of the culture such as excess light, lack of nutrients, or the presence of toxins or heavy metals (Ciardi et al., 2022). *S. almeriensis,* in the present study, maintains a quantum yield of 0.6 ± 0.2 throughout the entire operation. These results demonstrate that the antibiotics did not cause a clear inhibitory effect on the *Scenedesmus almeriensis* microalgae–bacteria consortia.

3.2. Removal of veterinary antibiotics in the photobioreactor

During the first 15 days of operation, the liquid and solid samples were analyzed to determine if the target contaminants were present in the liquid fraction of pig slurry dilution or adsorbed on the *Scenedesmus almeriensis* microalgae–bacteria consortia. Neither of the three antibiotics were detected. After a two-week stabilization period, a mixture of TET, CIP, and SDZ was added to the reactor feed at a concentration of 100 μg/L for each antibiotic, just at the inlet of the photobioreactor (Fig. 1).

Table 2
Presence of veterinary antibiotics at the inlet and outlet of the photobioreactor.

VA	Concentration (*)					
	Before doping			After doping		
	Influent Q_3	Effluent Q_6	Biomass Q_6	Influent Q_3	Effluent Q_6	Biomass Q_6
TET	0	0	0	100	23 ± 5	979 ± 382
CIP	0	0	0	100	10 ± 14	n.d.
SDZ	0	0	0	100	40 ± 27	192 ± 69

Influent (μg/L), Effluent (μg/L), Biomass (ng/g).

After the addition of the antibiotics, during 20 days of operation, the removal efficiency of VA in the thin layer photobioreactor by the microalgae–bacteria consortia (MBC) was 77 ± 5% for TET, 90 ± 14% for CIP, and 60 ± 27% for SDZ (Table 2). The results obtained in the present study for the removal of TET agree with the literature. de Godos et al. (2012) obtained 69 ± 1% TET removal by using synthetic wastewater in a 14 L HRAP inoculated with *C. vulgaris* culture for a 7 days HRT and an initial TET concentration of 2 mg/L. Norvill et al. (2017) obtained higher removal percentages of 97% for 4 days HRT and >99% for 7 days HRT by using pretreated fresh domestic wastewater spiked with 2 mg/L TET in a 180 L HRAP inoculated with microalgae–bacteria consortia. The higher removal rates obtained by Norvill et al. (2017) could be due to the use of a single antibiotic and domestic wastewater. The present study used a complex matrix of pig slurry and a mixture of antibiotics which might reduce the efficacy of the treatment; however, considering the kinetic constant of 0.1362 h^{-1} determined in our batch experiments (Zambrano et al., 2023) for an initial TET concentration of 2 mg/L for a 7 days HRT, our process achieved a 95.8% removal.

In the case of CIP, Hom-Diaz et al. (2017) used a 1000 L HRAP inoculated with a mixed culture from a wastewater treatment stabilization pond to treat primary domestic wastewater spiked with 2 mg/L CIP with an HRT of 10 days, obtaining 10.04 ± 0.91% CIP removal. The low CIP removal percentages were explained by Hom-Diaz et al. (2017) as a result of low light penetration in the HRAP and high nitrate concentrations which act as scavengers and inhibit CIP removal. In the present study, higher removal percentages were achieved because nitrate was not an issue and nitrification was not observed; the $N{-}NO_3^-$ concentrations at the effluent were lower than at the influent, and the use of a thin layer reactor allowed for good light penetration. Nitrification is not common in a thin-layer cascade because the lower depth of the culture maximizes light availability and promotes phototrophic growth (Ciardi et al., 2022).

The results obtained for SDZ in the present study cannot be compared to the existing literature, as to the best of our knowledge, this is the first analysis of SDZ removal by microalgae–bacteria consortia on a pilot scale. In this sense, a direct comparison with a pilot scale experiment could not be carried out. For comparison purposes, batch experiments performed by Xie et al. (2020) and Peng et al. (2020) will be consider by using the k values they obtained to calculate the removal efficiency they would have achieved using a continuous process, assuming a pseudo-first order irreversible kinetic model. For an initial SDZ concentration of 100 μg/L for a 5 day HRT, they would have achieved 32.5% and 24.9% removal, respectively, which is lower than the one obtained in the present study. This lower removal might be due to the use of *Chlamydomonas sp* and *C. vulgaris* since antibiotic removal have shown to be highly dependent on the algae specie (Leng et al., 2020).

3.3. Veterinary antibiotics retention in the photobioreactor biomass

Biomass from the reactor was collected to determine the amount of antibiotics retained in the *Scenedesmus almeriensis* microalgae–bacteria consortia. CIP was not detected, while 979 ± 382 ng/g of TET and 192 ± 69 ng/g of SDZ was found in the biomass (Table 2). There are few previously published comparable data for VA concentrations in biomass. López-Serna et al. (2022) determined the concentration of veterinary antibiotics in algae biomass from a thin layer photobioreactor fed with pig slurry diluted at 5% with H_2O and a 3 days HRT. The biomass was mostly composed of *Chlorella sp. and Scenedesmus sp.* López-Serna et al. (2022) made two assays; TET was no detected in any of them and CIP and SDZ were detected only in one assay with concentrations of 14 ng/g and < 5 ng/g, respectively.

3.4. Mass balance

The mass balance of the WWTP proposed herein (Table 3) was based on a microalgae production of 17.6 $g/m^2/d$ (Ciardi et al., 2022) and the results that were experimentally observed. The microalgae had a mass content of 59.1% carbon and 10.6% nitrogen while the protein, carbohydrate, and lipid content were 51.7, 22.3, and 13.4%, respectively. Moreover, the mass balance was carried out considering the removal efficiencies of TOC, TN, $N{-}NH_4^+$, and $P{-}PO_4^{3-}$, found experimentally (Section 3.1), which were: 65, 64, 89, and 30%, respectively.

The kinetic constants obtained in the batch experiments, reported in a previous study (Zambrano et al., 2023), were used in the mass balance to determine the theoretical concentration of each antibiotic in the effluent and in the biomass. Specifically, the pseudo-first order kinetic constants (k) of the global process listed in Table S2 were used (0.1362 h^{-1} for TET, 0.0119 h^{-1} for CIP and 0.0098 h^{-1} for SDZ). With these rate constants, the mass balance analysis for VA was

Table 3
Composition and flow rates of process streams.

Flow	Stream													
	Q_1	Q_2	Q_3	Q_4	Q_5	Q_6	Q_6 (Liquid fraction)	Q_6 (Solid fraction)	Q_7	Q_7 (Liquid fraction)	Q_7 (Solid fraction)	Q_8	Q_8 (Liquid fraction)	Q_8 (Solid fraction)
Q (m^3/d)	0.012	0.228	0.240	0.075	0.075	0.24			0.235			0.005		
Q (kg/d)	12.00	228.00	240.00	75.00	75.00	240.53			235.30			5.23		
Water (kg/d)	12.00	228.00	240.00	75.00	75.00	240.00	240.00	0	235.25	235.25	0	4.75	4.75	0
Microalgae (g/d)	0	0	0	0	0	528.00	0	528.00	52.80	0	52.80	475.20	0	475.20
TSS (g/d)	2.40	0	2.40	0	0	528.00	0	528.00	52.80	0	52.80	475.20	0	475.20
TOC (g/d)	336.96	0	336.96	0	−94.48	431.36	119.21	312.15	148.06	116.85	31.22	283.30	2.36	280.94
TN (g/d)	90.48	0	90.48	0	1.81	88.67	32.64	55.97	37.59	31.99	5.60	51.02	0.65	50.37
N-NH$_4$ (g/d)	5.66	0	5.66	0	1.81	3.85	0.65	0	3.78	0.64	0	0.076	0.013	0
TP (g/d)	1.75	0	1.75	0	0	1.75	1.22	0.53	1.25	1.20	0.05	0.50	0.02	0.47
TET (mg/d)	0		24.00	0	0	1.71	1.36	3.47E−01	1.37	1.33	3.47E−02	0.34	0.03	3.12E−01
CIP (mg/d)	0		24.00	0	0	10,05	9.76	2.87E−01	9.60	9.57	2.87E−02	0.45	0.19	2.59E−01
SDZ (mg/d)	0		24.00	0	0	11.10	10.97	1.28E−01	10.75	10.75	1.28E−02	0.33	0.22	1.15E−01
Proteins (g/d)						272.98		272.98	27.30		27.30	245.68		245.68
Carbohydrates (g/d)						117.74		117.74	11.77		11.77	105.97		105.97
Lipids (g/d)						70.75		70.75	7.08		7.08	63.68		63.68
Concentration	Stream													
	Q_1	Q_2	Q_3	Q_4	Q_5	Q_6	Q_6 (Liquid fraction)	Q_6 (Solid fraction)	Q_7	Q_7 (Liquid fraction)	Q_7 (Solid fraction)	Q_8	Q_8 (Liquid fraction)	Q_8 (Solid fraction)
Microalgae (mg/L)	0		0			2200	0		224.44			1.00E+05		
TSS (mg/L)	200		10.0			2200	0		224.44			1.00E+05		
TOC (mg/L)	28080		1404.0			1797.35	496.71		629.40	496.71		5.96E+04	496.71	
TN (mg/L)	7540		377.0			369.46	135.98		159.77	135.98		1.07E+04	135.98	
N-NH$_4$ (mg/L)	472		23.6			16.06	2.70		16.06	2.70		16.06	2.70	
TP (mg/L)	146		7.3			7.30	5.10		5.33	5.10		104.98	5.10	
TET (ug/L)[a] (ug/mg)[b]	0		100.0				5.68	6.57E−04		5.68	6.57E−04		5.68	6.57E−04
CIP (ug/L)[a] (ug/mg)[b]	0		100.0				40.67	5.44E−04		40.67	5.44E−04		40.67	5.44E−04
SDZ (ug/L)[a] (ug/mg)[b]	0		100.0				45.71	2.42E−04		45.71	2.42E−04		45.71	2.42E−04

[a] For the liquid fraction.

[b] For the solid fraction.

first done assuming that the adsorption equilibrium was reached. However, assuming the hypothesis that adsorption equilibrium was reached, the amounts of VA adsorbed on the biomass resulting from the mass balance were found to be much higher than those experimentally observed (Table S3). Assuming, then, that adsorption equilibrium was not reached, a mass balance was performed considering a pseudo second-order kinetic of adsorption with the kinetic constants shown in Table S2 (7.05 mg $\mu g^{-1} h^{-1}$ for TET, 1.65 mg $\mu g^{-1} h^{-1}$ for CIP and 8.12 mg $\mu g^{-1} h^{-1}$ for SDZ) for an HRT of 5 days, corresponding to that used in the photobioreactor. In this case, accepting the hypothesis that the adsorption equilibrium was not reached, the mass balance led to theoretical results much closer to the experimentally obtained values, as can be seen in Table S4.

Table S5 shows that the experimental values for TET and CIP concentrations in the effluent varied slightly from those obtained in the mass balance. The experimental value for CIP was lower than the theoretical one, which can be attributed to photolysis being one of the most significant processes contributing to CIP removal (Zambrano et al., 2023). In the pilot-scale experiment, the thin layer had a larger surface area that facilitated photodegradation, whereas batch experiments were conducted in beakers with a higher loss of radiant energy. Moreover, the light intensity in Almeria, at around 60 mW/cm^2, was higher than the intensity used in the batch experiments (12.6 mW/cm^2). As for TET, the experimental value was higher than the theoretical one. Zambrano et al. (2023) determined that biosorption was the main mechanism involved in TET removal while working with synthetic wastewater. However, in real samples, solids and ions present in the water might interfere with biosorption (Bai and Acharya, 2017).

In addition, it is important to notice that there is a negative mass flow of TOC in stream Q_5. This negative value appears due to the capture of CO_2 from the atmosphere because of the cellular synthesis of microalgae, demonstrating that microalgae-based technologies can help with the reduction of greenhouse gas emissions.

4. Conclusions

Microalgae-based technologies have been proven to be a good alternative for wastewater treatment processes. A thin-layer cascade reactor inoculated with microalgae bacteria consortia mainly composed of *Scenedesmus almeriensis* was tested for the treatment of real swine manure wastewater and antibiotic removal. This treatment process proved to be highly efficient in the removal of organic matter, nutrients, and antibiotics. Moreover, the antibiotics showed not to have a negative effect on the *Scenedesmus almeriensis* microalgae–bacteria consortia. The results were consistent with previous studies; they corroborate the laboratory-scale batch experiments with respect to veterinary antibiotic removal efficiencies and validate the proposed irreversible pseudo-first-order kinetics. Photobioreactors based on microalgae–bacteria consortia demonstrated to be a promising wastewater treatment process for the removal of antibiotics and swine manure remediation as they can remove up to 77% TET, 90% CIP, and 60% SDZ without the use of additional chemicals preventing the generation of additional wastes. Further research should focus on by-product formation and on obtaining deeper insights into the harvested biomass to discover the best biomass for further use.

CRediT authorship contribution statement

Johanna Zambrano: Conceptualization, Methodology, Investigation, Data curation, Formal analysis, Visualization, Writing – original draft. **Pedro Antonio García-Encina:** Conceptualization, Supervision, Writing – review & editing. **Juan J. Jiménez:** Methodology, Supervision, Writing – review & editing. **Martina Ciardi:** Methodology, Investigation, Data curation. **Silvia Bolado-Rodríguez:** Funding acquisition, Resources, Conceptualization. **Rubén Irusta-Mata:** Conceptualization, Methodology, Software, Validation, Formal analysis, Writing – review & editing, Supervision.

Declaration of competing interest

The authors 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.

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Appendix A. Supplementary data

Supplementary material related to this article can be found online at https://doi.org/10.1016/j.eti.2023.103190.

References

A, D., Chen, C., Zou, M., Deng, Y., Zhang, X., Du, J., Yang, Y., 2021. Removal efficiency, kinetic, and behavior of antibiotics from sewage treatment plant effluent in a hybrid constructed wetland and a layered biological filter. J. Environ. Manag. 288, 112435. http://dx.doi.org/10.1016/J.JENVMAN.2021.112435.

APHA, 2005. Standard Methods for the Examination of Water and Waste Water, twenty first ed. American Public Health Association, Washington, DC.

Argüeso-Mata, 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, http://dx.doi.org/10.1016/j.chemosphere.2021.130039.

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. http://dx.doi.org/10.1016/j.scitotenv.2016.12.192.

Ben, W., Qiang, Z., Yin, X., Qu, J., Pan, X., 2014. Adsorption behavior of sulfamethazine in an activated sludgeprocess treating swine wastewater. J. Environ. Sci. 26, 1623–1629. http://dx.doi.org/10.1016/j.jes.2014.06.002.

Casagli, F., Beline, F., Ficara, E., Bernard, O., 2022. Optimizing resource recovery from wastewater with algae-bacteria membrane reactors. Chem. Eng. J. 451, 138488. http://dx.doi.org/10.1016/j.cej.2022.138488.

Cheng, D., Ngo, H.H., Guo, W., Chang, S.W., Nguyen, D.D., Liu, Y., Wei, Q., Wei, D., 2020. A critical review on antibiotics and hormones in swine wastewater: Water pollution problems and control approaches. J. Hazard. Mater. 387, 121682. http://dx.doi.org/10.1016/j.jhazmat.2019.121682.

Ciardi, M., Gómez-Serrano, C., Lafarga, T., González-Céspedes, A., Acién, G., López-Segura, J.G., Fernández-Sevilla, J.M., 2022. Pilot-scale annual production of scenedesmus almeriensis using diluted pig slurry as the nutrient source: Reduction of water losses in thin-layer cascade reactors. J. Clean. Prod. 359, http://dx.doi.org/10.1016/j.jclepro.2022.132076.

de Godos, I., Muñoz, R., Guieysse, B., 2012. Tetracycline removal during wastewater treatment in high-rate algal ponds. J. Hazard. Mater. 229–230, 446–449. http://dx.doi.org/10.1016/j.jhazmat.2012.05.106.

de Souza Santos, L.V., Meireles, A.M., Lange, L.C., 2015. Degradation of antibiotics norfloxacin by Fenton, UV and UV/H2O2. J. Environ. Manag. 154, 8–12. http://dx.doi.org/10.1016/J.JENVMAN.2015.02.021.

Feng, L., Casas, M.E., Ottosen, L.D.M., Møller, H.B., Bester, K., 2017. Removal of antibiotics during the anaerobic digestion of pig manure. Sci. Total Environ. 603–604, 219–225. http://dx.doi.org/10.1016/J.SCITOTENV.2017.05.280.

Fiorentino, A., Esteban, B., Garrido-Cardenas, J.A., Kowalska, K., Rizzo, L., Aguera, A., Pérez, J.A.S., 2019. Effect of solar photo-fenton process in raceway pond reactors at neutral pH on antibiotic resistance determinants in secondary treated urban wastewater. J. Hazard. Mater. 378, 120737. http://dx.doi.org/10.1016/J.JHAZMAT.2019.06.014.

Hena, S., Znad, H., Heong, K.T., Judd, S., 2018. Dairy farm wastewater treatment and lipid accumulation by Arthrospira platensis. Water Res. 128, 267–277. http://dx.doi.org/10.1016/j.watres.2017.10.057.

Hom-Diaz, A., Norvill, Z.N., Blánquez, P., Vicent, T., Guieysse, B., 2017. Ciprofloxacin removal during secondary domestic wastewater treatment in high rate algal ponds. Chemosphere http://dx.doi.org/10.1016/j.chemosphere.2017.03.125.

Huang, A., Yan, M., Lin, J., Xu, L., He Gong Han, Gong, 2021. A review of processes for removing antibiotics from breeding wastewater. Int. J. Environ. Res. Public Health 18, http://dx.doi.org/10.3390/ijerph18094909.

Iakovides, I.C., Michael-Kordatou, I., Moreira, N.F.F., Ribeiro, A.R., Fernandes, T., Pereira, M.F.R., Nunes, O.C., Manaia, C.M., Silva, A.M.T., Fatta-Kassinos, D., 2019. Continuous ozonation of urban wastewater: Removal of antibiotics, antibiotic-resistant escherichia coli and antibiotic resistance genes and phytotoxicity. Water Res. 159, 333–347. http://dx.doi.org/10.1016/J.WATRES.2019.05.025.

Kanakaraju, D., Motti, C.A., Glass, B.D., Oelgemöller, M., 2015. TiO2 photocatalysis of naproxen: Effect of the water matrix, anions and diclofenac on degradation rates. Chemosphere 139, 579–588. http://dx.doi.org/10.1016/j.chemosphere.2015.07.070.

L. Opez-Ortiz, C.M., Sentana-Gadea, I., Var O-Galva, P.J., Maestre-P Erez, S.E., Prats-Rico, D., 2018. Effect of magnetic ion exchange (MIEX®) on removal of emerging organic contaminants. http://dx.doi.org/10.1016/j.chemosphere.2018.05.194.

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. http://dx.doi.org/10.1016/j.chemosphere.2019.124680.

Li, S.N., Zhang, C., Li, F., Ren, N.Q., Ho, S.H., 2023. Recent advances of algae-bacteria consortia in aquatic remediation. Crit. Rev. Environ. Sci. Technol. 53, 315–339. http://dx.doi.org/10.1080/10643389.2022.2052704.

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, http://dx.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. http://dx.doi.org/10.1016/J.Scitotenv.2019.07.126.

Lucas, D., Badia-Fabregat, M., Vicent, T., Caminal, G., Rodríguez-Mozaz, S., Balcázar, J.L., Barceló, D., 2016. Fungal treatment for the removal of antibiotics and antibiotic resistance genes in veterinary hospital wastewater. Chemosphere 152, 301–308. http://dx.doi.org/10.1016/J.CHEMOSPHERE.2016.02.113.

Norvill, Z.N., Toledo-Cervantes, A., Blanco, S., Shilton, A., Guieysse, B., Muñoz, R., 2017. Photodegradation and sorption govern tetracycline removal during wastewater treatment in algal ponds. Bioresour. Technol. http://dx.doi.org/10.1016/j.biortech.2017.02.011.

Ormeno-Cano, N., Radjenovic, J., 2022. Electrochemical degradation of antibiotics using flow-through graphene sponge electrodes. J. Hazard. Mater. 431, 128462. http://dx.doi.org/10.1016/J.JHAZMAT.2022.128462.

Peng, Y.Y., Gao, F., Yang, H.L., Wu, H.W.J., Li, C., Lu, M.M., Yang, Z.Y., 2020. Simultaneous removal of nutrient and sulfonamides from marine aquaculture wastewater by concentrated and attached cultivation of Chlorella vulgaris in an algal biofilm membrane photobioreactor (BF-MPBR). Sci. Total Environ. 725, http://dx.doi.org/10.1016/j.scitotenv.2020.138524.

Posadas, E., García-Encina, P.A., Soltau, A., Domínguez, A., Díaz, I., Muñoz, R., 2013. Carbon and nutrient removal from centrates and domestic wastewater using algal–bacterial biofilm bioreactors. Bioresour. Technol. 139, 50–58. http://dx.doi.org/10.1016/j.biortech.2013.04.008.

Rocha, D.C., da Silva Rocha, C., Tavares, D.S., de Morais Calado, S.L., Gomes, M.P., 2021. Veterinary antibiotics and plant physiology: An overview. Sci. Total Environ. 767, 144902. http://dx.doi.org/10.1016/j.scitotenv.2020.144902.

Sarmah, A.K., Meyer, M.T., Boxall, A.B.A., 2006. A global perspective on the use, sales, exposure pathways, occurrence, fate and effects of veterinary antibiotics (VAs) in the environment. Chemosphere 65, 725–759. http://dx.doi.org/10.1016/j.chemosphere.2006.03.026.

Saucier, C., Adebayo, M.A., Lima, E.C., Cataluña, R., Thue, P.S., Prola, L.D.T., Puchana-Rosero, M.J., Machado, F.M., Pavan, F.A., Dotto, G.L., 2015. Microwave-assisted activated carbon from cocoa shell as adsorbent for removal of sodium diclofenac and nimesulide from aqueous effluents. J. Hazard. Mater. 289, 18–27. http://dx.doi.org/10.1016/J.JHAZMAT.2015.02.026.

Stanier, R.Y., Kunisawa, R., Mandel, M., Cohen-Bazire, G., 1971. Purification and properties of unicellular blue–green algae (order Chroococcales). Bacteriol. Rev. 35, 171–205. http://dx.doi.org/10.1128/mmbr.35.2.171-205.1971.

Taheran, M., Brar, S.K., Verma, M., Surampalli, R.Y., Zhang, T.C., Valero, J.R., 2016. Membrane processes for removal of pharmaceutically active compounds (PhACs) from water and wastewaters. Sci. Total Environ. 547, 60–77. http://dx.doi.org/10.1016/J.Scitotenv.2015.12.139.

Tasho, R.P., Cho, J.Y., 2016. Veterinary antibiotics in animal waste, its distribution in soil and uptake by plants: A review. Sci. Total Environ. 563–564, 366–376. http://dx.doi.org/10.1016/j.scitotenv.2016.04.140.

Tian, R., Li, C., Xie, S., You, F., Cao, Z., Xu, Z., Yu, G., Wang, Y., 2019. Preparation of biochar via pyrolysis at laboratory and pilot scales to remove antibiotics and immobilize heavy metals in livestock feces. J. Soil. Sediments 19, 2891–2902. http://dx.doi.org/10.1007/s11368-019-02350-2.

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. http://dx.doi.org/10.1016/j.watres.2020.115475.

Xiong, Q., Hu, L.X., Liu, Y.S., Zhao, J.L., He, L.Y., Ying, G.G., 2021. Microalgae-based technology for antibiotics removal: From mechanisms to application of innovational hybrid systems. Environ. Int. 155, http://dx.doi.org/10.1016/j.envint.2021.106594.

Zambrano, J., García-Encina, P.A., Hernández, F., Botero-Coy, A.M., Jiménez, J.J., Irusta-Mata, R., 2023. Kinetics of the removal mechanisms of veterinary antibiotics in synthetic wastewater using microalgae-bacteria consortia. Environ. Technol. Innov. 29, 103031. http://dx.doi.org/10.1016/J.Eti.2023.103031.

Zambrano, J., García-Encina, P.A., Jiménez, J.J., López-Serna, R., Irusta-Mata, R., 2022. Photolytic and photocatalytic removal of a mixture of four veterinary antibiotics. J. Water Process Eng. http://dx.doi.org/10.1016/j.jwpe.2022.102841.