Process Safety and Environmental Protection 199 (2025) 107338

Contents lists available at ScienceDirect

Process Safety and Environmental Protection

journal homepage: www.journals.elsevier.com/process-safety-and-environmental-protection

Phycoremediation of ten sulfonamide antibiotics in swine wastewater: Microalgal tolerance, toxicity, and carbohydrate-rich biomass production

William Michelon [a,b,*], Vanessa Gressler [c], Micheli Colla Vieira [a], Mateus Gustavo Novello [a], Renato Eising [b], Estela de Oliveira Nunes [c], Alexandre Matthiensen [c], Aline Viancelli [a]

[a] Universidade do Contestado, Concórdia, SC 89711-330, Brazil
[b] Federal University of Technology – Paraná, Toledo, PR 85902-490, Brazil
[c] Embrapa Swine and Poultry, Concórdia, SC 89700-000, Brazil

ARTICLE INFO

Keywords:
Antibiotic removal
Biofuel potential
Environmental remediation
Genotoxicity
Pigment content

ABSTRACT

This study investigates the removal of ten sulfonamide antibiotic residues from swine wastewater using *Chlorella spp.* in a phycoremediation process. The effects of sulfonamides on microalgal biomass production, pigment content (chlorophyll *α*, chlorophyll *b*, and carotenoids), and the genotoxicity of treated water were evaluated. Sulfonamides were tested at concentrations of 0.1, 1.0, 10, 50, and/or 100 mg L^{-1} , with the antibiotic removal specifically assessed at 10 mg L^{-1} . Results showed that *Chlorella spp.* exhibited robust biomass growth and maintained stable pigment production, even at the highest concentrations, indicating the microalgae's tolerance to antibiotic exposure. The removal efficiency for sulfonamides was notably high, particularly for sulfamethoxazole (70 %), sulfachlorpyridazine (55 %), and sulfamerazine and sulfamethizole (50 %) at the 10 mg L^{-1} concentration. Genotoxicity assays with *Allium cepa* revealed minimal chromosomal aberrations, suggesting that the treated wastewater posed a low genotoxic risk. The microalgal biomass, characterized by high carbohydrate content, also holds promise for biofuel production. These findings highlight *Chlorella spp.* as an effective and sustainable solution for mitigating antibiotic pollution in agricultural wastewater, while simultaneously providing valuable biomass for renewable energy applications.

1. Introduction

Sulfonamides are a widely used class of synthetic antibiotics characterized by their functional group (-SO_2NH_2), which effectively treat a range of bacterial infections in both humans and animals (Rohilla and Sharma, 2023). Sulfonamides are bacteriostatic agents that selectively inhibiting folic acid synthesis in bacterial cells, a process essential for their growth and replication (Ovung and Bhattacharyya, 2021). They exhibit broad-spectrum activity, targeting gram-positive and gram-negative bacteria (Ovung and Bhattacharyya, 2021).

Chemically, sulfonamides are derivatives of para-amino benzene sulfonamides, with a general formula RSO_2NH_2, where the R group can vary, leading to diverse biological activities (Rohilla and Sharma, 2023). In veterinary medicine, sulfonamides have been extensively utilized since their discovery in 1939, particularly in livestock management, to prevent and treat infections in swine and poultry (Montone et al., 2024). However, the widespread use of these compounds has led to environmental challenges, as a significant portion of the antibiotics administered to animals is excreted unchanged and enters the environment through manure and wastewater (Muhammad et al., 2020; Bilal et al., 2020).

Their widespread use, especially in intensive animal farming, has resulted in the presence of these antibiotics in various environmental matrices, including surface water, groundwater, and agricultural soils. This contamination is of particular concern because it can lead to the development of antibiotic-resistant bacteria and negatively impact microbial communities in natural ecosystems (Manyi-Loh et al., 2018; Koch et al., 2021).

Several studies have investigated the occurrence of sulfonamides residues in wastewater, particularly those originating from animal farming. Common sulfonamides detected in swine wastewater include sulfamethoxazole, sulfadiazine, sulfadimethoxine, and sulfamethazine (Li et al., 2017; Chan et al., 2022; Tian et al., 2022). Moreover, these compounds are highly persistent in the environment, and conventional wastewater treatment methods have proven insufficient for their complete removal, requiring the development of more effective and

* Correspondence to: Victor Sopelsa, 3000, Concordia, SC 89711-330, Brazil.
E-mail address: william@unc.br (W. Michelon).

https://doi.org/10.1016/j.psep.2025.107338
Received 19 February 2025; Received in revised form 29 April 2025; Accepted 18 May 2025
Available online 22 May 2025

sustainable technologies to mitigate their environmental impact (Liu et al., 2023). As a result, bioremediation strategies, such as phycoremediation, have been explored as potential solutions (Jain et al., 2022).

Phycoremediation is a sustainable approach that employs microalgae to remove contaminants, including antibiotics, from wastewater (Dayana Priyadharshini et al., 2021). Microalgae can absorb and metabolize a wide range of pollutants, making them an attractive option for treating agricultural and municipal wastewaters. Despite promising results, the removal efficiencies of sulfonamides by microalgae vary widely depending on several factors, including the specific antibiotic, the species of microalgae used, and the cultivation conditions (Frascaroli et al., 2024; Wani et al., 2024). Additionally, most studies have focused on individual sulfonamides in controlled environments, leaving a significant gap in knowledge regarding the removal of multiple antibiotics in complex, real-world wastewater systems, such as those from swine farms (Table 1) (Zhuang et al., 2024).

Furthermore, the potential contamination of the biomass with residual antibiotics raises concerns about its direct application, particularly in agriculture or as animal feed, due to contamination risks. To address this issue, converting the biomass into bioenergy, such as bioethanol or biomethane, offers a way to transform potentially contaminated waste into a renewable energy source (Hussain et al., 2021). Phycoremediation processes can degrade antibiotics, potentially generating more toxic by-products (Viancelli et al., 2020) thus, assessing post-phycoremediation toxicity of the treated wastewater is essential to evaluate any remaining environmental or health risks.

This study aimed to address critical knowledge gaps in phycoremediation by examining the physiological responses (biomass and pigment production) of microalgae exposed to 10 distinct sulfonamide antibiotics. Following exposure, removal efficiency was assessed alongside the potential genotoxicity of the treated material.

2. Material and methods

2.1. Microalgae inoculum acclimation

The microalgae consortium, predominantly composed of *Chlorella spp.*, was originally obtained from a large-scale open pond system used for tertiary treatment of swine wastewater at the Brazilian Agricultural Research Corporation (EMBRAPA), located in Concordia, Brazil (Michelon et al., 2016). The collected microalgae inoculum experienced acclimation in 12 L glass photobioreactors (PBRs; 20 cm internal diameter), containing water mixed with 6 % v v^{-1} of non-sterile digestate from an Upflow Anaerobic Sludge Blanket (UASB) reactor treating swine wastewater. This UASB reactor, part of a swine waste treatment facility, has a working volume of 26 m^3 and treats 15 m^3 day^{-1} of manure, producing biogas at a rate of 14.3 ± 6.0 m^3 (Kunz et al., 2006). The PBRs were agitated continuously using recirculating mechanical pumps (Model SB2700, Sarlobetter brand, Brazil) at room temperature (23 °C) under mixotrophic conditions (12 hours of light and 12 hours of dark). Red light-emitting diode lamps (PGL-RBC 2500, Parus, Korea) with a wavelength of 630 nm [photosynthetic photon flux density (PPFD) of 148.5 μmol m^{-2} s^{-1}] were used to enhance biomass growth, as indicated in previous studies (Prandini et al., 2016). The lamps, measuring 2400 mm in length, 40 mm in width, and 60 mm in height, were sourced from Parus Co., Korea. The dominance of *Chlorella* spp. in the consortium was monitored throughout the cultivation period by regular visual inspections under an optical microscope (Fig. 1).

2.2. Wastewater characterization

Prior to inoculation (at time zero), the chemical composition of the diluted UASB effluent was analyzed, revealing concentrations (in mg L^{-1}) of: phosphate-P (10.5 ± 5.6), biological oxygen demand (BOD_5 91.8 ± 10.9), total organic carbon (101 ± 9.2), alkalinity as $CaCO_3$ (189 ± 20), ammonia-N (55.1 ± 1.1), and total nitrogen (60.1 ± 1.8). The pH value was recorded at 7.9 ± 0.6. Phosphate-P was determined using the ascorbic acid colorimetric method (4500-P). Nitrite (N–NO_2-) (4500-NO_2^- B), nitrate (N–NO_3-) (4500-NO_3^- D), and ammonia (N–NH_3) (4500-NH_3 D) were measured via flow injection analysis (Model 2500, FIAlab Instruments, USA) (APHA, 2012) Alkalinity (as mg $CaCO_3$) was determined through automatic titration (Model 848 Titrino Plus, Metrohm, Switzerland). Total organic carbon (TOC) was assessed using a TOC analyzer (Model TOC-LCPH/CPN, Shimadzu, Japan). The pH was monitored using a pH meter (Model pH/mV Meter - HI8424, Hanna Instruments, Brazil), and light intensity was estimated with a Luximeter (Model DX-100, Lux Meter, Japan).

2.3. Biomass concentration and pigments analysis

The biomass was calculated based on the strong correlation (r^2 = 0.989) between dry matter biomass concentration and suspended solids (2540-D) (APHA, 2012) using the optical density at 750 nm (OD_{750}) according to the equation: mg_{DW} L^{-1} = 238.93 × OD_{750}nm–37.432. As a result, microalgae growth was monitored using spectrophotometry analysis (Model Cary 50 Bio UV-Visible Spectrophotometer, Varian, USA) at a wavelength of 750 nm.

The extraction process for the wet microalgae involved mixing the biomass with 96 % methanol at a ratio of 50 mL per gram, followed by homogenization for 10 min at a speed of 11,000 rpm. The mixture was then centrifuged at 5000 rpm for 8 min (Model Universal 320, Hettich, Germany) and the clear supernatant was collected. This procedure was repeated three times until the solvent became completely clear. The combined supernatants were mixed thoroughly, and the absorbance was measured using a UV-spectrophotometer (Model Cary 50 Bio UV-Visible Spectrophotometer, Varian, USA). The concentrations of chlorophyll *α* was determined using equation Chlorophyll $\alpha = 16.72(A_{665.2}) - 9.16(A_{652.4})$, chlorophyll *b* was calculated as Chlorophyll $b = 34.09(A_{652.4}) - 15.28(A_{665.2})$, and total carotenoids were obtained from total carotenoid = [1000(A_{470}) − 1.63(Chlorophyll *α*) − 104.96(Chlorophyll *b*)] / 221 (Xing et al., 2022).

2.4. Experimental Procedure – sulfonamides removal

The experiments were conducted in 1-L reactors exposed to white light (PPFD of 99 μmol m^{-2} s^{-1}) under mixotrophic conditions, with a 12-hour light/12-hour dark cycle. Constant aeration was provided at an airflow rate of 106.80 ± 15.95 mL min^{-1}, and the reactors were maintained at room temperature (23 °C). The reactors were operated in fed-batch mode, utilizing swine wastewater from a UASB treatment. The wastewater was diluted by adding 0.06 L of swine wastewater to 0.79 L of chlorine-free tap water, along with 0.15 L of biomass inoculum. The initial concentration of microalgae in the reactors was 69 ± 0.6 mgDW L^{-1}. Each reactor received a standard solution of the test compound (sulfonamides), prepared in methanol, and a 4 mL aliquot was added to each reactor, resulting in a final concentration of 10 mg L^{-1}. Positive control reactors containing only methanol (4 mL) without sulfonamides were used to assess methanol's impact on microalgae growth, while negative controls (water with sulfonamides but no microalgae) were used to evaluate the natural photodegradation of the compounds. The *Chlorella* cultures were maintained for 11 days because this period corresponds to the exponential growth phase under optimized conditions. This timing was selected to maximize biomass yield before the onset of the stationary phase, which could negatively affect the metabolic performance of the microalgae (Yatirajula et al., 2019). Microalgae cultures (50 mL) were sampled and centrifuged at 3500 ×g for 10 minutes (Model Universal 320, Hettich, Germany) to remove the microalgae cells. The resulting supernatant was used for subsequent antibiotic analysis.

Table 1
Characterization of antibiotic removal efficiency and biomass parameters in aquatic cultures of microalgae.

Antibiotic	Initial Concentration (mg L^{-1})	Removal Efficiency (%)	Biomass (g L^{-1})	Chlorophyll *α* (μg mL^{-1})	Chlorophyll *b* (μg mL^{-1})	Carotenoids (μg mL^{-1})	Biomass Composition (Carbohydrates, Proteins, Lipids) (%)	Species	Culture Medium	Reference
Sulfamethoxazole	0.05	54.3	0.2	5.22	-	-	-	Microalgae-bacteria consortium (*Chlorella sorokiniana* predominant specie)	Wastewater treatment domestic	da Silva Rodrigues et al. (2020)
Sulfamethoxazole	0.1 and 5.0	60.3 and 86.5	1.5	-	-	-	-	*Chlorella sorokiniana*	Artificial wastewater	Chu et al., (2022)
Sulfamethoxazole + sulfamethazine (mixture 1:1 (w w^{-1}))	0.025, 0.075, 0.125, 0.175, and 0.25	31, 35, 49, 55, and 62 28, 29, 35, 41, and 47	0.5	-	-	-	25.04–20.8 (carbohydrates)	*Scenedesmus obliquus*	Sterilized Bold's Basal Medium	Xiong et al., (2019)
Sulfamethoxazole	5.0	49	0.25	-	-	-	-	*Chlorella pyrenoidosa*	BG−11 medium	Huang et al., (2023)
Sulfamethoxazole	5.0	16	0.15	-	-	-	-	*Scenedesmus quadricauda*	BG−11 medium	
Sulfamethoxazole	5.0	13	0.1	-	-	-	-	*Dictyosphaerium* sp.	BG−11 medium	
Sulfamethoxazole	5.0	10	0.09	-	-	-	-	*Haematoccocus pluvialis*	BG−11 medium	
Sulfamethoxazole	5.0	9	0.06	-	-	-	-	*Botryococcus braunii*	BG−11 medium	
Sulfadiazine	0.5 1.0, 10, and 100.0	54, 40, 34 and 19	0.03–0.1	-	-	-	-	*Chlorella* sp.	Artificial wastewater	Gao et al., (2023)
Sulfadiazine	1.0–160	2.1–5.85	1.3–1.9	1.1–2.0	0.25–0.75	0.55–0.75	-	Microalgae-bacteria consortium (*Scenedesmus obliquus* predominant specie)	BG−11 medium	Wang et al., (2022)
Sulfamethazine	1.0–50	16–33	0.55–0.7	4.0–4.5	-	-	-	*Chlorella vulgaris*	BG−11 medium	Chen et al., (2020b)
Sulfamethazine	1.0–50	14–27	0.58–0.75	1.5–1.75	-	-	-	*Chrysosporum ovalisporum*	BG−11 medium	
Sulfamonomethoxine	0.005–5.5	98.9–38.7	-	9.0–10.0	-	-	-	*Chlorella pyrenoidosa*	BG−11 medium	Li et al., (2024)
Sulfamonomethoxine	0.1–0.4	77.2–68.4	-	0.08–0.12	-	-	-	*Chlorella vulgaris*	Aquaculture wastewater	Wang et al. (2024a)
Sulfamethoxazole	10.0	70	0.45 ± 0.6	6.27 ± 0.17	4.168 ± 0.1	3.32 ± 0.05	52.8 ± 0.7, 41.3 ± 0.5, 1.2 ± 0.01	*Chlorella* spp.	Swine wastewater	This study
Sulfamerazine	10.0	50	0.52 ± 0.01	7.14 ± 0.57	3.951 ± 0.2	3.15 ± 0.18	51.1 ± 0.2, 40.4 ± 0.1, 1.0 ± 0.01	*Chlorella* spp.	Swine wastewater	This study
Sulfadimethoxine	10.0	40	0.46 ± 0.4	6.91 ± 0.24	3.95 ± 0.11	3.54 ± 0.13	50.2 ± 1.0, 41.6 ± 0.9, 0.8 ± 0.01	*Chlorella* spp.	Swine wastewater	This study
Sulfathiazole	10.0	30	0.44 ± 0.6	6.58 ± 0.54	4.73 ± 0.20	2.78 ± 0.35	50.9 ± 1.0, 40.8 ± 0.9, 0.8 ± 0.01	*Chlorella* spp.	Swine wastewater	This study
Sulfamonomethoxine	10.0	25	0.54 ± 0.03	6.87 ± 0.16	4.77 ± 0.07	3.16 ± 0.17	56.6 ± 0.2, 36.5 ± 0.1, 0.9 ± 0.02	*Chlorella* spp.	Swine wastewater	This study
Sulfadoxine	10.0	30	0.52 ± 0.07	5.56 ± 0.38	4.65 ± 0.09	2.97 ± 0.11	52.6 ± 0.7, 39.3 ± 0.5, 0.9 ± 0.4	*Chlorella* spp.	Swine wastewater	This study
Sulfamethazine	10.0	25	0.44 ± 0.2	5.71 ± 0.62	4.26 ± 0.63	2.90 ± 0.38	52.8 ± 0.7, 41.3 ± 0.5, 0.7 ± 0.2	*Chlorella* spp.	Swine wastewater	This study
Sulfamethizole	10.0	50	0.46 ± 0.4	7.73 ± 0.19	3.89 ± 0.92	3.17 ± 0.21	46.1 ± 0.5, 45.8 ± 1.0, 0.9 ± 0.01	*Chlorella* spp.	Swine wastewater	This study
Sulfachlorpyridazine	10.0	55	0.554 ± 0.01	6.59 ± 0.11	4.47 ± 0.05	3.12 ± 0.43	56.4 ± 0.2, 38.4 ± 0.1, 0.6 ± 0.02	*Chlorella* spp.	Swine wastewater	This study
Sulfaguanidine	10.0	25	0.579 ± 0.04	7.23 ± 0.09	4.12 ± 0.11	3.22 ± 0.29	53.2 ± 1.7, 38.6 ± 0.9, 1.2 ± 0.1	*Chlorella* spp.	Swine wastewater	This study

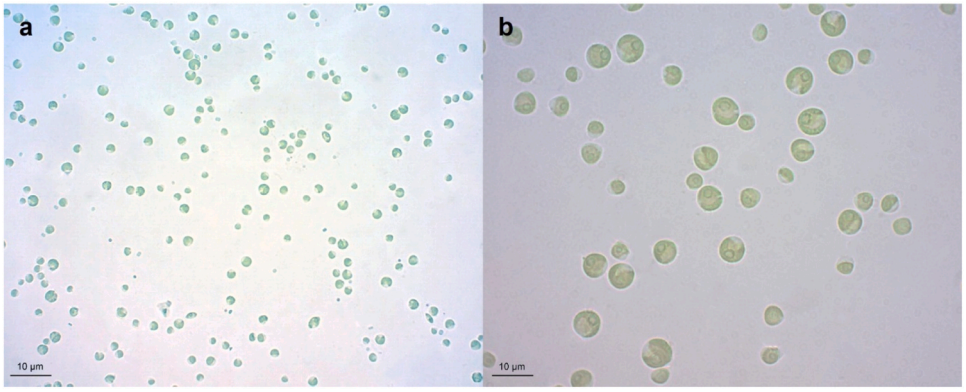


Fig. 1. Optical microscopy images of the microbial consortium showing the dominance of Chlorella spp. during cultivation. (a) Magnification of 400 × ; (b) Magnification of 1000 × .

2.5. Antibiotic measurement

2.5.1. Chemicals and materials

The standards (VETRANAL®, analytical standard grade) sulfamethoxazole, sulfamethazine, sulfathiazole, sulfadimethoxine, sulfamethizole, sulfamerazine, sulfamonomethoxine, sulfachlorpyridazine, sulfaguanidine, d6-sulfamethazine and (EP, European Pharmacopoeia Reference Standard grade) sulfadoxine were all purchased from Merck. Formic acid Optima LC-MS grade was obtained from Fisher Chemical (USA). All solvents used were HPLC grade and purchased from Panreac (Darmstadt, Germany) and J.T. Baker (USA). Ultrapure water was prepared using a Millipore Advantage A10 purification system. Solid phase extraction (SPE) cartridges (Oasis HLB, 60 mg, 30 cc) were purchased from Waters (Milford, USA).

2.5.2. Standard solutions

Individual stock standards of each analyte at 100 mg L^{-1} were prepared in methanol for all compounds and stored at 4 °C. Working standard solutions were prepared in methanol by diluting stock standard and stored at 4 °C.

Sulfonamides solutions were mixed and diluted, with ultrapure water (Milli-Q, Millipore, USA), to a final concentration of 10 mg L^{-1} of each antibiotic becoming the working solution for spiking the calibration curve samples. Serial dilution was further performed to generate the standard curves. Internal standards were prepared similarly.

2.5.3. Extraction procedure

Centrifuged microalgae cultures (2 mL) were sampled into polypropylene centrifuge tubes (15 mL) and fortified with the internal standard (50 µg L^{-1}). Before sample loading at SPE, the cartridges were conditioned with 3 mL of methanol and 3 mL of ultrapure water. The sample (2 mL) was added to the cartridge for analytes interaction and then the cartridges washed with 3 mL of ultrapure water and 1 min of vacuuming to remove possible chemical interferences. Sulfonamides were eluted using 3 mL of methanol and the extracts collected into a clean polypropylene tube for further dryness under nitrogen steam at less than 40 °C. Sulfonamides were re-dissolved in 1 mL of methanol, filtered (PTFE, 0.22 µm), and then an aliquot of 10 µL was injected onto the LC–MS/MS.

2.5.4. Instrumentation

The LC–MS/MS system was a TSQ Quantum Access Max coupled to a Surveyor Plus LC system. The instrument was controlled by Xcalibur software (Version 2.1). Separation was achieved using a (100 mm × 4.6 mm i.d.), 5 µm particle size, combined with a C18 guard column (2.0 mm, 3 µm), both from Phenomenex® (Torrance, USA). The oven temperature was set at 30 °C. The chromatographic separation was performed in gradient mode using water acidified with 0.1 % formic acid (mobile phase A) and methanol acidified with 0.1 % formic acid (mobile phase B), at a flow rate of 1.0 mL min^{-1}. The initial conditions (from 0 to 2 min) were 95 % A. Then the conditions were changed to 100 % B over 4 min from 2 to 6 min and these were maintained until 8 min. Finally, the conditions returned to 95 % A over 0.5 min from 8 to 8.5 min, and were maintained until the end of the run at 10 min. For all compounds, positive electrospray ionization (ESI+) was used in the MS utilized with the following parameters: spray voltage of 5.0 kV, sheath gas at 5 psi, auxiliary gas at 5 psi, cone temperature of 350 °C and vaporized temperature of 385 °C. High purity argon as the collision gas at 2.1 mTorr was used in the collision cell. Chemical structures, pK_a, precursor and product ions, and collision energies for each analyte investigated are listed in Table 2. To quantify sulfonamides in the cultured samples, the respective antibiotic analytical curve was performed with antibiotic free (blank) culture samples fortified at final levels of 10, 50, 100, 250, 500, 750 and 1000 (ηg L^{-1}) of each antibiotic prior to SPE extraction (matrix matched calibration curve). The analytical correlation coefficients (R^2) over the calibration range, retention times (Rt), limits of detection (LOD) and limits of quantification (LOQ) are described in Table 2.

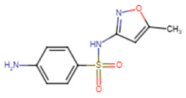
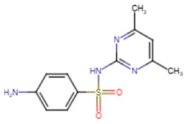
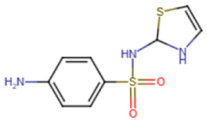
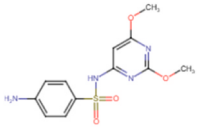
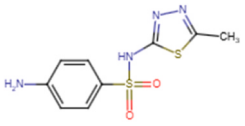
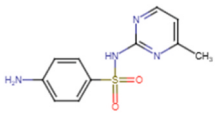
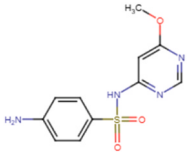
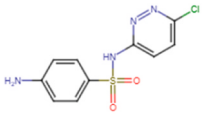
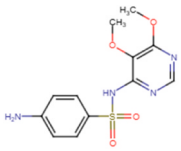
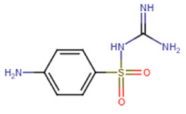
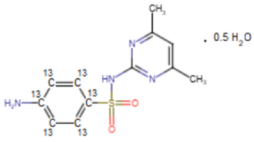
2.6. Biochemical composition of biomass from phycoremediation

Biomass samples were collected after centrifugation at 3500 ×g (Model Universal 320, Hettich, Germany) on the 11th day post-inoculation. The samples were immediately frozen at −20 °C and subsequently lyophilized using a Model 030-JJ LJI Scientific lyophilizer, Brazil. The biochemical composition of the microalgae biomass, including lipid, carbohydrate, protein, and mineral content, was determined following the methodology described by Michelon et al. (2016).

2.7. Genotoxicity assay

Healthy bulbs of *Allium cepa* were prepared by rooting in distilled water under continuous aeration and maintained at room temperature (± 25 °C) with a 12-hour light and 12-hour dark cycle, until roots of approximately 1.0 cm in length were obtained. For the genotoxicity assay, 200 mL of the centrifuged microalgae culture (supernatant) was collected, placed in a separate container, and the *Allium cepa* bulbs were exposed to this solution for 72 h. The test followed the genotoxicity protocol described in previous studies (Fonseca et al., 2021; Michelon et al., 2024). The chromosomal aberrations and mitotic cycle stages were observed and counted using an optical microscope at 400 × magnification (Model CX23, Olympus, USA). For each sample, 3000 cells were examined to calculate the mitotic index and aberration index. The mitotic index was calculated as the number of dividing cells per total number of cells, while the aberration index was determined as the number of aberrant cells per total number of mitotic cells (including

Table 2
Comparison of chemical structure, pK_a, retention time (Rt), precursor and product ions, collision energies and limits of detection (LOD) and quantification (LOQ) of all 10 sulfonamides analyzed.

Antibiotic	Structure	pK_a*	Log Kow	Rt (min)	Molecular weight (g mol^{-1})	Precursor ion $[M+H]^+$ (m/z)	Product ions (m/z)	Collision energy (EV)	LOD (µg L^{-1})	LOQ (µg L^{-1})
Sulfamethoxazole		pK_{a1} = 2.1 pK_{a2} = 5.3	0.89	6.42	253.28	254.3	156.3 92.5	15 15	0.9	3.0
Sulfamethazine		pK_{a1} = 2.3 pK_{a2} = 7.4	0.89	6.18	278.33	279.4	186.5 156.5	18 15	0.9	3.0
Sulfathiazole		pK_{a1} = 2.1 pK_{a2} = 7.1	0.05	5.74	255.30	256.3	156.5 108.3	15 15	0.9	3.0
Sulfadimethoxine		pK_{a1} = 2.9 pK_{a2} = 6.1	1.63	6.92	310.33	311.4	218.4 156.4	19 20	0.9	3.0
Sulfamethizole		pK_{a1} = 1.6 pK_{a2} = 5.7	0.54	6.19	270.30	271.3	156.5 92.5	14 14	0.9	3.0
Sulfamerazine		pK_{a1} = 2.2 pK_{a2} = 6.8	0.14	5.93	264.31	265.4	172.5 92.5	16 29	0.9	3.0
Sulfamonomethoxine		pK_{a1} = 2.0 pK_{a2} = 6.0	0.7	6.52	280.31	281.4	156.5 92.5	18 30	0.9	3.0
Sulfachlorpyridazine		pK_{a1} = 1.9 pK_{a2} = 5.6	0.31	6.41	284.72	285.4	108.5 92.5	24 27	1.5	5.0
Sulfadoxine		pK_{a1} = 1.5 pK_{a2} = 6.0	0.7	6.53	310.32	311.4	156.5 108.4	20 28	0.9	3.0
Sulfaguanidine		pK_{a1} = 0.5 pK_{a1} = 2.8 pK_{a2} = 12.1	−1.22	1.66	214.25	215.3	156.5 92.5	14 25	1.5	5.0
Sulfamethazine-(phenyl−13C6) hemihydrate		-	-	6.18	293.29 (284.4)	285.4	186.3 114.3	18 28	-	-

*The pK_{a1} values of sulfonamides are not usually mentioned once the double protonated form is a strong acid (pK_{a1} <2), so this exists only under very acidic conditions. pK_a

*The pK_{a1} values of sulfonamides are not usually mentioned once the double protonated form is a strong acid (pK_{a1} <2), so this exists only under very acidic conditions. pK_a values (Li et al., 2021), pk_a sulfaguanidine (Białk-Bielińska et al., 2012).

anaphase bridges, laggards, micronuclei, and stickiness).

2.8. Statistical analysis

One-way analysis of variance (ANOVA) was used to determine statistical differences among the treatment groups, with the significance level set at $p \leq 0.05$. Tukey's post-hoc test was performed to assess pairwise differences where variances were significant. Statistical analyses were conducted using Statistica® 8.0 software (STATSOFT trial version).

3. Results and discussion

3.1. Effect of sulfonamide antibiotics on microalgae biomass

The 11-day microalgae cultivation experiment with sulfonamide concentrations ranging from 0.1 mg L^{-1} to 100 mg L^{-1} revealed generally consistent biomass growth across treatments (Fig. 2). The impact of different sulfonamides on the biomass accumulation of *Chlorella* sp. was quantitatively assessed over an 11-day cultivation period. The specific growth rates (μ, d^{-1}) varied depending on the compound and its concentration. Sulfamethoxazole resulted in μ values close to 0.6 d^{-1} ; sulfamerazine ranged from 0.38 to 0.47 d^{-1} ; sulfamethazine from 0.56 to 0.60 d^{-1} ; sulfamonomethoxine from 0.44 to 0.47 d^{-1} ; sulfadimethoxine from 0.57 to 0.66 d^{-1} ; sulfadoxine from 0.44 to 0.48 d^{-1} ; sulfathiazole from 0.59 to 0.66 d^{-1} ; sulfaguanidine from 0.45 to 0.49 d^{-1} ; and sulfachlorpyridazine from 0.40 to 0.46 d^{-1} ; and sulfamethizole ranged from 0.59 to 0.63 d^{-1} .

Most antibiotics tested showed biomass levels in treated groups were similar to controls by day 11 ($p > 0.05$). For sulfamethoxazole (0.45 DW g L^{-1}), sulfamethazine (0.45 DW g L^{-1}), sulfathiazole (0.5 DW g L^{-1}), sulfadimethoxine (0.45 DW g L^{-1}), sulfachlorpyridazine (0.5 DW g L^{-1}), and sulfaguanidine (0.55 DW g L^{-1}), the biomass exhibited steady growth at all concentrations, with minimal to no inhibitory effects even at the highest doses (100 mg L^{-1}). Minor initial differences were observed for some antibiotics, such as a slight lag in biomass growth for sulfamethoxazole between days 3 and 5, but by the end of the experiment, treated and control groups showed comparable biomass levels. For sulfamerazine (0.5 DW g L^{-1}) and sulfadoxine (0.55 DW g L^{-1}), the biomass growth was relatively unaffected across concentrations, maintaining consistent levels even at high exposure (50 mg L^{-1}), further confirming the microalgae's tolerance to these antibiotics. In contrast, sulfamonomethoxine exhibited slight inhibitory effects at the highest concentrations. Sulfamonomethoxine delayed growth in the initial four days at 50 mg L^{-1} , though biomass levels aligned with controls by day 4.

The results suggest that the tested microalgal consortium demonstrates a remarkable tolerance to sulfonamide antibiotics, maintaining stable growth at concentrations that typically inhibit monocultures such as *Chlorella vulgaris*, as reported by Chen et al. (2020a). Their study observed substantial growth inhibition at similar antibiotic levels, with biomass declines of up to 45.9 % depending on the antibiotic concentration (Chen et al., 2020a).

This observation implies the presence of potentially more efficient tolerance and degradation mechanisms supported by interactions among multiple species within the consortium. Studies indicate that microalgal consortia may utilize a broader range of metabolic pathways due to the presence of multiple species, each contributing specific enzymes that assist in breaking down complex contaminants like sulfonamide antibiotics. This enzymatic diversity allows the consortium to turn antibiotics into potential secondary sources of carbon or nitrogen, facilitating the process of metabolization and reducing the inhibitory effects of these contaminants on cell growth (Eheneden et al., 2023).

Another important mechanism is the potential presence of bacteria associated with the consortium that can aid in breaking down sulfonamide compounds. Microalgae frequently form symbiosis with bacteria capable of degrading antibiotics and other toxic compounds. This symbiotic process may enhance antibiotic removal efficiency and mitigate toxic effects on microalgae growth, as demonstrated in other studies involving microalgae and emerging contaminants (Chan et al., 2022).

3.2. Effect of sulfonamide antibiotics on microalgae pigments

The results from the evaluation of the impact of the residual sulfonamides concentration on microalgae pigment production, specifically chlorophyll *α*, chlorophyll *b*, and carotenoids (Fig. S1). The chlorophyll a content in *Chlorella* spp. generally increased over time in treatments with 50 and 100 mg L^{-1} of sulfonamides, ranging from 6.0 to 6.5 μg mL^{-1} . Similarly, chlorophyll *b* production at these higher concentrations (50 and 100 mg L^{-1}) resulted in values between 3.5 and 4.5 μg mL^{-1} . Carotenoid concentrations also increased throughout the experiment, with all treatments at 50 and 100 mg L^{-1} showing values ranging from 2.5 to 3.5 μg mL^{-1} .

Higher concentrations of sulfamethoxazole, sulfathiazole, sulfadimethoxine, and sulfadoxine initially inhibited chlorophyll *α*, with recovery generally occurring by day 11, suggesting a temporary adaptive response. Chlorophyll *b* and carotenoids were less affected, indicating a potential protective role in stress adaptation. Carotenoids, in particular, are known antioxidants that can scavenge reactive oxygen species (ROS), potentially helping to stabilize cellular functions under sulfonamide exposure and contributing to the recovery of chlorophyll *α* (Chen and Xiong, 2024). In contrast, sulfathizole, sulfamerazine, sulfachlorpyridazine, sulfamonomethoxine, and sulfaguanidine had minimal impact on chlorophylls and carotenoids across all concentrations, maintaining pigment levels similar to controls ($p > 0.05$).

The stability observed in carotenoid levels suggests an intrinsic protective mechanism within the consortium that may mitigate oxidative stress more effectively than single-species systems, as supported by previous findings in *Chlorella vulgaris* (Chen et al., 2020a). In monoculture systems, sulfonamide exposure typically triggers higher oxidative stress markers (e.g., superoxide dismutase and hydrogen peroxide) resulting in notable pigment degradation and growth inhibition. In contrast, the consortium's stable carotenoid levels and quick pigment recovery suggest a more robust internal antioxidant system capable of countering ROS accumulation, reducing photosynthetic damage, and supporting pigment stability under antibiotic stress (Chen et al., 2020a).

3.3. pH dynamics during phycoremediation

The dynamics of pH during the phycoremediation process are significant for the solubility, ionization, and subsequent degradation of sulfonamides. pH levels in both experimental groups gradually increased over the 11-day period (Figs. 3A and 3B), with slight variations based on the specific antibiotic. By the end of the experiment, pH stabilized within a range of 9.5–9.8 across all treatments. In contrast, the control (water + antibiotics) maintained pH values between 8.5 and 9.5, suggesting that the combination of antibiotic presence and microalgae activity contributed to the pH rise observed in the experimental groups.

Sulfonamides are amphoteric compounds, indicating they have both acidic and basic properties, with pK_{a1} and pK_{a2} values typically ranging from 1.5 to 3.0 and 5.0–10.5, respectively (Table 2) that influence their ionization state in varying pH environments (Li et al., 2021a). In general, sulfonamides are positively charged at acidic conditions up to pH 2, neutral between pH 2 and 5, and negatively charged at pH above 5 (Hoff et al., 2016). As pH increases, these antibiotics transition from their protonated, less soluble form to a deprotonated, more soluble form, enhancing their degradation potential. In this study, the pH started

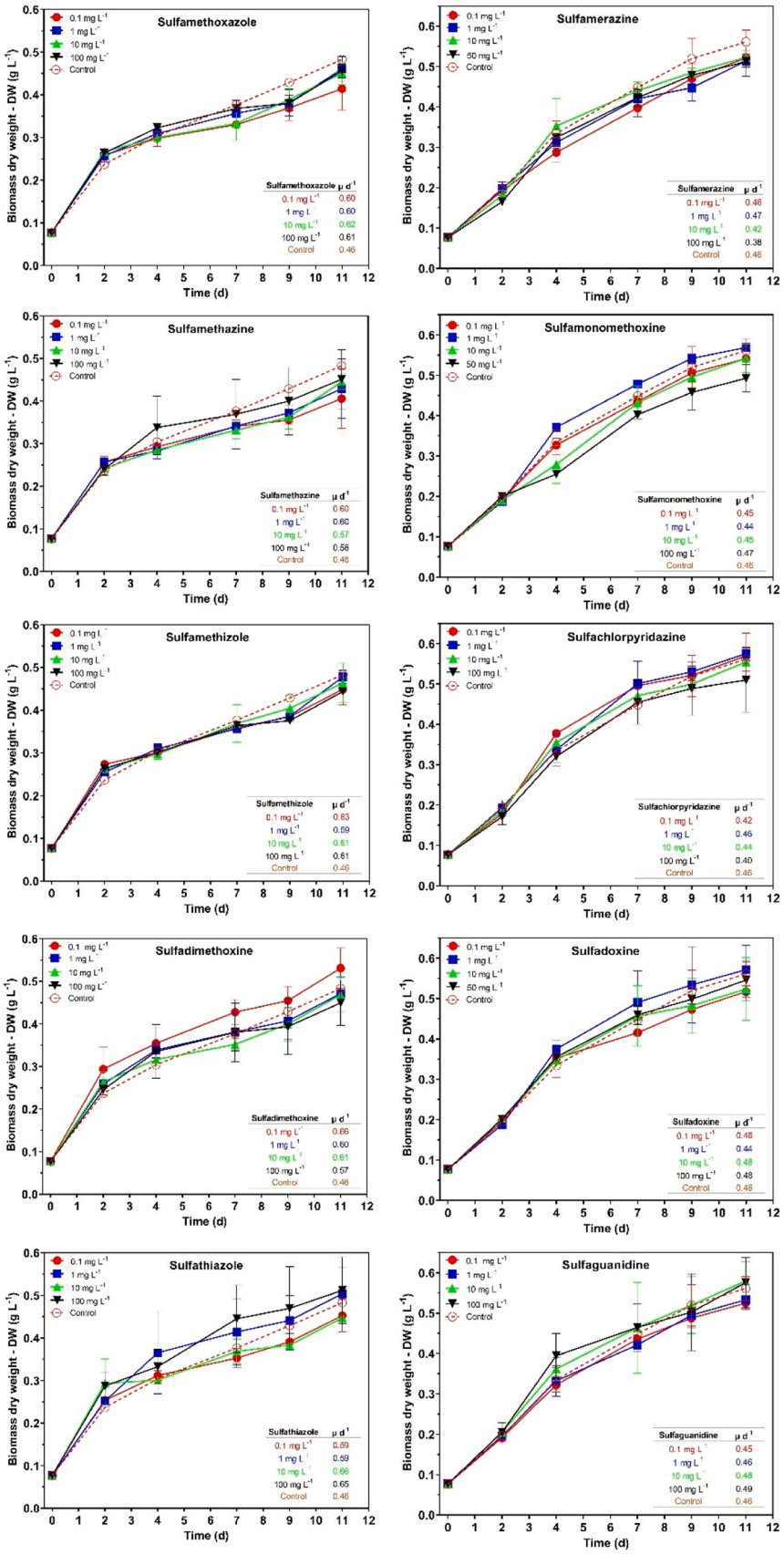


Fig. 2. Effect of sulfonamide antibiotics on growth of microalgae cultivated with swine wastewater.

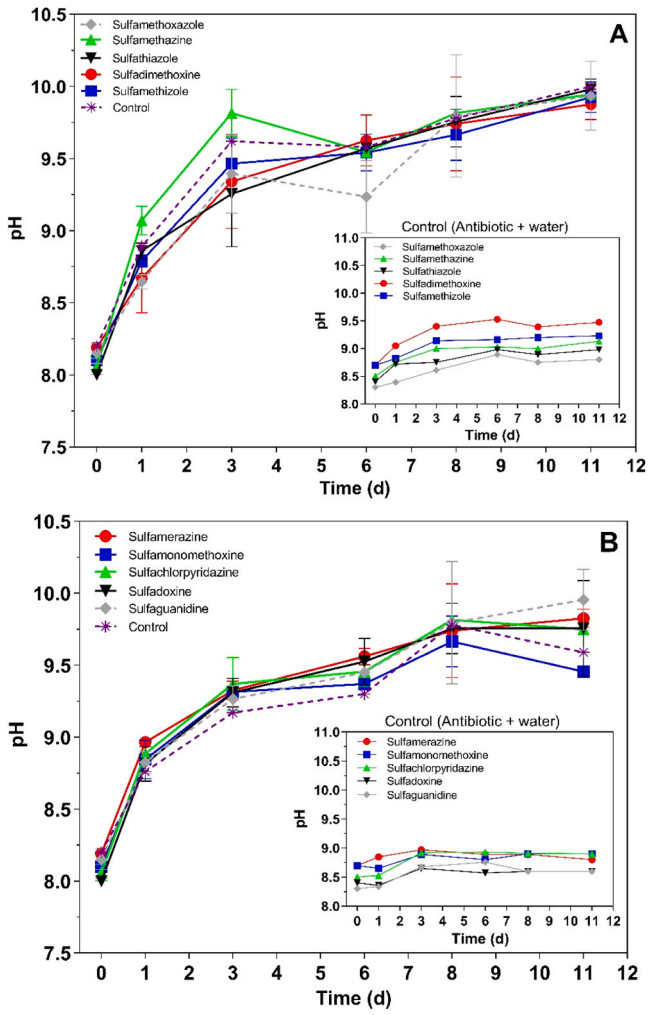


Fig. 3. pH oscillations during microalgae cultivation in the presence of sulfonamide antibiotics.

around 8.0 and progressively rose to between 9.5 and 10.5, depending on the antibiotic. When the pH exceeds these pK_a values, the antibiotics become deprotonated, making them more bioavailable and easier for microalgae to degrade.

Except for sulfaguanidine, the experimental pH range during microalgae growth (pH from 8.0 to 10.5) favored the deprotonated form of the antibiotics tested ($pK_{a2} < 7.4$) during all experiment; therefore, the pH variation probably has a low influence on the degradation kinetics compared with other molecule classes (e.g., tetracyclines, (Michelon et al., 2022)). However, the metabolic and enzymatic activity of microalgae is susceptible to pH changes, and in more alkaline conditions the degradation pathways for antibiotics may become more efficient, especially for compounds that are more soluble in such environments, like sulfonamides, which have Log K_{ow} values generally below 1 (Şanli et al., 2009) (Table 2).

Sulfaguanidine is an unusual sulfonamide compared to the other molecules from the group, as it has a pK_{a2} of 12.1; therefore, it does not have an acidic functional group. In the experimental pH range observed during microalgae growth, the neutral form of sulfaguanidine was favored, and this environment could corroborate with the low degradation results for this molecule observed. However, its lower Log K_{ow} (-1.22) could have exerted compensation on microalgae degradation efficiency.

3.4. Sulfonamide removal efficiency

Fig. 4 presents the degradation profiles of various sulfonamide antibiotics compared to their respective controls over time. To assess the potential removal of sulfonamides, we specifically analyzed a concentration of 10 mg L^{-1}, selecting this intermediate level as it aligns with the concentrations used in other tests. This approach allowed for comparative study among the sulfonamides, addressing a significant gap in the literature, as no previous studies have systematically compared the removal of such a wide range of antibiotics. Sulfamethoxazole exhibited effective removal, with the C/C_o ratio dropping to approximately 0.70 (30 % removal) by day 2 and further decreasing to around 0.30 (70 % removal) by day 6, indicating high removal efficiency by the microalgae. Similarly, sulfachlorpyridazine demonstrated significant removal when compared to control ($p < 0.05$), with the C/C_o ratio decreasing to about 0.80 (20 % removal) by day 1 and falling below 0.55 (55 % removal) by day 11. Sulfamerazine also showed notable degradation, with the C/C_o ratio dropping to approximately 0.50 (50 % removal) by day 4, maintaining this level until the experiment's conclusion. Sulfathiazole exhibited a C/C_o ratio of 0.70 (30 % removal) by day 1, with further reductions observed by day 3. In contrast, sulfadoxine's removal was more gradual, reaching a C/C_o ratio of around 0.70 (30 % removal) by day 3 and remaining stable thereafter.

Sulfamonomethoxine showed a gradual decrease, with the C/C_o ratio reaching about 0.75 (25 % removal) by day 11. Sulfaguanidine exhibited slower degradation, with the C/C_o ratio decreasing to approximately 0.75 (25 % removal) by day 7. In all these cases, the controls exhibited minimal degradation, maintaining a C/C_o ratio close to 1 throughout the experiment. Conversely, sulfamethazine and sulfamethoxine did not demonstrate substantial removal efficiency ($p > 0.05$), as indicated by C/C_o ratios that remained close to those of their controls, suggesting lower or negligible degradation in the presence of microalgae.

Table 1 presents various previous studies on the removal of sulfonamides by microalgae. This removal can occur through several mechanisms, including bioaccumulation, biosorption, and biodegradation (Chu et al., 2022; Eheneden et al., 2023). Bioaccumulation refers to the uptake of contaminants by microalgae from the surrounding environment, leading to their concentration within algal cells. This process can facilitate the removal of sulfonamides, but may also raise concerns regarding potential toxicity to the algae and the food web (Li et al., 2021b). Biosorption, on the other hand, involves the passive adsorption of sulfonamide molecules onto the surface of algal cells, which can occur through various physical and chemical interactions. This mechanism may not necessarily involve metabolic transformation, but it can effectively reduce the concentration of these contaminants in the aqueous phase (Long et al., 2024). Lastly, biodegradation is a more active process where microalgae, often in conjunction with associated microbial communities, enzymatically transform sulfonamides into by-products (Sharma et al., 2022). This process is important for the effective long-term removal of these contaminants, as it can lead to the complete mineralization of sulfonamides.

3.5. Genotoxicity assessment following phycoremediation of sulfonamides antibiotics

The data in Table 3 indicate that the mitotic index (MI) of *Allium cepa* meristematic cells remained high across all treatments with sulfonamide antibiotics after phycoremediation, ranging from 95.1 % to 99.3 %. The control group (microalgae + swine wastewater) exhibited a MI of 98.2 %, suggesting that the phycoremediated water had minimal cytotoxic effects. Notably the rate of genetic aberrations was below 1 % in all evaluated samples. The results suggest that microalgae-based phycoremediation effectively removes most sulfonamide antibiotics, with minimal genotoxic effects observed.

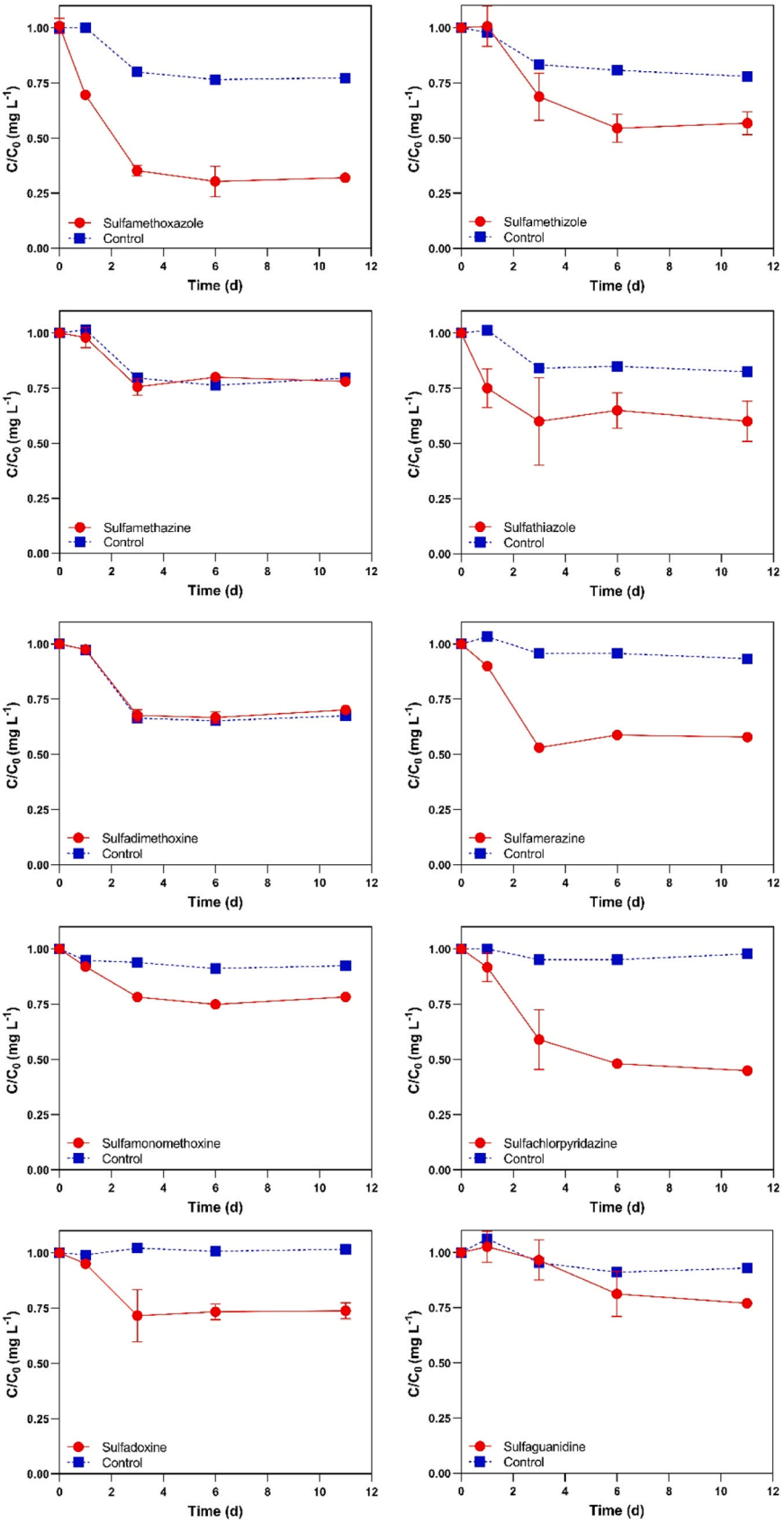


Fig. 4. Removal profile of sulfonamides by microalgae consortia. Except for sulfamethazine and sulfadimethoxine, all treatments removed significantly higher ($p < 0.05$) concentrations than the control.

Table 3
Mitotic index observed in meristematic cells of *Allium cepa* exposed to water post-phycoremediation of antibiotics.

Antibiotic	Mitotic index (%)
Sulfamethoxazole	99.3
Sulfamethazine	97.6
Sulfathiazole	98.9
Sulfadimethoxine	97.1
Sulfamethizole	98.8
Sulfamerazine	97.8
Sulfamonomethoxine	96.4
Sulfachlorpyridazine	97.2
Sulfadoxine	98.7
Sulfaguanidine	95.1
Control (microalgae + swine wastewater)	98.2

3.6. Biochemical composition and biofuel production theoretical

The biochemical composition of the microalgal biomass after phycoremediation was evaluated by analyzing the content of carbohydrates, proteins, lipids, and mineral matter across treatments with different sulfonamide antibiotics (Fig. 5). The carbohydrate content varied significantly among treatments ($p < 0.05$), with sulfamonomethoxine and sulfachlorpyridazine treatments leading to the highest carbohydrate levels (56 %). In contrast, treatments with sulfamethazine and sulfamethizole exhibited lower carbohydrate content, (45 %), which was similar to the control group ($p > 0.05$). The reduced carbohydrate accumulation in these treatments could be attributed to metabolic stress, where energy is diverted from carbohydrate storage toward other metabolic processes, such as protein synthesis or stress response mechanisms (Debnath et al., 2021).

The protein content analysis revealed that the microalgae group, which previously exhibited higher carbohydrate concentrations, now shows lower protein levels. The control displayed the highest protein content (~50 %), with similar levels observed in sulfamethazine and sulfamethizole treatments. Significant differences ($p < 0.05$) were noted for other antibiotics, suggesting that certain sulfonamide compounds may inhibit protein synthesis or enhance protein degradation in the microalgae cells, potentially as a response to oxidative stress or impaired metabolic pathways. Conversely, antibiotics like sulfamethazine and sulfamethizole had a smaller impact on protein accumulation, indicating that the microalgal consortium had a higher tolerance or more efficient metabolic adaptation in response to these specific antibiotics. A study by Chen et al. (2020b) measured the *Chlorella vulgaris* antioxidant enzymes and reported that this algae presents a higher tolerance to sulfonamide antibiotics.

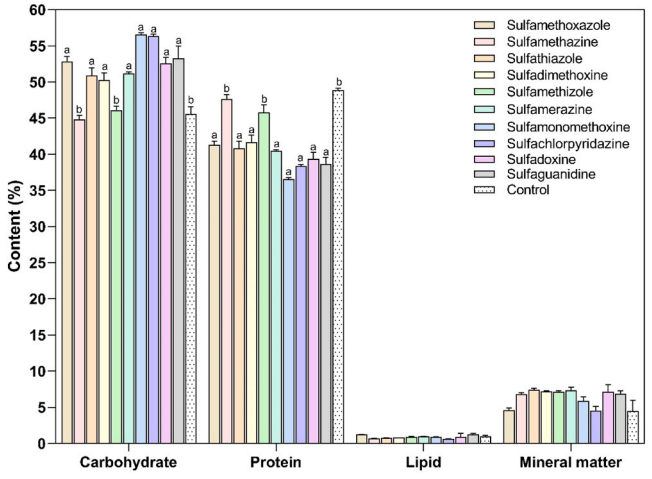


Fig. 5. Biochemical composition of microalgal biomass after phycoremediation with different sulfonamide antibiotics, showing carbohydrate, protein, lipid, and mineral matter contents. Different letters indicate statistically significant differences.

Lipid content remained low across all treatments, including the control, with no significant differences between the antibiotics ($p > 0.05$). This consistency suggests that sulfonamide antibiotics did not substantially influence lipid synthesis or accumulation during phycoremediation. The low lipid levels may reflect an energy allocation priority toward carbohydrate and protein synthesis, or it could be influenced by the nutrient composition of the medium, which may not have supported lipid accumulation under the experimental conditions (Michelon et al., 2016). The mineral matter content was relatively uniform across all treatments, with no statistically significant differences between antibiotics and control ($p > 0.05$). This uniformity indicates that sulfonamide antibiotics did not interfere with the uptake or assimilation of essential minerals during the remediation process.

Carbohydrates, along with proteins and lipids, are key components of microalgal biomass, primarily cellulose and starch found in the cell wall and plastids. In a study on *Scenedesmus obliquus*, the carbohydrate content decreased gradually from 25 % to 20 % as the concentration of the sulfamethazine and sulfamethoxazole mixture increased from 0 to 0.5 mg L^{-1}. This reduction in carbohydrate content was consistent with a decline in total chlorophyll levels, as carbon fixation through photosynthesis is the primary source of carbohydrates in microalgae (Xiong et al., 2019).

The biomass of microalgae recovered from swine wastewater after phycoremediation may still contain traces of antibiotics, which raises concerns about its potential applications (Wang et al., 2024b). Nevertheless, given its high carbohydrate and protein content, this biomass can serve as an economical feedstock for bioethanol production (Damayanti et al., 2025) or be utilized in biomethane generation (Vargas-Estrada et al., 2022), aligning well with circular economy principles.

Based on the method developed by Michelon et al. (2019) and applying the observed microalgal biomass production from the current study (average biomass yield of 0.5 g L^{-1} over an 11-day cultivation period), swine wastewater treated through phycoremediation could yield approximately 66.4 tons of microalgal biomass per hectare per year. If this biomass, containing about 50 % carbohydrates (Silva and Bertucco 2019), were used for bioethanol production at a conversion rate of 324 L per ton, it could generate about 21,501 L of bioethanol per hectare annually. Alternatively, converting the biomass into biomethane could result in an estimated production of 2920,000 L_N of biomethane per hectare per year, based on the methane yield of 44 ± 2.5 L per kilogram of biomass (Perazzoli et al., 2016). This biofuel production process can be further optimized by integrating substrates such as microalgae and swine manure and increasing changes in the cellular composition of microalgae (Dinnebier et al., 2021).

4. Conclusion

This study demonstrates the potential of *Chlorella* spp. for efficiently removing sulfonamide antibiotics from swine wastewater through phycoremediation. The microalgae consortium exhibited robust growth and effective degradation of various sulfonamides, with sulfamethoxazole and sulfachlorpyridazine showing the highest removal efficiencies. Furthermore, the treatment process had a minimal impact on the genotoxicity of wastewater, as evidenced by the low chromosomal aberrations observed in *Allium cepa* assays. The genotoxicity assessment confirmed that the treated wastewater poses minimal environmental risk. Additionally, the biochemical composition of microalgal biomass, particularly its carbohydrate content, suggests it could serve as a valuable resource for biofuel production. These findings provide strong evidence for the use of microalgae-based phycoremediation as a viable and sustainable approach to managing antibiotic contamination in agricultural wastewater while contributing to renewable energy solutions.

Statements and declarations

Competing interests

The authors have no relevant financial or non-financial interests to disclose.

Funding

The authors declare that no funds, grants, or other support were received during the preparation of this manuscript

CRediT authorship contribution statement

Aline Viancelli: Writing – review & editing, Writing – original draft, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Alexandre Matthiensen:** Writing – review & editing, Writing – original draft. **Estela de Oliveira Nunes:** Writing – review & editing, Writing – original draft. **Renato Eising:** Writing – review & editing, Writing – original draft. **Mateus Gustavo Novello:** Methodology, Investigation, Formal analysis. **Micheli Colla Vieira:** Methodology, Investigation, Formal analysis. **Vanessa Gressler:** Writing – review & editing, Writing – original draft, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **William Michelon:** Writing – review & editing, Writing – original draft, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

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.

Acknowledgements

The authors gratefully acknowledge the support provided by the Fundação Araucária de Apoio ao Desenvolvimento Científico e Tecnológico do Paraná and Biopark Educação.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.psep.2025.107338.

Data availability

The datasets generated for this study are available on request from the corresponding author.

References

Apha, 2012. Standard methods for the examination for water and wastewater, 22nd edn. American Water Works Association, Washington, DC.

Białk-Bielińska, A., Stolte, S., Matzke, M., et al., 2012. Hydrolysis of sulphonamides in aqueous solutions. J. Hazard Mater. 221–222 264–274. https://doi.org/10.1016/j.jhazmat.2012.04.044.

Bilal, M., Mehmood, S., Rasheed, T., Iqbal, H.M.N., 2020. Antibiotics traces in the aquatic environment: persistence and adverse environmental impact. Curr. Opin. Environ. Sci. Heal 13, 68–74. https://doi.org/10.1016/j.coesh.2019.11.005.

Chan, S.S., Khoo, K.S., Chew, K.W., et al., 2022. Recent advances biodegradation and biosorption of organic compounds from wastewater: Microalgae-bacteria consortium - A review. Bioresour. Technol. 344, 126159. https://doi.org/10.1016/j.biortech.2021.126159.

Chen, S., Zhang, W., Li, J., et al., 2020b. Ecotoxicological effects of sulfonamides and fluoroquinolones and their removal by a green alga (Chlorella vulgaris) and a cyanobacterium (Chrysosporum ovalisporum). Environ. Pollut. 263, 114554. https://doi.org/10.1016/j.envpol.2020.114554.

Chen, S., Wang, L., Feng, W., et al., 2020a. Sulfonamides-induced oxidative stress in freshwater microalga Chlorella vulgaris: Evaluation of growth, photosynthesis, antioxidants, ultrastructure, and nucleic acids. Sci. Rep. 10, 8243. https://doi.org/10.1038/s41598-020-65219-2.

Chen, Z., Xiong, J.-Q., 2024. Recovery mechanism of a microalgal species, Chlorella sp. from toxicity of doxylamine: Physiological and biochemical changes, and transcriptomics. J. Hazard Mater. 474, 134752. https://doi.org/10.1016/j.jhazmat.2024.134752.

Chu, Y., Zhang, C., Wang, R., et al., 2022. Biotransformation of sulfamethoxazole by microalgae: Removal efficiency, pathways, and mechanisms. Water Res 221, 118834. https://doi.org/10.1016/j.watres.2022.118834.

Damayanti, Megawati, A., Winaningsih, I., et al., 2025. Comparative study of pretreatment methods for the reverse enzymatic hydrolysis of Chlorella and Spirulina microalgae for bioethanol production. Biofuels 1–7. https://doi.org/10.1080/17597269.2025.2469384.

Dayana Priyadharshini, S., Suresh Babu, P., Manikandan, S., et al., 2021. Phycoremediation of wastewater for pollutant removal: A green approach to environmental protection and long-term remediation. Environ. Pollut. 290, 117989. https://doi.org/10.1016/j.envpol.2021.117989.

Debnath, C., Bandyopadhyay, T.K., Bhunia, B., et al., 2021. Microalgae: Sustainable resource of carbohydrates in third-generation biofuel production. Renew. Sustain Energy Rev. 150, 111464. https://doi.org/10.1016/j.rser.2021.111464.

Dinnebier, H.C.F., Matthiensen, A., Michelon, W., et al., 2021. Phycoremediation and biomass production from high strong swine wastewater for biogas generation improvement: An integrated bioprocess. Bioresour. Technol. 332, 125111. https://doi.org/10.1016/j.biortech.2021.125111.

Eheneden, I., Wang, R., Zhao, J., 2023. Antibiotic removal by microalgae-bacteria consortium: Metabolic pathways and microbial responses. Sci. Total Environ. 891, 164489. https://doi.org/10.1016/j.scitotenv.2023.164489.

Fonseca, T.G., Motta, E.A., Mass, A.P., et al., 2021. Toxicity and Enterobacteriaceae Profile in Water in Different Hydrological Events: a Case from South Brazil. Water, Air, Soil Pollut. 232, 278. https://doi.org/10.1007/s11270-021-05208-x.

Frascaroli, G., Roberts, J., Hunter, C., Escudero, A., 2024. Removal efficiencies of seven frequently detected antibiotics and related physiological responses in three microalgae species. Environ. Sci. Pollut. Res 31, 14178–14190. https://doi.org/10.1007/s11356-024-32026-5.

Gao, F., Zhou, J.-L., Zhang, Y.-R., et al., 2023. Efficient coupling of sulfadiazine removal with microalgae lipid production in a membrane photobioreactor. Chemosphere 316, 137880. https://doi.org/10.1016/j.chemosphere.2023.137880.

Hoff, R., Pizzolato, T.M., Diaz-Cruz, M.S., 2016. Trends in sulfonamides and their by-products analysis in environmental samples using mass spectrometry techniques. Trends Environ. Anal. Chem. 9, 24–36. https://doi.org/10.1016/j.teac.2016.02.002.

Huang, R., Liu, W., Su, J., et al., 2023. Keystone microalgae species determine the removal efficiency of sulfamethoxazole: a case study of Chlorella pyrenoidosa and microalgae consortia. Front Plant Sci. 14. https://doi.org/10.3389/fpls.2023.1193668.

Hussain, F., Shah, S.Z., Ahmad, H., et al., 2021. Microalgae an ecofriendly and sustainable wastewater treatment option: Biomass application in biofuel and biofertilizer production. A review. Renew. Sustain Energy Rev. 137, 110603. https://doi.org/10.1016/j.rser.2020.110603.

Jain, M., Khan, S.A., Sharma, K., et al., 2022. Current perspective of innovative strategies for bioremediation of organic pollutants from wastewater. Bioresour. Technol. 344, 126305. https://doi.org/10.1016/j.biortech.2021.126305.

Koch, N., Islam, N.F., Sonowal, S., et al., 2021. Environmental antibiotics and resistance genes as emerging contaminants: Methods of detection and bioremediation. Curr. Res Micro Sci. 2, 100027. https://doi.org/10.1016/j.crmicr.2021.100027.

Kunz, A., Schierholt, G., Menozzo, G.F., et al., 2006. Estação de tratamento de dejetos de suínos (ETDS) como alternativa na redução do impacto ambiental da suinocultura. Comun. Técnico 452, 1–6.

Li, D., Wang, P., Sun, M., et al., 2024. Effects of sulfamonomethoxine and trimethoprim co-exposures at different environmentally relevant concentrations on microalgal growth and nutrient assimilation. Aquat. Toxicol. 271, 106937. https://doi.org/10.1016/j.aquatox.2024.106937.

Li, J., Zhao, L., Feng, M., et al., 2021a. Abiotic transformation and ecotoxicity change of sulfonamide antibiotics in environmental and water treatment processes: A critical review. Water Res 202, 117463. https://doi.org/10.1016/j.watres.2021.117463.

Li, S., Yu, Y., Gao, X., et al., 2021b. Evaluation of growth and biochemical responses of freshwater microalgae Chlorella vulgaris due to exposure and uptake of sulfonamides and copper. Bioresour. Technol. 342, 126064. https://doi.org/10.1016/j.biortech.2021.126064.

Li, X., Guo, P., Shan, Y., et al., 2017. Determination of 82 veterinary drugs in swine waste lagoon sludge by ultra-high performance liquid chromatography–tandem mass spectrometry. J. Chromatogr. A 1499, 57–64. https://doi.org/10.1016/j.chroma.2017.03.055.

Liu, W., Wang, Y., Xia, R., et al., 2023. Occurrence and fate of antibiotics in swine waste treatment: An industrial case. Environ. Pollut. 331, 121945. https://doi.org/10.1016/j.envpol.2023.121945.

Long, S., Hamilton, P.B., Wang, C., et al., 2024. Bioadsorption, bioaccumulation and biodegradation of antibiotics by algae and their association with algal physiological state and antibiotic physicochemical properties. J. Hazard Mater. 468, 133787. https://doi.org/10.1016/j.jhazmat.2024.133787.

Manyi-Loh, C., Mamphweli, S., Meyer, E., Okoh, A., 2018. Antibiotic Use in Agriculture and Its Consequential Resistance in Environmental Sources: Potential Public Health Implications. Molecules 23, 795. https://doi.org/10.3390/molecules23040795.

Michelon, W., Da Silva, M.L.B., Mezzari, M.P., et al., 2016. Effects of Nitrogen and Phosphorus on Biochemical Composition of Microalgae Polyculture Harvested from Phycoremediation of Piggery Wastewater Digestate. Appl. Biochem Biotechnol. 178, 1407–1419. https://doi.org/10.1007/s12010-015-1955-x.

Michelon, W., Pirolli, M., Mezzari, M.P., et al., 2019. Residual sugar from microalgae biomass harvested from phycoremediation of swine wastewater digestate. Water Sci. Technol. 79, 2203–2210. https://doi.org/10.2166/wst.2019.226.

Michelon, W., Matthiensen, A., Viancelli, A., et al., 2022. Removal of veterinary antibiotics in swine wastewater using microalgae-based process. Environ. Res 207, 112192. https://doi.org/10.1016/j.envres.2021.112192.

Michelon, W., Nienov, F.A., Knoblauch, P.M., et al., 2024. Biochar produced from eggshell waste applied for removal of water-polluting substances and clayey soil stabilization: an environmental friendly application. Biomass-.-. Convers. Biorefinery 14, 13123–13134. https://doi.org/10.1007/s13399-022-03268-5.

Montone, C.M., Giannelli Moneta, B., Laganà, A., et al., 2024. Transformation products of antibacterial drugs in environmental water: Identification approaches based on liquid chromatography-high resolution mass spectrometry. J. Pharm. Biomed. Anal. 238, 115818. https://doi.org/10.1016/j.jpba.2023.115818.

Muhammad, J., Khan, S., Su, J.Q., et al., 2020. Antibiotics in poultry manure and their associated health issues: a systematic review. J. Soils Sediment. 20, 486–497. https://doi.org/10.1007/s11368-019-02360-0.

Ovung, A., Bhattacharyya, J., 2021. Sulfonamide drugs: structure, antibacterial property, toxicity, and biophysical interactions. Biophys. Rev. 13, 259–272. https://doi.org/10.1007/s12551-021-00795-9.

Perazzoli, S., Bruchez, B.M., Michelon, W., et al., 2016. Optimizing biomethane production from anaerobic degradation of Scenedesmus spp. biomass harvested from algae-based swine digestate treatment. Int Biodeterior. Biodegrad. 109, 23–28. https://doi.org/10.1016/j.ibiod.2015.12.027.

Prandini, J.M., da Silva, M.L.B., Mezzari, M.P., et al., 2016. Enhancement of nutrient removal from swine wastewater digestate coupled to biogas purification by microalgae Scenedesmus spp. Bioresour. Technol. 202, 67–75. https://doi.org/10.1016/j.biortech.2015.11.082.

Rohilla, S., Sharma, D., 2023. Sulfonamides, quinolones, antiseptics, and disinfectants. In: Medicinal Chemistry of Chemotherapeutic Agents. Elsevier, pp. 21–63.

Şanli, S., Altun, Y., Şanli, N., et al., 2009. Solvent Effects on p K a values of Some Substituted Sulfonamides in Acetonitrile−Water Binary Mixtures by the UV-Spectroscopy Method. J. Chem. Eng. Data 54, 3014–3021. https://doi.org/10.1021/je9000813.

Sharma, J., Joshi, M., Nigam, S., 2022. Role of microalgae in degradation of pharmaceutical compounds from water. In: An Integration of Phycoremediation Processes in Wastewater Treatment. Elsevier, pp. 75–102.

Silva, C.E.de F., Bertucco, A., 2019. Bioethanol from Microalgal Biomass: A Promising Approach in Biorefinery. Braz. Arch. Biol. Technol. 62. https://doi.org/10.1590/1678-4324-2019160816.

da Silva Rodrigues DA, da Cunha, C.C.R.F., Freitas, M.G., et al., 2020. Biodegradation of sulfamethoxazole by microalgae-bacteria consortium in wastewater treatment plant effluents. Sci. Total Environ. 749, 141441. https://doi.org/10.1016/j.scitotenv.2020.141441.

Tian, Y., Li, J., Li, X., et al., 2022. Sample pretreatment and analytical methodology for the determination of antibiotics in swine wastewater and activated sludge. Environ. Sci. Pollut. Res 29, 83671–83685. https://doi.org/10.1007/s11356-022-21595-y.

Vargas-Estrada, L., Longoria, A., Arenas, E., et al., 2022. A Review on Current Trends in Biogas Production from Microalgae Biomass and Microalgae Waste by Anaerobic Digestion and Co-digestion. BioEnergy Res 15, 77–92. https://doi.org/10.1007/s12155-021-10276-2.

Viancelli, A., Michelon, W., Rogovski, P., et al., 2020. A review on alternative bioprocesses for removal of emerging contaminants. Bioprocess Biosyst. Eng. 43, 2117–2129. https://doi.org/10.1007/s00449-020-02410-9.

Wang, K., Tong, L., Yu, J., et al., 2024a. Supplementation of diethyl aminoethyl hexanoate for enhancing antibiotics removal by different microalgae-based system. Bioresour. Technol. 408, 131231. https://doi.org/10.1016/j.biortech.2024.131231.

Wang, Y., Li, J., Lei, Y., et al., 2022. Bioremediation of sulfonamides by a microalgae-bacteria consortium – Analysis of pollutants removal efficiency, cellular composition, and bacterial community. Bioresour. Technol. 351, 126964. https://doi.org/10.1016/j.biortech.2022.126964.

Wang, Z., Hu, G., Hong, Y., 2024b. Strong Alliance of Microalgae and Bacteria: The State-of-the-Art Review and Future Prospects of Utilizing Microalgae-Bacteria Consortia for Comprehensive Treatment of Swine Wastewater. Curr. Pollut. Rep. 10, 744–764. https://doi.org/10.1007/s40726-024-00325-7.

Wani, A.K., ul Gani Mir, T., Akhtar, N., et al., 2024. Algae-Mediated Removal of Prevalent Genotoxic Antibiotics: Molecular Perspective on Algae-Bacteria Consortia and Bioreactor-Based Strategies. Curr. Microbiol 81, 112. https://doi.org/10.1007/s00284-024-03631-x.

Xing, C., Li, J., Yuan, H., Yang, J., 2022. Physiological and transcription level responses of microalgae AuxenoChlorella protothecoides to cold and heat induced oxidative stress. Environ. Res 211, 113023. https://doi.org/10.1016/j.envres.2022.113023.

Xiong, J.-Q., Govindwar, S., Kurade, M.B., et al., 2019. Toxicity of sulfamethazine and sulfamethoxazole and their removal by a green microalga, Scenedesmus obliquus. Chemosphere 218, 551–558. https://doi.org/10.1016/j.chemosphere.2018.11.146.

Yatirajula, S.K., Shrivastava, A., Saxena, V.K., Kodavaty, J., 2019. Flow behavior analysis of Chlorella vulgaris microalgal biomass. Heliyon 5, e01845. https://doi.org/10.1016/j.heliyon.2019.e01845.

Zhuang, L.-L., Qian, W., Wang, X., et al., 2024. General performance, kinetic modification, and key regulating factor recognition of microalgae-based sulfonamide removal. J. Hazard Mater. 475, 134891. https://doi.org/10.1016/j.jhazmat.2024.134891.