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Research article

Harnessing UV-C photoassisted AOPs: Amoxicillin degradation, disinfection by-products formation, and *Enterococcus faecalis* inactivation in aquaculture water

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ABSTRACT

The continuous growth of global population has promoted the development of new aquaculture techniques for enhancing food supply. Modern approaches, such as Recirculating Aquaculture Systems (RAS), present new challenges concerning the elimination of chemical substances and pathogens that appear because of their operation. In this study, the simultaneous removal of amoxicillin and water disinfection was evaluated using different UV-C photoassisted Advanced Oxidation Processes (AOPs). The processes involved oxidants such as hydrogen peroxide, peracetic acid, sodium hypochlorite, potassium peroxymonosulfate, and sodium persulfate. The optimization of radiation and oxidant dosage was assessed in a batch system photoreactor using simulated aquaculture water. Moreover, the disinfection potential of these treatments, as well of the production of disinfection by-products (DBPs), phytotoxicity generation and matrix interaction are also addressed. Finally, a first approximation to the reaction mechanism is carried out *via* scavenging studies. Results show that 10 W/m^2 of UV-C and optimized dosages in the 0.1–3.2 mM range of oxidant eliminate at least 80 % of amoxicillin in 2 h of operation. Disinfection was achieved in 1 min of treatment and maintained for at least two weeks after storage. While DBPs and matrix parameters variation were minimal. Scavenging study shows that $HO^{\bullet}$, $^1O_2$, and $SO_4^{\bullet-}$ radicals are the main ones involved in antibiotic degradation. The optimization of operation conditions sets the path towards scale-up of the process in continuous systems, with the objective of application of the process in real aquaculture water.

1. Introduction

As human population rises, food demand increases too (van Dijk et al., 2021). The pursuit of new techniques for food production is crucial to human development, and aquaculture is a key industry that can help to reach a solution to the challenge of feeding global population (López-Mas et al., 2021; Natale et al., 2013). However, in a context of land and water scarcity (Pueppke et al., 2020), traditional open-net aquaculture has faced growing scrutiny for its environmental impact, including excessive water intake, potential for disease propagation, and nutrient contamination (*i.e.* eutrophication) (Kang et al., 2021; Streicher et al., 2021). Recirculating aquaculture systems (RAS) emerge as a promising approach, featuring a closed-loop facility for intensive fish and algae production (Ahmad et al., 2022; Aich et al., 2020; Zhang et al., 2023).

RAS are based on the treatment and reuse of water within the system (Halvorson and Smolowitz, 2009). Water from the rearing tanks is treated by mechanical and biological filtration; the purpose of mechanical filtration is the removal of larger solids, while biological filtration is based on the conversion of ammonia, a toxic by-product of fish metabolism, into nitrite and nitrate by nitrifying bacteria (Tom et al., 2021). Oxidized forms of nitrogen are subsequently denitrified by other bacterial communities (Preena et al., 2021). The biofiltration process allows for a reduced water exchange, considerably reducing freshwater needs of the installation in comparison to conventional open-net systems (Bartelme et al., 2017).

However, the operation of RAS is also challenging (Badiola et al., 2012). While these systems offer a controlled, continuously monitored

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environment for the growing of fish (Lindholm-Lehto, 2023), the presence of organic matter (associated both to the feeding regime of the cultivated species and their excretion products) serves as the substrate for the proliferation of opportunistic, and often pathogenic, bacteria (Bugten et al., 2022). Control of these harmful microorganisms is often performed by the application of antibiotics, but their dosage may be miscalculated (Estrada-San Agustín et al., 2023). The presence of remanent antibiotic drugs in the water supposes great environmental and microbiological concern; the incomplete degradation of these molecules or their metabolites could contribute to the development of antimicrobial resistance (AMR) within the aquaculture installation, as well as disrupting the natural bacterial communities present in the biofiltration processes (Kulik et al., 2023).

Advanced Oxidation Processes (AOPs) are interesting approaches towards the elimination of these compounds (Zorzi et al., 2023). AOPs are a class of chemical treatment methods that involve the generation of highly reactive oxygen species (ROS), such as hydroxyl radicals ($HO^{\bullet}$), sulfate radicals ($SO_4^{\bullet-}$), and singlet oxygen ($^1O_2$) among others (Deng and Zhao, 2015a; Krystynik, 2022; Li et al., 2022). These ROS are powerful oxidants capable of degrading a wide range of organic, inorganic and biological contaminants in any water matrix (Khan et al., 2023; Ricardo et al., 2021). The generation of these ROS promotes the degradation of organic pollutants *via* several mechanisms, namely hydrogen abstraction, hydroxyl addition, and other redox pathways (Deng and Zhao, 2015b). Compared to other AOPs such as photocatalysis and ozonation, UV-C/oxidant systems offer several operational advantages that make them more suitable for aquaculture water treatment. Photocatalytic systems often require catalyst recovery or immobilization, complicating reactor design and maintenance (Liu et al., 2020). Ozonation, while effective for disinfection, is associated with high energy demands, safety concerns due to ozone handling, and substantial infrastructure costs (Gopalakrishnan et al., 2023). In contrast, homogeneous UV-C/Ox systems operate with simpler setups, generate reactive oxygen species *in situ* without the need for solids handling, and can be easily scaled or integrated into existing RAS designs with minimal space and maintenance requirements (Silva et al., 2021; Uyğun et al., 2024a). These features make UV-C/oxidant AOPs an attractive, practical solution for addressing antibiotic and microbial contamination in closed-loop aquaculture systems.

Beyond their oxidative efficiency, AOPs — especially photochemical systems — offer clear contributions to circular economy strategies in water management. These systems facilitate the *in-situ* regeneration and reuse of aquaculture water by removing both chemical and biological contaminants, minimizing solid waste production (Kurniawan et al., 2025). This regeneration approach supposes a lesser freshwater intake, reducing effluent volume discharge and lowering environmental burdens (García Rodríguez et al., 2025). For these reasons, homogeneous AOPs support a more sustainable aquaculture framework.

Photoassisted AOPs are profusely studied due to the versatility and flexibility in their application towards antibiotic and microorganisms' elimination in aquaculture systems (Moreno-Andrés et al., 2020a; Uyğun et al., 2024b; Zorzi et al., 2023). Their basic principle of operation is the interaction between radiation and matter in water systems, yielding molecular excited states whose reactivity promotes the generation of ROS. In these systems, energetic radiation (from the UV-C to the solar range) is applied to the water matrices, often in presence of oxidizing species (Li et al., 2023a; Rodriguez-Gonzalez et al., 2019). This interaction between radiation and oxidants may be synergistic, enhancing the disinfection and decontamination power of these techniques (Chin et al., 2023; Santana et al., 2017; Silva et al., 2023; Singh et al., 2022).

The application of photoassisted AOPs in aquaculture systems for pathogen inactivation and antibiotic removal has gained growing interest in the recent years. Studies have focused on the comparison of different photochemical technologies on disinfection performance (Blanchon et al., 2024; Hu et al., 2024; Li et al., 2023b; Moreno-Andrés et al., 2020b; Uyğun et al., 2024b) and the assessment of the removal of antibiotics by the combined effect of radiation, oxidants and/or catalysts. However, alongside their effectiveness, the potential formation of harmful disinfection by-products (DBPs) must be considered.

The formation of disinfection by-products (DBPs) is a well-documented concern in water treatment due to their potential adverse effects on human health (Li and Mitch, 2018). Traditionally, conventional disinfectants such as sodium hypochlorite (NaClO) have been widely used for pathogen removal in aqueous matrices. However, their reaction with organic matter and halogens present in water can lead to the formation of organic DBPs, such as trihalomethanes (THMs), and inorganic DBPs, such as chlorite (ClO_2^-), chlorate (ClO_3^-), and bromate (BrO_3^-) ions, which have been associated with carcinogenic and toxic effects in humans (Amin et al., 2013; Li et al., 2017).

In this context, advanced oxidation processes (AOPs), such as combined UV-C/oxidant treatments, have been investigated as alternatives to enhance disinfection and the degradation of contaminants of emerging concern (CECs). However, these processes may also induce the formation of DBPs through the interaction of the generated oxidant species with the components of the aqueous matrix. In particular, the activation of peroxymonosulfate (PMS) in the presence of halogenated species may contribute to the generation of inorganic DBPs (Zhang et al., 2022).

Given the potential impact of these compounds on water quality and public health, which in aquaculture matrices often remains underexplored this study will assess the formation of DBPs in different UV-C/Ox treatment combinations. A quantitative analysis of DBPs, featuring inorganic species such as ClO_2^-, ClO_3^-, and BrO_3^-, as well as organic compounds such as THMs, will be conducted to characterize the risks associated with these technologies and contribute to the development of safer and more efficient treatment strategies.

Despite growing interest in photochemical AOPs, most studies to date have focused either on antibiotic abatement or on disinfection performance assessment, rarely addressing both aspects in an integrated fashion. This study aims at filling a significant knowledge gap at the intersection of water reuse safety and sustainable aquaculture. To do so, five different homogeneous AOPs based on UV-C radiation using chemical oxidants (hydrogen peroxide, peracetic acid, sodium hypochlorite, potassium peroxymonosulfate, and sodium persulfate) have been assessed. After determination of their optimal oxidant concentration and radiation dosages (defined as the minimum doses required to remove 80 % of the antibiotic), a disinfection matrix study with *Enterococcus faecalis* (including a 1–2 weeks regrowth study) will be performed. The formation of disinfection by-products (DBPs) after the application of the studied treatments will be addressed. A scavenging study will explore the role of reactive oxygen species (ROS), alongside monitoring changes in the ionic profile and pH. Finally, the feasibility of applying optimal UV-C/oxidant conditions in real aquaculture water from a real facility in Madrid, Spain, will be investigated. This panoptic approach allows for a comprehensive knowledge of the studied UV-C/Oxidant processes, aiming at their (photo)chemical, microbiological and mechanistic understanding, with sight on their future scaling.

2. Materials and methods

2.1. Preparation of the simulated aquaculture water

Simulated aquaculture water (SAW) is the matrix employed for the experiments of amoxicillin (AMX) degradation, and its preparation is carried out according to previous water characterizations, simulating the composition of discharge effluents in recirculating aquaculture systems (summarized in Table S1 in *Supplementary Materials*). SAW is composed of yeast extract (Sigma; 193.35 mg/L), ammonium chloride (NH_4Cl, Labkem; 26.28 mg/L), potassium dihydrogen phosphate (KH_2PO_4, Scharlau; 1.96 mg/L), sodium hydrogen carbonate, ($NaHCO_3$, Sigma; 0.31 mg/L), iron citrate, $FeC_6H_6O_7$ (Acros Organics; 0.31 mg/L),

magnessium chloride ($MgCl_2$, Labkem; 50.0 mg/L), calcium chloride ($CaCl_2•2H_2O$, Scharlau; 55.86 mg/L) and zinc sulfate ($ZnSO_4$, Labkem; 0.25 mg/L). Amoxicillin ($C_{16}H_{19}N_3O_5S$, AMX; Sigma-Aldrich) is added to the SAW to an initial concentration of 25 mg/L. The fixed amoxicillin concentration, while higher than the typical values of the antibiotic in aquaculture effluents, is an acceptable starting point towards the knowledge of the homogeneous photochemical degradation process. The simulated matrix is also spiked with a strain of *Enterococcus faecalis,* prepared as detailed in section 2.2 below, to reach an approximate concentration of 10^6 CFU/mL.

2.2. Chemical reagents

Five chemical oxidants, namely hydrogen peroxide (H_2O_2, 30 % v/v; Chem-Lab), peracetic acid (PAA, $C_2H_4O_3$, 15 % v/v; PanReac), sodium hypochlorite (NaClO, 5–9 % Cl; Carlo-Erba), potassium peroxymonosulfate (PMS, $KHSO_5$-0.5$KHSO_4$-0.5K_2SO_4; Oxone®, Sigma-Aldrich) and sodium persulfate (PS, $Na_2S_2O_8$, Chem-Lab) are assessed in combination with UV-C radiation. The studied range of concentration for the oxidants is 0.01–6.4 mM and has been stablished based on the stoichiometric concentration of the antibiotic with respect to the oxidant.

After determining the optimal degradation conditions, a scavenging study is carried out. Methanol (MeOH, J.T. Baker), tert-butyl alcohol (t-BuOH, Sigma-Aldrich), and furfuryl alcohol (FFA, Sigma-Aldrich) are selected for their $HO^•$, $SO_4^{•-}$, and $^1O_2$ scavenging roles. The scavengers are added in a 20:1 concentration with respect to the oxidant to allow quantitative reaction with the generated radical species.

2.3. Experimental procedure

100 mL of SAW are placed in the batch UV-C photolysis system, which consists of a 250 mL beaker under constant stirring and is irradiated by a Philips TUV 6W G6T5 UV-C light source emitting short-wave radiation with a peak at the 254 nm band. The selected concentration of the oxidant under study (H_2O_2, PAA, NaClO, PMS, or PS) is added to the reactor, and the reaction time begins when the UV-C radiation source is turned on. Before that, AMX is added to the system at an initial concentration of 25.0 mg/L. Additionally, in experiments evaluating the simultaneous removal of AMX and *Enterococcus faecalis*, the bacterium is introduced into the SAW to reach a concentration of 10^6 CFU/mL. Radiation intensity is modulated by adjusting the separation distance between the batch reactor and the radiation source, using a Delta OHM HD 2102.1 Photoradiometer. After extraction, samples are mixed with 100 μL of MeOH to quench the reaction between the oxidant and the pollutants and prevent further degradation.

The study aimed at understanding the main species involved in the treatment is based on the use of different scavengers and is applied under the previously optimized treatment conditions. Concentrations of MeOH, tBuOH and FFA are added to the system prior to the starting of the photochemical reaction. The sampling pattern and sample post-treatment is analogous to the previously described experiments of AMX degradation.

2.4. Analytical determinations

For the determination of AMX in the samples, an Agilent 1260 Infinity II LC System HPLC/VWD is employed, with a ZORBAX Eclipse Plus C18 (4.6 × 100 mm, 5 μm) column as a stationary phase and a 2 % Acetic acid in H_2O/Acetonitrile 90:10 at 1.5 mL/min as a mobile phase, with no temperature control. 5 μL of sample were injected in the system, and the total analysis time was 5 min. The absorption wavelength was fixed at 254 nm. AMX concentration determinations were carried out by duplicate.

Ionic Chromatography (IC) measurements are performed at the beginning and ending of the AMX degradation experiments to assess the generation or abatement of anionic species in the aqueous matrix. For the IC measurements, an Methrom Eco IC Ion Chromatograph, with Na_2CO_3/$NaHCO_3$ 8.0/0.25 mM at a 0.7 mL/min flow as the mobile phase, is employed. The detection of the anionic analytes is performed *via* conductometric measurements.

The determination of THMs after the application of the treatments of study was performed *via* Gas Chromatography hyphenated to Mass Spectrometry (CG/MS) analysis according to Standard Method 6232B. THMs were analyzed based on the liquid-liquid extraction gas chromatographic method (Method 6232 B); by the employment of a PerkinElmer Clarus 600 Gas Chromatograph/Mass Spectrometer (GC-MS) equipped with TurboMass software version 5.4.0. Samples were collected in clean, baked 40-mL glass open screw-top TFE-faced vials. For the extraction process, 2.0 mL of pentane, containing a specified concentration of 1,2-dibromopropane as the internal standard, was added to 35 mL of the raw sample, and the mixture was mechanically shaken at 90–100 rpm for 1 min. The extracted sample was then injected with an autosampler to the GC column (30 m × 0.25 mm, 0.25 μm, Elite-5MS, PerkinElmer). The injection volume and split ratio were 2 μL and 50 %, respectively. High purity He was used as the carrier gas at a specified flow rate. The oven temperature was initially set at 30 °C for 5 min, then ramped up at a rate of 30 °C/min to a final temperature of 180 °C, where it was maintained for 5 min. The inlet line and source temperatures were 200 °C. For the MS detection, selective ion recording (SIR) was employed, targeting the m/z values of the specific THMs and their retention times.

For the determination of Total Organic Carbon (TOC) a Teledyne Tekmar TOC Torch equipped with combustion catalytic oxidation (700 °C) and non-dispersive infrared detection (NDIR) is employed.

2.4.1. Microbiological analysis

Disinfection validation studies were carried out to assess the disinfection potential of the UV-C/Oxidant treatments. *Enterococcus faecalis* strains (ATCC® 29212TM) were purchased in the form of MiSTraCon®Swabs2 pellets (Scharlau). The commercial strains, stored in cryovials at −20 °C, are growth in 10 mL of Luria Bertani broth (Condalab), and incubated at 37 °C for 24h at 190 rpm using a Biosan ES-20 Orbital Shaker-Incubator. After incubation is concluded, the bacterial suspension is centrifuged for 10 min at 4200 rpm, and the extracted pellet is reconstituted in 10 mL of 0.9 % w/V NaCl (Scharlau). The resuspended bacterial stock solution reaches a concentration in the order of 10^9 CFU/mL.

For the determination of bacteria in the disinfection validation experiments, the drop-plate method, with a detection limit of 100 CFU/mL is considered. A selective culture medium (Slanetz&Bartley; Condalab) is inoculated with serial 10-fold dilutions in sterile 0.9 % NaCl solution; the spread plate method (Standard Method 9215C) is employed to extend the detection limit to 10 CFU/mL. The counting of the appearing colonies is carried out after the incubation of the plates during 48h at 37 °C.

For the bacterial regrowth study, treated water from each UV-C/oxidant process is collected at the end of the application time and stored in the dark. The determination of bacteria is analogously carried out, after 7 and 14 days of storage, in triplicate.

2.5. Phytotoxicity study

To assess the toxicity of the generated effluents, a phytotoxicity study based on seed germination is carried out. This study is based on the differences on the germination of different vegetal species when exposed to the aqueous medium prior and after the advanced oxidation treatment. *Raphanus sativus* (radish), *Solanum lycopersicum* (tomato) and *Lactuca sativa* (lettuce) where the selected species. For carrying out the experiment, 15 seeds of each species are placed on a Petri dish with a previously placed filtering paper, and 10 mL of the target sample are afterwards located. The seeds are incubated for 72 h at 25 °C. After the

incubation period, the germinated seeds were counted and the average length of the stems of the species were measured. The germination index (GI) was calculated according to equation (1):

$$GI\ (\%) = \frac{G_S{}^* L_S}{G_C{}^* L_C} * 100 \quad (1)$$

Where G_S and G_C are the number of seeds which have germinated after application of the treatment effluent and the control, and L_S and L_C are the length of the stems of such germinated plants.

3. Results and discussion

3.1. Determination of the optimal radiation intensity. Application of the figures of merit

The photochemical system operates within a radiation intensity range of 1.0–10 W/m^2, within which AMX removal is evaluated. The progress of AMX removal throughout the reaction is shown in Fig. 1.

The determination of the optimal intensity of UV-C is first carried out. The optimal degradation is defined as the one that minimizes the electrical energy by reaction order (E_{EO}), which is a figure of merit that relates amount of energy (in kWh) that is needed to reduce the concentration of a contaminant in one order of magnitude. For batch operation, E_{EO} may be calculated according to equations (2) and (3) (Bolton et al., 2001):

$$E_{EO} = \frac{1000 \cdot P \cdot t}{V \cdot \log\left(\frac{C_f}{C_0}\right)} \quad (2)$$

$$E_{EO} = \frac{38.4 \cdot P}{V \cdot k_1} \quad (3)$$

Where P is the nominal power, t is the reaction time, V is the volume of the batch system, C_f and C_0 the final and initial concentrations and k_1 the first-order kinetic constant. In Equation (2), the $\log\left(\frac{C_f}{C_0}\right)$ term may be substituted by $0.4343 \cdot k_1 \cdot t$, yielding Equation (3).

Based on the results observed in Fig. 1, the research findings are fitted to a first-order kinetics model to calculate the removal rate constants, determine the half-life ($t_{1/2}$), and compute the figures of merit. Therefore, the summary of the kinetic and degradation parameters of the UV-C photolysis systems are summarized in Table 1.

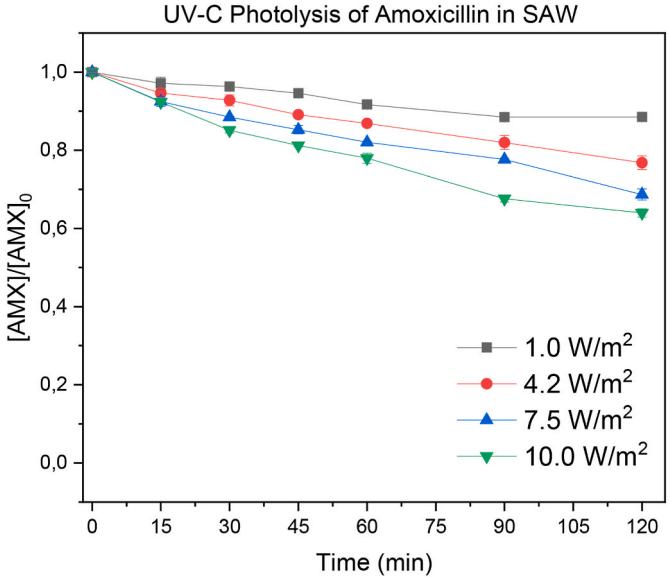

Fig. 1. Removal of AMX in presence of variable intensities of UV-C radiat'ion.

A greater removal degree of AMX is observed in presence of 10 W/m^2 UV-C radiation, as greater radiation intensities produce higher removal percentages of the antibiotic. Moreover, the application of 10 W/m^2 of UV-C radiation during 120 min of treatment minimizes the figure of merit. This intensity is thus selected as the optimal radiation for the studied system. A lower value of E_{EO} is interpreted as a lower energy requirement for the degradation of the antibiotic, which translates into a more efficient decontamination process. The other studied radiation intensities, as it can be seen, are associated to noticeably higher values of E_{EO} and are for this reason considered unsuitable in terms of efficiency. The selected intensity of 10 W/m^2 emerges as the optimal choice for the studied system, ensuring a balance between contaminant removal and energy efficiency.

However, the abatement of AMX for 10 W/m^2 of intensity is only of 36 % after 120 min of photolysis, which evidences the need of the intensification of the photochemical treatment. As proposed by Alegbeleye et al., the sole application of UV-C radiation is often not sufficient for achieving the quantitative antibiotic degradation, so the combined effect of oxidizing agents could be a much more effective process (Alegbeleye et al., 2022). For the oxidant optimization dosage study, the duration of the experiments will be stablished at 120 min; a timeframe considered long enough for the assessment of the effect of each oxidant after their application in the photochemical system.

3.2. Assessment of the intensified UV/C oxidant systems. Determination of the optimal oxidant dosage

After selecting 10 W/m^2 as the UV-C intensity and being aware of the need to intensify the process to achieve a higher AMX removal efficiency, the UV-C/oxidant process is studied. First, the effect of the oxidant dosage on AMX abatement is analyzed. The initial concentration of oxidant is calculated assuming specific and complete mineralization reaction between each oxidant and the antibiotic. The interactions between the organic species in the matrix and the oxidant, while possible, are neglected. For an initial concentration of 25 mg/L of AMX, the first studied concentrations for each oxidant were 1.64 mM, as detailed in the *Supplementary materials*, in Table S2. The removal of AMX under the stoichiometric conditions is presented in Fig. 2.

After assessing the AMX degradation for the oxidants of study, the initial hypothesis of the stoichiometric dose for the treatments appears to not have been correct: for some of the oxidants (H_2O_2 and PS) the dose that ensures the oxidation of the 80 % of amoxicillin is higher than the theoretical stoichiometric amount, while for others (PAA, NaClO and PMS) the experimental dose is far lower than the theoretical, as no AMX is observed after just 15 min of application of the treatment.

The discrepancy in the theoretical and the experimental dosage could be attributed to several factors. For H_2O_2 and PS, the positive difference between the operational and the theoretical dose could be attributed to the interference between the oxidants and the organic matter present in the SAW, as well as the possibility of the occurrence of self-scavenging reactivity (Honarmandrad et al., 2023; Zhao et al., 2021). Equations (4)–(7) explain the self-scavenging reactivity behind this decreased yield:

Table 1
Performance indicators for the different studied intensities in the UV-C photolysis system.

Intensity (W/m^2)	k_1 (min^{-1})	$t_{1/2}$ (min)	Removal (%)	E_{EO} (kWh·m^{-3})
1.0	0.00108	640.60	11.50 %	13.65
4.2	0.00211	329.00	23.21 %	7.01
7.5	0.00286	242.30	31.32 %	5.16
10.0	0.00374	185.20	36.04 %	3.95

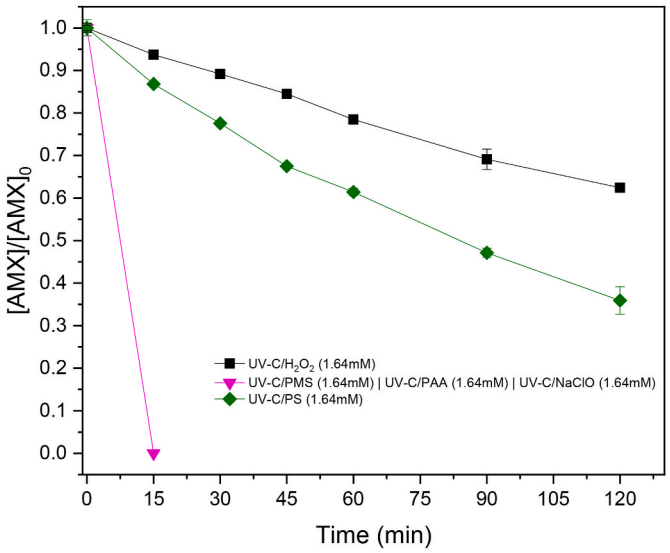

Fig. 2. Removal of AMX in presence of stoichiometric conditions of oxidant and 10 W/m^2 of UV-C radiation.

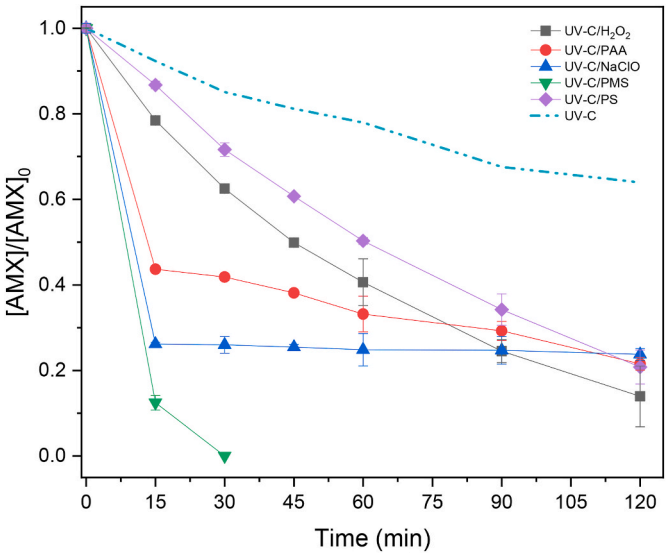

Fig. 3. Removal of AMX in presence of optimized conditions of oxidant and 10 W/m^2 of UV-C radiation.

$$HO^{\bullet} + H_2O_2 \rightarrow HO_2^{\bullet} + H_2O \quad (4)$$

$$SO_4^{\bullet -} + S_2O_8^{2-} \rightarrow SO_4^{2-} + S_2O_8^{\bullet -} \quad (5)$$

Equations (6) and (7) show the organic matter (OM) competition reactions for the ROS in the photochemical systems, for both $HO^{\bullet}$ and $SO_4^{\bullet -}$ radicals:

$$HO^{\bullet} + R{-}H \rightarrow H_2O + R^{\bullet} \quad (6)$$

$$SO_4^{\bullet -} + OM \rightarrow SO_4^{2-} + \text{degraded OM} \quad (7)$$

For PAA, NaClO and PMS, the negative difference may have its root in the contribution of the degradation of AMX by direct photolysis and the generation of additional ROS after oxidant-radiation interaction, which greatly enhance antibiotic degradation (Guan et al., 2020; Liu et al., 2022; Zhang et al., 2021). This enhanced performance may be explained by the following (photo)chemical reactivity:

$$CH_3COOOH \xrightarrow{\text{UV-C}} CH_3COO^{\cdot} + HO^{\cdot} \quad (8)$$

$$ClO^{-} \xrightarrow{\text{UV-C}} Cl^{\cdot} + O^{\cdot -} \quad (9)$$

$$O^{\cdot -} + H_2O \rightarrow HO^{\cdot} + HO^{-} \quad (10)$$

$$HSO_5^{-} \xrightarrow{\text{UV-C}} SO_4^{\cdot -} + HO^{\cdot} \quad (11)$$

After the first approximation to the dosage optimization, the concentrations of oxidants are fined-tuned to reach their optimal value in terms of AMX removal, by adjusting of the dosage of the oxidants until reaching of the 80 % target degradation. Assayed concentrations are summarized in Table S3 in *Supplementary materials*. Selected optimized concentrations are detailed below in Table 2.

The evolution of the concentration of AMX under the optimal conditions of oxidant and radiation concentration in SAW is presented in Fig. 3.

The oxidants, while fulfilling the 80 % AMX degradation threshold, present different reactivities. Both H_2O_2 and PS present analogous reactivities, assimilable to first-order kinetics, while PAA, NaClO and PMS systems achieve degradations of 55–90 % in the first stage of the degradation process, and their behaviour cannot be modelled as first-order like.

To assess the contribution of the oxidant to the degradation of AMX, dark-phase experiments are carried out. The selected dosages of the oxidants are the same of the optimization experiments. For each UV-C/Oxidant treatment, the percentual synergistic effect (% SE) is calculated following Equation (12):

$$\%\,SE = \frac{\%D_{UV-C/OX} - (\%D_{UV-C} + \%D_{OX})}{(\%D_{UV-C} + \%D_{OX})} \cdot 100 \quad (12)$$

Where $\%D_{UV\text{-}C/OX}$ is the degradation percentage for each UV-C/Oxidant treatment, $\%D_{OX}$ is the degradation percentage for each dark-phase treatment in presence of the studied oxidants, and $\%D_{UV\text{-}C}$ is the degradation percentage for the direct photolysis of AMX at 10 W/m^2 of radiation intensity. A positive value %SE indicates a synergistic effect for the oxidant and the radiation, while a negative value for the parameter is associated with an antagonistic effect between the two factors. A %SE value of zero would indicate a null interaction between radiation and oxidant. Table 3 summarizes the optimal dose of each UV-C/oxidant treatment to achieve the 80 % degradation of the AMX, the

Table 2
Experimental assayed concentrations for oxidant dosage optimization.

Treatment	Concentration range (mM)	Optimal concentration (mM)
UV-C/H_2O_2	1.64-6.40	3.20
UV-C/PAA	0.02-1.64	0.10
UV-C/NaClO	0.02-1.64	0.75
UV-C/PMS	0.02-1.64	0.10
UV-C/PS	1.64-2.20	2.20

Table 3
Optimization of the oxidant dosages, dark-phase treatment performance and synergistic effect.

Optimal conditions	Percentual removal (%)	SE (%)
10 W/m^2	36.04	–
0.10 mM PAA + 10 W/m^2	78.46	77.75 %
0.10 mM PAA	70.15	
3.20 mM H_2O_2 + 10 W/m^2	86.05	85.53 %
3.20 mM H_2O_2	18.88	
0.75 mM NaClO + 10 W/m^2	81.25	80.54 %
0.75 mM NaClO	76.22	
2.20 mM PS + 10 W/m^2	79.20	78.69 %
2.20 mM PS	8.70	
0.10 mM PMS + 10 W/m^2	100.00	99.34 %
0.10 mM PMS	87.01	

degradation of the corresponding dark-phase treatment, and the %SE.

As it can be observed, all treatments benefit from the application of radiation. A synergistic effect appears for all the studied treatments, which is an indicative of a cooperative interaction among the oxidant and the radiation. These photochemical interactions may be summarized in equations (13)–(20):

$$H_2O_2 \xrightarrow{\text{UV-C}} 2HO\cdot \quad (13)$$

$$CH_3COOOH \xrightarrow{\text{UV-C}} CH_3COO\cdot + HO\cdot \quad (14)$$

$$ClO^- \xrightarrow{\text{UV-C}} Cl^- + O\cdot \quad (15)$$

$$O^\bullet + H_2O \rightarrow 2HO^\bullet \quad (16)$$

$$SO_4^{-\bullet} + H_2O \rightarrow HO^\bullet + HSO_4^- \quad (17)$$

$$HSO_5^- \xrightarrow{\text{UV-C}} SO_4^{-\cdot} + HO\cdot \quad (18)$$

$$ClO^- \xrightarrow{\text{UV-C}} Cl^- + O\cdot \quad (19)$$

$$S_2O_8^{2-} \xrightarrow{\text{UV-C}} 2SO_4^{\cdot -} \quad (20)$$

The appearance of such synergistic behaviours in UV-C/Oxidant systems for the degradation of antibiotics has been previously reported in literature. Studies indicate that the combination of UV radiation with H_2O_2 and PS significantly improves AMX photodegradation (Zhang et al., 2021), boosts radical generation from PAA, thereby promoting micropollutant removal (Yang et al., 2022), and facilitates synergistic interactions in the degradation of micropollutants when using UV with sulfate-based oxidants (Antonopoulou et al., 2024; Montazeri et al., 2020).

3.3. Validation of disinfection potential and bacterial regrowth analysis

After the determination of the optimal concentrations for the degradation of AMX, the potential of the studied treatments for the simultaneous decontamination and disinfection of water is assessed. Table 4 presents the variation of the concentration of *E. faecalis* along the time of reaction for the different treatments.

After 1 min of the application of the treatment, all the studied processes guarantee the disinfection of the matrix. The disinfection of the matrix, in terms of dosage application of both UV-C and oxidants, is expected to be swiftly achieved, as the operational conditions are rather harsh for the biological contaminants of study.

Several studies have proposed the disinfection of pathogenic microbes by UV/oxidant AOPs. In the work carried out by Koivunen et al., where the potential of UV-C/H_2O_2, UV-C/PAA and UV-C/NaClO for enteric bacteria disinfection was addressed, where 2–3 log reduction of *E. faecalis* was obtained in 10 min of exposure, considered higher doses of the studied oxidants (1-2 orders of magnitude with respect to this study). However, the radiation intensity was far lower (2-3 orders of magnitude with respect to this study) (Koivunen and Heinonen-Tanski, 2005).

The superior performance of the UV-C/NaClO system observed in this work can be explained by the combined biocidal mechanisms of hypochlorous acid (HOCl) and UV-C light. HOCl, intensively generated from NaClO in aqueous conditions, is known to disrupt cellular structures and metabolic pathways in bacteria through oxidation of membrane proteins, lipids, and nucleic acids (Douterelo et al., 2014). Additionally, UV-C photolysis of HOCl generates highly reactive species such as chlorine radicals ($Cl^\bullet$) and hydroxyl radicals ($HO^\bullet$), as shown in the equation (21):

$$HOCl \xrightarrow{\text{UV-C}} Cl\cdot + HO\cdot \quad (21)$$

These radicals intensify oxidative stress on bacterial cells and enhance disinfection efficacy. The synergy between chemical and photolytic pathways, combined with the high UV-C fluence rate (10 W/m^2), leads to rapid microbial inactivation — a phenomenon reported in other studies involving halogenated AOPs under high-intensity UV (Khajouei et al., 2022).

Disinfection in aquaculture systems has been addressed by other authors, such as Moreno-Andrés et al., where they explored the UV-C interaction with H_2O_2 and PMS process for *Vibrio alginolyticus*. Applied oxidant dosages (10 mg/L for H_2O_2 and 2.5 mg/L for PMS) are lower than this study and satisfy quantitative disinfection in the 5 min range, which is coherent with the Gram-negative nature of the *Vibrionacenae* bacterium, presenting a thinner, more susceptible to advanced oxidation cell wall. Peptidoglycan layer in Gram-positive bacteria, in contrast, contributes to their greater resistance to the action of UV-C radiation (Kim et al., 2023; McKinney and Pruden, 2012). Rodríguez-Chueca et al. studied the performance of sulfate-based AOPs and observed that the required oxidant dosage for wastewater disinfection (0.01 mM) was markedly lower than that for antibiotic removal (5 mM). This discrepancy arises from the fundamentally different mechanisms involved in each process. Bacterial inactivation primarily targets cell membranes, proteins, and nucleic acids, which are rapidly oxidized by reactive oxygen species (ROS), even at low concentrations. In contrast, antibiotic degradation involves the breakdown of more stable molecular structures and often requires sustained exposure to higher ROS levels. As such, achieving significant pollutant removal demands considerably higher oxidant doses. The study also reported that UV-C/sulfate-based AOPs outperformed UV-C/H_2O_2 in terms of antibiotic abatement, likely due to the higher oxidation potential and selectivity of sulfate radicals ($SO_4^{-\bullet}$) under the tested conditions (Rodríguez-Chueca et al., 2019).

Matrix composition may also affect disinfection performance. Moreno-Andrés et al. studied the UV-C/PS system several water matrices: distilled water, mineral water, saltwater and marine saltwater. After PS dosage optimization (1 mM), the authors observed an antagonistic effect for increasing ionic concentration (particularly HCO_3^-), as the decrease in the inactivation rate was higher in mineral water than in saltwater and marine saltwater.

To evaluate the long-term disinfection effect of the studied treatments, Concentration of *E. faecalis* in the sample is monitored after 1 and 2 weeks from the application of the treatment. No *E. faecalis* was determined above the 10 CFU/mL threshold after either of the sampling periods, for all five studied treatments.

Table 4
Evolution of *E. faecalis* population after application of the optimized treatments (Initial population: 250,000 CFU/mL).

Treatment	$[E.\ faecalis]_{t=1\ min}$ (CFU/mL)
UV-C	55
UV-C/H_2O_2	75
UV-C/PAA	50
UV-C/NaClO	<10
UV-C/PMS	60
UV-C/PS	60

3.4. Determination of disinfection by-products following optimized treatments

After determination of the optimized UV-C/Oxidant treatments, the generation of disinfection by-products by the studied systems was assessed. Of all the treatments, only UV-C/NaClO (65.2 mg_{ClO3-}/L and 59.1 μg_{CHCl3}/L) and UV-C/PMS (0.64 mg_{ClO3-}/L) presented levels of DBPs above the analytical detection limit (stablished at 0.25 mg/L for ionic DBPs and 5.0 μg/L for $CHCl_3$). No ClO_2^- and BrO_3^- were detected at the end of the treatment application for any of the studied photolytic processes, and for the UV-C/H_2O_2, UV-C/PAA and UV-C/PS no DBPs whatsoever were determined after the end of the treatment.

The presence of DBPs in water systems has been regulated by the European Union. The Drinking Water Directive (Directive EU, 2020/2184 on the Quality of Water Intended for Human Consumption) stablishes a maximum concentration of 100 μg/L for all THMs, 10 μg/L for BrO_3^-, and 250 μg/L for ClO_2^- and ClO_3^- in drinking water. While no specific regulation exists for the control of DBPs concentration in advanced treatment of aquaculture water, the presence of trace levels of DBPs has reported to have negative effects towards different trophic levels in freshwater ecosystems (Cui et al., 2021, 2022; Yuan et al., 2022), highlighting the importance of monitoring their concentration and minimizing their production.

The obtained results are consistent with the nature of the employed oxidants. NaClO treatments, while effective (Sun et al., 2022; Dao et al., 2018), are often associated with DBPs generation, as oxidized forms of chlorine react with organic matter yielding inorganic (Hu et al., 2025) and organic (Carra et al., 2020) DBPs. Hu et al. detected lower levels of $CHCl_3$ (6.5 μg/L) and ClO_3^- (0.62 mg/L), but their experiments considered a higher a long incubation time (72 h) which could promote the volatilization of trihalomethanes (Thurnau and Clark, 2017). Carra et al. considered an UV/Chlorine system for the removal of pesticides, observing a production of trihalomethanes up to 60 μg/L in acidic medium, with 30 % lower values at natural pH of water. The authors also detected concentrations of Haloacetic Acids in concentrations around 20 μg/L in acidic and natural pH.

For the case of PMS, a concentration of 0.64 mg/L of ClO_3 ion is generated after the application of the treatment. However, chlorate is the only detected DBP. The formation of chlorate, as proposed by Hou et al., may be attributed to the oxidation of Cl^- to HOCl, which undergoes further oxidation to ClO_3^- (Hou et al., 2018) (equations (22)-(24)):

$$Cl^- \xrightarrow{SO_4^{\cdot -}} HOCl\,/\,OCl^- \quad (22)$$

$$HOCl\,/\,OCl \xrightarrow{SO_4^{\cdot -}} ClO_3^- \quad (23)$$

$$HOCl/OCl \xrightarrow{h\nu} ClO_3^- \quad (24)$$

The rest of the UV-C/oxidant treatments generate no detectable levels of either inorganic or organic DBPs, which constitute a promising result in terms of suitability for technology upscaling. The application of the UV-H_2O_2 may result in the elimination of $CHCl_3$, as proposed by Rudra et al., which observed degradation percentages of at least 92 % for several organic halides by application of the UV-C/H_2O_2 process. (Rudra et al., 2005). The non-significative formation of several chloro- and bromo-THMs and HAAs in presence of PAA compared with chlorine-based disinfectants, observed by Xue et al., opens the door to the viability of said oxidant (Xue et al., 2017). Finally, the study carried out by Hu et al., comparing the DBPs formation in advanced treatment of Iopromide show that UV/PS presents a lower $CHCl_3$ formation compared to the UV/Cl_2 process, reaching levels of 10 μg/L after treatment (Hu et al., 2022).

3.5. Phytotoxicity assessment

Being UV-C/NaClO and UV-C/PMS the two potentially most hazardous treatments (since they have detectable levels of the considered DBPs), a phytotoxicity study is carried out on their effluents as a first step towards the assessment of the toxicity of these advanced oxidation processes. The tests are selected for their low price, simplicity of use and versatility. The GI for each vegetal species is presented in Table 5.

Table 5
Percentual GI after the application of UV-C/NaClO and UV-C/PMS treatments to the studied vegetal species.

Process	GI (*R. sativus*)	GI (*S. lycopersicum*)	GI (*L. sativa*)
UV-C/NaClO	59.9 %	26.6 %	0 %
UV-C/PMS	104.4 %	72.8 %	0 %

The results show that the phytotoxicity effect of both treatments over *L. sativa* has a high impact on its growth, as no seeds have germinated after application of the photochemical treatment. For the case of *R. sativus* and *S. lycopersicum*, the results obtained for the GI are consistent with the DBPs generation results, since a greater GI is observed for UV-C/PMS in both species than in UV-C/NaClO. *R. sativus* appears to be a more resistant species than *S. lycopersicum* towards the exposure to the treatment effluents. A study by Pizzichetti et al., addressing the phytotoxicity of effluents generated by the UV-C treatment of diclofenac, is coherent with this tendency, as all GI values for *R. sativus* are greater than their *S. lycopersicum* counterparts (Pizzichetti et al., 2023).

Phytotoxicity studies for PMS treatment systems have been addressed by other authors. Ghanbari et al. shown that the PMS treatment itself reduced the phytotoxicity in both *R. sativus* and *S. Lycopersicum* species. The study assumed a value of 70 % of GI as an indicative of non-phytotoxicity. Values for the UV-C/PMS treatment would be acceptable in terms of phytotoxicity for both species.

3.6. Variation of ionic profile and water quality parameters

The variation in the ionic profile of the system, calculated as the difference of the concentration values of the SAW matrix before and after the treatment, is studied by analysis of the formate, acetate, nitrite and nitrate ions concentration evolution prior and after the treatment, as well as the evolution of the pH of the solution and the mineralization percentage in terms of removal of TOC with respect to SAW (summarized in Table S4 in the *Supplementary Materials*). These analytical parameters are common quality indicators of water (nitrite, nitrate, pH) or chemical byproducts that appear during AOP treatments (formate, acetate). They are also related to the chemical reactivity of the oxidants with the organic matter present in the system. The evolution in the concentration of ionic species is presented in Fig. 4.

Appearances of acetate and formate (short-chain carboxylic acids, SCCA) in the system are associated with the degradation of organic matter, being these species the result of the partial oxidation of carbon. For the case of the UV-C/PAA process, the apparition of acetate is also attributed to the reduction of the peracetic ion to acetate (Zhang and Huang, 2020). Apparition of SCCA in UV photolysis of organic pollutants processes have been described in previous works, *via* peroxidation pathways (Loraine, 1999) or pyridinium ring opening (Marien et al.,

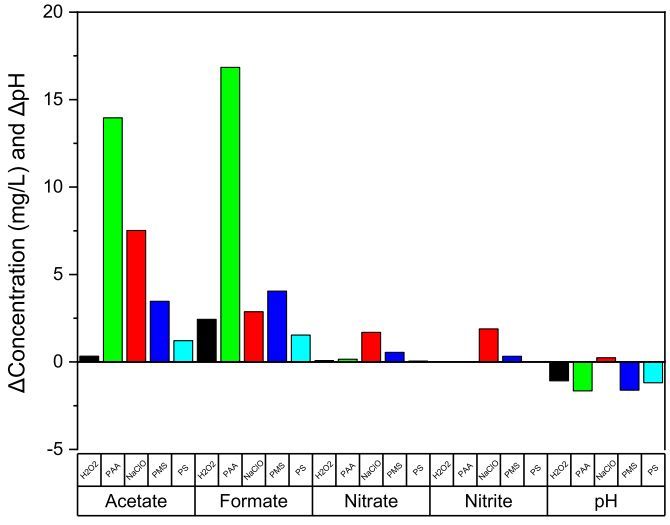

Fig. 4. Variation in the concentration of quality indicators and degradation by-products after application of the UV-C/oxidant treatments.

2019).

Oxidized nitrogen species (NO_2^- and NO_3^-) appear after hydrolysis and subsequent oxidation of organic nitrogen (present in both the organic matrix and the antibiotic) in oxidizing media. These species, which are UV active, may enhance pollutant degradation via generation of additional ROS and reactive nitrogen species (RNS) (Ao et al., 2024). However, they may act as a photo-competitor in the UV-C/PAA system, slowing the degradation process.

The generation of SCCA is greater in the PAA, NaClO and PMS treatments. High generated concentrations of acetate and formate by UV-C/NaClO and UV-C/PMS suggest a greater degradation of the organic matter present in SAW.

The variations of pH of the treatments of study are consistent with the expected chemical behaviour of the oxidants of use. NaClO, as an

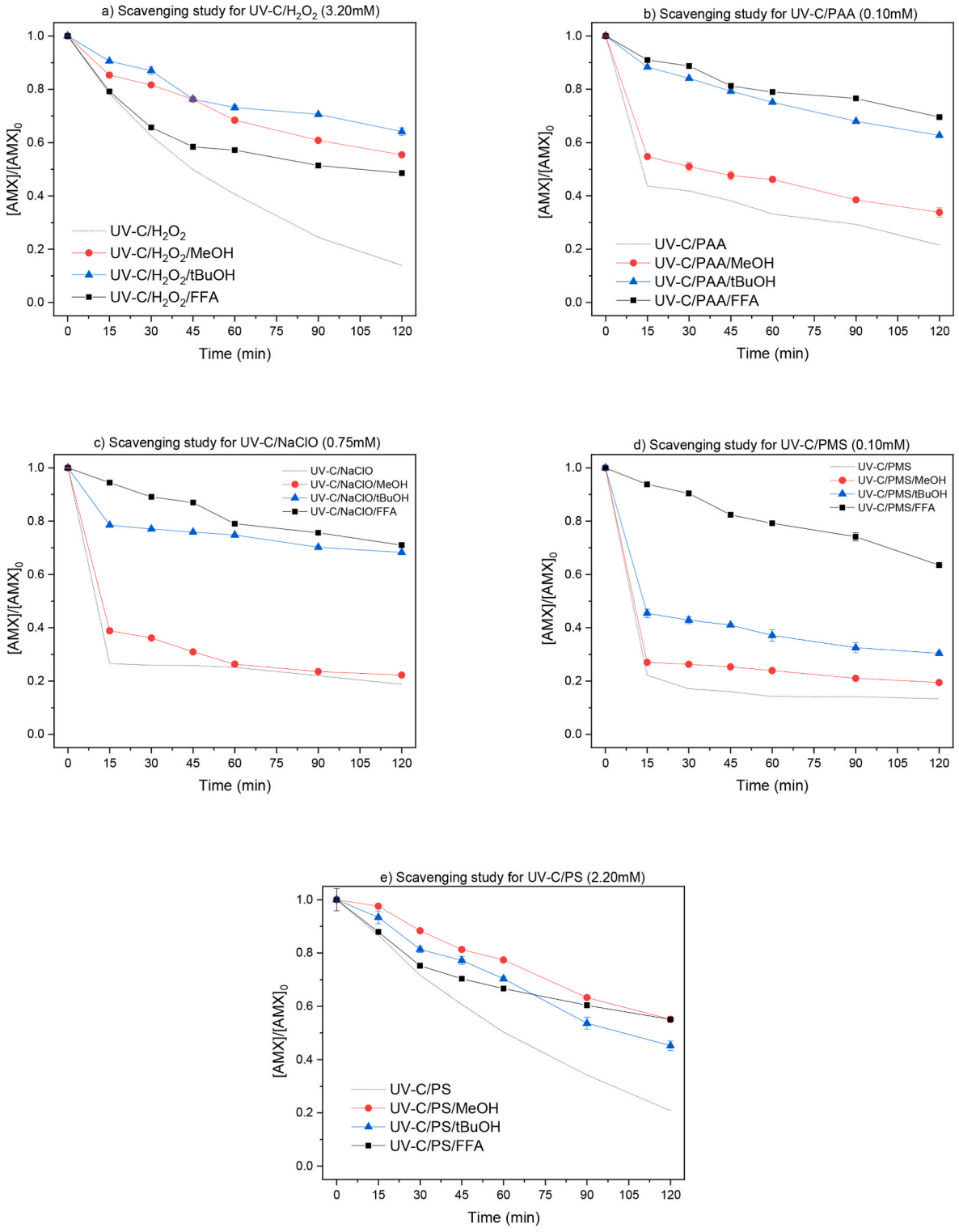

Fig. 5. Comparison of the scavenging effect of MeOH, tBuOH and FFA in UV-C/H_2O_2 (a), UV-C/PAA (b), UV-C/NaClO (c), UV-C/PMS (d) and UV-C/PS (e) optimized systems.

alkaline reagent, is responsible for the rise of the pH in its respective treatment. Notable pH decreases are shown for the UV-C/PAA and UV-C/PMS, which are in concordance with their acidic nature. Mild changes in the pH are observed for the UV-C/H_2O_2 and UV-C/PS treatments, which are accountable to the acidic nature of their respective oxidants.

3.7. Reactive species scavenging study

For the assessment of the main reactive species involved in the degradation process, a scavenging study is performed. The optimized treatments are assessed following the same sampling pattern, sample treatment and chromatographic measurement, in presence of radical scavengers. The evolution of AMX concentration with respect to time is presented in Fig. 5.

The scavenging study shows that the contribution of the considered ROS is different for each of the studied treatments. FFA appears to be the most effective scavenger for UV-C/PAA, NaClO, and PMS, which can be an indicative of the combined oxidative contribution of $^1O_2$, $SO_4^{\bullet-}$, and $HO^{\bullet}$ in these systems. The very similar scavenging effect of tBuOH and FFA in UV-C/PAA, NaClO and PS could be an indicative of the weak role role of $^1O_2$ on AMX degradation for these processes. Other studies report the sensitization of $^1O_2$ by peracetic acid radicals and chlorinated species in presence of UV radiation (Khajouei et al., 2022).

For the UV-C/H_2O_2 process, the exhibited degradation performance in presence of the three scavengers is similar, which is consistent with the absence of sulfate radicals participating in the reactivity.

Residual MeOH scavenging is observed for the UV-C/NaClO, as opposed to the strong scavenging effect of tBuOH and FFA. This result suggests a strong contribution of the $HO^{\bullet}$ radical in this system, and a negligible weight of the sulfate radical to AMX degradation. A similar effect is observed in the case of the UV-C/PAA system, which is consistent with the chemical structure of the oxidant and revealing that the contribution of S-based oxidants does not intervene in AMX degradation.

UV-C/PMS and UV-C/PS treatments present different behaviours in terms of antibiotic degradation. FFA has a greater scavenging effect on the antibiotic degradation for the case of PMS, while MeOH produces a higher scavenging effect for the UV-C/PS process. The dominant role of the sulfate radical in the UV-C/PS process is consistent with the work carried out by Yu et al., where $SO_4^{\bullet-}$ radicals are the radical species in degradation (Yu et al., 2024). A low scavenging effect of MeOH for PMS systems was also observed in the work of Wu et al., when exploring the role of the radical oxidants in the degradation of antibiotics. The significant role of $^1O_2$ in the PMS system may have its root in secondary reactions involving SO_5^{2-} and other PMS-derived intermediates.

Despite the results obtained using scavengers, advanced detection techniques such as electron paramagnetic resonance spectroscopy (EPR) should be applied to identify minor ROS contributions and validate the mechanistic hypotheses proposed in this work.

3.8. Experiments in real aquaculture water samples

After optimization of the optimal dosages of oxidant and radiation, the experiments have been conducted on a real aquaculture water matrix, from the aquaculture facility in the Community of Madrid, Spain. The abatement of AMX over time is presented on Fig. 6.

The carried-out experiments show that the degradation of the antibiotic in the real water matrix is much faster than in SAW, as all the treatments achieve the target 80 % degradation threshold in a maximum time of 45 min. A plausible explanation could be the difference between the organic matter concentration between both matrices (56.68 mg_{TOC}/L in SAW vs 2.25 mg_{TOC}/L in real water matrix). Organic matter can act as a competitor for ROS (Luo et al., 2024; Yang et al., 2022), due to its reducing nature, diminishing the concentration of radical species available for antibiotic degradation. The role of other water parameters, such as the ionic composition of each matrix, appears to be secondary.

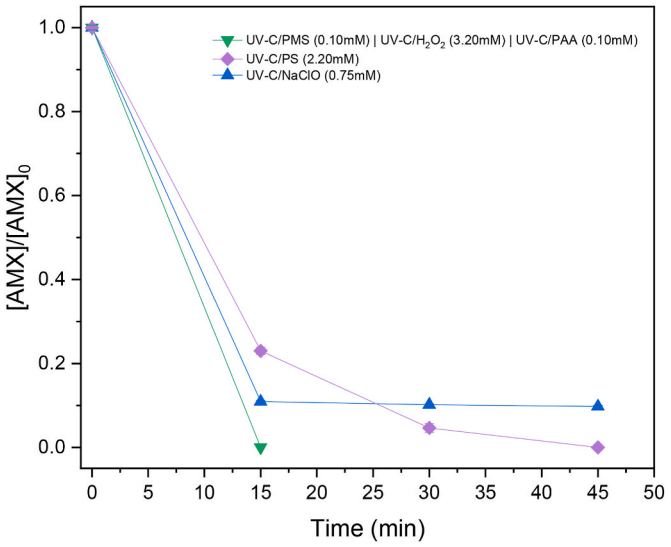

Fig. 6. Removal of AMX in presence of optimal dosages of UV-C and Oxidant in real aquaculture water.

4. Conclusions

A set of experimental conditions (oxidant and radiation dosages) for a batch photochemical system treating simulated aquaculture water has been optimized to achieve 80 % AMX degradation within 2 h. While direct photolysis of the antibiotic does not ensure effective degradation, the combined effect of different oxidants and radiation ensures successful antibiotic removal. All oxidants enhance the degradation process, while synergistic effects are more notable for UV-C/H_2O_2 and UV-C/PS systems.

The assessment of inorganic (ClO_2^-, ClO_3^-, and BrO_3^-) and organic ($CHCl_3$) DBP generation shows promising results. UV-C/H_2O_2, UV-C/PAA, and UV-C/PS treatments produce DBP levels below the analytical detection limits, UV-C/PMS yields only traceable chlorate, while UV-C/NaClO treatment shows less favorable results. The modification of the matrix's chemical composition is minimal for all oxidants, except for the increased production of acetate with UV-C/PAA.

All treatments ensure *E. faecalis* disinfection in less than 5 min, with no microbial regrowth observed after 2 weeks of storage.

The preliminary scavenging study indicates that the reactivity of the studied oxidants varies, consistent with their different chemical structures and behaviors. $HO^{\bullet}$ radicals are the primary ROS responsible for AMX degradation across all treatments, except for UV-C/PS, where $SO_4^{\bullet-}$ plays a larger role.

Future studies evaluating the effectiveness of UV-C/oxidant treatments in real matrices should be conducted as the first step towards scaling up the process. The next step will involve applying continuous, pilot-scale reactors for full technological development and eventual use in real industrial aquaculture facilities.

CRediT authorship contribution statement

Pablo Santiago-Espiñeira: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Patricia García-Muñoz:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition, Conceptualization. **Paula Campayo-Navarro:** Writing – original draft, Investigation, Data curation. **Jorge Rodríguez-Chueca:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition, 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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jenvman.2025.125983.

Data availability

Data will be made available on request.

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