Water Research 218 (2022) 118497

Contents lists available at ScienceDirect

Water Research

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

Antibiotics removal from aquaculture effluents by ozonation: chemical and toxicity descriptors

Ana M. Gorito [a,b,c], Ana R. Lado Ribeiro [a,b], Pedro Rodrigues [c], M. Fernando R. Pereira [a,b], Laura Guimarães [c], C. Marisa R. Almeida [c,*], Adrián M.T. Silva [a,b,*]

[a] LSRE-LCM - Laboratory of Separation and Reaction Engineering – Laboratory of Catalysis and Materials, Faculty of Engineering, University of Porto, Rua Dr. Roberto Frias, 4200-465 Porto, Portugal
[b] ALiCE - Associate Laboratory in Chemical Engineering, Faculty of Engineering, University of Porto, Rua Dr. Roberto Frias, 4200-465 Porto, Portugal
[c] Interdisciplinary Centre of Marine and Environmental Research (CIIMAR/CIMAR), Universidade do Porto, Terminal de Cruzeiros do Porto de Leixões, Av. General Norton de Matos s/n, 4450-208 Matosinhos, Portugal

ARTICLE INFO

Keywords:
Water pollution
aquaculture
antibiotics
ozone
zebrafish

ABSTRACT

Antibiotics are often applied in aquaculture to prevent fish diseases. These substances can cause disturbances on receiving waters, when not properly eliminated from the aquaculture effluents. In this work, ozone (O_3) was investigated as a possible oxidizing agent to remove fishery antibiotics from aquaculture effluents: florfenicol (FF), oxytetracycline (OTC), sulfadimethoxine (SDM), sulfamethoxazole (SMX), and trimethoprim (TMP). Batch experiments were performed using ultrapure water and aquaculture effluents spiked with a mixture of target antibiotics at relatively high concentrations (10 mg L^{-1} each). OTC, SMX and TMP were fully removed (< 30 min) regardless of the tested conditions, mainly by O_3 direct attack. In contrast, FF was partially removed in 30 min (~ 10 and 60%, in aquaculture effluents and ultrapure water, respectively), but only in the presence of hydroxyl radicals ($HO^{\bullet}$), the FF concentrations reaching levels below the detection limits in ultrapure water after 60 min. In the case of SDM, its degradation was highly influenced by the selected water matrix, but with removals always higher than 68%. In continuous-flow experiments applying more environmentally relevant antibiotic concentrations (100 ng L^{-1} each) and low O_3 doses (1.5 mg L^{-1}), ozonation highly removed (> 98%) all tested antibiotics from aquaculture effluents with a hydraulic retention time (HRT) of 10 min, except FF (68%). Although by-products were detected in treated samples, zebrafish (*Danio rerio*) embryotoxicity tests did not show a toxicity increase by applying this ozonation treatment. Ozonation is thus a possible solution to remove antibiotics from aquaculture effluents. Still, full-scale studies in aquaculture farms are needed, and generation of $HO^{\bullet}$ may be favoured to readily oxidize the FF antibiotic.

1. Introduction

In the last few years, the global fish consumption has increased more than that of any other animal protein food (*e.g.*, meat, dairy, and milk), and aquaculture is one of the fastest-growing food-producing sectors (FAO, 2020). Intensive aquaculture typically involves many cultured organisms in confined areas, which can promote the dissemination of parasites and bacterial infections and, consequently, considerable economic losses for the sector (Lulijwa et al., 2020; Rigos et al., 2010). In this context, to tackle infections and their undesirable effects, antibiotics such as tetracyclines, trimethoprim (TMP), sulfonamides, quinolones, β-lactams, fluoroquinolones, and phenicols have been widely supplied in aquaculture (Gorito et al., 2022).

The excessive application of antibiotics in aquaculture can bring serious threats to the environment and human health, such as their spread in the different environmental compartments and the possible proliferation of antibiotic-resistant bacteria and their related genes (ARB & ARGs), which is one of the main challenges that humans face today (Preena et al., 2020; Schar et al., 2020; Zheng et al., 2021). Indeed, high levels of ARB have been described in, and around, aquaculture farms (Choi et al., 2020). Antibiotics are generally added directly to the water through food pellets, and thus, both the unconsumed fraction of antibiotics and the excreta of farming organisms end up in outlet waters (Leal et al., 2017; Pereira et al., 2013; Serrano, 2005). Moreover, water

* Corresponding authors
E-mail addresses: calmeida@fc.up.pt (C.M.R. Almeida), adrian@fe.up.pt (A.M.T. Silva).

https://doi.org/10.1016/j.watres.2022.118497
Received 3 February 2022; Received in revised form 14 April 2022; Accepted 21 April 2022
Available online 25 April 2022
0043-1354/© 2022 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

treatment equipment installed in aquaculture farms is not commonly adequate for removing antibiotics, which are highly stable (Camera-Roda et al., 2019; Zhu et al., 2020).

One alternative process that has been used to improve water quality in aquaculture is ozonation. High removal rates of pathogens, organic matter, solids, colour, and inorganic species can be achieved with this treatment (Powell and Scolding, 2018; Schroeder et al., 2011; Tango and Gagnon, 2003). However, specific information regarding antibiotics removal in aquaculture is still very scarce – only two studies were found in the *Scopus* database using the keywords "*ozone/ozonation*", "*antibiotics*" and "*aquaculture*" (Choi et al., 2020; Kye et al., 2020). In these two studies, ozone (O_3) significantly removed the fishery antibiotics amoxicillin (Choi et al., 2020), oxytetracycline (OTC) (Choi et al., 2020) and oxolinic acid (Choi et al., 2020; Kye et al., 2020), but florfenicol (FF) was very refractory to the treatment (Choi et al., 2020; Kye et al., 2020). One of the main drawbacks of ozonation is the formation of oxidation by-products that might have toxicological effects similar or even higher than those of parent compounds (Prieto-Rodríguez et al., 2013). For instance, bromate ion is a known ozonation by-product of toxicological concern originated from O_3 reaction with bromide naturally found in water matrices (Camera-Roda et al., 2019; Gorito et al., 2021). Thus, both physico-chemical and toxicological criteria are crucial to conclude about ozonation efficiency and quality of treated water (García-Cambero et al., 2019; Prieto-Rodríguez et al., 2013). Specifically for aquaculture, guaranteeing the quality of ozonated effluents is essential when these effluents are discharged into the environment or recirculated to feed the aquaculture facilities. Despite that, studies involving aquaculture effluents are only focused on antibiotics degradation without assessment of toxicity and/or general quality of the treated effluent (Choi et al., 2020; Kye et al., 2020), as is also the case of many other studies dealing with ozonation for the removal of antibiotics from different water matrices.

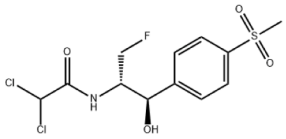
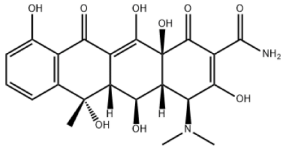
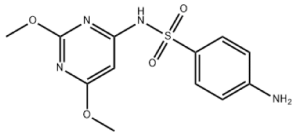
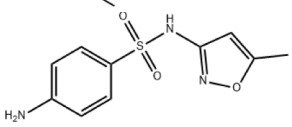
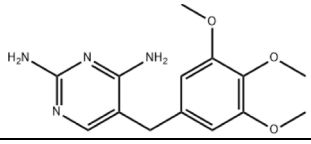
In this work, the possibility of applying ozonation to aquaculture effluents aiming at the removal of different antibiotics commonly used in these farms (FF, OTC, sulfadimethoxine (SDM), sulfamethoxazole (SMX), and TMP; Table 1) was investigated. Firstly, the degradation of target compounds was studied in batch mode, where different parameters such as the role of hydroxyl radicals ($HO^{\bullet}$) and water matrix were evaluated. Afterwards, the removal of target antibiotics was also assessed in a continuous-flow mode for which more realistic conditions were applied (environmentally relevant antibiotic concentrations, low O_3 concentration and short hydraulic retention time - HRT). The potential toxicity effects of the continuous ozonated effluents were then estimated using zebrafish embryo assays.

2. Materials and methods

2.1. Chemicals

All reference antibiotic standards (FF, OTC, SDM, SMX and TMP), as well as the internal standard SDM-d_6, were purchased from Sigma-Aldrich (Steinheim, Germany). Individual stock solutions of approximately 1000 mg L^{-1} were prepared by dissolving the appropriate mass of each antibiotic or internal standard in methanol (MeOH), which were then diluted to 10 and 1 mg L^{-1} for mass spectrometry analysis optimization. A working solution containing all antibiotic standards (2.5 mg L^{-1}) was also prepared by dilution in MeOH, which was used to spike the aqueous matrix for the ozonation experiments. Likewise, a working solution of SDM- d_6 (5 mg L^{-1}) was prepared to be added to each sample before the solid-phase extraction (SPE) procedure. MeOH and acetonitrile MS grade were purchased from VWR International (Oregon, USA), whereas formic acid and sulphuric acid were supplied by Merck (Darmstadt, Germany). Ethylenediaminetetraacetic acid disodium salt dihydrate (EDTA-$Na_2.2H_2O$) was acquired from PanReac (Castellar del Vallès, Barcelona) and ammonium formate (> 97% purity) was supplied by Honeywell Fluka (North Carolina, USA). 3,4-dichloroaniline (98%) was obtained from Alfa Aesar (Kandel, Germany). Ultrapure water (resistivity > 18.2 MΩ cm at 25°C) was supplied by a Milli-Q water system from Millipore (Massachusetts, USA). Dechlorinated water used in zebrafish embryo assays was obtained by a reverse osmosis system

Table 1
Target antibiotics and their properties: class, molecular structure, and reaction rate kinetic constants with O_3 (k_{O3}) and $HO^{\bullet}$ ($k_{HO\bullet}$) determined for each compound individually in ultrapure/deionized water.

Antibiotic Class	Compound	Molecular Structure	k_{O3} (M^{-1} s^{-1})	$k_{HO\bullet}$ (10^{9} M^{-1} s^{-1})
Phenicols	Florfenicol (FF)		< 10 (Choi et al., 2020)	-
Tetracyclines	Oxytetracycline (OTC)		7×10^5 (Hopkins and Blaney, 2014)***	5.6 (Jeong et al., 2010)**,+
Sulfonamides	Sulfadimethoxine (SDM)		2.7×10^6 (Ben et al., 2012)*,+	6.1 (Ikehata et al., 2006)
	Sulfamethoxazole (SMX)		2.5×10^6 (Huber et al., 2003)*,+	5.5 (Huber et al., 2003)*,+
Others	Trimethoprim (TMP)		2.7×10^5 (Dodd et al., 2006)+	6.9 (Dodd et al., 2006)+

+ * 20 °C; + pH 7; ** 22 °C; *** pH 9, 20-21 °C.

(LSRO-101-A Lan Shan, Changhua County, Taiwan).

2.2. Aquaculture water samples

Aquaculture effluents were collected in a trout farm located in Portugal. This farm is mainly designed for recreational fishing activities, with very low production rates of fish and a high dependency of natural conditions. After sampling, effluents were stored in pre-rinsed amber glass bottles, transported at 4°C to the laboratory and kept refrigerated until use (*i.e.,* within 3 days after sampling). In addition, collected effluents were characterised in terms of: (i) general physico-chemical parameters (*i.e.*, pH, dissolved organic carbon (DOC), chemical (COD) and biochemical (BOD) oxygen demand, total suspended (TSS) and dissolved (TDS) solids, and several inorganic ions: ammonium, bromate, bromide, calcium, chloride, magnesium, nitrate, nitrite, phosphate, potassium, sodium, and sulphate) (Table S1, Supplementary Material), (ii) concentrations of the selected antibiotics (Table S2, Supplementary Material), and (iii) toxicity, before and after continuous-flow ozonation.

2.3. Ozonation experiments

2.3.1. Batch mode ozonation experiments

Batch experiments were performed in a lab-scale glass reactor filled with 500 mL of solutions (ultrapure water or aquaculture effluents) spiked with the target antibiotics at 10 mg L^{-1} each. For spiking, 5 mg of each target compound were directly added to the matrices and subjected to sonication for 10 min. For $HO^{\bullet}$ scavenging experiments, apart from the antibiotics, a known amount of MeOH (commonly used as radical scavenger) (Cong et al., 2015; Leresche et al., 2021; Tachikawa and Yamanaka, 2014) was added (< 1%) to ultrapure water. The reaction medium was always magnetically stirred at 400 rpm, approximately at 18 °C. Ozone was produced from pure oxygen in a BMT 802X O_3 generator (BMT Messtechnik, Germany) at a constant flow rate (150 Ncm^3 min^{-1}) and inlet gaseous concentration of approximately 50 g Nm^{-3}, aiming to ensure that the degradation of the target antibiotics was conducted in the presence of an excess of O_3. The O_3 concentration in the gas phase was monitored with a BMT 964 ozone analyser (BMT Messtechnik, Germany), and the O_3 leaving the reactor was removed using gas washing bottles filled with potassium iodide solution. 1 mL aliquots were collected periodically from the reactor (*i.e.*, 0, 1, 2, 5, 10, 12, 15, 17, 20, 30, 40, 45, and 60 min) for analysis of antibiotics concentrations. To remove residual O_3, each sample was bubbled with air. The collected samples were placed immediately in the fridge until analysis.

2.3.2. Continuous-flow mode ozonation experiments

Experiments with continuous-flow ozonation were performed in a bubble column reactor (3.0 cm internal diameter × 70 cm height) at a temperature of approximately 17°C, applying conditions based on our previous study dealing with ozonation for the removal of other organic micropollutants from surface water (Gorito et al., 2021) and information regarding ozonation at full-scale aquaculture systems for water disinfection (Gonçalves and Gagnon, 2011; Summerfelt, 2003; Summerfelt and Hochheimer, 1997). Briefly, for spiking the aquaculture effluents (100 ng L^{-1}), 400 μL of a methanolic solution containing all target antibiotics (2.5 mg L^{-1}) was added to an empty 10 L ISO bottle blue cap and the residual solvent was evaporated using a nitrogen flow. Afterwards, a 10 L aliquot of the aquaculture effluent was added into the bottle and stirred for 2 min at 600 rpm. The spiked effluent was continuously pumped at the bottom of the reactor with a peristaltic pump (Watson-Marlow, UK) and constantly collected at its top after the respective treatment (*i.e.*, one single passage in the column). The reactor was packed with glass Raschig rings (3 mm diameter × 3 mm length, resulting in a filling volume of 355 mL after packing), in this way promoting the gas-liquid O_3 mass transfer during the treatment (Graça et al., 2020). The BMT 802X O_3 generator (BMT Messtechnik, Germany) was used to produce O_3 from pure oxygen at a constant flow rate (300 Ncm^3 min^{-1} in the gas phase) and inlet concentration (~ 20 g Nm^{-3}; 1.5 mg O_3 L^{-1}), which was fed to the reactor through a ceramic diffuser. It is important to highlight that O_3 concentrations were established according to the information mentioned above (Gonçalves and Gagnon, 2011; Gorito et al., 2021; Summerfelt, 2003; Summerfelt and Hochheimer, 1997). O_3 in the gas phase was monitored by a BMT 964 ozone analyzer (BMT Messtechnik, Germany), while dissolved O_3 was measured by a Q45H/64 probe (Analytical Technology, USA) configurated for exclusive use in continuous mode (*accuracy*: ± 0.02 mg L^{-1}). The O_3 leaving the reactor was eliminated as described in the previous section. In a typical run, the reactor was filled with distilled water and when the spiked effluent starts to be pumped (t_0), a dilution effect is observed until the steady state (t_s), which was determined using sodium chloride as tracer (Moreira et al., 2016). At this point (*i.e.*, when the t_s is achieved), a first sample of 1 L was collected (blank, t_1), and then the treatment began by bubbling O_3 to the spiked water fed into the reactor. After that, another period equivalent to that needed to achieve t_s was considered before the next sampling of the ozonated effluent (t_2). Three 1 L samples were withdrawn for antibiotic concentrations analysis in each experiment (t_{2a}, t_{2b}, t_{2c}), these samples being immediately bubbled with air to remove any possible residual O_3. The remaining volume of treated water was stored in the fridge for toxicity tests and other physico-chemical analyses. The experiments were performed in triplicate, and the removal efficiency of each antibiotic was estimated considering its average concentration abatement between t_1 (without O_3) and $t_{2(a, b, c)}$ (with O_3) for the three independent ozonation experiments.

2.4. Quantification of antibiotics concentrations

The selected antibiotics (FF, OTC, SDM, SMX and TMP) were analyzed using liquid chromatography coupled to tandem mass spectrometry (LC–MS/MS). Regarding continuous O_3 experiments (*i.e.*, with lower antibiotics concentration), SPE was needed prior to chromatographic analyses. For that, a SPE protocol was adapted from two previous studies on the determination of veterinary antibiotics in aqueous matrices (Tetzner and Rath, 2018; Zhou et al., 2012). In summary, samples of 1 L were filtered through 1.2 μm glass-fiber filters (47 mm GF/C, Whatman™, UK) and acidified to pH ~ 3 using sulphuric acid. Moreover, SDM-d_6 (20 μL) was added as internal standard and EDTA--$Na_2.2H_2O$ (0.2 g) as complexing agent. The samples were then extracted by Oasis® Hydrophilic-Lipophilic-Balanced (HLB) cartridges (150 mg, 6 mL) using an extraction manifold, both acquired from Waters (Milford, USA). After the total sample volume was loaded onto the cartridges (without preconditioning), they were rinsed with ultrapure water (10 mL) and dried using a vacuum pump (ILMVAC GmbH, Germany) for 60 min. The dried cartridges were eluted with 6 mL of MeOH (3 × 2 mL), which were then evaporated in a Labconco® CentriVap (Kansas City, USA). Finally, the residues were re-suspended in 250 μL of a MeOH/ultrapure water mixture (40:60, v/v). The average recovery during SPE, accuracy, precision, and matrix effect for each target analyte are described in Table S3 (Supplementary Material).

A Shimadzu Corporation apparatus (Tokyo, Japan) with LC (UHPLC, Nexera) and triple quadrupole tandem mass (MS/MS) detection (LCMS-8040) was used for chromatographic analyses. Separation was achieved using a Kinetex ™ 1.7 μm F5 100 Å column (2.1 × 100 mm) supplied by Phenomenex, Inc. (California, USA), applying a mobile phase composed of water and MeOH, both containing 0.1% of formic acid, at a flow rate of 0.2 mL min^{-1}. The chromatographic run was operated at gradient mode consisting of 27.5% of MeOH during 4 min, a linear gradient during 6.5 min up to 50%, after which the initial conditions were set again in 0.5 min to condition the column during 5 min, totalizing a 16 min run. Column oven and autosampler temperatures were set at 25°C and 4°C, respectively. The volume of injection was 5 μL. The mass spectrometer was operated using electrospray ionization (ESI) under positive- and negative modes, and multiple reaction monitoring (MRM)

was used to quantify the target compounds (selected reaction monitoring instrument parameters can be consulted in Table S4, Supplementary Material). Capillary voltage, drying gas flow rate, nebulizing gas flow rate, desolvation and source temperature of the mass spectrometer were 4.5 kV, 15 dm^3 min^{-1}, 3 dm^3 min^{-1}, 250°C and 400°C, respectively. The analysis was based on a triplicate matrix-matched calibrations curve, by adding selected amounts of each antibiotic standard solution and a constant amount of internal standard solution to 1 L of spiked matrix. Retention time, range, linearity, instrument and method detection and quantification limits of the target antibiotics are available in Table S5 (Supplementary Material).

2.5. Physico-chemical parameters determinations

A TOC-L analyser (Shimadzu Scientific Instruments, Japan) was used to determine the DOC content, following the procedure 5310B of the Standard Methods for Examination of Water and Wastewater (APHA et al., 1998). COD, BOD, and TSS were measured following procedures 5220D, 5210B and 2540D, respectively (APHA et al., 1998). The concentrations of cations (ammonium, calcium, magnesium, potassium, and sodium) and anions (bromide, chloride, nitrate, nitrite, phosphate, and sulphate) in solution were determined by ionic chromatography using Metrosep C4 Cationic Exchange and Metrosep A Supp 7 Anionic Exchange columns, respectively (250 mm × 4.0 mm) in a Metrohm 881 Compact IC Pro equipment. Bromate ions were determined by LC-MS/MS, using a Waters™ ACQUITY UPLC® BEH Amide 1.7 μm column (2.1 × 100 mm, Milford, USA), with a mobile phase of acetonitrile/10 mM ammonium formate (80/20, *v/v*), operating at isocratic mode. The pH values were measured using a pH meter pHenomenal® pH 1100 L (VWR, Oregon, USA).

2.6. Zebrafish embryotoxicity tests

Zebrafish (*Danio rerio*) breeders were maintained under standard culture conditions at *CIIMAR* (Matosinhos, Portugal) certified facilities for aquatic organisms. For reproduction, males and females were placed in a maternity inside a 30 L tank the day before reproduction. After reproduction, shortly after the beginning of light period, embryos (0-1 hpf (h post fertilisation)) were collected, cleaned, counted, and assessed for fertilization success (> 90%). The assays were carried out in 24 well plates (VWR, Oregon, USA). Ten embryos were placed in each well in a final volume of 2 mL of test solution. A total of 40 embryos (10 embryos/well) were exposed per test condition in each assay. Each assay plate was loaded with the test solutions for 24 h before the embryo assays were done, to minimise contaminant losses from the test solutions to the media by adsorption to the plates. During the assays the test solutions were completely renewed every day. Test solutions included: (i) aquaculture effluents as collected; (ii) aquaculture effluents spiked with FF, OTC, SDM, SMX and TMP (100 ng L^{-1} of each antibiotic); (iii) spiked aquaculture effluents after continuous ozonation treatment; (iv) dechlorinated water (negative control); and (v) 3,4-dichloroaniline solution at 4 mg L^{-1} (positive control). Control groups were used for quality control of the assay. Embryonic development was evaluated at 24, 48, 72, 96, 120 144 and 168 hpf using an inverted microscope (Nikon Eclipse TS100, Amsterdam, Netherlands). Mortality (24, 48, 72, 96, 120 144 and 168 hpf), hatching (24, 48, 72, 96, 120 hpf), malformation in the somites (24, 48, 72, 96 hpf), tail detachment (24, 48, 72, 96 hpf), yolk sac (24, 48, 72, 96, 120, 144 and 168 hpf), otoliths (48, 72, 96, 120, 144 and 168 hpf), eyes (48, 72, 96, 120, 144 and 168 hpf), heartbeat (48, 72, 96, 120, 144 and 168 hpf), blood circulation (48, 72, 96, 120, 144 and 168 hpf), skeleton (72, 96, 120, 144 and 168 hpf) and side-wise position (144 and 168 hpf) were the endpoints observed during the assays. Three independent repetitions of the assays were carried out to assess the potential embryotoxicity of the aquaculture effluent as collected and after treatment with continuous-flow ozonation, following the experiments described above (Section 2.3.2).

2.7. Statistical analysis

Removal of antibiotics by ozonation was compared by single-factor analysis of variance (ANOVA), followed by post-hoc Tukey's test using the software SPSS (Version 25.0 for Windows). The significance level was set to 0.05. In toxicity assays, mortality and hatching data are presented as cumulative frequency. The data were analyzed using the Pearson Chi-square test, taking the duration of the exposure and the experimental conditions as factors. When significant differences were identified, pairwise comparisons were done using either the Chi-square test or the Fisher Exact test, with a Bonferroni correction, to identify their origin. The median time to hatching (HT_{50}) was estimated using the Probit method (Finney, 1971). The statistical analyses were performed in SPSS 27, against a significance level of 0.05.

3. Results and discussion

3.1. Removal of antibiotics

3.1.1. Batch mode ozonation experiments

Fig. 1A shows the normalized concentrations of the target antibiotics spiked in ultrapure water (10 mg L^{-1} of each antibiotic) in the ozonation experiments (60 min; ~ 50 g Nm^{-3}; pH 6). Among the five tested antibiotics (FF, OTC, SDM, SMX and TMP), only SDM was not completely removed in 60 min (a_{SDM}*, $p < 0.05$), *i.e.*, SDM was the only compound with a concentration higher than the detection limit after 60 min of ozonation. OTC and SMX were the compounds with faster degradation, showing removals of 85% and 74%, respectively, in 10 min of contact time with O_3. These antibiotics and TMP were not detected in any of the 30 min treated samples. Regarding FF, a slower degradation was observed, but it was fully removed after 60 min. In the case of SDM, a fast removal is depicted in the first 10 min of reaction, followed by a slower removal after that period, a maximum removal of 82% being achieved in 60 min of ozonation.

It is well known that organic contaminants can be removed in ozonation by direct and indirect modes, depending on whether direct attack of O_3 or reaction with generated radicals (in particular $HO^{\bullet}$) respectively occurs (Peleg, 1976). As can be seen in Table 1, all the studied antibiotics have reaction rate kinetic constants above 10^5 M^{-1} s^{-1} with O_3 (k_{O3}), except FF (< 10 M^{-1} s^{-1}). This observation agrees with the slower degradation observed for FF in Fig. 1A. The k_{O3} are higher for the other antibiotics because the amino groups are expected to activate the aromatic rings and double bonds of the chemical structures (Ben et al., 2012; Von Sonntag and Von Gunten, 2012). In fact, sulfonamides, tetracyclines, and TMP are antibiotics for which ozonation has demonstrated high removal efficiencies, mainly by direct oxidation (Gireli et al., 2019; Ikehata et al., 2006; Kuang et al., 2013; Lin et al., 2009; Wang et al., 2011), whereas poor removals have been reported for FF (Choi et al., 2020; Khan et al., 2020; Kye et al., 2020; Zhang et al., 2016). Thus, the degradation of FF observed in Fig. 1A (closed circles) seems to be related to an indirect oxidation pathway by the reaction with $HO^{\bullet}$.

To infer about the role of $HO^{\bullet}$ (and other possible radicals) in the degradation of these antibiotics, MeOH was added to ultrapure water in the second set of experiments (Fig. 1B). The removal of FF was negligible (5%), indicating that the indirect oxidation pathway is crucial for its removal, as confirmed by the significant differences of ANOVA analysis between ultrapure water with and without addition of scavenger (b_{FF}, Fig. 1B). It is known that $HO^{\bullet}$ is generated after a series of reactions which starts with the primary reaction between O_3 and hydroxide ions present in water (Eqs. S1-14 in Supplementary Material), meaning that the formation of $HO^{\bullet}$ can be accelerated by increasing the pH (Von Gunten, 2003). In the present study, a pH of 6 was sufficient to remove FF in 60 min of ozonation (Fig. 1A). Unlike FF, OTC, SMX, and TMP were removed in less time in the presence of MeOH, but the differences were not significant (Fig. 1A *vs* 1B, $p < 0.05$). In any case, the radical

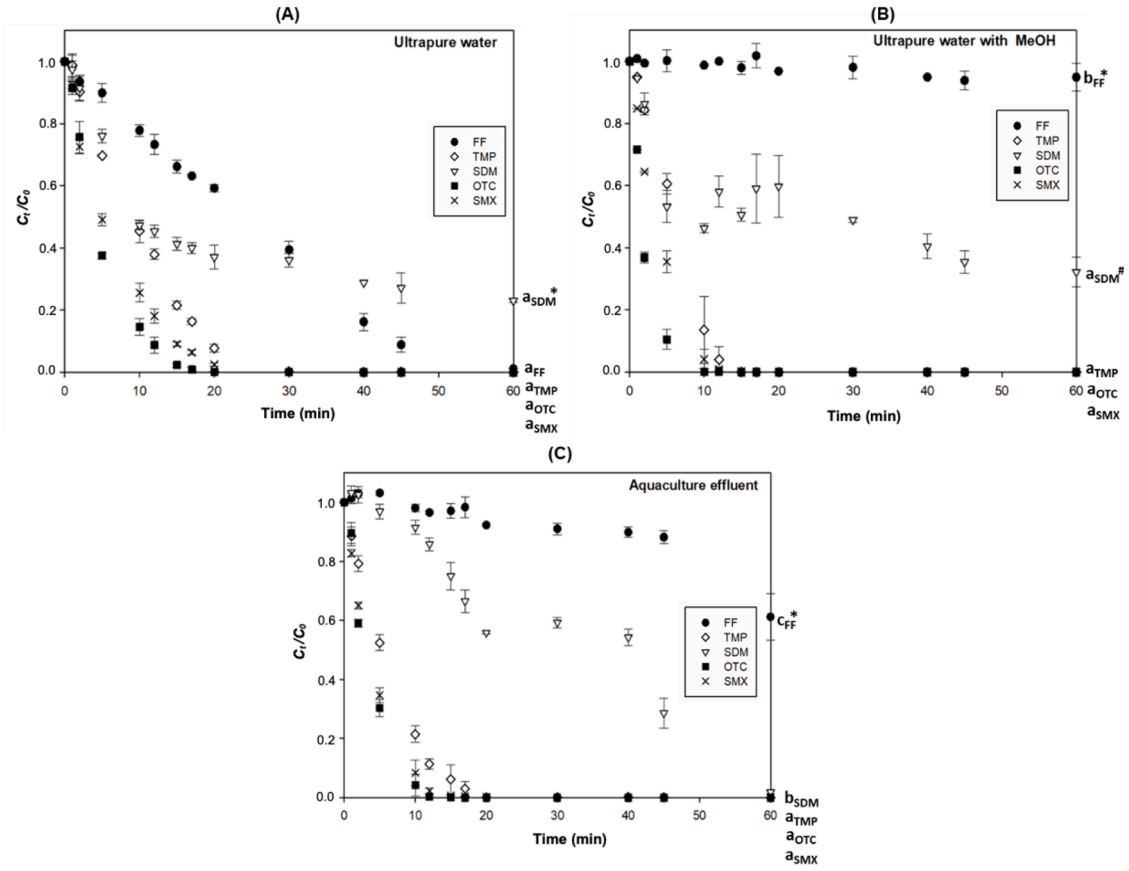


Fig. 1. Degradation of FF, TMP, SDM, OTC and SMX in batch ozonation treating ultrapure water (A), ultrapure water with MeOH as $HO^{\bullet}$ scavenger (B) and aquaculture effluent (C), spiked with the mixture of five antibiotics. Experimental conditions: $[FF, OTC, SDM, SMX, or TMP]_0 = 10$ mg L^{-1}, T =18 °C, $[O_3]_{gas\ phase} \sim 50$ g Nm^{-3}, pH = 6 in (A) and (B), 8 in (C). The results correspond to the mean removals of three replicates, with the respective standard deviation. For each compound, letters a, b and c represent significant different removals between the tested matrices, considering samples collected after 60 min of ozonation - assessed by One-Way ANOVA followed by Tukey *post hoc test* $p < 0.05$. $^{*,\#}$ represents significant different removals among samples for each compound in the same matrix.

scavenger consumes $HO^{\bullet}$ and, without regenerating the superoxide radical ion ($O_2^{\bullet-}$), the O_3 decomposition may be retarded (Eqs. S4 and S7, respectively), thus O_3 being available for direct attack (Langlais et al., 1991). ANOVA analysis also suggested that $HO^{\bullet}$ did not affect SDM degradation (a_{SDM}, Fig. 1B). Thus, these results confirmed that OTC, SMX, TMP, and SDM are preferentially removed by direct oxidation.

A set of additional experiments without MeOH was performed with FF alone and adding each one of the other antibiotics to FF in ultrapure water (Fig. S1a, Supplementary Material). Interestingly, these other antibiotics affected the FF degradation when simultaneously present in the solution, perhaps due to the readily O_3 consumption by direct attack of either parent (OTC, SMX, TMP, and SDM) or their intermediate compounds. Complementary experiments with SDM also suggested that the co-existence of the other four antibiotics affected its own removal (Fig. S1b, Supplementary Material), perhaps by generating by-products that can react with SDM.

Beyond the chemical properties of the compounds, the water matrix can also affect the degradation of antibiotics. As shown in Fig 1C, the matrix under analysis (*i.e.*, aquaculture effluents) impacted considerably the SDM removal (b_{SDM}). The degradation of SDM increased approximately 20% when the aquaculture effluent was tested instead of ultrapure water. However, the potential interference of $HO^{\bullet}$ favoured by the higher pH of aquaculture effluent (8 *vs* 6) does not justify this enhancement since these reactive species did not have a remarkable removal effect in the SMD removal (Fig. 1B). These results are in accordance with a recent study on ozonation of SDM (Shad et al., 2018), where the important role of the water matrix in its degradation was also highlighted. The co-existence of cations and anions (*e.g.*, Fe^{3+}, Cu^{2+}, NH_4^+, HCO_3^-, and NO_3^-) in the aqueous medium may play a catalytic role in ozonation of SDM (Shad et al., 2018), which might result in its larger removal in the aquaculture effluents (Fig. 1C). Furthermore, FF degradation was also affected by water matrix (c_{FF}), following the tendency of the experiments carried out in the presence of MeOH (Fig. 1C). Despite the pH of this matrix (pH of 8) being more favourable to the decomposition of O_3 into $HO^{\bullet}$, the matrix constituents may have acted as inhibitors of the formation of $HO^{\bullet}$ or instead, they might have consumed the generated radicals. This also supports the higher removal of SDM in the more complex matrix, since such $HO^{\bullet}$ inhibitors can reduce the decay of O_3, leading to an accelerated degradation of SDM which is mainly removed by O_3 direct attack. The complexity and diversity of water matrices turn difficult to predict their impact as initiator, promoter, or inhibitor during ozonation (Cai and Lin, 2016; Lado Ribeiro et al., 2019). As such, the fraction of O_3 and $HO^{\bullet}$ (and other possible reactive radicals) which effectively oxidizes the antibiotics is highly dependent on the action of all co-existing substances in the water matrix (Staehelin and Hoigne, 1985). Thus, the collected aquaculture effluent (with physico-chemical characterization shown in Table S1, Supplementary Material) probably suppressed the effect of $HO^{\bullet}$ on FF removal, which did not exceeded 40%. In contrast, OTC, SMX, TMP, and SDM were efficiently eliminated from the aquaculture effluent in 60 min (to levels below method detection limits). Moreover, it is also perceptible that aquaculture effluents had good water quality indicators (Table S1, Supplementary Material) and, therefore, a more complex matrix (*i.e.*, with higher organic content and/or ammonia concentration, among others) could affect antibiotics kinetic degradation since a higher

competition could occur between the species susceptible to oxidation by O_3 .

3.1.2. Continuous-flow mode ozonation experiments

The degradation of antibiotics in continuous-flow ozonation was investigated for an HRT of 10 min (corresponding to a t_s of 33 min), applying an O_3 concentration of 1.5 mg L^{-1}. As referred above, these selected conditions were based on the information available in the literature regarding ozonation at full-scale aquaculture systems for disinfection (Gonçalves and Gagnon, 2011; Summerfelt, 2003; Summerfelt and Hochheimer, 1997) and our previous study focused on ozonation for the removal of several other organic micropollutants from surface water (Gorito et al., 2021). For these experiments, the aquaculture effluent was spiked with 100 ng L^{-1} of each antibiotic (FF, OTC, SDM, SMX and TMP), *i.e.*, the spiked level was considerably lower than in batch experiments (hundreds of ng L^{-1} instead of tens of mg L^{-1}), mimicking concentrations frequently detected in aquaculture effluents (Pereira et al., 2015; Tetzner and Rath, 2018; Zou et al., 2011). In Fig. 2 (closed circles, top axis), it is possible to verify some deviations between the predicted and achieved concentrations of these spiked compounds (*i.e.*, ranging from 80 to 220 ng L^{-1}), which can be, for instance, related to the occurrence of the antibiotics in the collected effluent (Table S2, Supplementary Material), matrix effects or other possible interferences.

Removals above 98% were observed for TMP, SDM, OTC, SMX, whereas FF did not surpass 68% (Fig. 2, grey bars, $p < 0.05$). Therefore, in these experiments, FF was the only compound recalcitrant to O_3, its degradation being highly influenced by the presence of $HO^{\bullet}$, as confirmed in the previous sub-section. The action of $HO^{\bullet}$ will depend on the composition of the matrix to be treated, and thus, it is very difficult to predict the removal extent of FF. The degradation of FF in aquaculture waters has been also investigated by applying UV and UV-photocatalysis, removals in the ranges of 4-85% and 98-100% being respectively registered (Gorito et al., 2022). Thus, combining UV or H_2O_2 with ozonation can be an interesting alternative in future works to enhance the production of $HO^{\bullet}$ and, consequently, the more efficient degradation of FF; however, the respective costs should also be considered when selecting one of these alternatives (Cuerda-Correa et al., 2020).

3.2. Influence of continuous ozonation on the general quality of aquaculture effluent

Among the target ions (ammonium, bromate, bromide, calcium, chloride, magnesium, nitrate, nitrite, phosphate, potassium, sodium and sulphate), nitrite and ammonium were never detected, while magnesium was always below the method quantification limit (0.4 mg L^{-1}) (Table S1, Supplementary Material). Before treatment, calcium, chloride, nitrate, phosphate, potassium, sodium and sulphate ions were detected at concentrations of respectively 0.2, 6.3, 3.8, 3.2, 1.3, 6.3 and 1.1 mg L^{-1} (Table S1, Supplementary Material); values which were similar after ozonation, except a slight difference for potassium ion (0.3 mg L^{-1}). Moreover, bromide ions were oxidized to bromates after reaction with O_3 (Fig. S2, Supplementary Material). Likewise, if nitrites were present above the detection limits in the aquaculture effluent, it would also be expectable that they were oxidised to nitrates (Baozhen et al., 1989; Lin and Wu, 1996). As expected, results of ions analysis suggested that apart from bromides and bromates, ozonation had a low impact on the ionic composition of the spiked aquaculture effluent. The aquaculture effluent had a very low organic load. COD was lower than the method detection limit (3.5 mg L^{-1}), and DOC was quantified at approximately 2 mg L^{-1} (Table S1, Supplementary Material). The protein rich wastes from aquaculture systems typically have COD, DOC, and ammonia concentrations often higher than those detected in the current study. However, the selected aquaculture farm is characterized by low rates of fish production, with an adequate distribution of organisms by ample ponds, and the water supplied to this farm comes from a location very close to the source of the river, which probably contributed to these originally unexpected values. Still DOC slightly

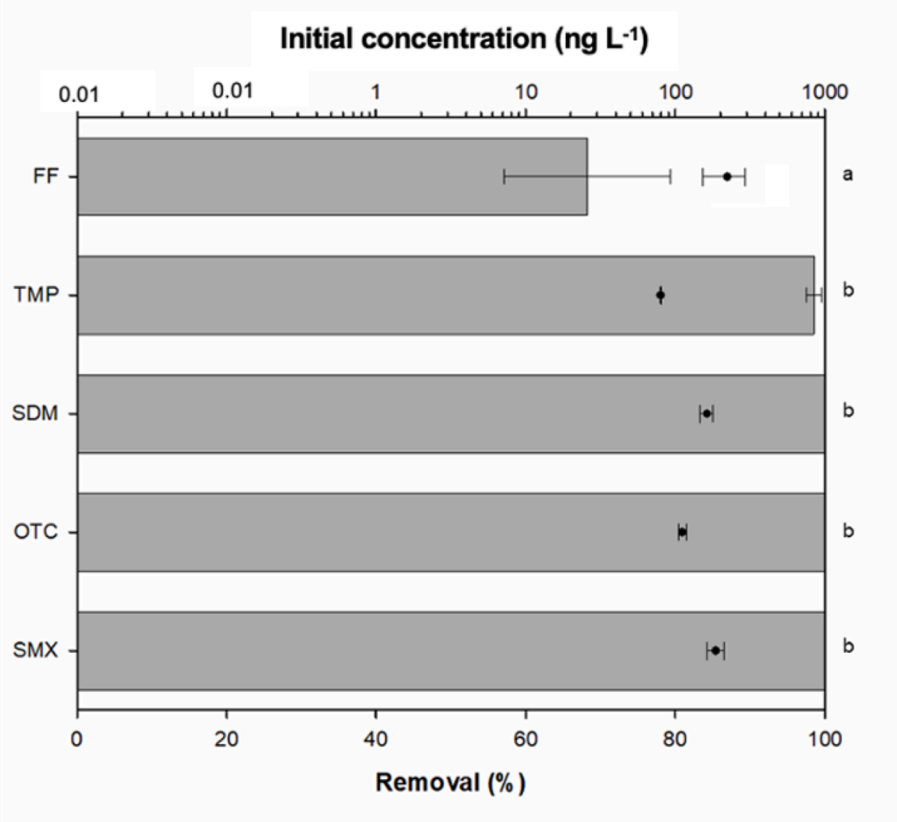


Fig. 2. Removal percentages of FF, TMP, SDM, OTC and SMX (grey bars, average of three independent experiments) in continuous-flow ozonation of spiked aquaculture effluent (100 ng L^{-1} of each antibiotic). Antibiotic initial concentrations after spiking (closed circles) are also shown for comparison purposes (top axis). Experimental conditions: T = 17 °C, HRT = 10 min, $[O_3]_{gas\ phase} \sim 20$ g Nm^{-3}, $[O_3]_{liquid\ phase} = 1.5$ mg L^{-1}, pH = 8. For each compound, letters a and b represent significant differences among samples assessed by One-Way ANOVA followed by Tukey *post hoc test* $p < 0.05$.

decreased (~ 4%) after ozonation. Typically, O_3 is a powerful oxidant of aromatic compounds. Still, it reacts slowly with low-molecular-weight carboxylic acids that are often formed from these parent compounds, which can lead to the accumulation of several by-products that cannot be easily oxidized by molecular O_3 (Moreira et al., 2015). In any case, the theoretical DOC due to the five MPs corresponds to less than 0.3% of the initial DOC content, since they are present at very low concentrations (Fig, 2). Regarding the particulate matter, O_3 eliminated approximately 60% of TSS.

3.3. Toxicity evaluation before and after continuous ozonation

Bromates (6 ug L^{-1}) and antibiotics by-products (Figs. S2 and S3, Supplementary Material) were detected by LC-MS/MS in the samples collected from the continuous ozonation reactor, highlighting the importance of toxicological evaluation. Zebrafish is a very popular model vertebrate used to evaluate the toxicity of water and it was used here to verify the potential risks of removing antibiotics from aquaculture effluents with O_3. The assays carried out in this work fulfilled all the validation criteria of the OECD guidelines 212 and 236 (OECD, 1998; 2013). Namely, at the end of the exposure period, the negative control group had a low mortality (< 10%) and a hatching success above 80%, while embryos exposed to 3,4-dichloroaniline (positive control) registered a mortality above 30% (Fig. 3). These results allowed to completely validate the applied methodology. Results for mortality and hatching obtained for the treated and non-treated aquaculture effluent are presented in Fig. 3.

Focusing on the mortality (Fig. 3a), no significant mortality was elicited either by the aquaculture effluent, the spiked effluent or the ozonated effluent treatment. For the negative control and the tested effluent (squares and triangles, Fig. 3a), mortality occurred mostly during the first 48 h of exposure, which is in accordance with the expected outcome of these types of toxicological assays. The first 48 h of zebrafish development are critical. Not only all the major gastrulation and segmentation processes occur in this period, but also defence mechanisms against external contamination are not fully maturated at this time frame (Chen et al., 2020; Fischer et al., 2013; Kimmel et al., 1995).

Hatching (Fig. 3b) occurred mainly between 48 hpf and 72hpf, as described in the literature (Kimmel et al., 1995). However, significant differences among experimental conditions were found in the time to hatching, compared to the controls (Chi-square = 66.0, degrees of freedom $df = 6$, $p < 0.00001$). The subsequent pairwise comparisons showed that hatching was significantly anticipated in the spiked aquaculture effluent (Chi-square = 24.8, $df = 2$, $p < 0.00001$) and the treated aquaculture effluent (Chi-square = 39.4, $df = 2$, $p < 0.00001$). Table 2 shows the median time to hatching (HT_{50}) obtained for each experimental condition. According to these results, embryos exposed to the spiked effluent took 10 h less than the control to hatch, while those exposed to the treated effluent took 13 h less.

The implications of premature hatching are yet to be fully understood. It has been observed previously that premature hatching in zebrafish may occur upon exposure to ionic stress (Ord, 2019), hypoxia (Small et al., 2020) or toxic substances such as tributyltin (Liang et al., 2017). The work of Ord (2019) seems to support that premature hatching may be an adaptive response allowing for animals to escape unfavourable conditions. In contrast, the investigation of Leite-Ferreira et al. (2019) suggests differential sensitivity to alcohol of premature and late hatching zebrafish larvae, with stronger responses of premature larvae to high exposure concentrations, compared to late hatching ones. Further investigations will help understanding the implications of such anticipation in relation to treatment benefits to water quality.

Statistically significant differences among test conditions were observed for the rate of malformations (Chi-square = 72.9, $df = 3$, $p < 0.00001$) (Fig. 4). The pairwise comparisons indicated a significantly higher proportion of abnormal embryos at 168 hpf in the aquaculture effluent (Fisher Exact test, $p < 0.00001$, compared to control embryos) and the spiked aquaculture effluent (Fisher Exact test, $p = 0.0005$, compared to controls) (Fig. 4d). Yolk sac malformations, side-wide position and skeletal deformities were the most prevalent abnormalities found, all interfering with larvae swimming, and thus their ability to feed and escape predators (Fig. 4a,b,c). Though less prevalent, alterations in the blood circulation and heartbeat could also be observed (data not shown). Nevertheless, in the group exposed to the effluent treated with continuous ozonation, the rate of malformations was very low and comparable to the control group (Fisher Exact test, $p = 0.672$). Overall, the results suggest that the ozonation treatment would effectively reduce the toxicity of the aquaculture effluent, improving water

Table 2
Median time to hatching (HT_{50}) of embryos exposed to the tested experimental conditions.

Experimental condition	HT_{50} (hours) (95% Confidence Interval)
Negative control	68 (66, 71)
Aquaculture effluent	68 (65, 70)
Spiked aquaculture effluent	58 (56, 60)
Treated aquaculture effluent	55 (53, 57)

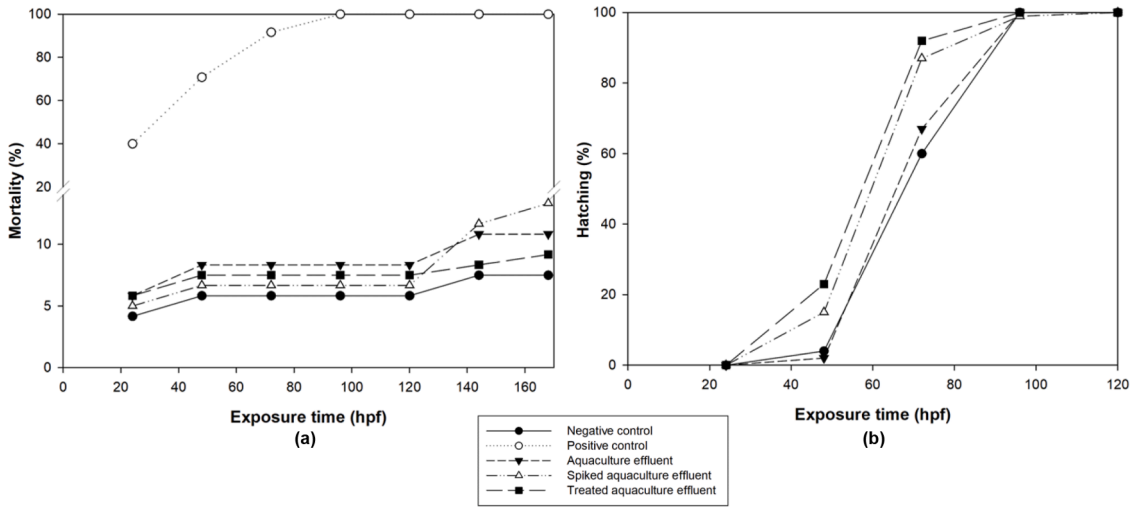


Fig. 3. Mortality **(a)** and hatching **(b)** recorded for embryos exposed to the aquaculture effluent and the same effluent treated with continuous ozonation. Data are presented as cumulative frequency.

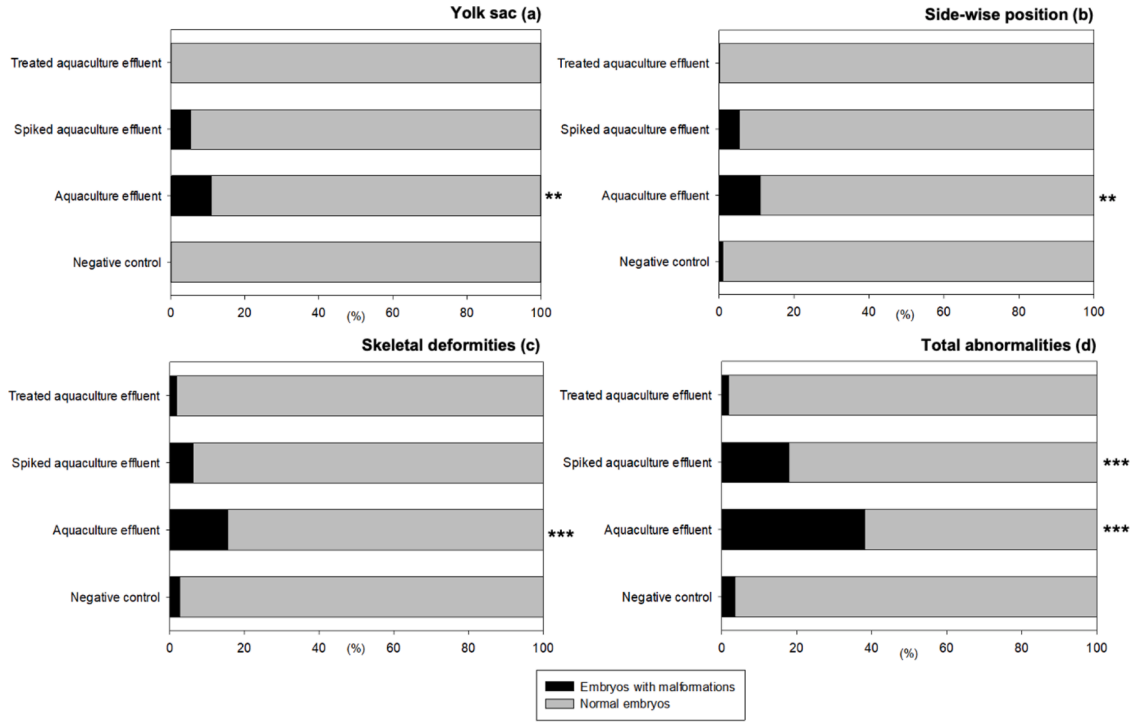


Fig. 4. Rate of embryonic malformations detected at 168 hpf in embryos exposed to the different experimental conditions. Statistically significant differences relative to the control group are indicated by the stars according to the results of the Fisher Exact test (** $p < 0.01$, *** $p < 0.001$).

quality for the development of the embryos.

Therefore, the by-products generated during ozonation were not harmful to zebrafish. Bromate was detected at 6 μg L^{-1} in treated effluent samples; however, in marine aquaculture, O_3 may result in bromate concentrations considerable higher because of the large amount of bromide in seawater (~ 60 mg L^{-1}) (Camera-Roda et al., 2019; Kye et al., 2020). Investigation on toxicity effects of bromate ions in zebrafish are still scarce, but studies for sodium and potassium bromate have reported embryotoxic effects only at very high concentrations (> 1 g L^{-1}) (Teixidó et al., 2015; Wang et al., 2016). It is important to refer that zebrafish toxicity tests for ozonated aquaculture effluents were performed in this study for the first time, with encouraging results.

4. Conclusions

Batch ozonation experiments (with antibiotics at 10 mg L^{-1} each; 60 min of treatment) showed that this process was efficient for removing TMP, OTC and SMX from aqueous matrices (< 30 min), and that direct O_3 attack is the main degradation pathway. In contrast, FF was not removed by direct O_3 attack; however, the results of this work reveal that FF can be successfully oxidized by the indirect reaction pathway (*i.e.*, through $HO^{\bullet}$ generation). SDM was fully eliminated from the aquaculture effluent, but its degradation was highly influenced by the selected water matrix. Additionally, in continuous-flow ozonation experiments (with antibiotics at 100 ng L^{-1} each; 10 min of treatment), all antibiotics were successfully removed from the aquaculture effluent (> 98%), except FF (68%). Thus, FF removal should be enhanced by promoting the formation of $HO^{\bullet}$. Complementary analysis showed that bromates (6 μg L^{-1}) and other reaction by-products are formed in the medium during ozonation treatment. However, endpoints of the zebrafish assays (mortality, hatching and malformations) did not reveal toxicity after O_3 treatment. Molecular, biochemical, and genetic biomarkers (*e.g.*, genotoxicity, oxidative stress, endocrine disruption, and neurotoxicity) can also be used to assess the possible toxicity effects on zebrafish, and thus, more specific analysis can be addressed in the future.

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

This work was financially supported by LA/P/0045/2020 (ALiCE), UIDB/50020/2020 and UIDP/50020/2020 (LSRE-LCM), funded by national funds through FCT/MCTES (PIDDAC). CIIMAR supported this work by Strategic Funding UIDB/04423/2020 and UIDP/04423/2020, both funded by national funds through FCT/MCTES (PIDDAC). This work is also a result of project "Healthy Waters – Identification, Elimination, Social Awareness and Education of Water Chemical and Biological Micropollutants with Health and Environmental Implications", with reference NORTE-01-0145-FEDER-000069, and included in Ocean 3R (ref. NORTE-01-0145-FEDER-000064) both supported by Norte Portugal Regional Operational Programme (NORTE 2020), under the PORTUGAL 2020 Partnership Agreement, through the European Regional Development Fund (ERDF) and NATURE (Aquatic/0004/2020) financed by FCT under AquaticPollutants ERA-NET Cofund (GA N° 869178). ARLR acknowledges FCT funding under DL57/2016 Transitory Norm Programme. AMG and PR acknowledge the research grants from FCT (Ref. COVID/BD/151974/2021 and SFRH/BD/134518/2017, respectively), with financing from the European Social Fund and the Human Potential Operational Programme.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.watres.2022.118497.

References

APHA, AWWA and WEF, 1998. Standard Methods for the Examination of Water and Wastewater Washington.

Baozhen, W., Jinzhi, T., Jun, Y.I.N., Guangmei, S.H.I, 1989. Ammonia, Nitrite and Nitrate Nitrogen Removal from Polluted Source Water with Ozonation and BAC Processes. Ozone: Science & Engineering 11 (2), 227–244. https://doi.org/10.1080/01919518908552438.

Ben, W., Qiang, Z., Pan, X., Nie, Y., 2012. Degradation of Veterinary Antibiotics by Ozone in Swine Wastewater Pretreated with Sequencing Batch Reactor. Journal of Environmental Engineering 138, 272–277. https://doi.org/10.1061/(ASCE)EE.1943-7870.0000404.

Cai, M.-J., Lin, Y.-P, 2016. Effects of effluent organic matter (EfOM) on the removal of emerging contaminants by ozonation. Chemosphere 151, 332–338. https://doi.org/10.1016/j.chemosphere.2016.02.094.

Camera-Roda, G., Loddo, V., Palmisano, L., Parrino, F., 2019. Photocatalytic ozonation for a sustainable aquaculture: A long-term test in a seawater aquarium. Applied Catalysis B: Environmental 253, 69–76. https://doi.org/10.1016/j.apcatb.2019.04.048.

Chen, Q., Di, Z., García Roger, E.M., Li, H., Richmond, P., Roehner, B.M, 2020. Magnitude and significance of the peak of early embryonic mortality. Journal of Biological Physics 46 (3), 233–251. https://doi.org/10.1007/s10867-020-09555-4.

Choi, S., Sim, W., Jang, D., Yoon, Y., Ryu, J., Oh, J., Woo, J.-S., Kim, Y.M., Lee, Y., 2020. Antibiotics in coastal aquaculture waters: Occurrence and elimination efficiency in oxidative water treatment processes. Journal of Hazardous Materials 396, 122585. https://doi.org/10.1016/j.jhazmat.2020.122585.

Cong, J., Wen, G., Huang, T., Deng, L., Ma, J., 2015. Study on enhanced ozonation degradation of para-chlorobenzoic acid by peroxymonosulfate in aqueous solution. Chemical Engineering Journal 264, 399–403. https://doi.org/10.1016/j.cej.2014.11.086.

Cuerda-Correa, E.M., Alexandre-Franco, M.F., Fernández-González, C., 2020. Advanced Oxidation Processes for the Removal of Antibiotics from Water. An Overview. Water 12 (1), 102. https://doi.org/10.3390/w12010102.

Dodd, M.C., Buffle, M.-O., von Gunten, U., 2006. Oxidation of Antibacterial Molecules by Aqueous Ozone: Moiety-Specific Reaction Kinetics and Application to Ozone-Based Wastewater Treatment. Environmental Science & Technology 40 (6), 1969–1977. https://doi.org/10.1021/es051369x.

FAO, 2020. The State of World Fisheries and Aquaculture 2020. Sustainability in action. Rome.

Finney, D.J., 1971. Probit Analysis. Cambridge University Press, Cambridge.

Fischer, S., Klüver, N., Burkhardt-Medicke, K., Pietsch, M., Schmidt, A.-M., Wellner, P., Schirmer, K., Luckenbach, T., 2013. Abcb4 acts as multixenobiotic transporter and active barrier against chemical uptake in zebrafish (Danio rerio) embryos. BMC Biology 11 (1), 69. https://doi.org/10.1186/1741-7007-11-69.

García-Cambero, J.P., Beltrán, F.J., Encinas, A., Rivas, F.J., Oropesa, A.L, 2019. The added value of a zebrafish embryo–larval model in the assessment of wastewater tertiary treatments. Environmental Science: Water Research & Technology 5 (12), 2269–2279. https://doi.org/10.1039/C9EW00411D.

Gireli, G.A.S., Maniero, M.G., Guimarães, J.R., 2019. Influence of pH value on sulfonamide ozonation using caffeine as a contamination indicator. Water Supply 20 (2), 508–515. https://doi.org/10.2166/ws.2019.182.

Gonçalves, A.A., Gagnon, G.A, 2011. Ozone Application in Recirculating Aquaculture System: An Overview. Ozone: Science & Engineering 33 (5), 345–367. https://doi.org/10.1080/01919512.2011.604595.

Gorito, A.M., Lado Ribeiro, A.R., Pereira, M.F.R., Almeida, C.M.R., Silva, A.M.T, 2022. Advanced oxidation technologies and constructed wetlands in aquaculture farms: What do we know so far about micropollutant removal? Environmental Research 204, 111955. https://doi.org/10.1016/j.envres.2021.111955.

Gorito, A.M., Pesqueira, J.F.J.R., Moreira, N.F.F., Ribeiro, A.R., Pereira, M.F.R., Nunes, O.C., Almeida, C.M.R., Silva, A.M.T, 2021. Ozone-based water treatment (O_3, O_3/UV, O_3/H_2O_2) for removal of organic micropollutants, bacteria inactivation and regrowth prevention. Journal of Environmental Chemical Engineering 9 (4), 105315. https://doi.org/10.1016/j.jece.2021.105315.

Graça, C.A.L., Lima, R.B., Pereira, M.F.R., Silva, A.M.T., Ferreira, A., 2020. Intensification of the ozone-water mass transfer in an oscillatory flow reactor with innovative design of periodic constrictions: Optimization and application in ozonation water treatment. Chemical Engineering Journal 389, 124412. https://doi.org/10.1016/j.cej.2020.124412.

Hopkins, Z.R., Blaney, L., 2014. A novel approach to modeling the reaction kinetics of tetracycline antibiotics with aqueous ozone. Science of The Total Environment 468-469, 337–344. https://doi.org/10.1016/j.scitotenv.2013.08.032.

Huber, M.M., Canonica, S., Park, G.-Y., Von Gunten, U., 2003. Oxidation of pharmaceuticals during ozonation and advanced oxidation processes. Environmental science & technology 37 (5), 1016–1024. https://doi.org/10.1021/es025896h.

Ikehata, K., Jodeiri Naghashkar, N., Gamal El-Din, M., 2006. Degradation of Aqueous Pharmaceuticals by Ozonation and Advanced Oxidation Processes: A Review. Ozone: Science & Engineering 28 (6), 353–414. https://doi.org/10.1080/01919510600985937.

Jeong, J., Song, W., Cooper, W.J., Jung, J., Greaves, J., 2010. Degradation of tetracycline antibiotics: Mechanisms and kinetic studies for advanced oxidation/reduction processes. Chemosphere 78 (5), 533–540. https://doi.org/10.1016/j.chemosphere.2009.11.024.

Khan, W., Nam, J.-Y., Byun, S., Kim, S., Han, C., Kim, H.-C, 2020. Emerging investigator series: quaternary treatment with algae-assisted oxidation for antibiotics removal and refractory organics degradation in livestock wastewater effluent. Environmental Science: Water Research & Technology 6 (12), 3262–3275. https://doi.org/10.1039/D0EW00634C.

Kimmel, C.B., Ballard, W.W., Kimmel, S.R., Ullmann, B., Schilling, T.F, 1995. Stages of embryonic development of the zebrafish. Developmental dynamics: an official publication of the. American Association of Anatomists 203 (3), 253–310. https://doi.org/10.1002/aja.1002030302.

Kuang, J., Huang, J., Wang, B., Cao, Q., Deng, S., Yu, G., 2013. Ozonation of trimethoprim in aqueous solution: identification of reaction products and their toxicity. Water Research 47 (8), 2863–2872. https://doi.org/10.1016/j.watres.2013.02.048.

Kye, H., Oh, H., Jung, Y., Kwon, M., Yoon, Y., Kang, J.W., Hwang, T.M, 2020. Oxidation of florfenicol and oxolinic acid in seawater by ozonation. Applied Sciences (Switzerland) 10 (14). https://doi.org/10.3390/app10144944.

Lado Ribeiro, A.R., Moreira, N.F.F., Li Puma, G., Silva, A.M.T, 2019. Impact of water matrix on the removal of micropollutants by advanced oxidation technologies. Chemical Engineering Journal 363, 155–173. https://doi.org/10.1016/j.cej.2019.01.080.

Langlais, B., Reckhow, D.A., Brink, D.R., 1991. Ozone in water treatment: application and engineering. CRC press.

Leal, J.F., Henriques, I.S., Correia, A., Santos, E.B.H., Esteves, V.I, 2017. Antibacterial activity of oxytetracycline photoproducts in marine aquaculture's water. Environmental Pollution 220, 644–649. https://doi.org/10.1016/j.envpol.2016.10.021.

Leite-Ferreira, M.E., Araujo-Silva, H., Luchiari, A.C, 2019. Individual Differences in Hatching Time Predict Alcohol Response in Zebrafish. Front Behav Neurosci 13, 166. https://doi.org/10.3389/fnbeh.2019.00166. -166.

Leresche, F., Torres-Ruiz, J.A., Kurtz, T., Von Gunten, U., Rosario-Ortiz, F.L., 2021. Optical properties and photochemical production of hydroxyl radical and singlet oxygen after ozonation of dissolved organic matter. Environmental Science: Water Research & Technology 7 (2), 346–356. https://doi.org/10.1039/D0EW00878H.

Liang, X., Souders II, C.L., Zhang, J., Martyniuk, C.J, 2017. Tributyltin induces premature hatching and reduces locomotor activity in zebrafish (Danio rerio) embryos/larvae at environmentally relevant levels. Chemosphere 189, 498–506. https://doi.org/10.1016/j.chemosphere.2017.09.093.

Lin, A.Y.-C., Lin, C.-F., Chiou, J.-M., Hong, P.K.A., 2009. O_3 and O_3/H_2O_2 treatment of sulfonamide and macrolide antibiotics in wastewater. Journal of Hazardous Materials 171 (1), 452–458. https://doi.org/10.1016/j.jhazmat.2009.06.031.

Lin, S.H., Wu, C.L, 1996. Removal of nitrogenous compounds from aqueous solution by ozonation and ion exchange. Water Research 30 (8), 1851–1857. https://doi.org/10.1016/0043-1354(95)00329-0.

Lulijwa, R., Rupia, E.J., Alfaro, A.C, 2020. Antibiotic use in aquaculture, policies and regulation, health and environmental risks: a review of the top 15 major producers. Reviews in Aquaculture 12 (2), 640–663. https://doi.org/10.1111/raq.12344.

Moreira, N.F.F., Orge, C.A., Ribeiro, A.R., Faria, J.L., Nunes, O.C., Pereira, M.F.R., Silva, A.M.T, 2015. Fast mineralization and detoxification of amoxicillin and diclofenac by photocatalytic ozonation and application to an urban wastewater. Water Research 87, 87–96. https://doi.org/10.1016/j.watres.2015.08.059.

Moreira, N.F.F., Sousa, J.M., Macedo, G., Ribeiro, A.R., Barreiros, L., Pedrosa, M., Faria, J.L., Pereira, M.F.R., Castro-Silva, S., Segundo, M.A., Manaia, C.M., Nunes, O.C., Silva, A.M.T, 2016. Photocatalytic ozonation of urban wastewater and surface water using immobilized TiO_2 with LEDs: Micropollutants, antibiotic resistance genes and estrogenic activity. Water Research 94, 10–22. https://doi.org/10.1016/j.watres.2016.02.003.

OECD, 1998. Test No. 212: Fish, Short-term Toxicity Test on Embryo and Sac-Fry Stages.

OECD, 2013. Test No. 236: Fish Embryo Acute Toxicity (FET) Test.

Ord, J., 2019. Ionic Stress Prompts Premature Hatching of Zebrafish (Danio rerio) Embryos. Fishes 4 (1), 20. https://doi.org/10.3390/fishes4010020.

Peleg, M., 1976. The chemistry of ozone in the treatment of water. Water Research 10 (5), 361–365. https://doi.org/10.1016/0043-1354(76)90052-X.

Pereira, A.M., Silva, L.J., Meisel, L.M., Pena, A., 2015. Fluoroquinolones and Tetracycline Antibiotics in a Portuguese Aquaculture System and Aquatic Surroundings: Occurrence and Environmental Impact. Journal of toxicology and environmental health. Part A 78 (15), 959–975. https://doi.org/10.1080/15287394.2015.1036185.

Pereira, J.H.O.S., Reis, A.C., Queirós, D., Nunes, O.C., Borges, M.T., Vilar, V.J.P., Boaventura, R.A.R, 2013. Insights into solar TiO_2-assisted photocatalytic oxidation of two antibiotics employed in aquatic animal production, oxolinic acid and oxytetracycline. Science of The Total Environment 463-464, 274–283. https://doi.org/10.1016/j.scitotenv.2013.05.098.

Powell, A., Scolding, J.W.S, 2018. Direct application of ozone in aquaculture systems. Reviews in Aquaculture 10 (2), 424–438. https://doi.org/10.1111/raq.12169.

Preena, P.G., Swaminathan, T.R., Rejish Kumar, V.J., Bright Singh, I.S., 2020. Unravelling the menace: detection of antimicrobial resistance in aquaculture. Letters in Applied Microbiology 71 (1), 26–38. https://doi.org/10.1111/lam.13292.

Prieto-Rodríguez, L., Oller, I., Klamerth, N., Agüera, A., Rodríguez, E.M., Malato, S., 2013. Application of solar AOPs and ozonation for elimination of micropollutants in municipal wastewater treatment plant effluents. Water Research 47 (4), 1521–1528. https://doi.org/10.1016/j.watres.2012.11.002.

Rigos, G., Bitchava, K., Nengas, I., 2010. Antibacterial drugs in products originating from aquaculture: Assessing the risks to public welfare. Mediterranean Marine Science 11 (1), 33–41. https://doi.org/10.12681/mms.89.

Schar, D., Klein, E.Y., Laxminarayan, R., Gilbert, M., Van Boeckel, T.P, 2020. Global trends in antimicrobial use in aquaculture. Scientific Reports 10 (1), 21878. https://doi.org/10.1038/s41598-020-78849-3.

Schroeder, J.P., Croot, P.L., Von Dewitz, B., Waller, U., Hanel, R., 2011. Potential and limitations of ozone for the removal of ammonia, nitrite, and yellow substances in

marine recirculating aquaculture systems. Aquacultural Engineering 45 (1), 35–41. https://doi.org/10.1016/j.aquaeng.2011.06.001.
Serrano, P.H., 2005. Responsible use of antibiotics in aquaculture. Food & Agriculture Org.
Shad, A., Li, C., Zuo, J., Liu, J., Dar, A.A., Wang, Z., 2018. Understanding the ozonated degradation of sulfadimethoxine, exploration of reaction site, and classification of degradation products. Chemosphere 212, 228–236. https://doi.org/10.1016/j.chemosphere.2018.08.050.
Small, C.D., el-Khoury, M., Deslongchamps, G., Benfey, T.J., Crawford, B.D, 2020. Matrix Metalloproteinase 13 Activity is Required for Normal and Hypoxia-Induced Precocious Hatching in Zebrafish Embryos. Journal of Developmental Biology 8 (1), 3. https://doi.org/10.3390/jdb8010003.
Staehelin, J., Hoigne, J., 1985. Decomposition of ozone in water in the presence of organic solutes acting as promoters and inhibitors of radical chain reactions. Environmental Science & Technology 19 (12), 1206–1213. https://doi.org/10.1021/es00142a012.
Summerfelt, S.T., 2003. Ozonation and UV irradiation—an introduction and examples of current applications. Aquacultural Engineering 28 (1), 21–36. https://doi.org/10.1016/S0144-8609(02)00069-9.
Summerfelt, S.T., Hochheimer, J.N., 1997. Review of Ozone Processes and Applications as an Oxidizing Agent in Aquaculture. The Progressive Fish-Culturist 59 (2), 94–105. https://doi.org/10.1577/1548-8640(1997)059<0094:ROOPAA>2.3.CO;2.
Tachikawa, M., Yamanaka, K., 2014. Synergistic disinfection and removal of biofilms by a sequential two-step treatment with ozone followed by hydrogen peroxide. Water Research 64, 94–101. https://doi.org/10.1016/j.watres.2014.06.047.
Tango, M.S., Gagnon, G.A, 2003. Impact of ozonation on water quality in marine recirculation systems. Aquacultural Engineering 29 (3), 125–137. https://doi.org/10.1016/S0144-8609(03)00061-X.
Teixidó, E., Piqué, E., Gonzalez-Linares, J., Llobet, J.M., Gómez-Catalán, J., 2015. Developmental effects and genotoxicity of 10 water disinfection by-products in zebrafish. Journal of Water and Health 13 (1), 54–66. https://doi.org/10.2166/wh.2014.006.
Tetzner, N.F., Rath, S., 2018. Veterinary antimicrobials and antiparasitics in fee-fishing ponds: analytical method and occurrence. International Journal of Environmental Analytical Chemistry 98 (15), 1354–1369. https://doi.org/10.1080/03067319.2018.1531395.
Von Gunten, U., 2003. Ozonation of drinking water: Part I. Oxidation kinetics and product formation. Water Research 37 (7), 1443–1467. https://doi.org/10.1016/S0043-1354(02)00457-8.
Von Sonntag, C., Von Gunten, U., 2012. Chemistry of Ozone in Water and Wastewater Treatment: From Basic Principles to Applications. IWA Publishing.
Wang, Y., Zhang, H., Zhang, J., Lu, C., Huang, Q., Wu, J., Liu, F., 2011. Degradation of tetracycline in aqueous media by ozonation in an internal loop-lift reactor. Journal of Hazardous Materials 192 (1), 35–43. https://doi.org/10.1016/j.jhazmat.2011.04.086.
Wang, Z.W., Liu, D.M., Zhang, W.J., Cui, F.Y, 2016. Acute toxic effects of bromate on aquatic organisms. Huanjing Kexue/Environmental Science 37 (2), 756–764. https://doi.org/10.13227/j.hjkx.2016.02.047.
Zhang, Y., Shao, Y., Gao, N., Chu, W., Chen, J., Li, S., Wang, Y., Xu, S., 2016. Chlorination of florfenicol (FF): reaction kinetics, influencing factors and by-products formation. RSC Advances 6 (109), 107256–107262. https://doi.org/10.1039/C6RA23342B.
Zheng, D., Yin, G., Liu, M., Chen, C., Jiang, Y., Hou, L., Zheng, Y., 2021. A systematic review of antibiotics and antibiotic resistance genes in estuarine and coastal environments. Science of The Total Environment 777, 146009. https://doi.org/10.1016/j.scitotenv.2021.146009.
Zhou, L.J., Ying, G.G., Liu, S., Zhao, J.L., Chen, F., Zhang, R.Q., Peng, F.Q., Zhang, Q.Q, 2012. Simultaneous determination of human and veterinary antibiotics in various environmental matrices by rapid resolution liquid chromatography-electrospray ionization tandem mass spectrometry. Journal of Chromatography A 1244, 123–138. https://doi.org/10.1016/j.chroma.2012.04.076.
Zhu, Y., Wei, M., Pan, Z., Li, L., Liang, J., Yu, K., Zhang, Y., 2020. Ultraviolet/peroxydisulfate degradation of ofloxacin in seawater: Kinetics, mechanism and toxicity of products. Science of The Total Environment 705, 135960. https://doi.org/10.1016/j.scitotenv.2019.135960.
Zou, S., Xu, W., Zhang, R., Tang, J., Chen, Y., Zhang, G., 2011. Occurrence and distribution of antibiotics in coastal water of the Bohai Bay, China: Impacts of river discharge and aquaculture activities. Environmental Pollution 159 (10), 2913–2920. https://doi.org/10.1016/j.envpol.2011.04.037.