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Removal of a mixture of veterinary medicinal products by adsorption onto a *Scenedesmus almeriensis* microalgae-bacteria consortium

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ABSTRACT

The adsorption of the veterinary medicinal products (VMP) tetracycline (TET), ciprofloxacin (CIP), sulfadiazine (SDZ) and sulfamethoxazole (SMX) onto a dried *Scenedesmus almeriensis* microalgae-bacteria consortium was studied at several equilibrium concentrations (20 to 1000 μg/L). Scanning electron microscopy (SEM) and Fourier-transform infrared spectroscopy (FTIR) analysis were performed to investigate the surface morphology of the microalgae and to identify the effect of the antibiotics´ functional groups on the surface of the consortium of *S. almeriensis* and bacteria. Ultra-high performance liquid chromatography tandem mass spectrometry (UHPLC-MS/MS) was used to determine the feasibility of this consortium for the removal of antibiotics via biosorption. Freundlich and Langmuir adsorption models were used for the mathematical description of the adsorption equilibrium. Pseudo-first order and pseudo-second order kinetic models were applied to fit the biosorption experimental data. Relative antibiotic removal was higher at low equilibrium concentrations. In the range of the initial VMP concentration studied, ciprofloxacin and tetracycline exhibited the highest removal efficiency of 43–100% and 75–82%, respectively. Likewise, ciprofloxacin and tetraciclyne presented the highest adsorption rates of 0.11–26.66 and 1.78–27.09 $mg \cdot \mu g^{-1} \cdot h^{-1}$, respectively. This study revealed that the *S. almeriensis*-bacteria consortium has a high biosorption power and proved that biosorption is an important mechanism in the removal of ciprofloxacin and tetraciclyne using a microalgae-based water treatment process. However, sulfadiazine and sulfamethoxazole removals did not exceed 32%.

1. Introduction

Demand for veterinary antibiotics has increased significantly in the last decades. The global total consumption in 2017 was estimated to be 93,309 tons and it is expected to increase by 11.5% by 2030 [1]. Fluoroquinolones, sulfonamides and tetracycline were the most abundant VMP found in water in the United States in 2017 [2] and were the most sold VMP in European countries in 2018 [3]. Moreover, the most frequently found VMP in manure worldwide were fluoroquinolones, sulfonamides and tetracyclines [4], a determination which was reinforced by a critical review of Cheng et al. [5], who stated that these antibiotic families were the most common VMP used in swine production. About 70 to 90% of antibiotics administered are excreted via urine or feces either in unchanged forms or as metabolites. Via sludge storage or by using manure as fertilizer, veterinary antibiotics are released into the environment, which affects soil and water quality. Antibiotics have been detected in different water samples, such as surface water, groundwater and municipal wastewater [4–6].

Various techniques, such as adsorption, coagulation, electrolysis, membrane separation, photocatalysis, coagulation, advanced oxidation and biological degradation have been used for antibiotic removal. These methodologies have various limitations, such as high energy consumption, high materials costs, and secondary pollution through the use of additional chemicals. However, among these, adsorption is the most versatile and widely used due to its high efficiency, high removal capacity, simplicity of design and ease of operation [7–9]. In this respect,

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Table 1
S. almeriensis characteristics.

Appearance	Cell size	Diameter: 3 μm Large: 6 μm	[45]
	Morphotype	Oval	
	Disposition	Clusters of six to ten individuals	
	Color	Intense green	
Elemental analysis	Carbon	36.5–47.9%	[25,45,46]
	Oxygen	34.5–52.2%	
	Hydrogen	5.3–6.6%	
	Nitrogen	5.7–7.9%	
	Sulphur	0.0–0.8%	
Major components	Proteins	44.2–49.4%	[45,46]
	Carbohydrates	24.6–25.2%	
	Lipids	12.0–24.6%	
	Ash	2.0–19.4%	
Growing conditions	pH	7–10	[18]
	Temperature	26–40 °C	
	Salinity	0–5 g NaCl/L	
	Photoinhibition	No signs up to 1625 μE/m^2	

biosorption has emerged as an eco-friendly, effective and economically feasible process for the removal of antibiotics, and is based on the properties of different kinds of live and inactive dead biomasses (heat, dried, chemically treated) to bind and concentrate pollutants from aqueous solutions [9,10].

Moreover, regarding biological processes, the fate of the antibiotics has been widely investigated, and results demonstrated that biosorption plays a critical role in antibiotic removal routes [11]. Microalgae-based wastewater treatment technologies have attracted researcher's attention, as these technologies have proved their ability to remove almost all types of emerging contaminants to some extent. Microalgae have demonstrated a biosorption capacity for antibiotics such as 7-amino cephalosporanic acid [12], cephalexin [13], tilmicosin [14], and ceftazidime [15], among others. Specifically, green algae have cellulosic on its cell wall which provide binding sites such as hydroxyl, carboxyl, amino and sulfhydryl, which play an essential role in the biosorption process [7,16,17]. Residual algal biomass have been proposed for certain uses such as biofertilizers and animal feed. However, its use might be discouraged without first knowing the concentration of some adsorbed VMP compounds.

Few studies have been published on antibiotic removal using microalgae at low concentrations, similar to those found in real samples. In addition, limited adsorption data is available for antibiotics. In order to broadly use microalgae in wastewater treatment applications, it is compulsory to determine the mechanisms involved in antibiotics removal by biomass. Biosorption is expected to be one of the predominant removal mechanisms. Hence, to determine the biosorption capacity of a microalgae-bacteria consortium, inactive dead biomass was used (for the first time, as far as we know) to disengage biosorption from biodegradation/bioassimilation. Additionally, it is easy to store and use for longer periods of time, it is not subject to toxicity limitations, and it does not need nutrient supplies. The aim of this study was to determine the biosorption power of a Scenedesmus almeriensis microalgae-bacteria consortium for a mixture of four antibiotics at different concentrations: tetracycline (TET), ciprofloxacin (CIP), sulfadiazine (SDZ) and sulfamethoxazole (SMX). These antibiotics were selected as representative compounds of three antibiotics families – fluoroquinolones, tetracyclines and sulfonamides – which have diverse physicochemical properties and are the VMP most frequently found in piggery wastewater samples [3–6]. Adsorption isotherms were obtained for the four antibiotics studied.

2. Materials and methods

2.1. Microalgae culture

Lyophilized biomass, mainly composed of a consortium of the microalgae *Scenedesmus almeriensis* and bacteria, was provided by the Department of Chemical Engineering of the University of Almería (Spain). *S. almeriensis* (Table 1) is a fast-growing and highly productive strain which easily adapts to stressful conditions [18]. The biomass was recovered from a high-rate algae pond (HRAP) used for piggery wastewater treatment with an operating volume of 11,800 L and a land surface of 80 m^2. The HRAP operational conditions were solar radiation within 1500–1600 μE/m^2 and temperature between 30 and 35 °C.

Lyophilization was used as the inactivation method of the microalgae bacteria consortium in order not to damage the cell structure, instead of using regular methods as the thermal or chemical inactivation (sodium azide). In this respect, by using dead biomass it is possible to focus solely on the study of biosorption, as mechanism of VMP removal from water, by avoiding biodegradation as no biological activity will be observed.

2.2. Chemicals and reagents

Tetracycline (TET), ciprofloxacin (CIP), sulfadiazine (SDZ) and sulfamethoxazole (SMX) were selected as target analytes. The standards for the drugs were of high purity grade (N95%) and purchased from Sigma-Aldrich (Tres Cantos, Madrid, Spain). All of them were acquired as neutral non-solvated molecules, except for tetracycline (hydrochloride). Individual stock solutions of 1 g/L for all the standards were prepared on a weight basis in methanol (MeOH), except for ciprofloxacin (which was dissolved in H_2O/MeOH (1:1)) containing 0.2% *v*/v hydrochloric acid (HCl) due to its low solubility in pure MeOH. Mixture stock solutions were subsequently prepared from individual stocks and stored at −80 °C in darkness to avoid uncontrolled degradation.

Ultrapure water was generated in-house by a Milli-Q (MQ) Advantage Ultrapure Water purification system and filtered through a 0.22 μm Millipak Express membrane and an LC-Pak polishing unit by Merck Millipore (Billercia, MA, USA). MeOH of high analytical grade was acquired from Sigma-Aldrich (Stockholm, Sweden), and HCl (37%) was supplied by Sigma-Aldrich (Tres Cantos, Madrid, Spain).

2.3. Experimental set-up for physical processes

Tests were performed in 1 L amber glass beakers containing 1 L Milli-Q water spiked with a mixture of the aforementioned 4 antibiotics to a final concentration of 1000, 500, 100 or 20 μg/L per antibiotic. After taking a first sample to determine the initial antibiotic concentration in each reactor, the lyophilized microalgae-bacteria consortium was added under continuous mixing to obtain a concentration of 1 g/L. The microalgae/bacteria concentration was chosen as a means of comparison with common microalgae concentration in HRAPs used for wastewater treatment. All reactors were mixed continuously at 200 rpm. Samples were taken at different time intervals until 4 days of operation to simulate the common residence time of biomass in a HRAP. pH was

Table 2
MS/MS transitions.

Compound	Quantification (Q)	Confirmation (q1)	Confirmation (q2)	Isotope-labelled internal standard (ILIS)
Sulfadiazine	251 > 156	251 > 92	251 > 108	257 > 162
Sulfamethoxazole	254 > 156	254 > 108	254 > 92	260 > 162
Ciprofloxacin	332 > 245	332 > 231	332 > 294	340 > 235
Tetracycline	445 > 154	445 > 410	445 > 427	451 > 416

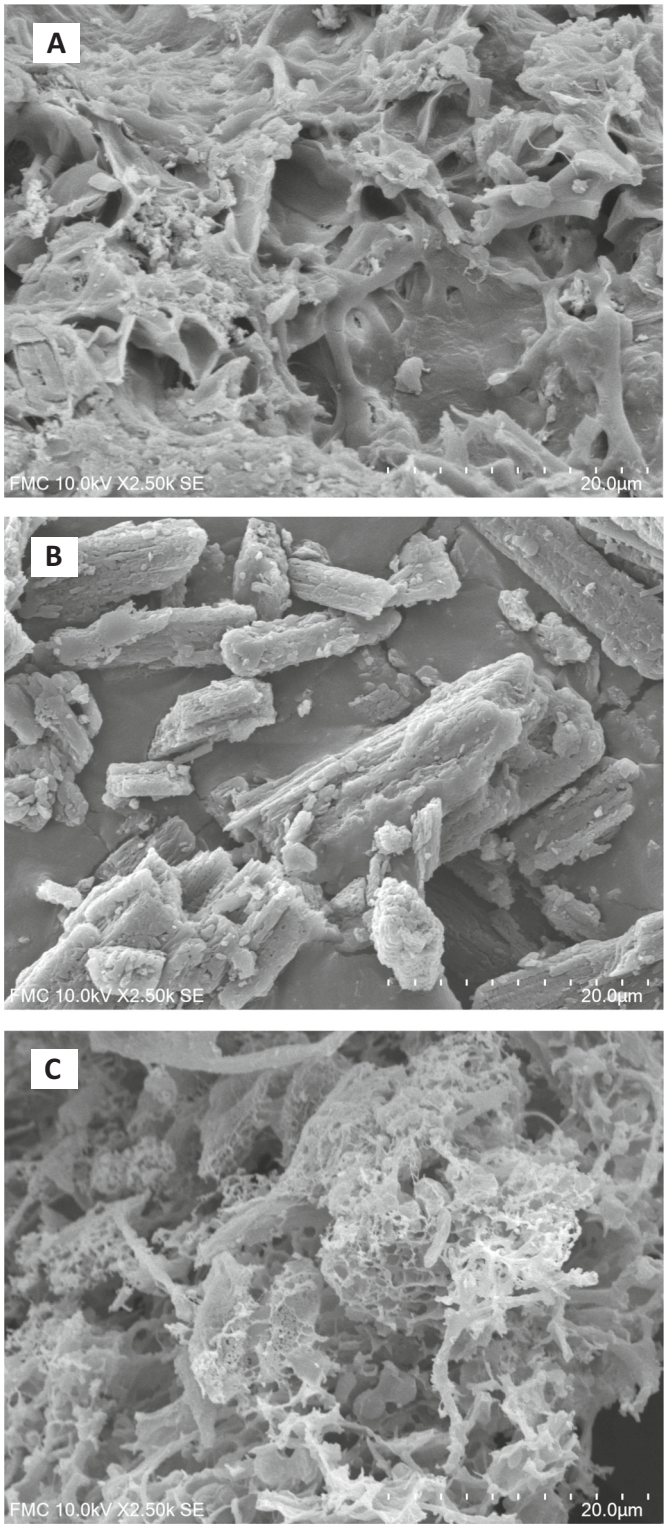


Fig. 1. SEM image of (A) *Scenedesmus almeriensis*-bacteria consortium, (B) mixture of antibiotics and (C) biomass exposed to antibiotics.

monitored during the whole operation process, remaining constant around 8.6 ± 0.2. Samples were filtered immediately through 0.22 mm pore-size nylon syringe filters (Fisherbrand) and stored at 4 °C before analysis of the aqueous phase antibiotics concentration. Experiments were conducted in triplicate.

2.4. Analytical method

Microalgae surface groups were identified by Fourier transform infrared (FTIR) spectroscopy, using a Bruker Tensor 27 spectrometer in the attenuated total reflectance (ATR) method. Morphological characterization was carried out using a Hitachi FlexSEM 1000 scanning electron microscope (SEM). FTIR and SEM analysis were done at the Industrial Testing Laboratory of Castilla y León (LEICAL/UVa), located in Valladolid University (Spain).

The quantitative determination of selected antibiotics was performed by liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) with triple quadrupole (Waters Acquity H-Class UPLC, equipped with a binary pump system, interfaced to a triple quadrupole Xevo TQ-STM mass spectrometer, with ESI source (Waters Corp)). The procedure was based on those previously developed by Botero-Coy et al. [19], employing direct injection of the (diluted) samples, without any pre-concentration step. In this work, a dilution ×100 (samples fortified at 1000 and 500 ng/mL) or dilution ×10 (samples fortified at 100 and 20 ng/mL) with Milli-Q water was made in order to reduce matrix complexity and to adjust the sample concentrations to the linear dynamic range of the calibration curve. Isotope-labelled internal standards (ILIS) were added to the four analytes under study (ciprofloxacin-d8, sulfamethoxazole-d6, sulfadiazine-13C6, tetracycline-d6) and were used for potential matrix effects correction. Three MS/MS transitions were acquired for each compound – one for quantification and the remaining ones for confirmation of the identity of the compound (Table 2).

The analytical procedure was as follows: a 100 µL aliquot of the sample was mixed with 800 µL Milli-Q water and a 100 µL mixture of four isotope labelled internal standards (ILIS) at 2 µg/L (the samples fortified at 1000 and 500 ng/mL were previously diluted ×10 before applying the procedure by adding 900 µL Milli-Q water to the 100 µL sample). Finally, 50 µL of the mixture were injected into the LC-MS/MS.

2.5. Zero-point charge (ZPC)

50 mL of Milli-Q water were taken in 100 mL Erlenmeyer flasks, adjusting the pH of each solution between 3 and 11, adding the appropriate amounts of 0.1 M HCl and 0.1 M NaOH. 0.5 g of lyophilized biomass to each Erlenmeyer, and the final pH value was measured after 48 h under stirring at room temperature. To calculate pH_{ZPC} the values of the final pH are plotted versus the initial pH. The pH_{ZPC} corresponds to the point where the curve of final pH versus initial pH cuts the graph diagonal [20].

2.6. Adsorption kinetics

Pseudo first-order (Eq. (1)) and pseudo second-order (Eq. (2)) equations were used for the description of the adsorption kinetics. The linear form of the equations are expressed as [10]:

$$ln\,(q_e - q_t) = ln\,q_e - k_1 \cdot t \tag{1}$$

$$\frac{t}{q_t} = \frac{1}{k_2 q_e^2} + \frac{1}{q_e} t \tag{2}$$

where, q_e (µg/mg) and q_t (µg/mg) are the amounts of adsorbed antibiotics at equilibrium and at time t, respectively, k_1 (h^{-1}) is the rate constant of pseudo first order adsorption and k_2 ($mg \cdot \mu g^{-1} \cdot h^{-1}$) is the rate constant of pseudo second order adsorption. The q_t values are calculated as follows (Eq. (3)) [10]:

$$q_t = \frac{C_0 - C_t}{W} \cdot V \tag{3}$$

where, C_o (µg/L) is the antibiotic concentration in the initial solution and C_t (µg/L) is the antibiotic concentration in the solution at time t, W (mg) is the amount of biosorbent used in the experiment, and *V* (L)

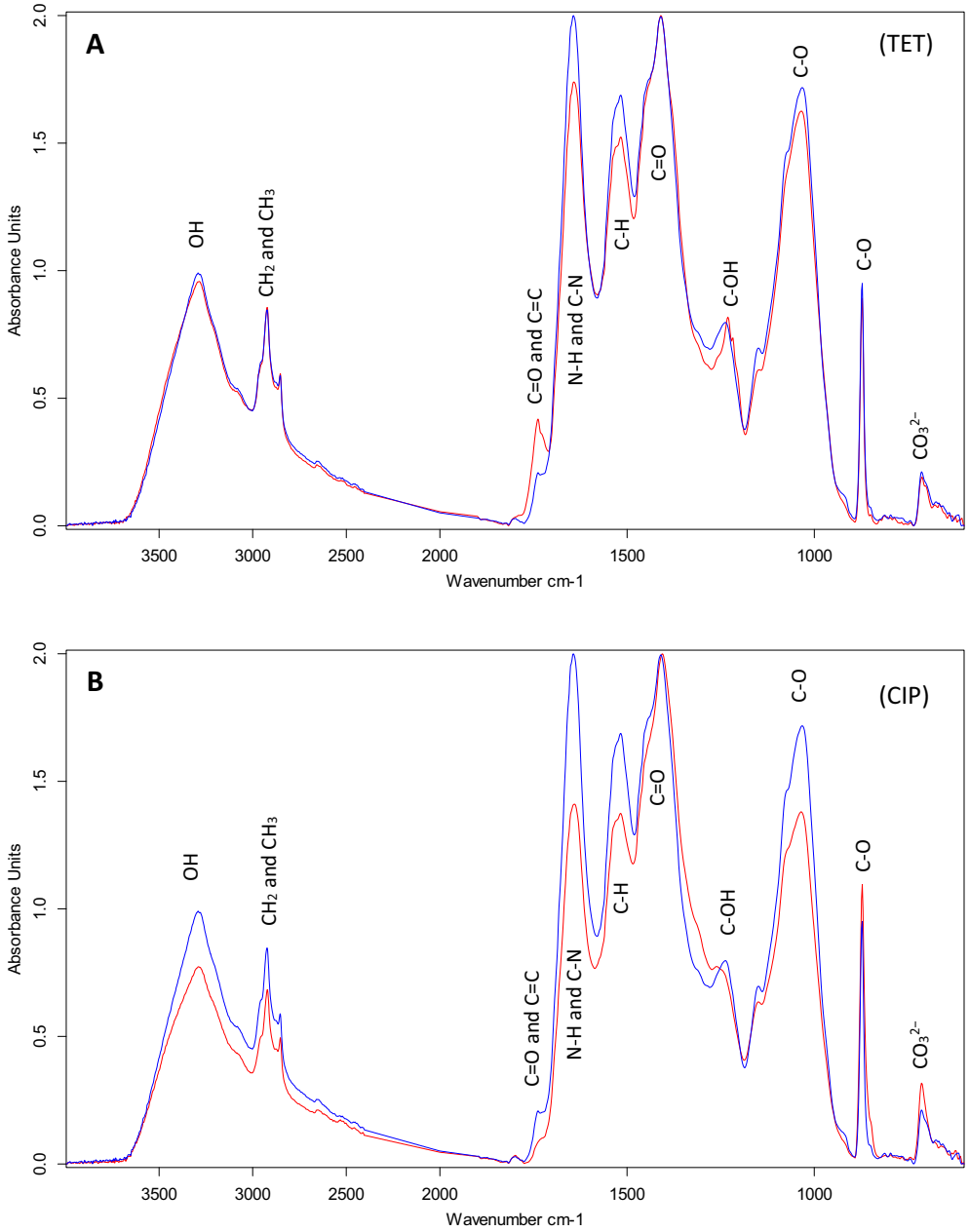


Fig. 2. FT-IR spectrum of *S. almeriensis*-bacteria consortium before (blue line) and after (red line) (A) tetracycline, (B) ciprofloxacin, (C) sulfadiazine and (D) sulfamethoxazole adsorption. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

corresponds to the volume of the solution. Therefore, for the pseudo first-order equation, a plot of ln (q_e-q_t) versus t gives a straight line of slope k_1 and intercepts ln q_e. While, for the pseudo second-order equation, a plot of t/q_t versus t gives a linear relationship, from which q_e and k_2 can be determined from the slope and intercept of the plot [21].

2.7. Adsorption isotherms

The Freundlich and Langmuir adsorption models were used for the mathematical description of the adsorption equilibrium. The linear form of Langmuir isotherm (Eq. (4)) and the linear form of Freundlich isotherm (Eq. (5)) are represented as follows [8,22]:

$$\frac{1}{q_e} = \frac{1}{q_m K_L C_e} + \frac{1}{q_m} \tag{4}$$

$$log\ q_e = log\ K_F + \frac{1}{n} log\ C_e \tag{5}$$

where, q_e (μg/mg) is the amount of adsorbed antibiotic per unit weight of biomass, C_e (μg/L) is the antibiotic concentration in the solution at equilibrium, K_L (L/μg) is the Langmuir equilibrium constant, q_m (μg/mg) is the theoretical monolayer saturation capacity, K_F (L/μg) is the Freundlich constant, and n (μg/L) is the adsorption intensity. Therefore, for Langmuir isotherm, a plot of $1/q_e$ versus $1/C_e$ gives a straight line of slope $1/(q_m K_L)$ and intercepts $1/q_m$. While for Freundlich isotherm, a plot of log q_e versus log C_e enables the constant K_F and exponent n to be determined [21].

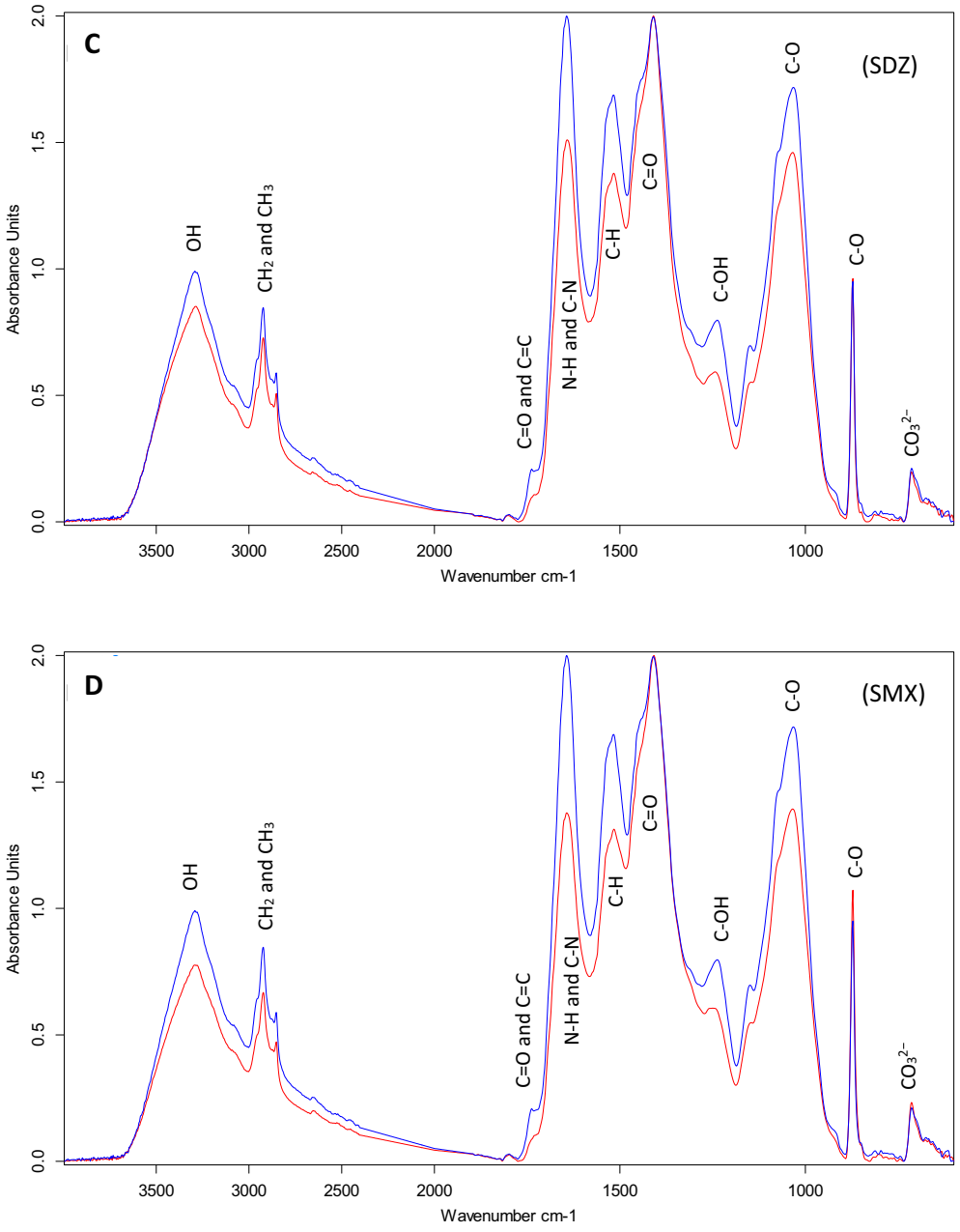


Fig. 2. (*continued*).

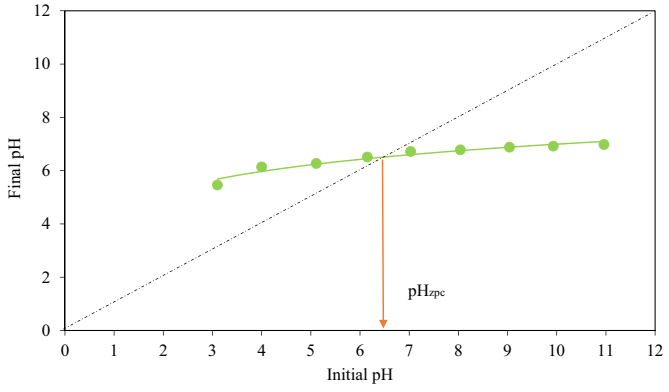


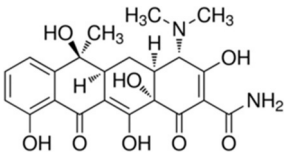
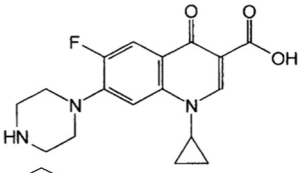
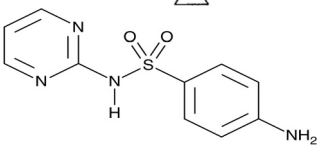
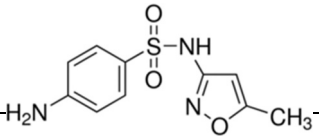
Fig. 3. Evaluation of zero-point charge (pH_{zpc}) for *Scenedesmus almeriensis*-bacteria consortium.

3. Results and discussions

3.1. Surface morphology and functional groups of Scenedesmus almeriensis-bacteria consortium

Biosorption may be accomplished by the functional groups and polymer assemblages present in microalgae and bacteria cell walls. The effectiveness of the adsorption process will depend significantly on the hydrophilicity, functionality and structures of the biomass (Fig. 1A) and the antibiotics (Fig. 1B). A *Scenedesmus almeriensis*-bacteria consortium can be an effective biosorbent for antibiotic removal as it presents an especially tough cell wall with cellulose, algaenan and glycoproteins [18,23]. Scanning electron microscopy (SEM) was used to investigate the surface morphology of the *Scenedesmus almeriensis*-bacteria consortium (Fig. 1A). It can be observed that the surface of the lyophilized biomass possessed pore structures, which makes them a good biosorbent. SEM provides a qualitative evaluation of the cell wall structure

Table 3
Dissociation constants (pK_a) and other physico-chemical properties of antibiotics.

Compound	Molecular formula	Molecular weight (g/mol)	Chemical structure	log K_{ow}	pK_a	Reference
Tetracycline	$C_{22}H_{24}N_2O_8$	444.4		−1.37	8.3, 10.2	[47]
					3.32 ± 0.30, 7.78 ± 0.05, 9.58 ± 0.30	[30]
					6.09, 8.74	[48]
					6.3	[47]
Ciprofloxacin	$C_{17}H_{18}FN_3O_3$	331.3		1.32	3.0 ± 0.30, 6.14 ± 0.13, 8.70 ± 0.09, 10.58 ± 0.30	[30]
					6.52	[49]
					6.52, 6.55	[50]
Sulfadiazine	$C_{10}H_{10}N_4O_2S$	250.3		−0.09	2.10, 6.28	[51]
					5.9	[47]
					5.70–5.73	[50]
					1.83, 5.57	[51]
Sulfamethoxazole	$C_{10}H_{11}N_3O_3S$	253.3		0.89	1.85 ± 0.30, 5.60 ± 0.04	[30]

after antibiotic adsorption to the algae (Fig. 1C). A clear alteration in the cell wall was observed (morphological changes of biomass surface) after adsorption, which was confirmed with FTIR spectroscopy [24].

FTIR analyses were conducted to identify the effect of the antibiotics on the functional groups on the surface of the *Scenedesmus almeriensis*-bacteria consortium. The FTIR spectrums of the *S. almeriensis*-bacteria consortium showed broad bands over a wavenumber range of 3600–800 cm^{-1} (OH band (3200–3400 cm^{-1}), CH_2 and CH_3 stretching (2930 cm^{-1}), aromatic conjugated C═O and C═C bonds (1640 cm^{-1}), N—H bending and C—N stretching (1520 cm^{-1}), C—H bending (1430 cm^{-1}), C═O carboxylate ion stretching (1375 cm^{-1}), C—OH phenolics (1270 cm^{-1}), C—O single bonds (1150 cm^{-1}), C—O stretching (1049 cm^{-1}), and CO_3^{2-}, (875 cm^{-1})) (Fig. 2). These results showed a similar pattern as others previously reported for *S. almeriensis* [25], showing stretching vibrations of the characteristic bands of polysaccharides and proteins.

The FTIR spectrums in Fig. 2 of the *S. almeriensis*-bacteria consortium before antibiotic adsorption are represented by a blue color while after adsorption by a red one. The strong spectrum of the *S. almeriensis*-bacteria consortium decreased after the incorporation of antibiotics into the system. For TET (Fig. 2A) peak modifications were observed at C═O and N—H stretching, which are related to proteins, suggesting that TET was bounded on the surface of the *S. almeriensis*-bacteria consortium via interactions with these functional groups. CIP (Fig. 2B) showed sharp peak modifications at C═O and N—H stretching as well as to the band in the range of 3500–3000 cm^{-1}, corresponding to the hydrogen bonds of carbohydrates and proteins. These results indicate that lower peaks were associated with CIP adsorption to biomass surface via these functional groups. While SDZ (Fig. 2C) and SMX (Fig. 2D) showed important peak modifications at C═O and N—H stretching, hydrogen bonds and C—O—C stretching (1200–1100 cm^{-1}) of polysaccharides suggest that these functional groups were involved in SDZ and SMX adsorption. FTIR results showed changes in chemical groups related to polysaccharides and proteins. Proteins are highly hydrophobic and can contribute to organic pollutant adsorption [11].

3.2. Zero-point charge (ZPC)

To determine the interactions between the antibiotics and the microalgae, the zero-point charge for the *S. almeriensis*-bacteria consortium was determined (Fig. 3). The zero-point charge (ZPC) is the pH for which the biomass surface is globally neutral. Consequently, the electrostatic attraction between the biomass particles and antibiotics is minimal. Below ZPC value, the microalgae-bacteria consortium surface is positively charged and exerts an electrostatic force towards the negatively charged antibiotics. In contrast, beyond this value, the surface is negatively charged and repels the anionic antibiotics [10,26]. pH_{ZPC} for the *S. almeriensis*-bacteria consortium was found at pH 6.5. The preset study was performed at pH 8, and therefore the biomass was negatively charged.

The pH of the solution is a major and sensitive factor that will control the biosorption of the antibiotics into the biomass. Aggregation, hydrophobicity, electrostatic attraction or repulsion of the antibiotics will be influenced by the pH. In addition, the physicochemical characteristics of the antibiotics – like the octanol-water partition coefficient (log K_{ow}) and the acid-base dissociation constant (pK_a) of the antibiotics – (Table 3) are important parameters which will directly affect biosorption. The microalgae-bacteria consortium could remove the antibiotics based on the lipophilic property of the antibiotics. The higher the log K_{ow}, the higher the lipophilicity. Meanwhile, pK_a influences lipophilicity, solubility, protein binding and permeability. The lower the pK_a, the greater the lipophilicity [27–29].

The four antibiotics used in the present study have low log K_{ow}, showing that these antibiotics are hydrophilic and will tend to be dissolved in the solution. However, all of the antibiotics studied have more than one pK_a values. This causes difficulty in determining the adsorption mechanism of a compound because it has different dissociated species (cationic, neutral, or anionic). Different chemical species may present different properties in regards to water solubility, volatility, UV absorption, and reactivity with chemical oxidants [29,30].

The following are predictions of the different interaction mechanisms based on the pH of the solution, the antibiotics pK_a and the pH_{ZPC} of the microalgae-bacteria consortium:

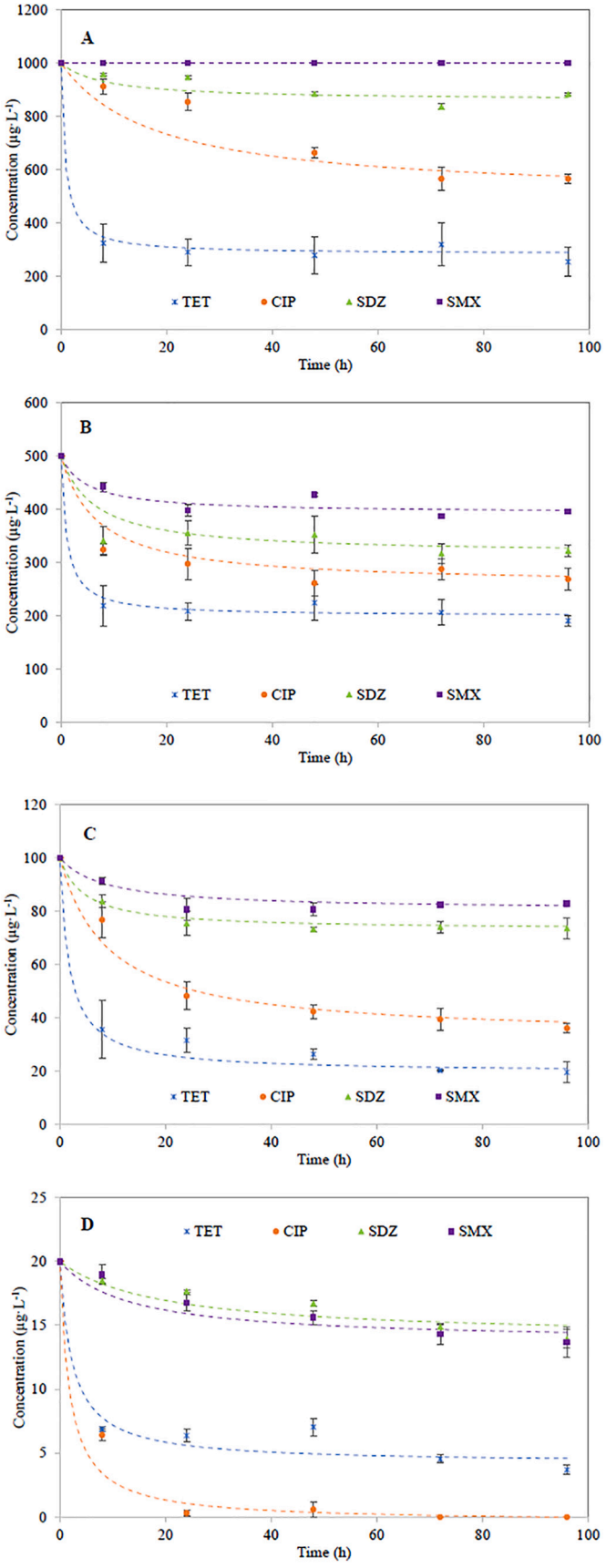


Fig. 4. Antibiotic removal by biosorption at: (A) 1000 μg L^{-1}, (B) 500 μg L^{-1}, (C) 100 μg L^{-1} and (D) 20 μg L^{-1} initial concentrations. Error bars represent ± standard error of mean.

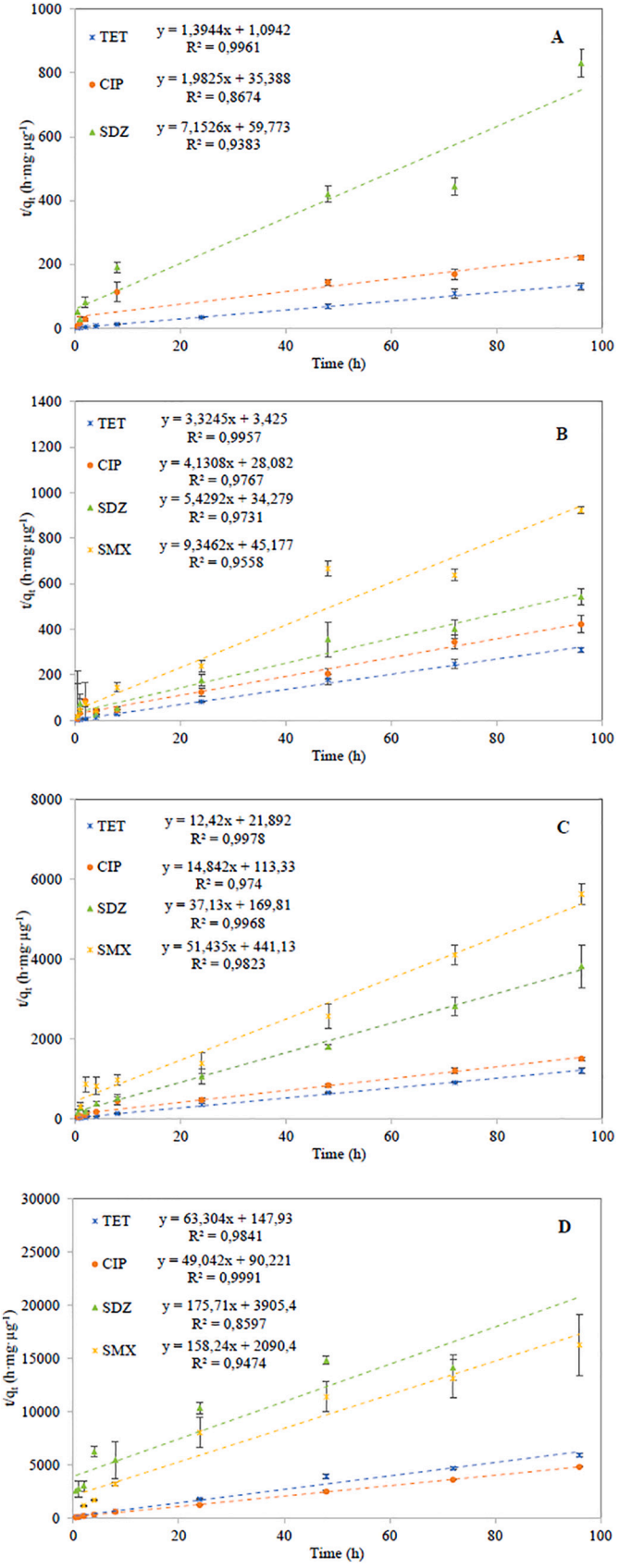


Fig. 5. Kinetic analysis for pseudo second-order reaction for antibiotic removal by biosorption at: (A) 1000 μg L^{-1}, (B) 500 μg L^{-1}, (C) 100 μg L^{-1} and (D) 20 μg L^{-1} initial concentrations. Error bars represent ± standard error of mean.

Table 4
Kinetic parameters for the biosorption of antibiotics by the *Scenedesmus almeriensis*-bacteria consortium

Compound	C_o (μg/L)	$C_{e,exp}$ (μg/L)	$q_{e,exp}$ (μg/mg)	Pseudo first-order model				Pseudo second-order model			
				k_1 (h^{-1})	$q_{e,calc}$ (μg/mg)	$C_{e,calc}$ (μg/mg)	r^2	k_2 ($mg \cdot \mu g^{-1} h^{-1}$)	$q_{e,calc}$ (μg/mg)	$C_{e,calc}$ (μg/mg)	r^2
Tetracycline	1000	254	0.75	0.03	0.31	692.17	0.65	1.78	0.72	282.85	0.99
	500	190	0.31	0.02	0.09	410.99	0.35	3.22	0.30	199.20	0.99
	100	20	0.08	0.01	0.01	88.79	0.89	7.05	0.08	19.48	0.99
	20	4	0.02	0.02	0.01	15.45	0.86	27.09	0.02	4.20	0.98
Ciprofloxacin	1000	566	0.43	0.03	0.37	626.37	0.85	0.11	0.50	495.59	0.87
	500	269	0.23	0.03	0.15	349.59	0.46	0.61	0.24	257.92	0.98
	100	36	0.06	0.03	0.02	83.33	0.89	1.65	0.07	32.62	0.97
	20	0	0.02	0.06	0.01	10.27	0.56	26.66	0.02	0	0.99
Sulfadiazine	1000	884	0.12	0.04	0.09	900.03	0.88	0.86	0.14	860.19	0.94
	500	322	0.18	0.01	0.08	417.57	0.07	0.86	0.18	315.81	0.97
	100	74	0.03	0.04	0.05	51.05	0.72	8.12	0.03	73.07	0.99
	20	14	0.01	0.02	0.01	14.37	0.99	8.49	0.01	13.97	0.92
Sulfamethoxazole	1000	1000	–	–	–	–	–	–	–	–	–
	500	396	0.10	0.01	0.05	452.45	0.02	1.93	0.11	393.00	0.96
	100	83	0.02	0.08	0.01	85.11	0.80	5.99	0.02	80.56	0.98
	20	14	0.01	0.03	0.01	14.57	0.99	11.98	0.01	13.68	0.95

i) at pH 8, TET ($pK_{a1} < pH < pK_{a2}$) will be in its neutral form and present hydrophobic interactions with the biomass that will be negatively charged [29].
ii) CIP pK_a are in the ranges of 3.0–6.3 for the carboxylic acid group and 8.7–10.6 for the nitrogen on the piperazinyl ring, showing that the acid will be dissociated at pH values higher than 6.3, and the nitrogen will be protonated at pH values lower than 8.7. Thus, at pH 8, CIP will be mostly dissociated, both positively and negatively charged, and its cations might enhance its sorption capacity on the negatively charged microalgae-bacteria consortium through electrostatic attraction [31,32].
iii) sulfonamides pK_a values are in the ranges of 1.8–2.1 and 5.6–6.6. Therefore, in the pH range of 2.1–5.6, the compound will be in its neutral form, while at higher pH the compound will be predominantly anionic. At pH 8, SDZ and SMX ($pH > pK_a$) will be negatively charged which will cause an electrostatic repulsion with negatively charged microalgae [29].

3.3. Biosorption

Batch experiments containing the dead biomass (lyophilized algae-bacteria consortium) under dark conditions help to understand to what extent the investigated VMP are eliminated from the solution solely by biosorption, which would mean that they are not being destroyed during the microalgae wastewater treatment process, but are transferred to the biomass, having important implications for its possible subsequent use as biofertilizer or animal feed. After 96 h of operation, the antibiotic removal by biosorption at an initial VMP concentration of 1000 μg/L was 75% TET, 43% CIP and 12% SDZ; SMX did not indicate removal by biosorption (Fig. 4A). At an initial VMP concentration of 500 μg/L, removals of 62, 46, 36 and 21% were presented by TET, CIP, SDZ and SMX (Fig. 4B), respectively. At initial 100 μg/L, TET, CIP, SDZ and SMX showed 80, 64, 26 and 17% of biosorption, respectively (Fig. 4C). Finally, at an initial concentration of 20 μg/L, the removal percentages were 80% TET, 30% SDZ and 30% SMX; CIP was completely removed by biosorption after 24 h (Fig. 4D).

Biosorption acts as an important mechanism for antibiotic removal, especially at low concentrations. Among the three spiked levels, higher removal was observed for 20 μg/L. One of the reasons why this happened was because there was a limited amount of biomass, 1000 mg/L (typical value at which raceways operate) which could not adsorb large amounts of VMP. This quantity will be determined in the adsorption isotherm tests in which we will find the adsorption capacity of each VMP per unit mass of adsorbent at equilibrium. TET and CIP presented a rapid decrease in their concentration in the first 24 h, while SDZ and SMX decrease gradually during the whole operation time. In general, TET and CIP showed a better affinity to the microalgae-bacteria consortium, which may explain why at high antibiotic concentrations, TET and CIP use the available sorption sites in microalgae and bacteria walls first, leaving few spaces for SDZ and SMX. Furthermore, SDZ and SMX showed low removal percentages by bioadsorption as they are highly water soluble and under the current pH condition they presented electrostatic repulsion with the negatively charged microalgae, which could explain the limited binding of sulfonamides to the algal cells [33].

The results obtained in the present study are comparable with previous studies on antibiotic removal by microalgae; for TET at a 2 mg/L initial concentration, de Godos et al. [34] using *C. vulgaris* (44–355 mg/L TSS) after 43 h observed 50–71% removal. Likewise, the present study, with a mixture of antibiotics, obtained around 72% for the half of the concentration used by de Godos et al. at 43 h. The lower removal obtained by de Godos et al. might be due to the concentration of microalgae present in the solution. Similarly, for the 2 mg/L TET initial concentration, Norvill et al. [35] achieved 97% TET removal after 14 h using algae-bacteria consortia (0.95 ± 0.02 g/L TSS) from a HRAP. However, the present study obtained around 70% of TET removal for 1 mg/L at 14 h. The higher removal obtained by Norvill et al. could be because they used just TET and not a mixture of different antibiotics; hence, the biomass surface will have more free available sites for sorption.

Hom-Diaz et al. [36], using an algae-bacteria consortia from an HRAP with an initial concentration of 2 mg/L CIP obtained 52.9% removal due to sorption after 111 h. Comparably, the present study using a mixture of antibiotics with 1 mg/L of CIP obtained around 43% removal after 96 h operation. This showed that biosorption is an important mechanism in CIP removal, which goes along with the conclusions of Hom-Diaz et al. On the other hand, Xie et al. [11] investigated the use of 80 mg/L *Chlamydomonas* sp. to remove CIP and SDZ with an initial concentration of 10 mg/L. After 9 days of operation, only 5.5% CIP and 0.5% SDZ removal was observed. This initial concentration of antibiotics was extremely high in relation to those that can be found habitually. In addition, the concentration of *Chlamydomonas* sp. was small, which would cause active sites of the microalgae to be rapidly exhausted. The present study did not use a concentration as high as Xie et al. [11], but the analysis of CIP and SDZ at different initial concentrations shows that sulfonamides have a lesser tendency to be adsorbed than other antibiotic families, such as fluoroquinolones, and that at higher antibiotic concentrations sorption decreases, which corroborates Xie et al. findings.

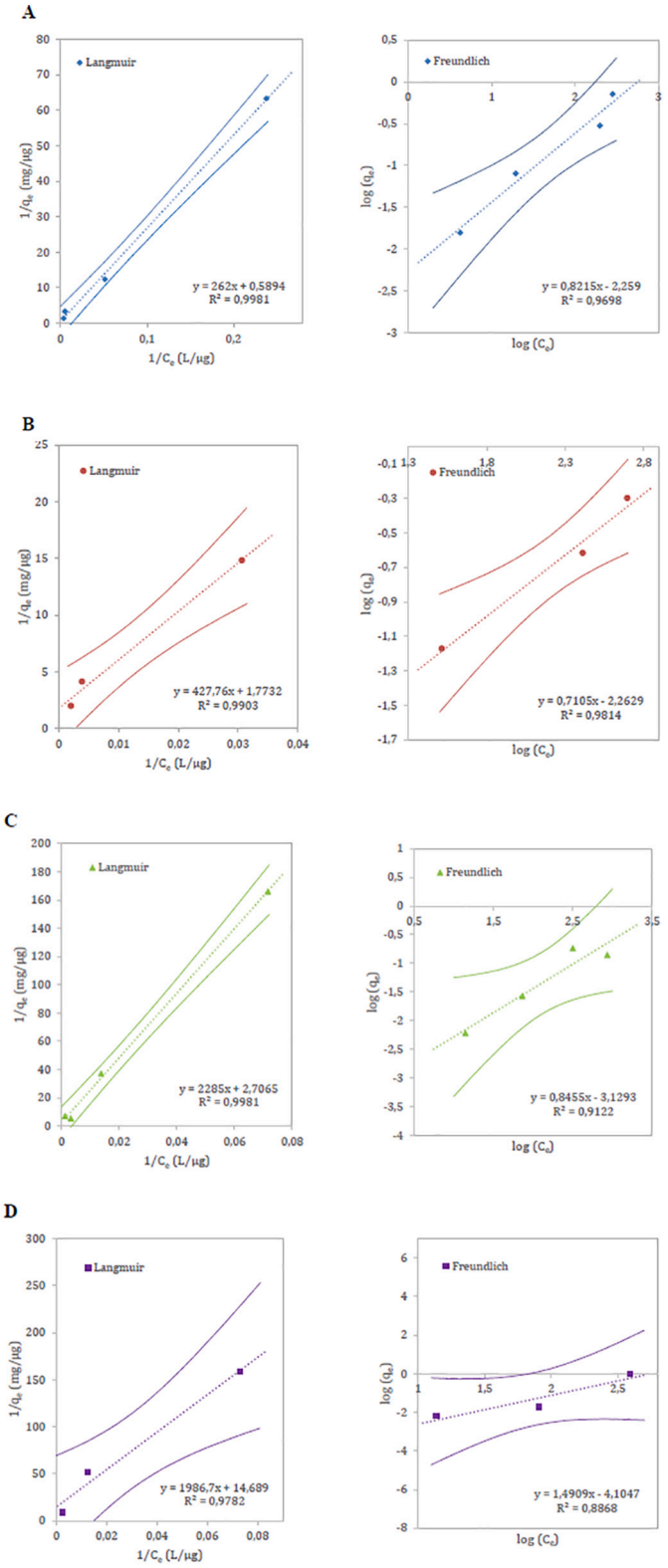


Fig. 6. Adsorption isotherms of (A) TET, (B) CIP, (C) SDZ and (D) SMX onto *Scenedesmus almeriensis* microalgae-bacteria consortium (With a Confidence Interval of 95%).

Bai and Acharya [31], using $2.0{\cdot}10^5$ cells/ml of a green algae, *Nannochloris* sp., and an initial concentration of 10 µg/L SMX obtained 11% of removal due to sorption after 14 days operation. In contrast, the present study obtained a higher SMX removal of 31% with a higher initial concentration of 20 µg/L in just 4 days of operation. This could be due the use of *S. almeriensis*, which showed the best affinity for SMX sorption. Likewise, Kiki et al. [37] investigated the biosorption of SMX using *H. pluvialis*, *S. capricornutum*, *S. quadricauda* and *C. vulgaris*. After 40 days of operation, 0–1.31% and 0.37–3.35% removal were obtained for initial concentrations of 20 and 100 µg/L, respectively. Comparisons with these results confirm that *S. almeriensis* has a better removal efficiency by biosorption when treating water polluted with SMX. Thus, by employing a literature review in which the amounts of microalgae used in each study and their removal efficiencies of VMP were compared, it has been demonstrated the importance of determining the corresponding adsorption isotherms of each VMP.

Table 5
Isotherm constants of antibiotics.

Compound	Langmuir model			Freundlich model		
	K_L (L/µg)	q_m (µg/mg)	r^2	K_F (L/µg)	n (µg/L)	r^2
Tetracycline	$5.43{\cdot}10^{-3}$	0.83	0.99	$5.98{\cdot}10^{-3}$	1.22	0.97
Ciprofloxacin	$3.87{\cdot}10^{-3}$	0.52	0.99	$5.38{\cdot}10^{-3}$	1.46	0.99
Sulfadiazine	$1.71{\cdot}10^{-3}$	0.26	0.99	$8.51{\cdot}10^{-4}$	1.25	0.89
Sulfamethoxazole	$5.36{\cdot}10^{-2}$	0.01	0.96	$7.65{\cdot}10^{-5}$	0.67	0.86

Additionally, Scenedesmus almeriensis microalgae-bacteria consortium capacity to adsorb VMP can be compared with other organic materials. Zeng et al. [38] achieved 50 to 80% biosorption from 20 mg/L CIP by using various concentrations of rice straw biochar from 0.2 to 1.6 g/L. Huang et al. [39] studied the biosorption of 10 mg/L CIP by using rabbit manure biochar, and they achieved removals of 76 to 97% varying the amount of biochar used from 0.2 to 3 g/L. Wu et al. [40] observed 21 to 35% biosorption of 25 mg/L CIP by using *Enteromorpha prolifera* in concentrations from 0.5 to 2 g/L. Guoting Li et al. [41] obtained 73.8% biosorption of 20 mg/L TET by layered carbon particles from seaweed *Sargassum* sp. Ahsan et al. [42] prepared saw dust derived functionalized graphitic carbon and obtained removals around 90% for100 mg/L TET and SMX. Pi et al. [43] achieved 47 to 70% of 0.4 to 1.8 mg/L SDZ biosorption by the utilization of extracelular polymeric substances extracted from *Klebsiella* sp. *J1*.

3.4. Kinetic analysis

The biosorption kinetic of the four aforementioned antibiotics was investigated with pseudo first-order and pseudo second-order kinetic models (Fig. 5). The values of the specific reaction rates, k_1 and k_2, were obtained by linear regression of the experimental data (Table 4). From the studied kinetic equations, r^2 values of the pseudo second-order equation were closer to unity, and the calculated adsorbed antibiotics at equilibrium were closer to the experimental values when compared with the pseudo first-order equation. The pseudo second-order rate constant, k_2, for the removal of antibiotics by the *Scenedesmus almeriensis*-bacteria consortium are 1.78–27.09 $mg{\cdot}\mu g^{-1}{\cdot}h^{-1}$ for TET, 0.11–26.66 $mg{\cdot}\mu g^{-1}{\cdot}h^{-1}$ for CIP, 0.86–8.49 $mg{\cdot}\mu g^{-1}{\cdot}h^{-1}$ for SDZ, and 1.93–11.98 $mg{\cdot}\mu g^{-1}{\cdot}h^{-1}$ for SMX. The k_2 rate constant increased with the decrease in the initial antibiotic concentration, due to the limited amount of biomass present in the solution, the reaction slows down as the available sorption sites get occupied. The k_2 rate const was higher for CIP; the higher rate constant for CIP is likely due to the log K_{ow} of CIP (1.32) which is higher than that of TET (−1.37), SDZ (−0.09) and SMX (0.89), indicating that CIP was more easily adsorbed. CIP was dissociated thus its cations enhanced its sorption capacity on the negatively charged microalgae and bacteria through electrostatic attraction [32]. These results denoted that sorption of CIP to *S. almeriensis* was easier and faster than that of TET, SDZ and SMX.

As far as we know, there are few publications about biosorption kinetics on the *S. almeriensis*-bacteria consortium for the four antibiotics

considered in the present study. From the bibliography available, Choi et al. [7] used *Spirulina* sp. for biochar production and determined a constant reaction kinetic of TET adsorption of 0.014 h^{-1}. Likewise, for SMX, Kiki et al. [37] determined constant reaction kinetics of $2.08{\cdot}10^{-3}$ to $5.83{\cdot}10^{-3}$ h^{-1} using *H. pluvialis*, *S. capricornutum*, *S. quadricauda* and *C. vulgaris*. Similarly, Xie et al. [11], using *Chlamydomonas* sp., obtained constant reaction rates of $1.82{\cdot}10^{-4}$ h^{-1} for CIP and $1.01{\cdot}10^{-5}$ h^{-1} for SDZ. Likewise, Hom-Diaz et al. [36] got a $7.3{\cdot}10^{-3}$ h^{-1} CIP removal rate constant by using an algae-bacteria consortia from an HRAP. Xiong et al. [44] for the biosorption of CIP by *Chlamydomonas mexicana*, obtained rate constants ranging from 5.04 10^{-4} to $3.29{\cdot}10^{-3}$ h^{-1}. The increase in removal rate was due to the addition of sodium acetate to enhance CIP removal by co-metabolism. In this case, biosorption onto active biomass can mask other removal processes that pure adsorption. These studies showed first or pseudo first-rate constants; thus, quantitative comparisons cannot be done with the values obtained in the preset study. Qualitatively, we can say that the present study obtained higher constant rates without the need of any additional substance. The comparisons of these results indicate that adsorption of antibiotics is faster when *S. almeriensis* is used, confirming that it has a better performance for antibiotic removal by biosorption.

3.5. Adsorption isotherms

To compare the adsorptive capacity of the *S. almeriensis*-bacteria consortium to TET, CIP, SDZ and SMX, Freundlich and Langmuir adsorption models were used (Fig. 6). The Freundlich isotherms assume a multilayer adsorption and are based on interactions between adsorbed molecules and non-ideal adsorption on heterogeneous surfaces. Nevertheless, the Langmuir isotherms are based upon an assumption of a finite number of binding sites, constant energies of adsorption and no interaction between adsorbed species [8,10,22]. In this work, the Langmuir model presented a higher correlation coefficient for all the antibiotics studied. The values of saturation capacity (q_m) from Langmuir isotherm (Table 5) were 0.83, 0.52, 0.26 and 0.01 μg/mg for TET, CIP, SDZ and SMX, respectively. These results indicated, assuming a monolayer saturation, that the *S. almeriensis*-bacteria consortium had a better affinity for TET.

The values of saturation capacity obtained from the Langmuir isotherm are comparable with the amounts of adsorbed antibiotic at equilibrium calculated by the pseudo second-order kinetic model. For the initial concentration of 1000 μg/L, the q_m values for TET and CIP are close to the q_e calculated of 0.72 and 0.50 μg/mg, respectively. However, the q_m value for SDZ is closer to the q_e calculated at the initial concentration of 500 μg/L which was 0.14 μg/mg. Finally, for SMX, the q_m value was the same as the q_e calculated at the initial concentration of 20 μg/L.

The results are consistent with previous findings [7], which determined that the Langmuir model presented a higher correlation coefficient than the Freundlich for TET removal by *Spirulina* sp. derived biochar. The isotherm constant values obtained for K_L and q_m were $1.81{\cdot}10^{-4}$ L/μg and 147.9 μg/mg, respectively [8]. The current study obtained a higher K_L of $5.43{\cdot}10^{-3}$ L/μg but a lower q_m of 0.83 μg/mg, probably due to high temperatures at which pyrolysis takes place to produce biochar which enhanced hydrophobicity and the specific surface area of the microalgae. To the best of our knowledge, no other authors have published isotherm constants for CIP, SDZ and SMX using microalgae-bacteria consortium, so further comparisons with literature could not be done.

4. Conclusions

Biosorption has been proven to be an important mechanism in the removal of TET and CIP by microalgae-based water treatment processes. For the first time, the biosorption capacity of the *Scenedesmus almeriensis* microalgae-bacteria consortium was analyzed and proven to be highly efficient in the removal of a mixture of four widely used VMP. Fluoroquinolones and tetracyclines turned out to have a better affinity to the *S. almeriensis* microalgae-bacteria consortium, showing high adsorption removal rates. Sulfonamides presented limited biosorption in the range of 20 to 1000 μg/L. The Langmuir isotherm better described the adsorption process of tetracycline, ciprofloxacin, sulfadiazine and sulfamethoxazole, defining a monolayer adsorption with no interaction between adsorbed species. Further research must focus on the other removal mechanisms of VMP during the microalgae wastewater treatment process to find the best conditions to enhance mechanisms, which completely eliminate VMP in order to find a further use for the harvested microalgae.

Declaration of competing interest

All the authors have declared no competing interest.

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