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Kinetics of the removal mechanisms of veterinary antibiotics in synthetic wastewater using microalgae–bacteria consortia

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ABSTRACT

The mechanisms involved in the removal of a mixture of four veterinary antibiotics (VA) – tetracycline (TTC), ciprofloxacin (CPF), sulfadiazine (SDZ) and sulfamethoxazole (SMX) – in synthetic wastewater using microalgae–bacteria consortia (MBC) dominated by *Scenedesmus almeriensis* was studied at different initial concentrations of 1000, 500, 100 and 20 μg/L per antibiotic. Ultra-high performance liquid chromatography and tandem mass spectrometry (UHPLC-MS/MS) were used to determine the removal of the VA for each mechanism. For a hydraulic retention time of 4 days, the overall removal of antibiotics by the MBC was 99.9% for TTC, 78.0% for CPF, 52.6% for SDZ and 5.0% for SMX. A pseudo-first order irreversible model was applied to best fit the experimental data. The degradation constant rates were 0.136 h^{-1} for TTC, 0.012 h^{-1} for CPF, 0.010 h^{-1} for SDZ and 0.0007 h^{-1} for SMX. Under all the evaluated conditions, CPF and TTC exhibited the highest removal efficiency. Biosorption was the main mechanism for all four antibiotics, followed by biodegradation in the cases of TTC and SDZ. CPF did not show removal via biodegradation. SMX did not show removal via hydrolysis or photolysis. This study (i) integrates and evaluates individually the mechanisms involved in VA removal using an MBC; (ii) determines an overall removal rate constant for a wide array of TTC, CPF, SDZ and SMX concentrations; and (iii) demonstrates the high removal capacity and potential use of microalgae as an ecofriendly wastewater treatment process.

1. Introduction

Emerging contaminants (ECs) are a group of pollutants associated with serious ecological and human health problems. However, their ecotoxicological effects are relatively unknown. ECs include endocrine disrupting chemicals (EDCs), pharmaceuticals and personal care products (PPCPs), perfluorinated compounds (PFCs), polycyclic aromatic hydrocarbons (PaHs), surfactants, pesticides, dyes and alkyl phenolic compounds, among others (Ahmed et al., 2021). Antibiotics are an EC which have attracted considerable attention since they are widely used in human and animal medicine. Antibiotics are commonly found in wastewater since they are minimally metabolized, only about 10 to 20% and its major fraction is evacuated in urine and feces (Cheng et al., 2020; Khalatbary et al., 2022). However, conventional wastewater treatment

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plants (WWTPs) are not intended to remove antibiotics specifically, leading to the liberation of antibiotics into the environment (Ahmed et al., 2017; Matamoros et al., 2015; Norvill et al., 2017).

Conventional physical, chemical and biological technologies have been tested at lab and industrial scales to remove ECs from wastewaters. Conventional wastewater treatment technologies have been recognized to be insufficient for the removal of ECs (Ahmadpour et al., 2021). However, adsorption using activated carbon (Azari et al., 2021; Jaafari et al., 2018; Zazouli et al., 2016) and advanced oxidation processes coupled with light irradiation (Badi et al., 2019; Hashemi et al., 2020) were found to be very effective for the removal of ECs. Additionally, hybrid systems combining ozonation with biological activated carbon showed good removal efficiencies for pharmaceuticals (Rodriguez-Narvaez et al., 2017). Similarly, a hybrid ozonation-ultrasound system can successfully remove up to 100% of many ECs. However, the absence of consistent analytical data of the resulting transformation products, together with their toxicity and the high costs of these technologies, has restricted their industrial application in WWTPs (Ahmed et al., 2017; López-Serna et al., 2019).

Microalgae-based wastewater treatment technologies have aroused the interest of researchers as they have been shown to be able to remove a broad range of ECs. Being photosynthetic microorganisms, microalgae provide oxygen via photosynthesis, lowering the need of external mechanical aeration. Additionally, microalgae use CO_2 for their growth which reduces CO_2 emissions and converts solar energy in valuable biomass. Moreover, microalgae extract resources, such as carbon, nitrogen and phosphorus, from wastewater. The main benefits of microalgae treatment processes are their ability to combine macro pollutant removal with biomass valorization (resource recovery from algal biomass as fertilizer, protein-rich feed or biofuel). This in turn produces high-quality effluent while potentially biodegrading hazardous pollutants (Gojkovic et al., 2019; Hom-Diaz et al., 2017; Matamoros et al., 2015).

Microalgae-based wastewater treatment technologies have the capacity to remove organic contaminants, such as antibiotics through both abiotic (sorption, volatilization or photodegradation) and biotic (biosorption, bioaccumulation and biodegradation) processes. The application of microalgae to remove antibiotics from wastewater has industrial potential as a green and sustainable sunlight-driven technology (Gojkovic et al., 2019; Hom-Diaz et al., 2017; Matamoros et al., 2015). However, the relatively high cost of conventional systems for the separation of the microalgal biomass from the treated wastewater is still a challenging matter (Lefoulon et al., 2016). The most widely used microalgae-based treatment system are high rate algae ponds (HRAP) which require large land areas and, being natural systems, have some variability in treatment performance (Ahmed et al., 2017; López-Serna et al., 2019).

The fate of antibiotics in microalgae-based systems is largely unknown. It is critical to assess the mechanisms involved in this process in order to widely use microalgae in wastewater treatment applications for the removal of antibiotics. With this objective, the aim of this study is to determine the processes involved in TTC, CPF, SDZ and SMX removal using a MBC from a high-rate algae pond (HRAP) used for swine manure wastewater treatment. The four antibiotics were chosen as representative compounds of the three families of antibiotics most commonly found in water samples (fluoroquinolones, sulfonamides, and tetracyclines). Different initial concentrations, like those found in real water samples, were analyzed. For the removal of TTC, CPF, SDZ and SMX using an MBC, this study has integrated and evaluated individually the different elimination mechanisms present in the process; plus, as far as we know, it has published for the first time the rate constants for the global process and the main mechanisms involved in the removal of this VA by adjusting the experimental data of a broad array of concentrations to a single general kinetic equation. A general equation was used for the whole set of analyzed concentrations rather than individual equations for each initial concentration which is a misinterpretation often found in the literature.

2. Materials and methods

2.1. Biomass

Active biomass and lyophilized biomass, essentially constituted of the microalgae *Scenedesmus almeriensis* (Table S1), was provided by the University of Almería (Spain) and stored at 4 °C until further use. *S. almeriensis* is a strain that grows fast and has high productivity; as well, *S. almeriensis* easy adapts to stressful conditions (Morillas-España et al., 2020). The biomass was harvested from an HRAP with an operating volume of 11 800 L, a land surface of 80 m^2, a solar radiation within 1500–1600 $\mu E/m^2$ and a temperature between 30–35 °C.

2.2. Chemicals

High-purity grade (>95%) reference standards of TTC, CPF, SDZ and SMX were acquired as neutral non-solvated molecules — except for TTC (hydrochloride) (Sigma-Aldrich Madrid, Spain). Individual stock solutions of 1 g/L for each antibiotic were prepared in methanol (MeOH). CPF was prepared in H_2O/MeOH (1:1) containing 0.2% v/v hydrochloric acid (HCl). Mix solutions were prepared from individual stock solutions and stored at <−20 °C in the dark. High analytical grade MeOH and HCl (37%) from Sigma-Aldrich (Madrid, Spain). Ultrapure water was obtained from Milli-Q (MQ) Advantage Ultrapure Water purification system and filtered through a 0.22 μm Millipak Express and LC-Pak polishing unit by Merck Millipore (Billercia, USA).

2.3. Experimental configuration

Tests were performed in 1 L glass beakers. Each beaker contained 1 L Milli-Q water spiked with a mixture of TTC, CPF, SDZ and SMX to a final concentration of 1000, 500, 100 and 20 μg/L for each compound. Firstly, an aliquot of the sample

was collected to determine the initial VA concentration in each reactor, and then the MBC was added (in the reactors selected for biosorption and biodegradation) under continuous mixing until a concentration of 1 g/L was obtained. The microalgae concentration selected was similar to the usual concentrations of microalgae in HRAPs used for wastewater treatment. Samples were collected at different time intervals (up to 4 days) to simulate the common residence time of microalgae in an HRAP. Samples were filtered through 0.22 mm nylon syringe filters (Fisherbrand) and stored at 4 °C until antibiotic analysis. All experiments were performed in triplicate.

2.3.1. Experimental configuration for physical removal processes

Three types of reactors were used in order to distinguish between hydrolysis, biosorption and photolysis. Amber glass beakers were used to study the removal of antibiotics only by biosorption (Reactor A) or only by hydrolysis (Reactor B). A transparent glass beaker was used for the removal of antibiotics only by photolysis (Reactor C). Reactor A (biosorption) contained a lyophilized MBC (dead biomass) without light exposure. Reactor B (hydrolysis) had neither a MBC nor light exposure. Reactor C (photolysis) was exposed to light but without a MBC. LED lights were used for illumination with a light intensity of 12.62 mW/cm^2. Reactors were mixed at 200 rpm continuously.

2.3.2. Experimental configuration for the biological removal process

The whole process for antibiotic removal using a MBC was studied in batch experiments by using 1 g/L active biomass in 1 L Erlenmeyer flask containing 1 L BG11 medium (Stanier et al., 1971). All reactors were mixed continuously by pumping air to the system, temperature was maintained at 25 °C and LED lights were used for illumination at 12 h light/12 h dark cycle with a light intensity of 12.62 mW/cm^2. To determine the biodegradation efficiency of VA by the MBC, the results obtained for hydrolysis, photolysis and biosorption were subtracted from the results obtained for the whole process (Fig. S1).

2.4. Analytical methods

Liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) (Waters Acquity H-Class UPLC), interfaced to a triple quadrupole Xevo TQ-STM mass spectrometer, with ESI source (Waters Corp) was used for the determination of TTC, CPF, SDZ and SMX. The sample procedure was based on Botero-Coy et al. (2018) and Bijlsma et al. (2021), using direct injection of the (diluted) samples. A previous dilution x100 (samples spiked at 1000 and 500 ng/mL) or x10 (samples spiked at 100 and 20 ng/mL) with Milli-Q water was applied to reduce matrix complexity and to adjust the sample concentrations to the calibration curve. Analyte isotope-labeled internal standards (ILIS) (CPF-d8, SMX-d6, SDZ-13C6, TTC-d6) were added to the samples and calibration standards for matrix effects corrections. Three MS/MS transitions were acquired per compound for quantification and reliable identification in the samples (Table S2). For more details, see Zambrano et al. (2021)

2.5. Kinetic analysis

The kinetics for the whole process and each mechanism involved (hydrolysis, photolysis and biodegradation) in VA removal via the MBC was described by using three kinetic models: zero-order (Eq. (1)), pseudo-first order irreversible (Eq. (2)) and pseudo-first order reversible (Eq. (3)):

$$r = -\frac{dC}{dt} = k_Z \tag{1}$$

$$r = -\frac{dC}{dt} = k_I \cdot C \tag{2}$$

$$r = -\frac{dC}{dt} = k_R \cdot (C - C_e) \tag{3}$$

where, r is the rate of antibiotic degradation ($\mu g\ L^{-1}\ h^{-1}$), C is the antibiotic concentration at any time ($\mu g\ L^{-1}$), C_e is the antibiotic concentration at equilibrium ($\mu g\ L^{-1}$), k_Z is the zero-order rate constant ($\mu g\ L^{-1}\ h^{-1}$), k_I is the pseudo-first order irreversible rate constant (h^{-1}) and k_R is the pseudo-first order reversible rate constant (h^{-1}).

Integrating Eqs. (1), (2) and (3) with respect to the limits $C = C_0$ for $t = t_0$ and $C = C_t$ for $t = t$, Eqs. (4), (5) and (6) can be obtained:

$$C_t = C_0 - k_Z \cdot t \tag{4}$$

$$C_t = C_0 \cdot e^{-k_I \cdot t} \tag{5}$$

$$C_t = C_e + (C_0 - C_e) \cdot e^{-k_R \cdot t} \tag{6}$$

Eq. (5) is a solution of Eq. (6) when $C_e = 0$; hence, the pseudo-first order kinetics is not reversible.

3. Results and discussions

3.1. Removal of VA by the MBC

The whole process for VA removal using a MBC was studied in batch experiments for 96 h (Fig. 1(A)–(D)). For a mixture of the four aforementioned VA, the initial concentration decreased over time. The removal efficiency of VA by the MBC

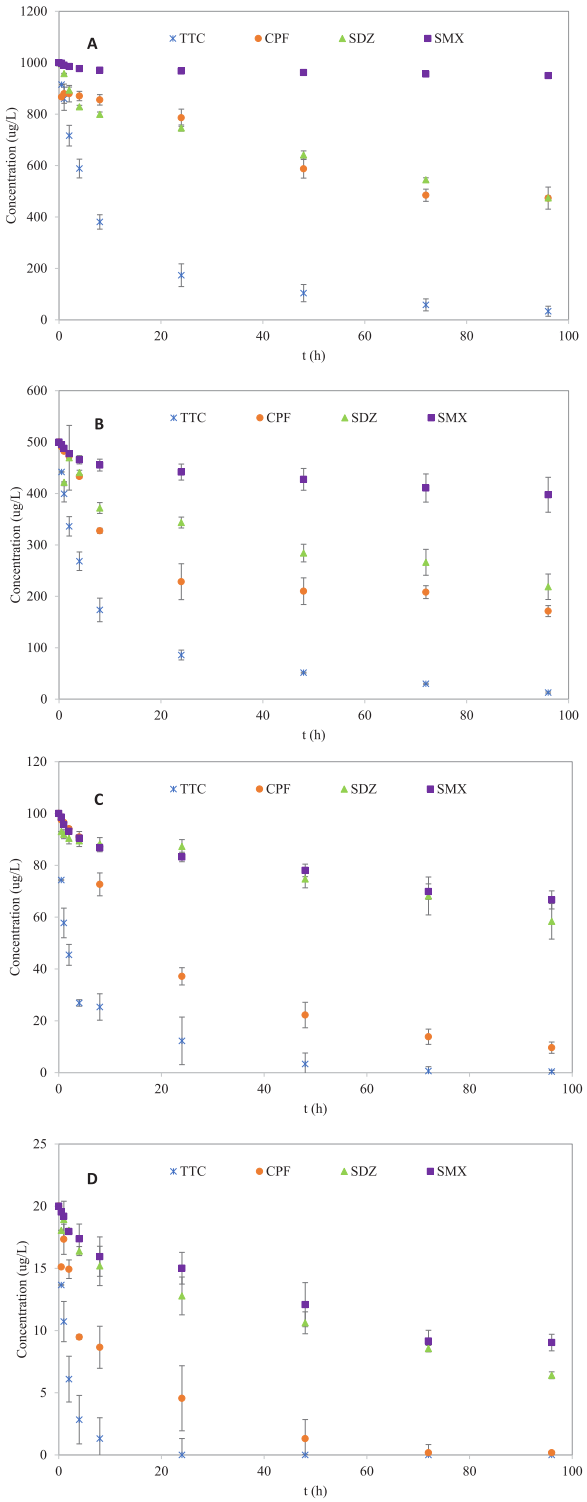

Fig. 1. VA removal using MBC (A) 1000, (B) 500, (C) 100 and (D) 20 μg L^{-1} initial concentration. Each data point represents the mean and standard deviation from triplicate assays.

is highly dependent on the antibiotic classes, as was indicated by other authors (Leng et al., 2020). In all the conditions studied, only TTC was completely removed.

In order to understand the mechanisms of VA removal, hydrolysis, photolysis and biodegradation were studied. As biosorption was expected to be the most important removal process, biosorption data was reported separately in a

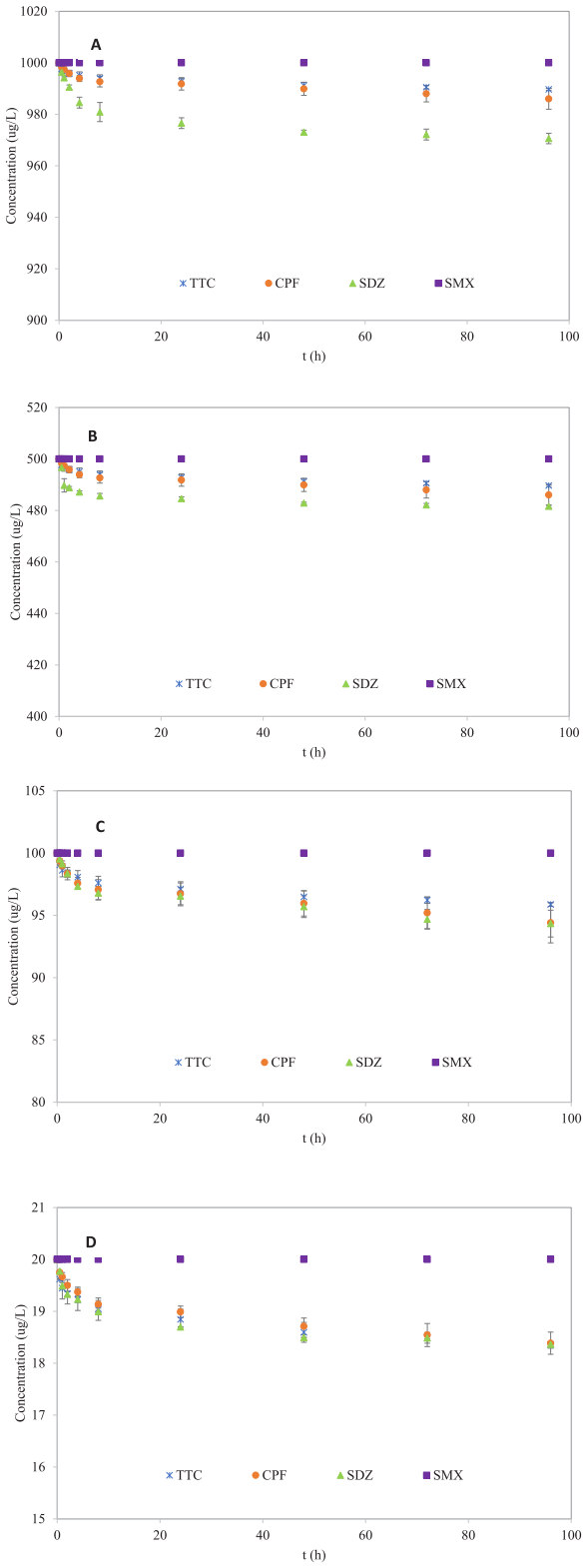

Fig. 2. VA removal by hydrolysis (A) 1000, (B) 500, (C) 100 and (D) 20 μg L^{-1} initial concentration. Each data point represents the mean and standard deviation from triplicate assays.

previous study (Zambrano et al., 2021). Data showed biosorption as the main removal process for CPF and TTC; however, it was not significant for SDZ and SMX removal.

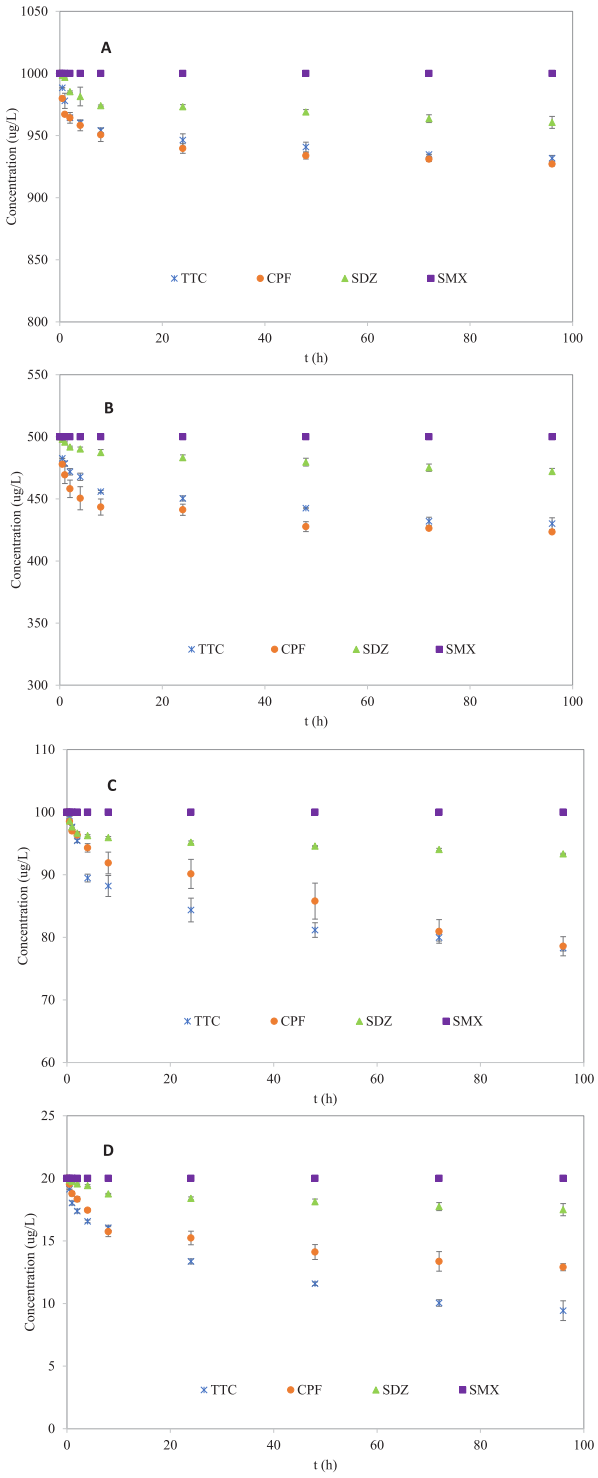

Fig. 3. VA removal by photolysis (A) 1000, (B) 500, (C) 100 and (D) 20 μg L^{-1} initial concentration. Each data point represents the mean and standard deviation from triplicate assays.

Batch experiments without algae under dark conditions indicated that antibiotic hydrolysis took place (Fig. 2(A)–(D)). The initial concentration decreased over time for three antibiotics, except for SMX which was not removed by hydrolysis in any of the studied conditions.

Antibiotic photolysis was studied in batch experiments without algae under illuminated conditions (Fig. 3(A)–(D)). SMX was not removed by photolysis in any of the studied conditions. For the other three antibiotics, a decrease in the

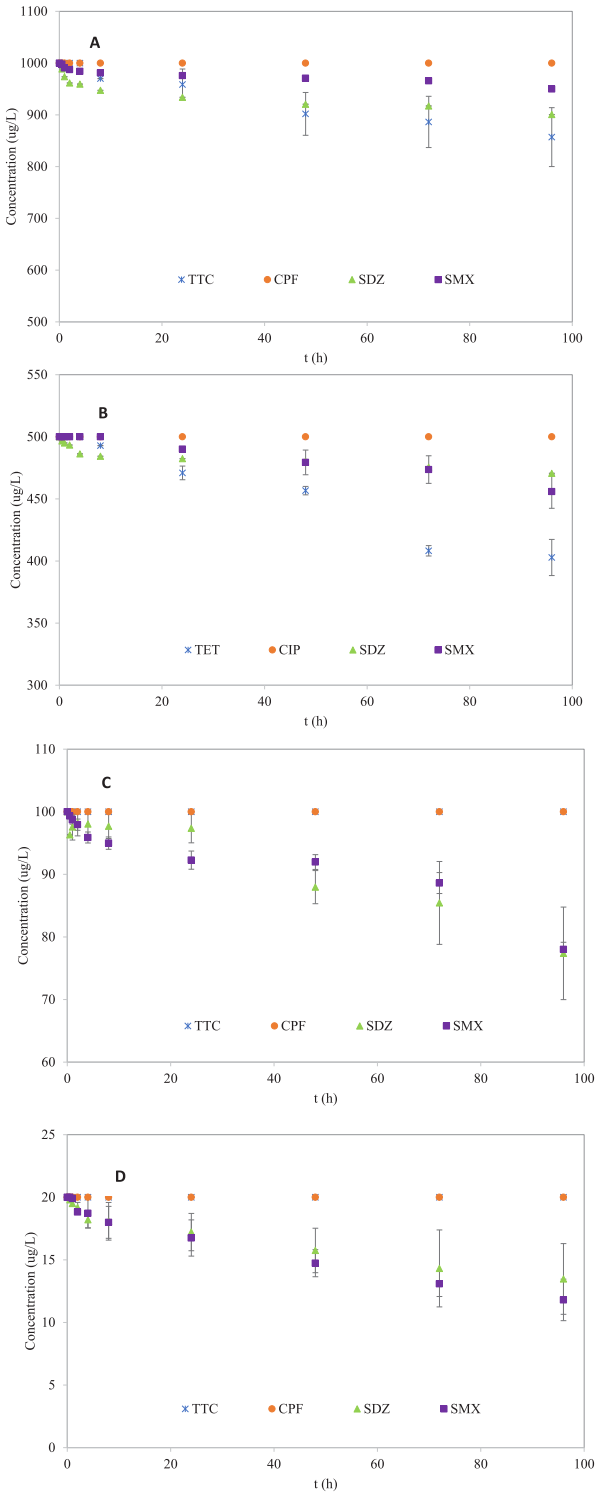

Fig. 4. VA removal by biodegradation (A) 1000, (B) 500, (C) 100 and (D) 20 μg L^{-1} initial concentration. Each data point represents the mean and standard deviation from triplicate assays.

initial concentration over time was more prominent for 20 μg/L. Likewise, Kiki et al. (2020) observed a higher removal of antibiotics at 20 μg/L for sulfonamides, obtaining a 5 to 8% mean removal by photodegradation in 20, 50, and 100 μg/L over 40 days of operation. Photolysis turned out to be an important process for antibiotic degradation for TTC and CPF at

low concentrations. Similarly, Niu et al. (2013) concluded that the lower the initial concentration of TTC, the higher the photolysis efficiency.

VA biodegradation was also studied in batch experiments for 96 h (Fig. 4(A)–(D)). CPF did not biodegrade under any of the studied conditions. From the four antibiotics, SMX had the highest biodegradation capacity, possible due to a good adaptation of *S. almeriensis* to this antibiotic because other algae of the genus *Scenedesmus*, such as *Scenedesmus obliquus*, have been shown to have an efficient adaptation mechanism for SMX (Leng et al., 2020). In the following sections, the kinetics of the removal of VA using a MBC and each of the removal modes involved in this process will be determined.

3.2. Kinetics for the removal of VA using MBC

The removal of TTC, CPF, SDZ and SMX was investigated with zero-order and pseudo-first order kinetic models (the latter being both an irreversible and a reversible reaction) (Table S3). First, the experimental data was adjusted to a zero-order kinetic by drawing the concentration data against time, as shown in Eq. (4). When the kinetic is a zero-order, the experimental data fit to a straight line with the intersection point at the origin C_0 and slope $-k_Z$, which was the case for all the VA analyzed in the present study for the three mechanisms (hydrolysis, photolysis and biodegradation). However, when analyzing the whole process of VA removal using MBC, the experimental data did not fit a zero-order kinetic. In this respect, a pseudo-first order irreversible kinetic was investigated by transforming Eq. (5) to obtain Eq. (7):

$$-\ln\left(\frac{C_t}{C_0}\right) = k_I \cdot t \tag{7}$$

the representation of $-\ln(C_t/C_0)$ against t will fit to a straight line with intersection point at the origin and slope k_I when the kinetics is a pseudo-first order irreversible. Additionally, by converting Eq. (6), Eq. (8) is obtained for a pseudo-first order reversible reaction:

$$-\ln\left[\frac{C_t - C_e}{C_0 - C_e}\right] = k_R \cdot t \tag{8}$$

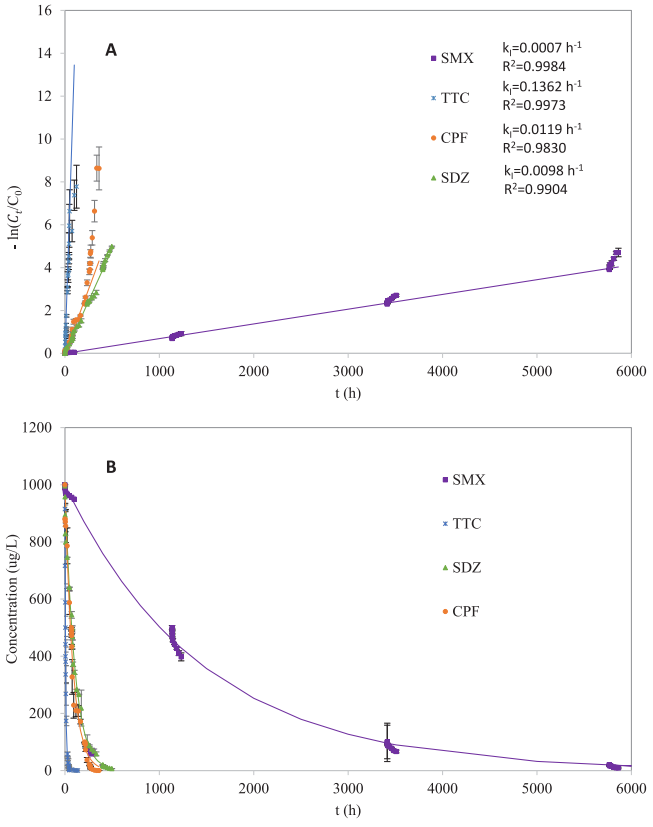

Fig. 5. (A) Linear representation of pseudo-first order kinetics for VA removal using MBC. (B) VA concentration variation using MBC along time. Each data point represents the mean and standard deviation from triplicate assays.

the representation of $-\ln[(C_t - C_e)/(C_0 - C_e)]$ against t will fit to a straight line with intersection point at the origin and slope k_R when the kinetic is a pseudo-first order reversible. The parameters k_2 and C_e were obtained by the Generalized Reduced Gradient (GRG) Nonlinear Solving method. Fitting the experimental data to a pseudo-first order reversible kinetic, the C_e obtained was 0, what meant that the kinetic model with the best fit was the pseudo-first order irreversible model. The coefficient of determination (R^2) was used to decide if the experimental data fit the best to a zero-order, pseudo-first order irreversible or pseudo-first order reversible model. R^2 values closer to unity were presented by the pseudo-first order irreversible kinetic model. This model fit the best to the experimental data for the whole process of VA removal via the MBC as well as for hydrolysis, photolysis and biodegradation (Fig. S2.). In the whole process of VA removal (Fig. 5) it was observed that TTC, CPF and SDZ were removed faster than SMX. The highest rate constant was presented by TTC. The pseudo-first order irreversible rate constant for the removal of VA by the MBC were 0.1362 h^{-1} for TTC, 0.0119 h^{-1} for CPF, 0.0098 h^{-1} for SDZ and 0.0007 h^{-1} for SMX. There are scarce studies on the reaction rates for the removal of VA using a MBC. Moreover, the literature found and used in the following discussion worked with just one VA and not with a mixture of different VA, which might have an influence on the results. Slower reaction rates were reported by Xiong et al. (2017) who obtained a k value of 0.0005 h^{-1} for 2 mg/L CPF removal using *C. Mexicana* and Xie et al. (2020) who worked with *Chlamydomonas sp.* for the removal of 10 mg/L SDZ and reported a k value of 0.004 h^{-1}. The higher removal rate obtained by the present study might be due to the use of *S. almeriensis*, because the VA removal rate is highly dependent on the algae specie (Leng et al., 2020). In the case of CPF, Xie et al. (2020) reported a higher k value of 0.028 h^{-1} which might be due to the use of synthetic wastewater. The presence of ions and cations in water can improve the efficiency of VA degradation (Eskandarloo et al., 2014; Loftin et al., 2008).

The pseudo first-order irreversible rate constant k for the removal of VA by hydrolysis (Fig. 6) were 0.0001 h^{-1} for TTC, 0.0002 h^{-1} for CPF and 0.0004 h^{-1} for SDZ. Higher reaction rates were found in the literature. De Godos et al. (2012), for the removal of 2 mg/l TTC by hydrolysis, obtained a k value of 0.002 h^{-1}, and Hom-Diaz et al. (2017) reported a k value of 0.0011 h^{-1} for hydrolysis of 2 mg/l CPF. The higher rate constants obtained by De Godos et al. (2012) and Hom-Diaz et al. (2017) might be due to the use of synthetic wastewater and domestic wastewater, respectively. The presence of salts in water can have a catalytic effect in the hydrolysis of VA (Loftin et al., 2008; Mabey and Mill, 1978).

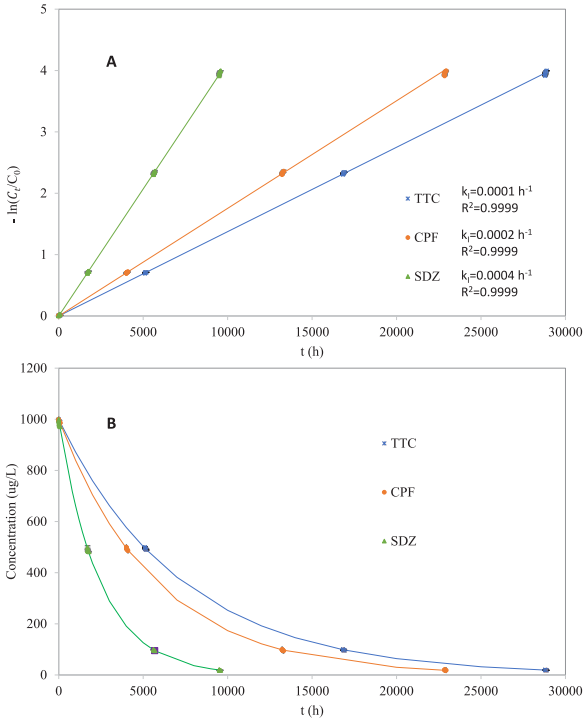

Fig. 6. (A) Linear representation of pseudo-first order kinetics for VA removal by hydrolysis. (B) VA concentration variation by hydrolysis along time. Each data point represents the mean and standard deviation from triplicate assays.

The pseudo first-order irreversible rate constant k for the removal of VA by photolysis (Fig. 7) were 0.0011 h^{-1} for TTC, 0.0012 h^{-1} for CPF and 0.0005 h^{-1} for SDZ. Xie et al. (2020) reported a similar k value for CPF removal of 0.0015 h^{-1} and a higher k value for SDZ of 0.002 h^{-1}. De Godos et al. (2012) reported a higher k value of 0.038 h^{-1} for the photodegradation of TTC. Bai and Acharya (2017) established a k value of 0.054 h^{-1} for the removal of 10 μg/L CPF. The higher rate constants obtained by Xie et al. (2020), De Godos et al. (2012), and Bai and Acharya (2017), could be because they used synthetic wastewater and lake water. The presence of ions in water can improve the efficiency of VA degradation due to the

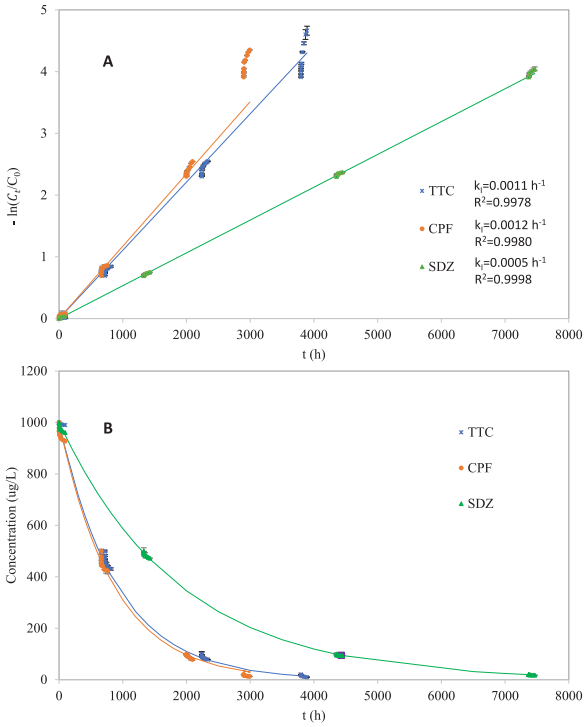

Fig. 7. (A) Linear representation of pseudo-first order kinetics for VA removal by photolysis. (B) VA concentration variation by photolysis along time. Each data point represents the mean and standard deviation from triplicate assays.

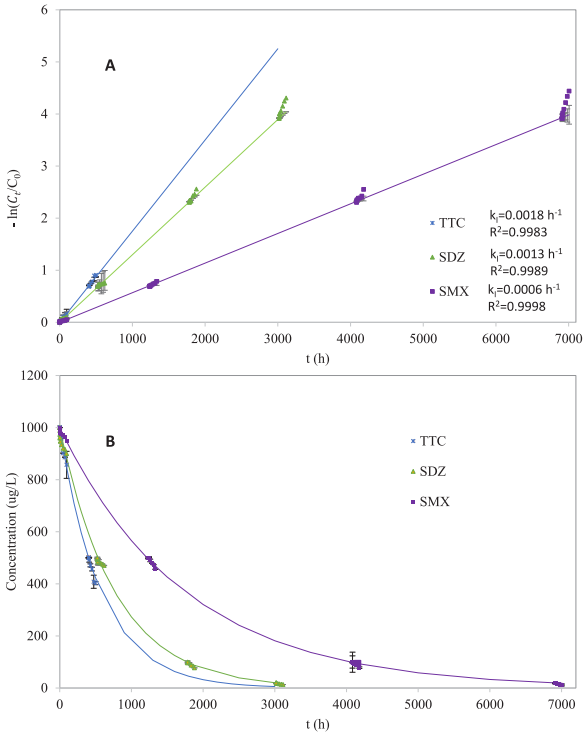

Fig. 8. (A) Linear representation of pseudo-first order kinetics for VA removal by biodegradation. (B) VA concentration variation by biodegradation along time. Each data point represents the mean and standard deviation from triplicate assays.

Table 1
Efficiency for the different removal mechanisms of veterinary antibiotics in synthetic wastewater using microalgae–bacteria consortia (4 day hydraulic retention time).

Compound	Removal (%)				
	Global process	Hydrolysis	Photolysis	Biodegradation	Biosorption[a]
Tetracycline	99.93	1.03	6.83	14.29	74.61–81.53
Ciprofloxacin	78.01	1.39	7.29	0	43.45–100
Sulfadiazine	52.58	2.94	3.94	9.94	11.62–30.35
Sulfamethoxazole	5.01	0	0	5.01	0–31.48

[a]Data reported in a previous study (Zambrano et al., 2021).

generation of highly reactive radical species (Eskandarloo et al., 2014; Mirzaei et al., 2016). Norvill et al. (2017) obtained a k value of 0.12 h^{-1} for the photodegradation of TTC in outdoor experiments using open-top plastic tanks, where direct sunlight, temperature and evaporation might have had an influence on the results (Wang et al., 2017). For the removal of 10 mg/L TTC, Niu et al. (2013) obtained k values of 0.3 to 1.2 h^{-1} using different light sources (solar, xenon and UV lamps) and light intensities from 6 to 19 mW/cm^2. In CPF's case, Hom-Diaz et al. (2017) reported a k value of 0.0355 h^{-1} for the photodegradation of 2 mg/l ciproofloxacin with a sunlight intensity of 0–90 mW/cm^2. Light has a significant effect on the photolytic rate constant depending on the wavelength and light intensity (Ahmed and Haider, 2018; Belver et al., 2020).

On the other hand, k values found for biodegradation (Fig. 8) were 0.0018 h^{-1} for TTC, 0.0026 h^{-1} for SDZ and 0.0006 h^{-1} for SMX. Similarly, low reaction rates for VA biodegradation was found by Xie et al. (2020) who reported a k value of 0.0008 h^{-1} for the biodegradation of 10 mg/L SDZ when working with *Chlamydomonas sp.* A comparison of the literature and the results presented in this study revealed that *S. almeriensis* showed a better affinity for SDZ removal than other algae specie.

In our previous work, biosorption kinetics were evaluated and a pseudo second-order kinetic model best fit the biosorption experimental data (Table S3). The results suggested that the rate constant increased with a decrease in the initial VA concentration and that CPF presented the highest k value (Zambrano et al., 2021).

3.3. Removal of VA using MBC

3.3.1. Global process

The removal of the four VA MBC is shown in Fig. 5B. The VA results are consistent with the ones previously reported in the literature (Table S4). In the present study, TTC removal was complete after 5.7 days. Using a High Rate Algae Process (HRAP) with an initial antibiotic concentration of 100 μg/L, Norvill et al. (2017) achieved 93% and 99% TTC removal after 4 and 7 days operation, respectively. De Godos et al. (2012) fed an HRAP with 2 mg/L TTC. After 62 days of operation, TTC removal stabilized to around 69%. The efficiency differences between these studies might be associated to the water sources used in the experiments. De Godos et al. (2012) used livestock wastewater, Norvill et al. (2017) worked with domestic wastewater, and the present study was done in Milli-Q water. Solids and ions present in the water could enhance or interfere in biosorption and photolysis which turned out to be important mechanisms in TTC removal.

Current results showed that CPF removal was complete after 15 days. Bai and Acharya (2017), working with *Nannochloris sp.*, reported complete removal of CPF after 14 days; and Xie et al. (2020) obtained complete CPF removal using *Chlamydomonas sp.* after 9 days. Xiong et al. (2017) obtained 13% removal of 2 mg/L CPF by using *C. mexicana* after 11 days; and Gentili and Fick (2017) obtained removals of 11% for CPF after 7 days using a mixed population of wild freshwater green algal species.

In the case of SDZ, removal was 88.3 and 92.5% after 9 and 12 days, respectively. Peng et al. (2020) studied SDZ removal using *C. vulgaris* and obtained removals of 32.1% in a batch reactor after 12 days. Xie et al. (2020) investigated the removal of SDZ using *Chlamydomonas sp.*, obtaining 54.5% removal after 9 days. Finally, SMX removal was 10.9, 16.6 and 20.6% after 7, 11 and 14 days, respectively. Bai and Acharya (2017) worked with *Nannochloris sp.*, and after 14 days reported that >60% of the applied dose of SMX remained in the water. Gentili and Fick (2017) obtained a removal of 6% for SMX after 7 days using a mixed population of wild freshwater green algal species. Xiong et al. (2017), by using *S. obliquus,* removed 15.7 to 17.3% of 0.05 to 1 mg/L SMX after 11 days.

The comparison of the results obtained in the present study with those previously reported in the literature showed that the VA removal efficiency is highly dependent on the algae strain used for the water treatment process and that the *S. almeriensis* algae–bacteria consortium presented good removal efficiencies.

Hydrolysis, photolysis and biodegradation will be described in the following sections. For comparison purposes, removal efficiencies are reported in Table 1.

3.3.2. Biosorption

Biosorption data was reported in our previous study (Zambrano et al., 2021), where CPF and TTC exhibited the highest removal efficiencies. Removal efficiencies of biosorption ranged between 74.6–81.5% for TTC, 43.5%–100% for CPF, 11.6–30.4% for SDZ, and 0–31.5% for SMX.

3.3.3. Hydrolysis

The removal of the four VA by hydrolysis is shown in Fig. 6B. SMX was not removed by hydrolysis. Using the kinetic parameters obtained in the previous section, it can be estimated that the complete removal of TTC, CPF and SDZ using only hydrolysis could be achieved after 1204, 956 and 400 days, respectively. In view of these results, and due to the hydrophobic characteristics of the four antibiotics analyzed in this study, hydrolysis is not representative in VA removal.

Moreover, Niu et al. (2013) obtained a removal of less than 5% of TTC by hydrolysis after 60 min and determined that it can be neglected. Also, studying the degradation of TTC, Norvill et al. (2017) and De Godos et al. (2012) reported that hydrolysis was not significant. As well, in the case of CPF, Xiong et al. (2017), Bai and Acharya (2017), and Hom-Diaz et al. (2017) did not observe any removal via hydrolysis. Similarly, after 9 days of hydrolysis, Xie et al. (2020) only obtained CPF and SDZ removals of 2.4 and 1.3%, respectively. For the removal of SMX by hydrolysis, Xiong et al. (2019) and Bai and Acharya (2017, 2016) observed less than 2% of removal after 11 days or no hydrolysis. After a literature review, Leng et al. (2020) determined that some antibiotics, such as fluoroquinolones and sulphonamides, were resistant to hydrolysis.

3.3.4. Photolysis

The removal of the four VA using a MBC by photolysis is shown in Fig. 7B. The complete removal of TTC, CPF and SDZ can be achieved after 162, 125, and 311 days, respectively, while SMX was not removed by photolysis.

For comparison purposes with the literature, TTC removal after 43 h was 0.9%; CPF removal was 17.7 and 22.2% after 7 and 9 days, respectively; and SDZ removal after 9 days was 10.85%.

De Godos et al. (2012) obtained 81% removal of 2 mg/L TTC by photolysis after 43 h. Bai and Acharya (2017) achieved the complete removal of 10 μg/L CPF after 7 days. Moreover, Hom-Diaz et al. (2017) observed 97.3% removal by photolysis from 2 mg/L CPF after 48 h. The higher removal efficiencies obtained by De Godos et al. (2012), Bai and Acharya (2017), and Hom-Diaz et al. (2017) could be because they worked with synthetic wastewater, lake water and domestic wastewater, respectively. Bai and Acharya (2017) determined that a photolysis reaction occurred to a greater extent using lake water compared with using pure water, which suggests that the process was enhanced by natural organic matter or other ingredients in the lake water such as nitrate (Bai and Acharya, 2017). However, after 9 days, Xie et al. (2020) obtained CPF and SDZ removals by photolysis of 27.1 and 35.6%, respectively. Xie et al. (2020) worked with synthetic wastewater and found that as nitrate was consumed, the photolysis rate of CPF and SDZ decreased, demonstrating that the photolysis of nitrate in the solution may lead to the formation of hydroxyl radicals (·OH) which are powerful oxidants (Xie et al., 2020). As in the present study, Bai and Acharya (2017, 2016) observed that SMX is resistant to light exposure, so no removal by photolysis was observed.

3.3.5. Biodegradation

The removal of the four veterinary antibiotics using a microalgae–bacteria consortium by biodegradation is shown in Fig. 8B. The complete removal of TTC, SDZ and SMX can be achieved after 125, 31 and 292 days, respectively. CPF was not removed by biodegradation.

For comparison purposes with the literature, SDZ removal was 24.4% after 9 days. SMX removal after 12 and 14 days were 15.1 and 17.4%, respectively.

Research on VA biodegradation using a microalgae–bacteria consortium is limited, so the results found in the literature are few and uneven. De Godos et al. (2012) reported the absence of biodegradation for TTC at a concentration of 2 mg/L using *C. vulgaris*. Xiong et al. (2017) studied the removal of 2 mg/L CPF using *C.mexicana, C. pitchmannii, C. vulgaris,* and *O. multisporus,* obtaining removals of 13, 1.6, 0 and 2%, respectively, using biotic factors (biodegradation, bioaccumulation, and biosorption) after 11 days. Moreover, Hom-Diaz et al. (2017) achieved a removal of 36.7% using biotic factors of 2 mg/L CPF by using a MBC from an HRAP after 48 h. Additionally, Xie et al. (2020) obtained CPF and SDZ biodegradation of 65.1 and 17.1%, respectively, after 9 days. Xiong et al. (2019) observed removals from 15.7 to 17.3% for 0.05 to 1 mg/L SMX removal by biotic factors after 11 days. Likewise, Bai and Acharya (2016) after 14 days, working with *Nannochloris sp*, reported removals of 32 to 37% of 10 μg/L SMX using a algae mediated process. A comparison of the results presented in the literature with the ones obtained in the present study showed the different efficiencies that can be achieved while working with different algae species. Moreover, the conclusion to be drawn from this comparison is that much more research is needed in this regard. The literature cited in this section does not exclusively consider elimination by biodegradation. The experiments performed were mainly based on the presence or absence of microalgae in the media. That is why the authors referred to the antibiotic elimination when microalgae are present in the media as removal by biotic factors or algae mediated processes, which means that the authors attribute the removal to biodegradation, bioaccumulation, biosorption or a combination of the three.

4. Conclusions

To our better knowledge for the first time, the mechanisms and rate constants for the removal of TTC, CPF, SDZ, and SMX from synthetic wastewater using a Scenedesmus almeriensis microalgae–bacteria consortium were analyzed. Using a pH of 8 and low concentrations of the four VA that resembled real water samples, the MBC was highly effective at removing the mixture of VA. The overall removal of the VA followed an irreversible pseudo-first order kinetic, with biosorption being the most important removal process for all four antibiotics and hydrolysis being insignificant. Hydrolysis

and photolysis took place in the removal of the VA except for SMX. Biodegradation was an important mechanism for TTC, SDZ and SMX but was not present in CPF removal. Further research should focus on optimizing operational parameters to improve photolysis and biodegradation and increase VA degradation, as well as studying byproduct formation to make the microalgae-based technology for VA removal more practical at an industrial scale.

CRediT authorship contribution statement

Johanna Zambrano: Conceptualization, Methodology, Investigation, Data curation, Formal analysis, Visualization, Writing – original draft. **Pedro Antonio García-Encina:** Funding acquisition, Resources, Supervision, Writing – review & editing. **Félix Hernández:** Methodology, Investigation, Data curation. **Ana M. Botero-Coy:** Methodology, Investigation, Data curation. **Juan J. Jiménez:** Methodology, Supervision, Writing – review & editing. **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 Figures and tables related to this article can be found online at https://doi.org/10.1016/j.eti.2023.103031.

References

Ahmadpour, N., Sayadi, M.H., Nowrouzi, M., 2021. Recent Nano-Based Technologies in the Removal of Pharmaceutical Compounds from Aquatic Environment, Vol. 1. Austin Publ. Gr., pp. 49–70.

Ahmed, S.N., Haider, W., 2018. Heterogeneous photocatalysis and its potential applications in water and wastewater treatment: A review. Nanotechnology 29, 342001. http://dx.doi.org/10.1088/1361-6528/aac6ea.

Ahmed, S.F., Mofijur, M., Nuzhat, S., Chowdhury, A.T., Rafa, N., Uddin, A., Inayat, A., Mahlia, T.M.I., Chyuan Ong, H., Chia, W.Y., Loke Show, P., 2021. Recent developments in physical, biological, chemical, and hybrid treatment techniques for removing emerging contaminants from wastewater. http://dx.doi.org/10.1016/j.jhazmat.2021.125912.

Ahmed, M.B., Zhou, J.L., Ngo, H.H., Guo, W., Thomaidis, N.S., Xu, J., 2017. Progress in the biological and chemical treatment technologies for emerging contaminant removal from wastewater: A critical review. J. Hazard. Mater. 323, 274–298. http://dx.doi.org/10.1016/j.jhazmat.2016.04.045.

Azari, A., Nabizadeh, R., Mahvi, A.H., Nasseri, S., 2021. Magnetic multi-walled carbon nanotubes-loaded alginate for treatment of industrial dye manufacturing effluent: adsorption modelling and process optimisation by central composite face-central design. Int. J. Environ. Anal. Chem. 00, 1–21. http://dx.doi.org/10.1080/03067319.2021.1877279.

Badi, M.Y., Esrafili, A., Pasalari, H., Kalantary, R.R., Ahmadi, E., Gholami, M., Azari, A., 2019. Degradation of dimethyl phthalate using persulfate activated by UV and ferrous ions: Optimizing operational parameters mechanism and pathway. J. Environ. Heal. Sci. Eng. 17, 685–700. http://dx.doi.org/10.1007/s40201-019-00384-9.

Bai, X., Acharya, K., 2016. Removal of trimethoprim, sulfamethoxazole, and triclosan by the green alga *Nannochloris* sp.. J. Hazard. Mater. 315, 70–75. http://dx.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. http://dx.doi.org/10.1016/j.scitotenv.2016.12.192.

Belver, C., Bedia, J., Peñas-Garzón, M., Muelas-Ramos, V., Gómez-Avilés, A., Rodriguez, J.J., 2020. Structured photocatalysts for the removal of emerging contaminants under visible or solar light. In: Visible Light Active Structured Photocatalysts for the Removal of Emerging Contaminants. Elsevier, pp. 41–98. http://dx.doi.org/10.1016/b978-0-12-818334-2.00003-1.

Bijlsma, L., Pitarch, E., Fonseca, E., Ibáñez, M., Botero, A.M., Claros, J., Pastor, L., Hernández, F., 2021. Investigation of pharmaceuticals in a conventional wastewater treatment plant: Removal efficiency, seasonal variation and impact of a nearby hospital. J. Environ. Chem. Eng. 9, http://dx.doi.org/10.1016/j.jece.2021.105548.

Botero-Coy, A.M., Martínez-Pachón, D., Boix, C., Rincón, R.J., Castillo, N., Arias-Marín, L.P., Manrique-Losada, L., Torres-Palma, R., Moncayo-Lasso, A., Hernández, F., 2018. An investigation into the occurrence and removal of pharmaceuticals in Colombian wastewater. Sci. Total Environ. 642, 842–853. http://dx.doi.org/10.1016/j.scitotenv.2018.06.088.

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.
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.
Eskandarloo, H., Badiei, A., Behnajady, M.A., 2014. Study of the effect of additives on the photocatalytic degradation of a triphenylmethane dye in the presence of immobilized TiO2/NiO nanoparticles: Artificial neural network modeling. Ind. Eng. Chem. Res. 53, 6881–6895. http://dx.doi.org/10.1021/ie500253q.
Gentili, F.G., Fick, J., 2017. Algal cultivation in urban wastewater: an efficient way to reduce pharmaceutical pollutants. J. Appl. Phycol. 29, 255–262. http://dx.doi.org/10.1007/s10811-016-0950-0.
Gojkovic, Z., Lindberg, R.H., Tysklind, M., Funk, C., 2019. Northern green algae have the capacity to remove active pharmaceutical ingredients. Ecotoxicol. Environ. Saf. 170, 644–656. http://dx.doi.org/10.1016/j.ecoenv.2018.12.032.
Hashemi, S.Y., Badi, M.Yegane., Pasalari, H., Azari, A., Arfaeinia, H., Kiani, A., 2020. Degradation of Ceftriaxone from aquatic solution using a heterogeneous and reusable O3/UV/ Fe3O4@TiO2 systems: operational factors, kinetics and mineralisation. Int. J. Environ. Anal. Chem. 00, 1–17. http://dx.doi.org/10.1080/03067319.2020.1817909.
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.
Jaafari, J., Ghozikali, M.G., Azari, A., Delkhosh, M.B., Javid, A.B., Mohammadi, A.A., Agarwal, S., Gupta, V.K., Sillanpää, M., Tkachev, A.G., Burakov, A.E., 2018. Adsorption of p-Cresol on Al2O3 coated multi-walled carbon nanotubes: Response surface methodology and isotherm study. J. Ind. Eng. Chem. 57, 396–404. http://dx.doi.org/10.1016/j.jiec.2017.08.048.
Khalatbary, M., Sayadi, M.H., Hajiani, M., Nowrouzi, M., Homaeigohar, S., 2022. Green, sustainable synthesis of γ-Fe2O3/MWCNT/Ag nano-composites using the viscum album leaf extract and waste car tire for removal of sulfamethazine and bacteria from wastewater streams. Nanomaterials 12, http://dx.doi.org/10.3390/nano12162798.
Kiki, C., Rashid, A., Wang, Y., Li, Y., Zeng, Q., Yu, C.P., Sun, Q., 2020. Dissipation of antibiotics by microalgae: Kinetics, identification of transformation products and pathways. J. Hazard. Mater. 387, http://dx.doi.org/10.1016/j.jhazmat.2019.121985.
Lefoulon, A., Beelen, V., Coolsaet, C., Sonnenholzner, S., Van Den Hende, S., Julien, L., Vervaeren, H., Rousseau, D.P.L., Vanhoucke, T., 2016. Technical potential of microalgal bacterial floc raceway ponds treating food-industry effluents while producing microalgal bacterial biomass: An outdoor pilot-scale study. Bioresour. Technol. http://dx.doi.org/10.1016/j.biortech.2016.07.065.
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.
Loftin, K.A., Adams, C.D., Meyer, M.T., Surampalli, R., 2008. Effects of ionic strength, temperature, and pH on degradation of selected antibiotics. J. Environ. Qual. 37, 378–386. http://dx.doi.org/10.2134/jeq2007.0230.
López-Serna, R., Posadas, E., García-Encina, P.A., Muñoz, R., 2019. Removal of contaminants of emerging concern from urban wastewater in novel algal–bacterial photobioreactors. Sci. Total Environ. http://dx.doi.org/10.1016/j.scitotenv.2019.01.206.
Mabey, W., Mill, T., 1978. Under environmental conditions contents-continued. J. Phys. Ref. Data 7, 383–415.
Matamoros, V., Gutiérrez, R., Ferrer, I., García, J., Bayona, J.M., 2015. Capability of microalgae-based wastewater treatment systems to remove emerging organic contaminants: A pilot-scale study. J. Hazard. Mater. http://dx.doi.org/10.1016/j.jhazmat.2015.02.002.
Mirzaei, A., Chen, Z., Haghighat, F., Yerushalmi, L., 2016. Removal of pharmaceuticals and endocrine disrupting compounds from water by zinc oxide-based photocatalytic degradation: A review. Sustain. Cities Soc. 27, 407–418. http://dx.doi.org/10.1016/j.scs.2016.08.004.
Morillas-España, A., Lafarga, T., Gómez-Serrano, C., Acién-Fernández, F.G., González-López, C.V., 2020. Year-long production of *Scenedesmus almeriensis* in pilot-scale raceway and thin-layer cascade photobioreactors. Algal Res. 51, 102069. http://dx.doi.org/10.1016/j.algal.2020.102069.
Niu, J., Li, Y., Wang, W., 2013. Light-source-dependent role of nitrate and humic acid in tetracycline photolysis: Kinetics and mechanism. Chemosphere 92, 1423–1429. http://dx.doi.org/10.1016/j.chemosphere.2013.03.049.
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.
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.
Rodriguez-Narvaez, O.M., Peralta-Hernandez, J.M., Goonetilleke, A., Bandala, E.R., 2017. Treatment technologies for emerging contaminants in water: A review. Chem. Eng. J. 323, 361–380. http://dx.doi.org/10.1016/j.cej.2017.04.106.
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.
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. http://dx.doi.org/10.1007/s11157-017-9446-x.
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, J.Q., Kim, S.J., Kurade, M.B., Govindwar, S., Abou-Shanab, R.A.I., Kim, J.R., Roh, H.S., Khan, M.A., Jeon, B.H., 2019. Combined effects of sulfamethazine and sulfamethoxazole on a freshwater microalga, *Scenedesmus obliquus*: toxicity, biodegradation, and metabolic fate. J. Hazard. Mater. 370, 138–146. http://dx.doi.org/10.1016/j.jhazmat.2018.07.049.
Xiong, J.Q., Kurade, M.B., Kim, J.R., Roh, H.S., Jeon, B.H., 2017. Ciprofloxacin toxicity and its co-metabolic removal by a freshwater microalga *Chlamydomonas* mexicana. J. Hazard. Mater. 323, 212–219. http://dx.doi.org/10.1016/j.jhazmat.2016.04.073.
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. http://dx.doi.org/10.1016/j.jwpe.2021.102226.
Zazouli, M.A., Azari, A., Dehghan, S., Malekkolae, R.S., 2016. Adsorption of methylene blue from aqueous solution onto activated carbons developed from eucalyptus bark and Crataegus oxyacantha core. Water Sci. Technol. 74, 2021–2035. http://dx.doi.org/10.2166/wst.2016.287.