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Application of Ozone-based oxidative processes (O_3, O_3/H_2O_2 & O_3/UV) in the removal of antibiotics

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ABSTRACT

Currently, the presence of organic pollutants in water bodies is a significant concern. This type of contamination is generated by various industrial, agricultural, and urban activities, and it has been linked to adverse effects, including endocrine disruption in fish and mammals. In addition, there is no extensive regulation that controls the introduction of many chemical substances into the water, implying that it is necessary to implement new control measures.

Antibiotics, including **azithromycin (AZT)**, **cephalexin (CPX)**, and **doxycycline (DOX)** are drugs used against pathogenic microorganisms in animals and humans. However, because of their incomplete absorption during their metabolization, together with their extensive and continuous use, they have mainly been released into the environment, resulting in their presence in a variety of environmental matrices such as surface waters, sediments, and soils. In particular, AZT, CPX, and DOX are some of the most widely consumed antibiotics worldwide, and their presence in different water bodies has been reported. When antibiotics enter bodies of natural water, they can select for a range of microorganisms and induce the production of resistant bacterial strains, which, when in contact with living beings, can cause diseases that are not treatable with conventional antibiotics, thereby diminishing the ability to stop epidemics.

For its part, **advanced oxidation technologies (AOTs)** are commonly applied to reduce the concentration of different toxic substances that cannot be eliminated using conventional water treatment processes, due to its demonstrated capacity to remove several biological and chemical pollutants. AOTs are characterized by the generation of the hydroxyl radicals (HO•), which can remove a high number of organic pollutants present in different water matrices.

Ozonation is a technology widely used in the treatment of drinking water. During ozonation, pollutants can be oxidized by ozone (O_3) and/or HO• formed from the natural decomposition of O_3 in water. Besides, the removal of contaminants could be enhanced by activating O_3 with other agents, such as hydrogen peroxide (H_2O_2), UV radiation, and ultrasound.

This study presents the main results obtained after evaluating the use of O_3, O_3/H_2O_2, O_3/UV, and O_3/H_2O_2/UV in the removal of a mixture of AZT, CPX, and DOX in both deionized water and hospital wastewater. The results indicated that O_3 is the primary agent responsible for removing contaminants. However, the O_3/H_2O_2/UV treatment was the one that allowed a higher mineralization of the organic matter. Additionally, the findings indicated that the presence of ions, turbidity, and organic matter in the wastewater could limit the removal of the antibiotics.

This research also involved optimizing certain reaction conditions, analyzing the extent of antibiotics elimination and the reduction of the organic matter present in the treated samples, monitoring the organic carbon, and considering the reaction kinetics.

Finally, the project results promote the development of techniques that favor the conservation of water resources and the minimization of pollution. They also contribute to strengthening national scientific capacity and human capital formation.

Keywords: Advanced oxidation technologies; antibiotics; azithromycin; cephalexin; doxycycline; ozone; and wastewater treatment.

1. PROBLEM STATEMENT & STATE OF ART

Water is an economic and ecological resource of vital importance, and the most essential component of the Earth. Unfortunately, its quality has been negatively affected due to rapid population growth. Some of the significant sources of pollution in aquatic systems are wastewater emissions that contain a considerable number of toxic substances. In this way, a high quantity of chemical and biological compounds has been classified as potentially dangerous by different international organizations, including the European Commission (EU) and the United States Environmental Protection Agency (EPA) [1,2]. In recent decades, attention to water pollution research has expanded from conventional pollutants to newly emerging contaminants (ECs), whose presence in environmental matrices is not necessarily new. Still, the consequences of this fact have not been fully clarified. These kinds of contaminants have been found in wastewater and surface water at concentrations that may be smaller than those of classical persistent and/or priority substances [3]. In addition, the development of analytical methods for its detection and quantification is limited [4,5].

A wide variety of products for daily use, with both domestic and industrial applications, such as pharmaceutical products, personal care products, antibiotics, surfactants, industrial additives, plasticizers, pesticides, and many other chemical compounds that can affect the endocrine functions of living beings, have been catalogued as ECs. Also, these substances could be bioaccumulated and are challenging to remove through conventional water treatment processes [1,6,7].

Antibiotics represent a class of organic substances produced through the secondary metabolism of living microorganisms or synthesized, used to kill, or inhibit the growth of pathogenic microorganisms. These compounds, widely used in medicine and agriculture, have benefited public health and agricultural productivity for a long time [8,9]. While antibiotics are among the most essential tools in medicine, their misuse and overuse can stimulate the evolution of microorganisms and the spread of bacterial resistance, which is recognized as one of the principal threats to public health in the 21st century [10–12]. Besides, in Latin America, due to the lack of regulatory laws (free sale), it is common for its inhabitants to obtain antibiotics without a medical prescription, which together with self-medication, and the lack of mechanisms of inspection, surveillance, and control constitute a serious problem that facilitates the appearance of bacterial resistance [13]**.** After being ingested and metabolized, pharmaceuticals, either in their original form or as metabolite compounds, are excreted through urine and feces and introduced to wastewater treatment plants (WWTPs) or are directly released into septic tanks [14]. Due to their antibacterial nature, they are not entirely removed in the biological wastewater treatment plants and reach the aquatic environment. In this way, effluents from wastewater treatment plants are one of the most important sources of pharmaceuticals, including antibiotics, to the environment [15].

Macrolides, β-lactams, and tetracycline antibiotics are highly consumed worldwide, which has led to their introduction into different bodies of water, including the influents and effluents of WWTPs, surface waters, and even drinking water [16–21]. In addition, some studies have indicated that β-lactam (penicillins and cephalosporins), tetracyclines, and macrolides are, respectively, the first, third, and fourth most used antibiotic families in Colombia. **Cephalexin (CPX)**, **doxycycline (DOX)**, and **azithromycin (AZT)** rank first inside each

group [13,22] and have been detected in WWTPs' influents and effluents, and hospital wastewater (range between 3.88 and 27.9 µg/L) in cities such as Bogotá, Medellín, and Cali [22].

A particular situation associated with AZT is that its use increased considerably during the COVID-19 pandemic because this antibiotic was considered an alternative for treating the disease. Many people worldwide began to consume it without a prescription to protect themselves. In this sense, it is to be expected that the concentrations of AZT in bodies of water have increased exponentially [23–27]. Colombia was one of the countries with the most confirmed cases of COVID-19 globally [28].

Different techniques have been evaluated for the potential treatment of water containing antibiotics. Technologies such as chemical oxidation, ionic treatment, photodegradation, adsorption, and electrochemical processes have been studied. However, in some cases, these methods could present some limitations, including a low extent of elimination of the pollutants, and the potential production of more toxic intermediate substances [29–33].

Advanced oxidation technologies (AOT) constitute one of the most used technological resources in the treatment of water contaminated with organic products that are not treatable by conventional techniques due to its high chemical stability and low biodegradability [34]. AOTs are designed to enhance the degradation and mineralization (transformation of the organic compounds into CO_2, H_2O, and inorganic ions) of micropollutants, or to transform them into less toxic compounds [7]. Likewise, AOTs have also been used in processes of disinfection, discoloration, and deodorization of effluents [35,36]. AOTs' mechanisms of action include the production of the hydroxyl free radical (HO•), a potent oxidant agent ($E°$=2.8 V) capable of oxidizing a wide range of organic compounds [37].

Ozonation is a technology widely used in drinking water treatment. During ozonation, pollutants can be oxidized by ozone (O_3) and/or HO• radicals formed from the natural decomposition of O_3 in water. O_3 oxidation is highly selective for compounds with electron-rich functional groups such as olefins, aromatics, and tertiary amines. In contrast, the HO• radical is a less selective agent that can oxidize almost any organic compound. Therefore, to improve the removal of refractory contaminants, a variety of advanced O_3-based oxidation processes have been developed, in which the combination of conventional ozonation with other agents such as ultraviolet light (UV) and hydrogen peroxide (H_2O_2) could improve the production of HO• and the elimination of pollutants [38,39].

Considering the above, this work presents the main results of the assessment of the potential application of O_3, O_3/H_2O_2, O_3/UV, and O_3/H_2O_2/UV treatments in the removal of AZT, CPX, and DOX from aqueous solutions. The effects of the initial ozone concentration, the pH of the solution, the reaction time, and the type of aqueous matrix (experiments were using deionized and hospital water) were evaluated.

From an environmental perspective, this study enabled the evaluation of new alternatives for wastewater remediation and the conservation of water resources and ecosystems. The project execution aimed to remove

organic compounds whose presence in bodies of water could compromise not only the quality of the aqueous matrices but also human health (bacterial resistance). The project results contribute to developing methodologies aimed at detecting, monitoring, and eliminating organic contaminants in water, ultimately leading to the conservation of natural resources and their associated ecosystems.

Implementing the results obtained in this research will contribute to the objectives set by the World Health Organization regarding reducing the probability of disseminating resistant microorganisms, their negative impact on patients, and the associated healthcare costs.

2. THEORETICAL FRAMEWORK

Emerging contaminants refer to chemicals and microorganisms detected in the environment, which may pose risks to human health and ecosystems. Among these, antibiotics are of particular concern due to their potential to generate bacterial resistance. The widespread use of antibiotics in human and veterinary medicine has led to their release into the environment through wastewater. These compounds are not fully eliminated in conventional wastewater treatment plants, and therefore end up reaching rivers, lakes, and aquifers [40,41].

The main sources of this contamination include human and animal excretion, discharge of wastewater from hospitals and pharmaceutical plants, and the use of organic fertilizers in agriculture. Antibiotics in water contribute to the proliferation of antibiotic-resistant bacteria, posing a serious public health problem. Antimicrobial resistance makes it difficult to treat infections, increasing the risk of serious illness and even death. In addition, antibiotics can have toxic effects on aquatic organisms, disrupting the balance of ecosystems [40,41].

The presence of antibiotics in wastewater is a global phenomenon, with significant concentrations found in many countries. Research on this issue is ongoing, and new technologies are being developed to eliminate antibiotics in WWTPs.

2.1. Antibiotics

Antibiotics represent a class of organic substances that inhibit the growth of pathogenic microorganisms, whose use in animal and human medicine has benefited public health and the eradication of many diseases. The human body does not fully metabolize a significant portion of ingested antibiotics. These unmetabolized compounds are then excreted in urine and feces, entering sewage systems. Antibiotics are widely used in livestock and aquaculture to prevent and treat diseases and to promote growth. Animal waste containing antibiotics can contaminate soil and water through runoff. Wastewater from pharmaceutical manufacturing plants can contain high concentrations of antibiotics if not adequately treated. Hospitals are a significant source of antibiotic-containing wastewater [42,43].

Antibiotics in the environment create a selection that promotes the development and spread of antibiotic-resistant bacteria. This poses a serious threat to public health, as antibiotic-resistant infections are more difficult and expensive to treat. These substances can have toxic effects on aquatic organisms, such as algae, bacteria, and fish, disrupting the balance of aquatic ecosystems, and could contaminate surface water, groundwater, and soil, potentially posing risks to human health and the environment [40,41].

Detecting AZT, CPX, and DOX antibiotics in wastewater streams represents a growing environmental and public health concern. These compounds, widely prescribed in human and veterinary medicine, find their way into sewage systems through various pathways, including human and animal excretion and discharges from hospitals and pharmaceutical manufacturing facilities [40,41].

Macrolides were introduced into medicinal use almost seventy years ago, following the discovery of erythromycin in 1949. They are a group of effective and safe antibiotics used to treat infectious diseases such as bacterial pneumonia and gonorrhea. However, its wide use in hospitals and communities has contributed to the development of bacterial resistance, mainly in gram-positive cocci (*Staphylococcus*, *Streptococcus,* and *Pneumococcus*) and in gram-negative bacteria (Enterobacteria, *Haemophilus influenzae*) [40]. Also, recent studies indicate that the concentration of this type of antibiotics in different bodies of water can range between 1.0 and 500.0 ng L^{-1} [40,41].

Azithromycin (AZT, $C_{38}H_{72}N_2O_{12}$, Figure 1a) is a macrolide antibiotic widely used to treat various bacterial infections. Its spectrum of action ranges from respiratory infections like pneumonia and bronchitis to skin and sexually transmitted infections like chlamydia. One of its advantages is its convenient dosing regimen, often administered in short-course doses. However, like all antibiotics, its indiscriminate use contributes to the rise of bacterial resistance. While subject to some degree of metabolism, this macrolide antibiotic exhibits persistent characteristics within wastewater environments, thereby contributing to the proliferation of antibiotic-resistant bacteria [16,19].

β-lactams are a group of antibiotics of natural or semi-synthetic origin that are characterized by having a β-lactam ring in their structure. They constitute the largest family of antimicrobials and are the most used in clinical practice. These antibiotics are compounds with slow bactericidal action, that present low toxicity and have a wide therapeutic margin of application. However, β-lactams presence in water bodies can harm the microbial community, inducing the appearance of resistant bacteria [16,19].

Cephalexin (CPX, $C_{16}H_{17}N_3O_4S$, Figure 1b) belongs to the β-lactam family, specifically the first-generation cephalosporins. It is effective against a variety of gram-positive and some gram-negative bacteria. It is commonly prescribed for skin, urinary tract, and respiratory infections. β-lactams work by inhibiting bacterial cell wall synthesis, leading to bacterial death. As with other antibiotics, it is crucial to complete the prescribed course of treatment to prevent bacterial resistance. CPX in wastewater potentially harms aquatic microorganisms and significantly elevates the risk of widespread antibiotic resistance [16,19].

Tetracycline antibiotics constitute a group of natural and semi-synthetic products derived from different species of *Streptomyces spp*. They act by inhibiting the synthesis of bacterial proteins, and are bacteriostatic with activity against a wide variety of microorganisms [44].

Doxycycline (DOX, $C_{22}H_{24}N_2O_8$, Figure 1c) is a broad-spectrum antibiotic in the tetracycline family. It treats various infections, including respiratory, urinary tract, skin, and sexually transmitted infections. It is also used in malaria prevention and acne treatment. DOX works by inhibiting bacterial protein synthesis. It is important to note that tetracyclines can cause photosensitivity. Thus, prolonged sun exposure is not recommended during treatment. This tetracycline antibiotic has also been consistently detected in wastewater samples, raising substantial concerns regarding its environmental impact and long-term ecological consequences [44].

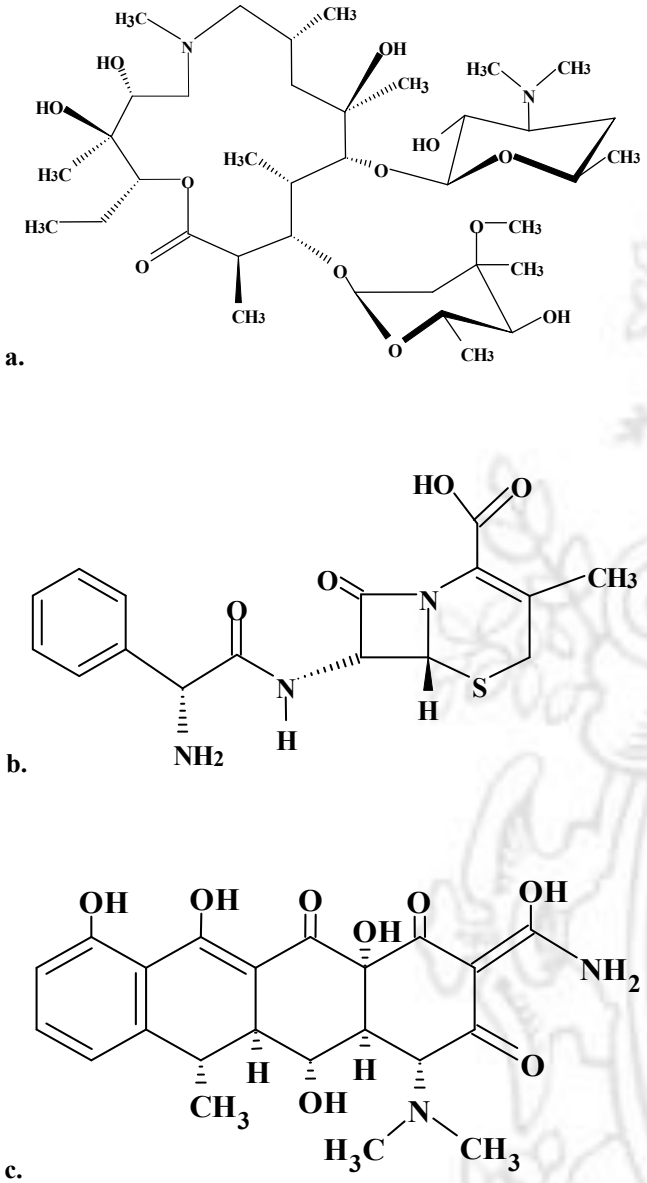


Figure 1. Molecular structure of antibiotics a. azithromycin, b. cephalexin and c. doxycycline.

Conventional wastewater treatment processes, encompassing sedimentation and biological digestion, demonstrate limited efficacy in removing antibiotic compounds. Consequently, implementing advanced treatment technologies, such as advanced oxidation processes and membrane filtration, is indispensable for the effective elimination of these contaminants from wastewater effluents. Adopting advanced treatment methodologies is of great importance in safeguarding public health and environmental integrity. The presence of antibiotics in wastewater significantly contributes to the dissemination of antibiotic resistance, thereby complicating the treatment of infections and augmenting the incidence of severe illnesses [44]. Furthermore, these compounds may exert toxic effects on aquatic organisms, disrupting ecosystem equilibrium. Therefore, it is imperative to allocate resources towards research and development initiatives aimed at advancing wastewater treatment technologies capable of efficiently removing antibiotics and other emerging contaminants. Additionally, enforcing stringent regulatory measures about the discharge of antibiotics into the environment is essential. Monitoring antibiotic levels in the environment and implementing regulations to control their discharge are crucial for protecting public

health and the environment [40,41]. **Continued research is needed to better understand the fate and transport of antibiotics in the environment and to develop more effective strategies for their removal.**

2.2. Advanced Oxidation Technologies

Advanced oxidation technologies (AOTs) are a set of chemical processes that can remove recalcitrant organic pollutants in wastewater by oxidation reactions involving a powerful, non-selective hydroxyl radical (HO•), which can eliminate organic substances resistant to conventional treatments generating simple and non-toxic inorganic compounds, which are essential for effective wastewater treatment enhancing the biodegradability of wastewater [39]. Under ideal conditions, AOTs can convert contaminants into stable byproducts like carbon dioxide (CO_2) and water (H_2O) [39].

In recent years, several AOTs have been successfully applied to remove different contaminants of emerging concern, including antibiotics, from aqueous solutions. Among these technologies, a special emphasis has been given to processes such as ozonation, the Fenton and photo-Fenton treatments, the heterogeneous photocatalysis, sonochemical and electrochemical processes, and the combination of hydrogen peroxide and ultraviolet light (UV/H_2O_2) [39]. However, one of the limitations of the application of AOTs on a pilot or real scale are the costs associated with the use of catalysts, the energy requirements, and problems related to mass transfer under heterogeneous reaction conditions [39,45].

AOTs have innovative methods for treating antibiotics, with fast reaction speed, high efficiency, and simple reaction mechanisms [39].

2.2.1. Ozonation

The use of ozone (O_3) for water treatment dates from the early 20th century, with the implementation of O_3 in a drinking water facility for disinfection purposes in 1906 (Nice, France). Since then, ozone has been used in effluents purification for multiple purposes, including disinfection, water taste and odor minimization, the reduction of the formation of disinfection byproducts, and the oxidation of contaminants. Thus, during the last decades, the popularity of ozone for wastewater treatment and/or reuse, as well as other industrial applications, has increased due to its ability to provide pathogen inactivation and the oxidation of recalcitrant organic pollutants. In addition, it offers the advantage that it is a technology used in many WWTPs and purification plants, where O_3 is generated *in situ* [39].

The benefits of ozonation in WWTPs include reducing sludge and removing recalcitrant organic contaminants from wastewater. It is seen that ozonation leads to sludge solubilization, reducing overall biomass yield. But, O_3 mass transfer is influenced by many factors, which can be divided into hydrodynamic and physicochemical effects. O_3 itself is unstable and can quickly decompose into molecular oxygen, leading to a low utilization rate, and does not cause a complete oxidation of some refractory pollutants [39]

In aqueous systems, ozone can eliminate organic pollutants using two mechanisms: *i.* direct electrophilic attack by molecular ozone, and *ii.* indirect attack by HO• radicals produced through the O_3 decomposition process

[39]. Equations 1 to 5 present the mechanism of HO• radicals formation from the O_3 decomposition in water [39,46].

$$OH^- + O_3 \rightarrow HO_4^- \quad (1)$$

$$HO_4^- \Leftrightarrow HO_2\bullet + O_2\bullet^- \quad (2)$$

$$O_2\bullet^- + O_3 \rightarrow O_2 + O_3\bullet^- \quad (3)$$

$$O_3\bullet^- \rightarrow O_2 + O\bullet^- \quad (4)$$

$$O\bullet^- + H_2O \rightarrow HO\bullet + OH^- \quad (5)$$

Despite the above, one limitation of the HO• generation from ozone decomposition is the need for a basic solution pH, implying that including O_3 in treatment systems is not enough in many cases. In this way, the O_3/H_2O_2 (peroxone) and O_3/UV processes have been used as alternatives for the removal of organic contaminants [39,46,47].

The O_3/H_2O_2 process involves a radical chain mechanism based on the ozone decomposition initiated by the hydroperoxide anion (HOO^-). The synergistic effect of O_3 and H_2O_2 promotes the production of HO• radicals (equation 6). However, excess H_2O_2 leads to HO• scavenging and the formation of HOO^-. An optimal peroxide concentration is one that favors the reaction with O_3 and not the consumption of HO• radicals [48]. This combination produces higher conversion yields than ozonation in those cases in which the direct ozone-pollutant reaction follows a slow kinetic regime due to gas–liquid matter transfer problems [48].

$$2H_2O_2 + 2O_3 \rightarrow H_2O + 3O_2 + HO_2\bullet + HO\bullet \quad (6)$$

In the case of the O_3/UV treatment, UV radiation has enough energy to break the ozone molecule (optimum wavelength ~260 nm) and promote the generation of radicals as presented in equation 7 (aqueous systems) [48]. UV radiation can excite the organic molecules of the pollutant, increasing their susceptibility towards an attack by the hydroxyl radicals [48].

$$O_3 + H_2O + h\nu \rightarrow O_2 + 2HO\bullet \quad (7)$$

The O_3, O_3/H_2O_2, and O_3/UV processes have been used to treat water containing pharmaceutical compounds, personal care products, and microorganisms; its use had been supported by the fact that the decomposition of ozone is speeded up by the simultaneous presence of hydrogen peroxide and UV irradiation, thus yielding an increased rate of generation of HO• radicals [48].

In general, it has been reported that the pH of the solution, the O_3 and/or H_2O_2 dose, and the presence of external agents (matrix effect) can inhibit or accelerate the elimination of the organic contaminants. For this reason, when evaluating the potential application of this type of technologies in the removal of a particular contaminant, it is important to establish the effect that each of these parameters has, as well as the kind of by-products generated, and the toxicity of the solution [38,39,46].

Table 1 shows reports on using O_3-based processes to remove AZT, CPX, and DOX in different matrices. In this aspect, it is important to highlight that in most of the studied cases, each antibiotic was eliminated individually, not in a mixture, as in this project, and that the extent of pollutants removal depends on the reaction conditions and treated matrix.

Table 1. AZT, CPX, and DOX removal ozone-based process.

Process	Antibiotic(s)	Reaction conditions	Efficiency	Reference
O_3	DOX	pH: 5.7 - 5.9, O_3: 4.6 - 6.29 mg/L, DOX: 50.0 mg/L, time: 60 min, matrix: deionized water.	> 99.0%	[38,39,46]
	AZT	pH: 7.2 - 7.8, O_3: 0.125 - 0.250 $gO_3/gDOC$, AZT: 0.184 - 0.358 µg/L, time: 40 min, matrix: wastewater.	> 99.0%	[38,39,46]
	AZT / DOX	O_3: 27.5 g h^{-1}, AZT / DOX: 1.1 / 1.4 µg/L, time: 10 min, matrix: hospital wastewater.	> 99.0%	[38,39,46]
	DOX	pH: 7.0 - 11.0, O_3: 7.0 mg/L, DOX: 500.0 µg/L, time: 25 min, matrix: ultrapure water.	> 99.0%	[38,39,46]
	AZT	O_3: 2.3-9 mg/L, AZT: 2.27 µg/L, time: 10 - 20 min, matrix: hospital wastewater.	~74.0%	[38,39,46]
O_3/H_2O_2	AZT	O_3: 450.0 mg/L, H_2O_2: 200.0 mg/L, AZT: 1.1 µg/L, time: 15 min, matrix: hospital wastewater.	> 99.0%	
O_3/UV	DOX	pH: 6.8, O_3: 0.15 - 0.75 mg/L, DOX: 1.0 mg/L, time: 2 - 10 min, matrix: distilled water, UV-C Lamp (350 nm).	~95.0%	[38,39,46]
	AZT	pH: 6.5 - 6.8, O_3: 1.0 – 6.0 mg/L, AZT: 0.06 ng/L, time: 5 - 10 min, matrix: wastewater, UV-C Lamp (254 nm).	~95.0%	[38,39,46]
	CPX	pH: 8.5, O_3: 0.5 g/h, CPX: 12.5 mg/L, time: 45 min, matrix: ultrapure water, UV-C Lamp (254 nm).	62.53%	[38,39,46]

3. RESEARCH QUESTION & OBJECTIVES

3.1. Research question:

How efficient is the use of the ozone-based technologies O_3, O_3/H_2O_2 & O_3/UV in the removal, from aqueous solutions, of the antibiotics azithromycin, cephalexin, and doxycycline?

3.2. Objectives

3.2.1. General Objective

To evaluate the potential application of O_3, O_3/H_2O_2 & O_3/UV in the removal, from aqueous solutions, of azithromycin, cephalexin, and doxycycline.

3.2.2. Specific objectives

- To assess the effects of operational parameters such as pH, reaction time, O_3, H_2O_2, and pollutants concentration, on AZT, CPX, and DOX removal using O_3, O_3/H_2O_2 & O_3/UV.
- To analyze the matrix effect on the AZT, CPX, and DOX elimination using O_3, O_3/H_2O_2 & O_3/UV in deionized and hospital wastewater.
- To determine the reaction kinetics of the AZT, CPX, and DOX removal using O_3, O_3/H_2O_2 & O_3/UV.
- To evaluate the mineralization of samples containing AZT, CPX, and DOX when treated with O_3, O_3/H_2O_2 & O_3/UV.

4. MATERIALS & METHODS

Research was developed considering a quantitative approach. It was necessary to carry out preliminary tests that allowed for establishing the ranges of experimental parameters that favor the removal of the target pollutants. Considering the particularities of each treatment studied, operational parameters were evaluated under three different experimental levels (low, medium, and high). In this way, the effects of the antibiotics' initial concentration, the O_3 initial dose, and the solution pH were assessed. Furthermore, the effect of the H_2O_2 initial concentration was evaluated in the O_3/H_2O_2 and O_3/H_2O_2/UV treatments.

4.1. Chemicals and solvents

A standard solution containing an equal concentration of each pollutant was prepared using analytical grade AZT, CPX, and DOX (purity > 99.9%) and ultra-pure deionized water (18.2 MΩ·cm resistivity). Solutions pH was adjusted using concentrated solutions of sodium hydroxide (NaOH) and hydrochloric acid (HCl) according to the requirements of each experiment. Hydrogen peroxide (H_2O_2, 30.0% v/v) was employed for O_3/H_2O_2, O_3/H_2O_2/UV and H_2O_2/UV tests and controls.

On the other hand, sodium azide (NaN_3), tert-butanol ($C_4H_{10}O$), and isopropanol (C_3H_8O) were used as scavenging agents to determine the role of O_3 and radicals in removing pollutants.

The solvents used in analytical methods were LC-MS-grade acetic acid ($C_2H_4O_2$), acetonitrile (MeCN), and methanol (MeOH).

Sodium thiosulfate ($Na_2S_2O_3$) was employed to stop the reaction during the sampling stage. $Na_2S_2O_3$ has been reported as an adequate O_3 and H_2O_2 quenching agent [49].

4.2. Analytic methods

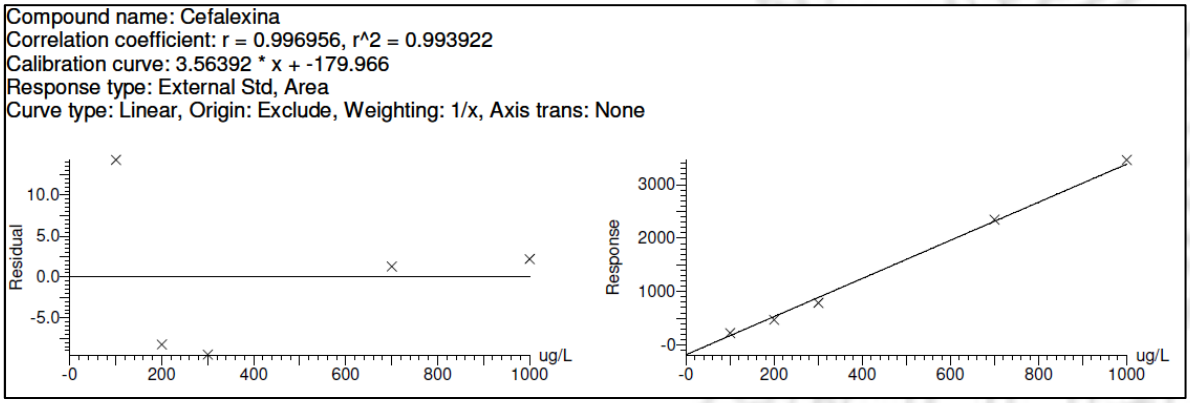
An analytical method was developed to determine simultaneously the AZT, CPX, and DOX concentrations employing liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) with triple quadrupole (QqQ) analyzers using an Acquity UPLC system (Waters Corporation, USA) and an Agilent 6460 triple quadrupole mass spectrometer. A Kinetex C18 2.6 µm 2.1 x 100 mm Synergi HPLC column (Phenomenex, USA) was used for chromatographic separation. The mobile phases were water (0.1% acetic acid) and MeCN:MeOH (1:1) as presented in Table 2. The injection mode was gradient type (30.0 µL). The flow rate was 0.3 mL min^{-1}, the average temperature of the samples was 15.2°C, and the column temperature was 40.0°C. The calibration curve range was between 100.0 and 1000.0 µg L^{-1}, with a correlation coefficient (R^2) greater than 99.9%. The retention times for AZT, CPX, and DOX were 4.45, 4.57, and 4.76 min, respectively.

Chromatographic spectra and calibration curves for pollutants quantification using LC-MS/MS are shown in Figure 2.

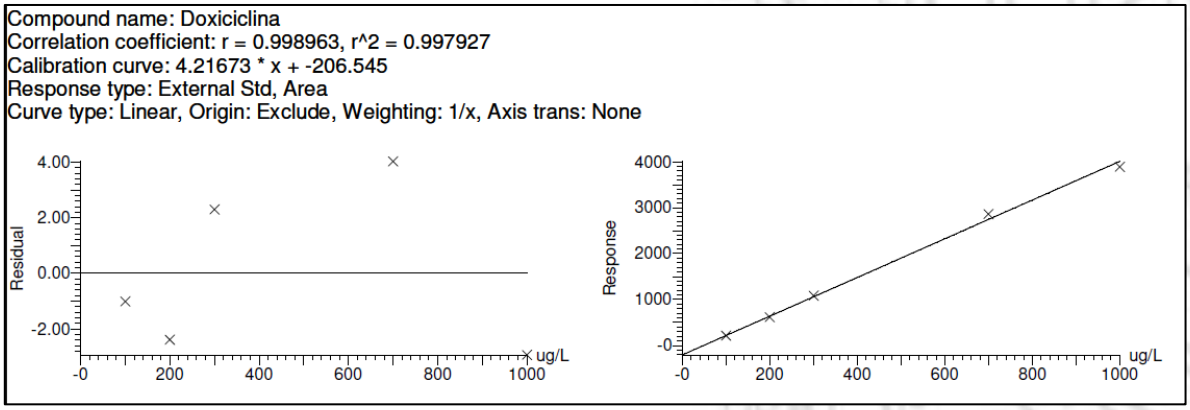
Table 2. Mobile phases proportions during antibiotics AZT, CPX, and DOX quantification using LC-MS/MS.

Time (min)	Mobile phase A: water (0.1% $C_2H_4O_2$) (%)	Mobile phase B: MeCN/MeOH (1:1) (%)
Initial	98.0	2.0
1.0	98.0	2.0
3.0	5.0	95.0
8.0	5.0	95.0
8.1	98.0	2.0
11.0	98.0	2.0

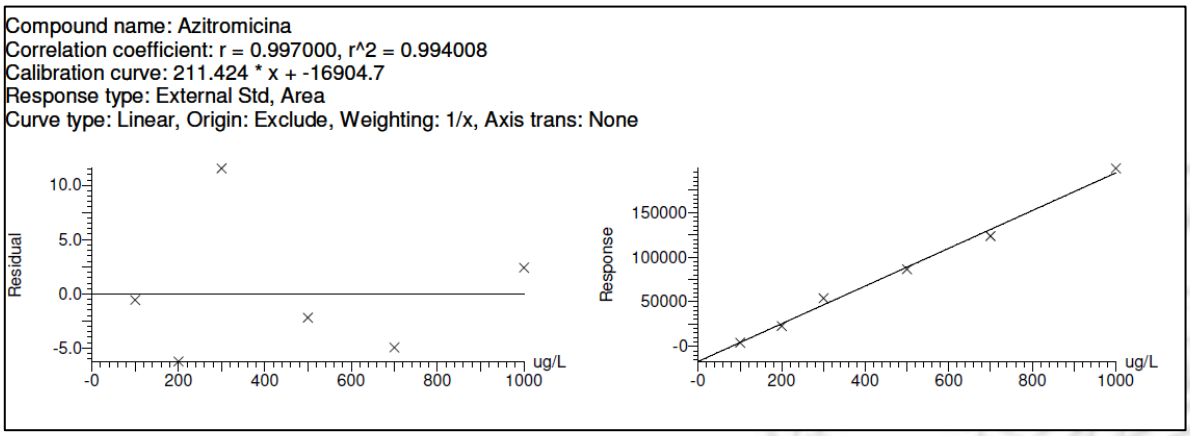
- **Calibration curve for cephalexin detection and quantification**



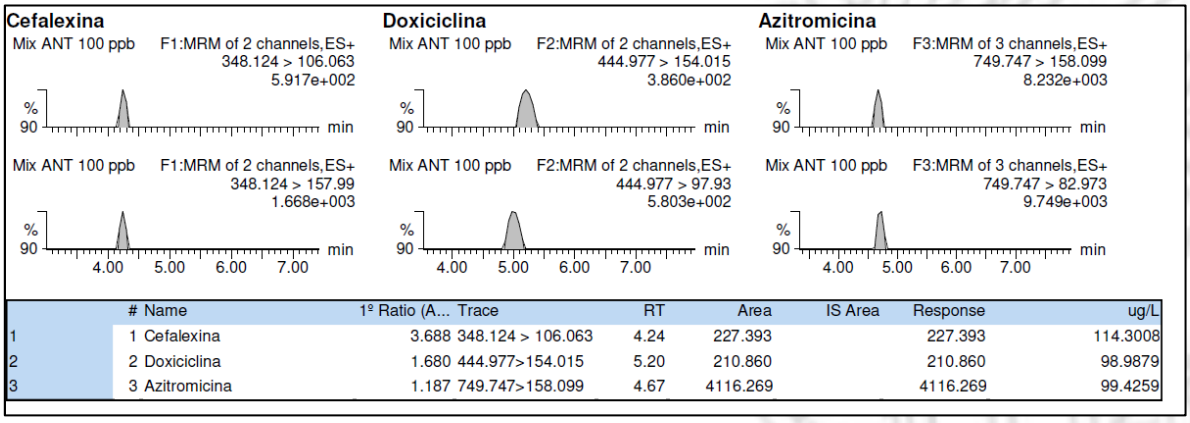
- **Calibration curve for doxycycline detection and quantification**



- **Calibration curve for azithromycin detection and quantification**

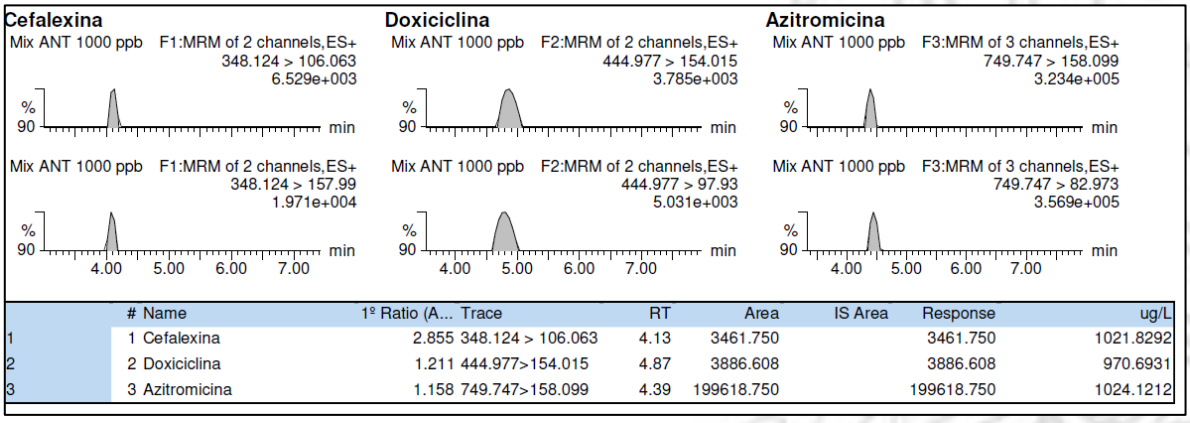


- **Chromatographic spectra for a standard (100 µg/L) of azithromycin, cephalexin, and doxycycline in deionized water**



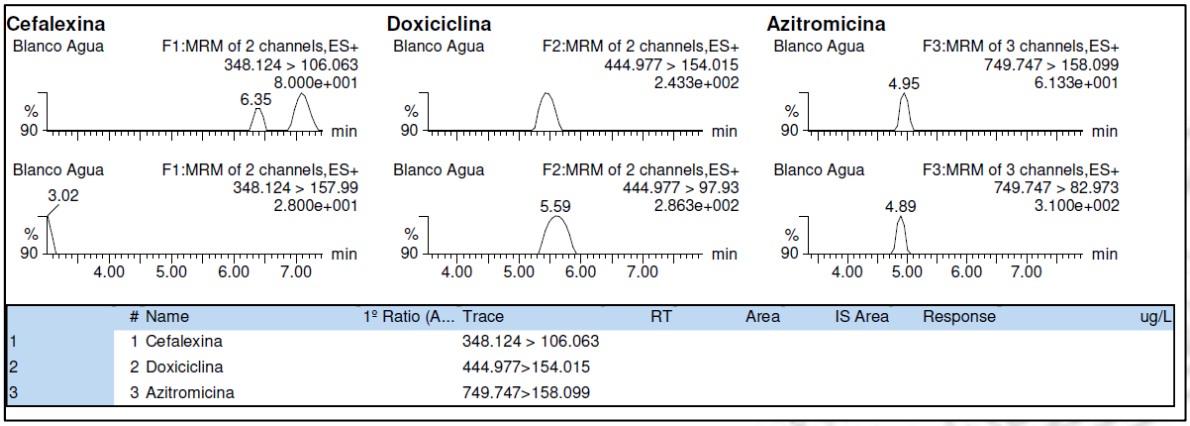
	#	Name	1º Ratio (A...	Trace	RT	Area	IS Area	Response	ug/L
1	1	Cefalexina	3.688	348.124 > 106.063	4.24	227.393		227.393	114.3008
2	2	Doxiciclina	1.680	444.977>154.015	5.20	210.860		210.860	98.9879
3	3	Azitromicina	1.187	749.747>158.099	4.67	4116.269		4116.269	99.4259

- **Chromatographic spectra for a standard (1000 µg/L) of azithromycin, cephalexin, and doxycycline in deionized water**



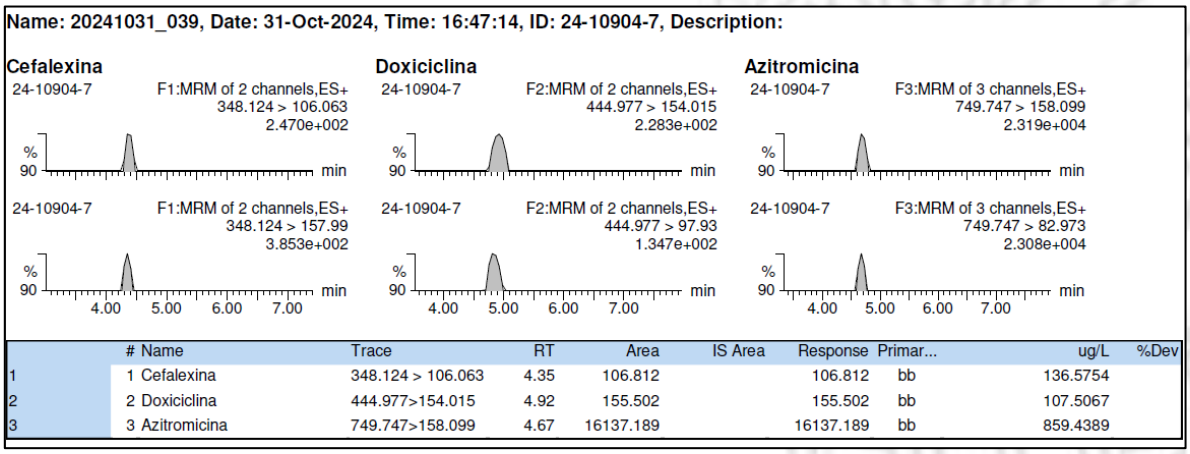
	#	Name	1º Ratio (A...	Trace	RT	Area	IS Area	Response	ug/L
1	1	Cefalexina	2.855	348.124 > 106.063	4.13	3461.750		3461.750	1021.8292
2	2	Doxiciclina	1.211	444.977>154.015	4.87	3886.608		3886.608	970.6931
3	3	Azitromicina	1.158	749.747>158.099	4.39	199618.750		199618.750	1024.1212

- **Chromatographic spectra for deionized water as control for azithromycin, cephalexin, and doxycycline detection and quantification**



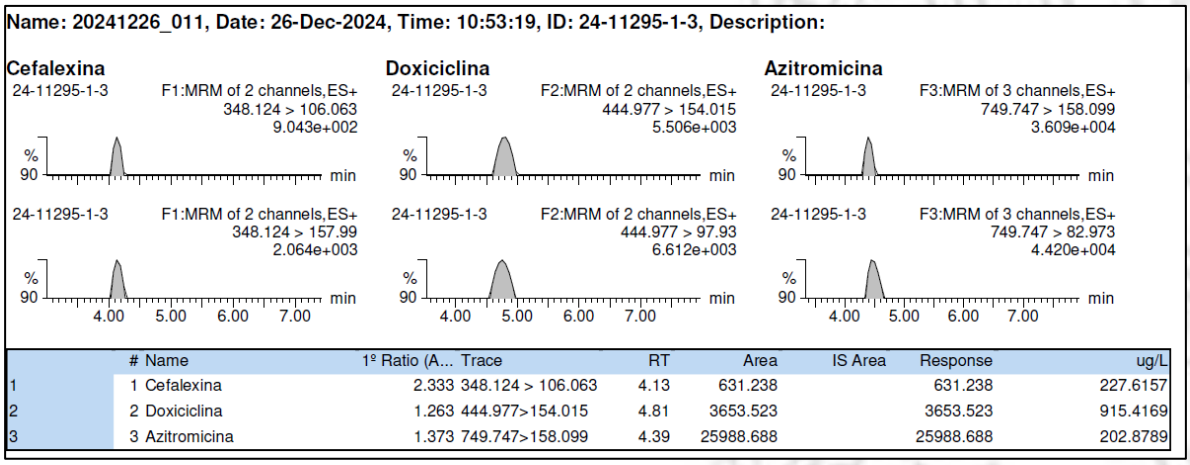
	#	Name	1º Ratio (A...	Trace	RT	Area	IS Area	Response	ug/L
1	1	Cefalexina		348.124 > 106.063					
2	2	Doxiciclina		444.977>154.015					
3	3	Azitromicina		749.747>158.099					

- **Example of a chromatographic spectrum for a sample of deionized water containing azithromycin, cephalexin, and doxycycline**



	#	Name	Trace	RT	Area	IS Area	Response	Primar...	ug/L	%Dev
1	1	Cefalexina	348.124 > 106.063	4.35	106.812		106.812	bb	136.5754	
2	2	Doxiciclina	444.977>154.015	4.92	155.502		155.502	bb	107.5067	
3	3	Azitromicina	749.747>158.099	4.67	16137.189		16137.189	bb	859.4389	

- **Example of a chromatographic spectrum for a sample of hospital wastewater containing azithromycin, cephalexin, and doxycycline**



	#	Name	1º Ratio (A...	Trace	RT	Area	IS Area	Response	ug/L
1	1	Cefalexina	2.333	348.124 > 106.063	4.13	631.238		631.238	227.6157
2	2	Doxiciclina	1.263	444.977>154.015	4.81	3653.523		3653.523	915.4169
3	3	Azitromicina	1.373	749.747>158.099	4.39	25988.688		25988.688	202.8789

Figure 2. Chromatographic spectra and calibration curves for quantifying AZT, CPX, and DOX using LC-MS/MS.

4.3. Ozone-based experiments

Ozone was generated using a domestic system provided by Pinus Longaeva (Shenzhen) Industrial Company (Guangdong, China), which can produce up to 5.0 g/h of O_3. The operation of the device is based on the corona effect using 316L stainless steel electrodes and quartz as the dielectric material. In this research, ambient air was used as an oxygen source.

During the experiments, 250.0 mL of a solution containing an initial concentration of 1.0 mg/L of each contaminant was treated considering different levels of ozone content. The modified parameter was the concentration of O_3 dissolved in water, which was established using the Standard Methods for the Examination of Water and Wastewater SM4500-O3B Ozone by indigo colorimetric method [50], which is a colorimetric technique based on ozone's decolorization of indigo blue. O_3 is a strong oxidant that reacts rapidly with indigo blue, causing it to decolorize. The intensity of the decolorization is proportional to the ozone concentration in the sample.

The residual ozone concentration can be determined by measuring the absorbance of the indigo blue solution before and after the reaction with ozone using a calibration curve or stoichiometric calculations. This method is helpful in controlling the effectiveness of ozonation processes used in water treatment [49].

In a conventional experiment, the solution was prepared considering the required pH, the concentration of the contaminants, and the O_3 dose. 1.0 mL samples were withdrawn at different intervals, and $Na_2S_2O_3$ was added to stop the oxidation reactions. Then, samples were analyzed using LC-MS/MS.

4.4. Anions and mineralization analysis

Mineralization is defined as the complete degradation of a compound to its inorganic constituents, where the organic carbon is oxidized to carbon dioxide. Mineralization is essential for eliminating persistent and toxic organic pollutants that conventional treatments cannot remove. This includes recalcitrant organic compounds, such as pharmaceuticals, pesticides, and industrial chemicals. Oxidation of the organic matter was evaluated by measuring the Total Organic Carbon (TOC) and the anions (nitrates and sulfates) concentration in the treated samples using an APOLLO 9000 TOC Combustion Analyzer (Teledyne Tekmar) and a Dionex Integrion HPIC system (Thermo Scientific), respectively. Standard Methods 526D (High temperature combustion method) and 4110B (Ion chromatography with chemical suppression of effluent conductivity) were also employed [49].

5. RESULTS AND DISCUSSIONS

5.1. Preliminary results

Initially, experiments were carried out to evaluate the removal of AZT, CPX, and DOX using ozone and similar technologies, considering the stoichiometric amount of ozone required to oxidize each contaminant completely. Equations 8, 9, and 10 show that 35.0, 15.7, and 17.7 moles of O_3 are required to oxidize completely one mole of AZT, CPX, and DOX, respectively.

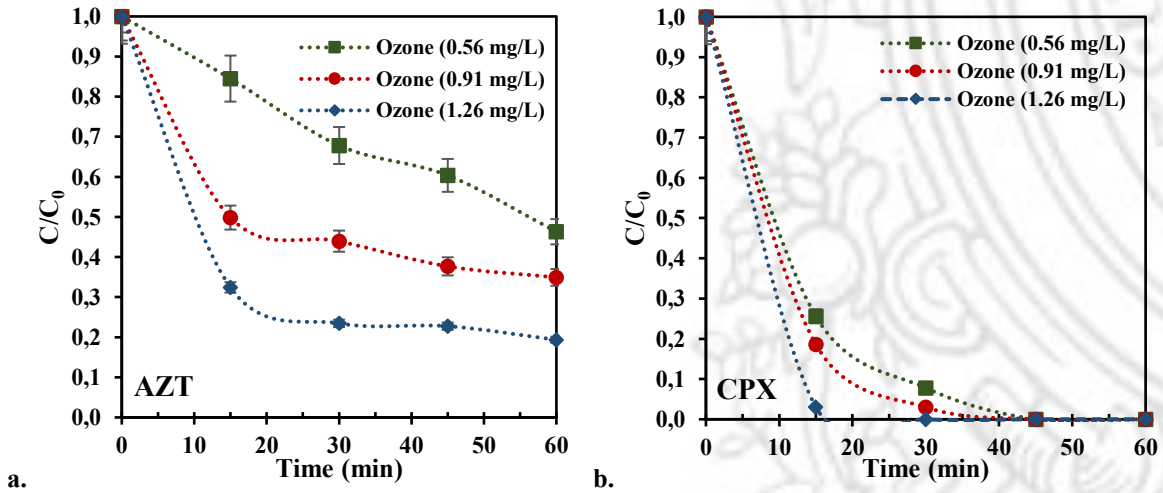
$$C_{38}H_{72}N_2O_{12} + 35O_3 \rightarrow \rightarrow \rightarrow 38CO_2 + 35H_2O + 2HNO_3 \quad (8)$$

$$C_{16}H_{17}N_3O_4S + 15.7O_3 \rightarrow \rightarrow \rightarrow 16CO_2 + 6H_2O + 3HNO_3 + H_2SO_4 \quad (9)$$

$$C_{22}H_{24}N_2O_8 + 17.7O_3 \rightarrow \rightarrow \rightarrow 22CO_2 + 11H_2O + 2HNO_3 \quad (10)$$

The experiments were conducted in 250.0 mL aqueous solutions (deionized water) with a 1.0 mg/L concentration of each antibiotic. The total concentration of contaminants in the water was 3.0 mg/L (mixture).

Based on the above, three different concentrations of dissolved O_3 (0.56, 0.91, and 1.26 mg/L) were evaluated. The system's pH was maintained at approximately 7.0. The results (Figure 3) underscore the crucial role of ozone concentration in the removal process. Higher O_3 doses promote higher pollutants elimination. After 60 minutes of reaction, the total removal of contaminants (mixture) ranged from 77.1% to 91.7%. Notably, under the highest O_3 condition, CPX was removed entirely in just 30 minutes, while the removal of AZT and DOX in 60 minutes was 80.6% and 94.4%, respectively.



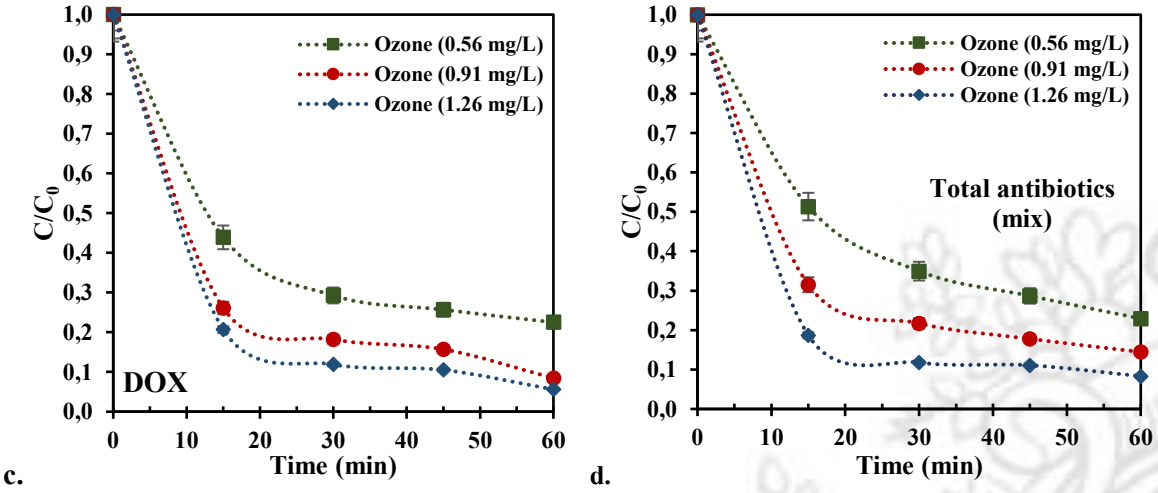


Figure 3. AZT, CPX, and DOX removal using O_3 (pollutants initial concentration 3.0 mg/L (1.0 mg/L per antibiotic), solution pH 7.0, and temperature 25.0°C).

5.2. AZT, CPX, and DOX removal optimization

Based on the above results, the reaction conditions were optimized considering three experimental factors: the dissolved O_3 concentration, the solution pH, and the reaction time. Table 3 indicates the experimental levels evaluated for each variable.

A face-centered central composite design (FCCD) was chosen to analyze the effect of the parameters of interest on eliminating AZT, CPX, and DOX and establishing the optimized reaction conditions. The total number of experiments was 17 (three central points), with equal intervals between values, and three replicates for each combination. The response variable was the removal of each pollutant. The results were analyzed using the Statgraphics Centurion 19 software.

Table 3. Experimental factors and levels evaluated for antibiotics AZT, CPX, and DOX removal using ozone.

Factor	Low level	Medium level	High level
Dissolved O_3 (mg/L)	0.21	0.56	0.91
Solution pH	3.0	6.0	9.0
Reaction time (min)	10.0	20.0	30.0

Table 4 presents the results obtained after carrying out the experimental design. The extent of the pollutants' removal was between 2.0 and 95.0%, suggesting a correlation between the evaluated factors and the response variable.

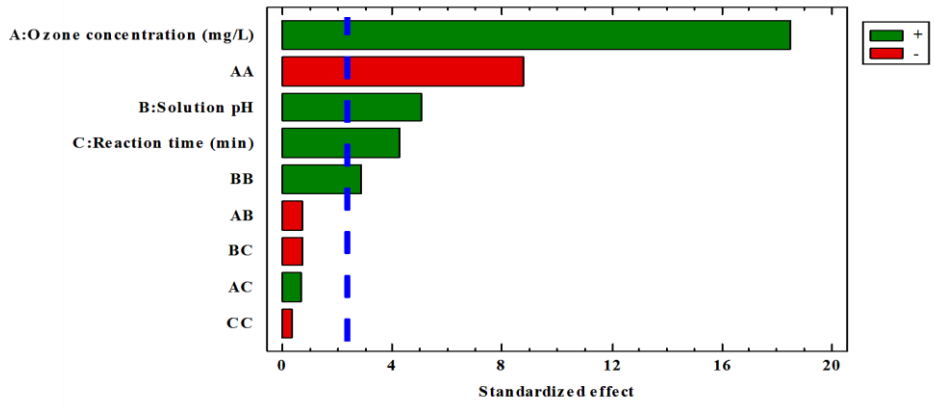
Table 4. Experimental design for antibiotics AZT, CPX, and DOX removal using ozone (pollutants initial concentration 3.0 mg/L (1.0 mg/L per antibiotic) and temperature 25.0°C).

Test	O_3 concentration (mg/L)	Solution pH	Reaction time (min)	AZT removal (%)	CFX removal (%)	DOX removal (%)	Total antibiotics removal (%)
1	0.21	3.0	10	9.63	5.32	2.49	5.81
2	0.21	3.0	30	33.61	7.33	10.52	17.15
3	0.56	3.0	20	30.28	87.85	67.36	61.83
4	0.91	3.0	10	35.46	86.40	59.41	60.42
5	0.91	3.0	30	77.63	88.26	67.63	77.84
6	0.21	6.0	20	1.18	2.11	7.14	3.48
7	0.56	6.0	10	9.52	87.68	65.99	54.40
8	**0.56**	**6.0**	**20**	**35.79**	**88.16**	**83.29**	**69.08**
9	**0.56**	**6.0**	**20**	**22.84**	**88.01**	**86.98**	**65.94**
10	**0.56**	**6.0**	**20**	**21.13**	**89.24**	**88.75**	**66.37**
11	0.56	6.0	30	38.17	92.49	**91.36**	74.00
12	0.91	6.0	20	55.04	**92.79**	73.24	73.69
13	0.21	9.0	10	18.87	1.80	51.08	23.92
14	0.21	9.0	30	32.68	6.60	55.76	31.68
15	0.56	9.0	20	91.85	85.65	79.93	**85.81**
16	0.91	9.0	10	72.00	87.37	65.41	74.93
17	0.91	9.0	30	**95.65**	88.10	73.37	85.70

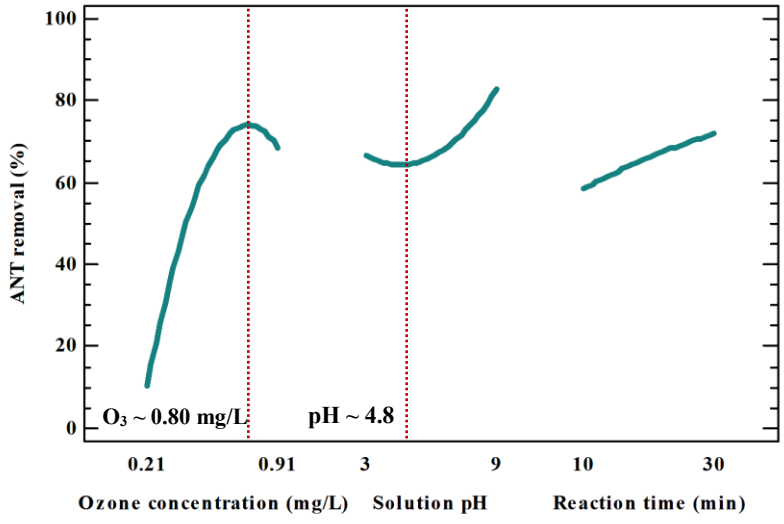
Figure 4 represents the statistical graphs associated with the effect of the factors of interest on the total removal of the pollutants (antibiotics total concentration decrease). Figure 4a. corresponds to the Pareto chart, which indicates that the three assessed variables significantly affect the removal of AZT, CPX, and DOX. However, the initial concentration of O_3 dissolved in the solution (A) seems to be the one with the greatest impact on the obtained results, which is to be expected considering that a higher presence of O_3 would promote a greater removal of the pollutants either directly or by the generation of HO• radicals. Despite this, the negative effect of the A-A interaction indicates that the most favorable initial dose of O_3 for removing the contaminants would correspond to an intermediate value, which suggests that an excessive or insufficient amount of ozone could hinder the removal process, information that is also supported by Figure 4b. (main effects plot).

Figure 4b. indicates that a higher concentration of O_3 initially promotes a greater removal of antibiotics, but under conditions higher than ~0.80 mg/L, an inhibitory effect occurs. In this regard, a higher presence of O_3 in the solution implies a higher flow rate of gas entering the system, which, according to some authors, would cause an excess of turbulence and a higher shear stress that would affect the stability of ozone in the solution, causing the O_3 molecules to decompose more quickly into O_2 and O•, reducing its available concentration for the contaminants oxidation [51]. In the case of the generation of HO• radicals, an excess of O_3 molecules could have a scavenger effect, meaning they could react with the HO•, reducing their concentration and thus their effectiveness in oxidizing the contaminants [52].

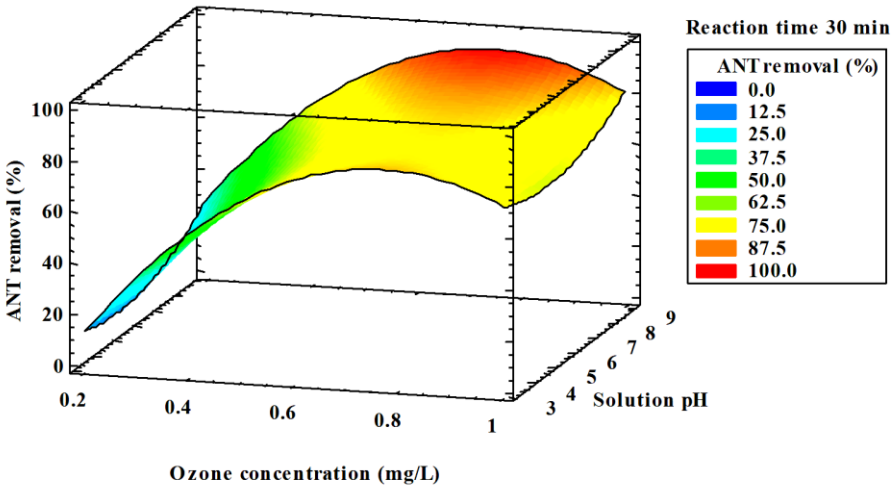
In the case of the solution's initial pH, Figure 4b. indicates that under acidic conditions, this variable does not significantly affect the elimination of the contaminants, which changes at pH above ~5.0 since O_3 is more unstable under more alkaline conditions, and the generation of HO• in the solution is promoted (equations 1 to 5), leading to an eventual oxidation of AZT, CPX, and DOX. Regarding the pH of the solution, it has been reported that it can promote the decomposition of O_3, the formation of radicals, and modify the stability of organic molecules [51,53].


A:Ozone concentration (mg/L)
AA
B:Solution pH
C:Reaction time (min)
BB
AB
BC
AC
CC
+
-
0
4
8
12
16
20
Standardized effect

a.


ANT removal (%)
100
80
60
40
20
0
O3 ~ 0.80 mg/L
pH ~ 4.8
0.21
0.91
3
9
10
30
Ozone concentration (mg/L)
Solution pH
Reaction time (min)

b.


Reaction time 30 min
ANT removal (%)
0.0
12.5
25.0
37.5
50.0
62.5
75.0
87.5
100.0
ANT removal (%)
100
80
60
40
20
0
0.2
0.4
0.6
0.8
1
Ozone concentration (mg/L)
3
4
5
6
7
8
9
Solution pH

c.

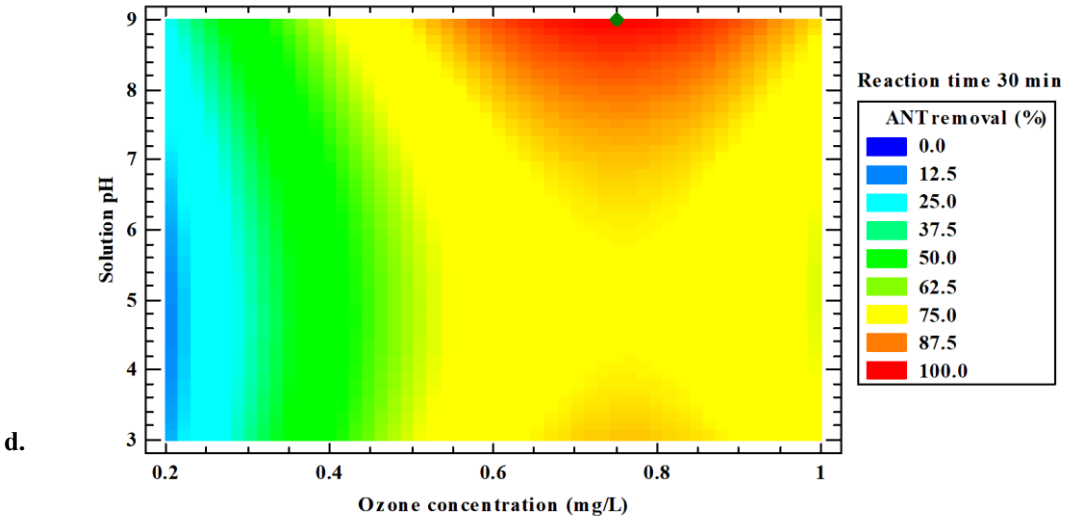


Figure 4. a. Pareto chart, b. Main effects plot, c. Response surface, and d. Contour surface for AZT, CPX, and DOX removal using O_3 (pollutants initial concentration 3.0 mg/L (1.0 mg/L per antibiotic) and temperature 25.0°C).

On the other hand, according to the reported acid dissociation constants (pK_a) for AZT (8.74 and 9.45), CPX (2.56 and 6.88), and DOX (3.5, 7.07, and 9.13) (Figure 5) [54–56], under basic pH the pollutants molecules are in their ionic form, which could promote a higher reactivity with molecular ozone and/or radicals [52]. Additionally, it is possible to infer that the longer the reaction time, the greater the elimination of antibiotics due to a longer contact time of these with ozone and the other oxidizing species in the solution.

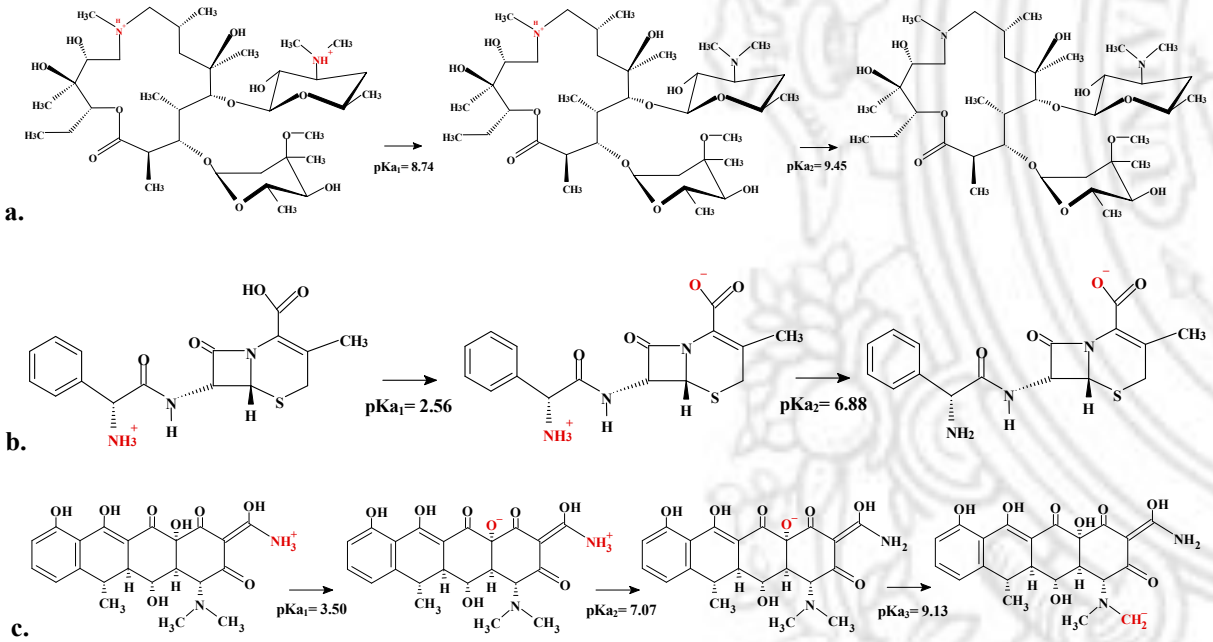


Figure 5. Molecular behaviour as a function of the solution pH for a. AZT, b. CPX, and c. DOX.

Figure 4c. and Figure 4d. shows the response and contour surface for the elimination of the antibiotics under a reaction time of 30.0 min (the most favorable).

Analyzing the experimental design results and the obtained response surface allows for determining a mathematical model that relates the total removal of the antibiotics (ANT removal (%)) and the evaluated factors.

Equation 11 corresponds to the found model, while Figure 6 indicates the fit between the experimental data and those predicted by the model. As can be seen in the figure, the fit is adequate (R^2 0.9824).

$$\textbf{ANT removal (\%)} = -45.895 + 329.749(\textbf{O}_3) - 8.370(\textbf{pH}) + 0.669(\textbf{t}) - 220.301(\textbf{O}_3)^2 + 0.917(\textbf{pH})^2 \quad (11)$$

O_3 is the ozone dissolved in the solution (mg/L), **pH** is the solution's initial pH, and **t** is the reaction time (min).

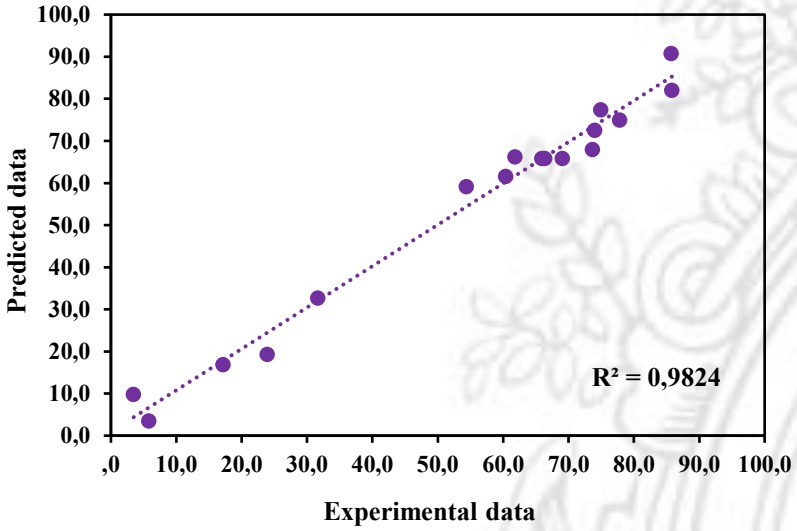


Figure 6. Correlation between experimental and predicted data for AZT, CPX, and DOX removal using O_3 (pollutants initial concentration 3.0 mg/L (1.0 mg/L per antibiotic) and temperature 25.0°C).

Finally, it can be established that **the optimized conditions for removing AZT, CPX, and DOX under the evaluated conditions are an ozone concentration in the solution of 0.75 mg/L, pH 8.0, and a reaction time of 30.0 minutes.**

5.3. AZT, CPX, and DOX removal under optimized conditions

The results regarding the removal of AZT, CPX, and DOX under the optimized reaction conditions are presented in Figure 7. Under the applied conditions, it was possible to remove more than 95.0% of CPX and DOX in 30 min of treatment. In the case of AZT, after 60 minutes, its removal was close to 80.0%. In this regard, it is important to note that from minute 30, the concentration of this antibiotic stayed almost constant.

Different authors have reported AZT as a rather recalcitrant organic contaminant that requires high doses of oxidizing agents for its total elimination and mineralization, even in deionized water [57,58]. In this study, it should also be considered that the joint presence of AZT, CPX, and DOX could limit the elimination of the contaminants due to aspects such as *i.* the availability of O_3 and other oxidants to react with each antibiotic, *ii.* some reactions could take place at the same time, and *iii.* the presence of different intermediates of organic nature (competitive aspects).

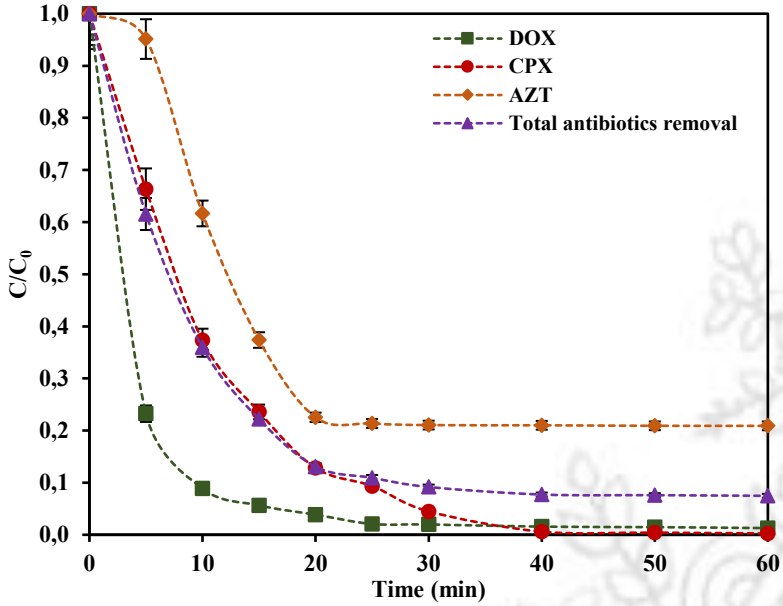


Figure 7. AZT, CPX, and DOX removal using O_3 under optimized conditions (pollutants initial concentration 3.0 mg/L (1.0 mg/L per antibiotic), O_3 initial concentration 0.75 mg/L, solution pH 8.0, and temperature 25.0°C).

5.4. AZT, CPX, and DOX removal using O_3/H_2O_2

As shown in equation 6, combining O_3 with H_2O_2 contributes to an increase in the removal of organic contaminants in water. This is due to the eventual generation of hydroxyl radicals, which, when combined with ozone, can achieve higher levels of pollutants degradation and mineralization. Additionally, H_2O_2 is an oxidizing agent widely used in water treatment and disinfection processes [59].

With the above, different experiments were carried out by adding H_2O_2 to the treated solutions. According to equation 6, 0.75 mg/L (0.016 mmol/L) of O_3 requires 0.53 mg/L of H_2O_2 to react completely. In this sense, experiments were done by adding quantities of H_2O_2 equivalent to 0.25, 0.5, 1.0, 1.5, and 2.0 times the stoichiometric ratio.

Figure 8 indicates that it is impossible to generalize the effect of H_2O_2 on removing each contaminant. Increasing or decreasing the peroxide concentration can favor or inhibit the elimination of the antibiotics, and depending on each compound, one effect is more predominant than the other. In addition, the increase in peroxide concentration directly affects the reduction of the reaction time to achieve the same percentage of removal of each compound in the analyzed samples.

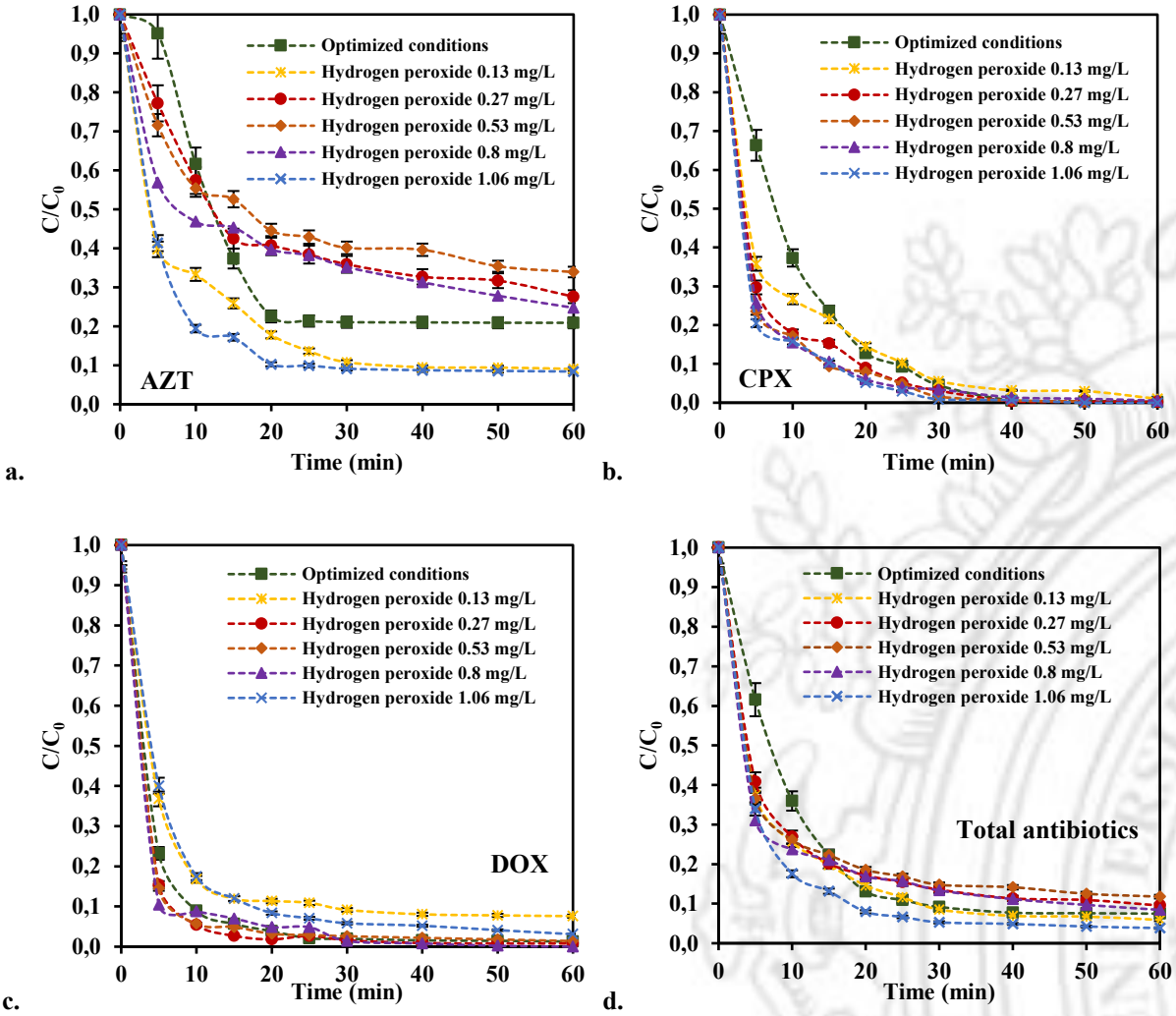


Figure 8. AZT, CPX, DOX, and mixture removal using O_3/H_2O_2 (pollutants initial concentration 3.0 mg/L (1.0 mg/L per antibiotic), O_3 initial concentration 0.75 mg/L, solution pH 8.0, and temperature 25.0°C).

In general, Figure 8d. shows that during the first minutes of the reaction, adding H_2O_2 improves the removal of antibiotics compared to the results under optimized conditions (no H_2O_2). However, after 20 min, the differences in the evolution of the concentration of contaminants are minor, and the kinetics of the reaction become slower, reaching, in all cases, elimination percentages higher than 88.0% after 60 min.

If the behavior of each antibiotic is analyzed individually, Figure 8a. indicates that for AZT, in the first 10 min of reaction, the presence of peroxide improves the elimination of the contaminant; after that time, only the doses of H_2O_2 of 0.13 and 1.06 mg/L have a better performance than the tests without peroxide, and that between 0.27 and 0.8 mg/L there seems to be an inhibitory effect, which could be associated with aspects linked to the presence of other contaminants in the solution and the competition that occurs by reacting with ozone and the generated radicals. In this study, it has been shown that AZT has been the most difficult to remove of the contaminants of interest.

For CPX, Figure 8b shows that contaminant elimination is improved regardless of the amount of H_2O_2 added. In fact, for concentrations between 0.27 and 1.06 mg/L of H_2O_2, the contaminant is completely removed after 40 min of reaction.

In the case of DOX, this seems to be the easiest antibiotic to remove. Figure 8c. indicates that at concentrations of 0.53 and 0.8 mg/L of H_2O_2, its elimination is faster during the first minutes, and that a dose of 1.06 mg/L (the highest evaluated) seems to inhibit the elimination of the antibiotic.

In conclusion, the presence of H_2O_2 can enhance the contaminants removal, particularly in the initial stages of the reaction. However, it is crucial to note that high H_2O_2 concentrations can lead to inhibitory effects depending on the specific contaminant. For instance, an excess of H_2O_2 can trap the hydroxyl radicals, leading to a scavenger effect [60,61]. Furthermore, in intermediate ranges, there is no significant difference in the improvement of the removal of AZT, CPX, and DOX.

5.5. AZT, CPX, and DOX removal using O_3/UV and O_3/H_2O_2/UV

Experiments were conducted by irradiating the solutions with UV light at 254.0 nm (lamp on top of the reactor). The incorporation of UV light in ozonation processes has been reported as an alternative to promote the removal of organic contaminants after the generation of HO• radicals [52]. However, Figure 9a. indicates that under the conditions evaluated in this study, the effect of light on removing the contaminants is practically negligible. During the first 15 min of the reaction, the pollutants removal was similar in the tests with only O_3 (optimized conditions) and in those with UV radiation. Even between 15 and 30 min of reaction, the extent of antibiotics removal was higher in the absence of UV light. Thus, it is presumed that although UV light can promote the generation of HO•, the decomposition of O_3 (equation 7) would limit the oxidation of contaminants, which possibly have a greater affinity to react directly with O_3. Liu et al. (2021) [62] reported similar results for removing the antibiotic sulfadiazine using UV/O_3. Sulfadiazine was removed more efficiently with only O_3.

Regarding the use of O_3/H_2O_2/UV in the removal of AZT, CPX, and DOX, it was decided to use an initial H_2O_2 concentration of 0.13 mg/L since, as previously discussed, the effect of increasing or decreasing the presence of peroxide may not be significant in terms of removal of the contaminants. In addition, from a practical point of view, working with a lower concentration of H_2O_2 is more convenient (lower operating costs and chemicals use). Thus, Figure 9a. allows to infer that the O_3/H_2O_2/UV combination promotes a more significant removal of contaminants in the first 10 min of reaction; subsequently, its performance decreases compared to ozonation. The combination of O_3/H_2O_2/UV brings several advantages, including the generation of additional HO• radicals after the homolytic breakdown of the peroxide molecule due to the presence of UV [63], plus the decomposition of O_3 via photolysis and by reaction with H_2O_2. However, it seems that this last effect would condition the elimination of AZT, CPX, and DOX.

On the other hand, Figure 9b. presents the results obtained regarding the variation of the TOC in treated solutions. The O_3/H_2O_2/UV treatment achieves higher mineralization (~60.0%) after 120 min, meaning that hydroxyl radicals or H_2O_2 could oxidize some of the reaction by-products.

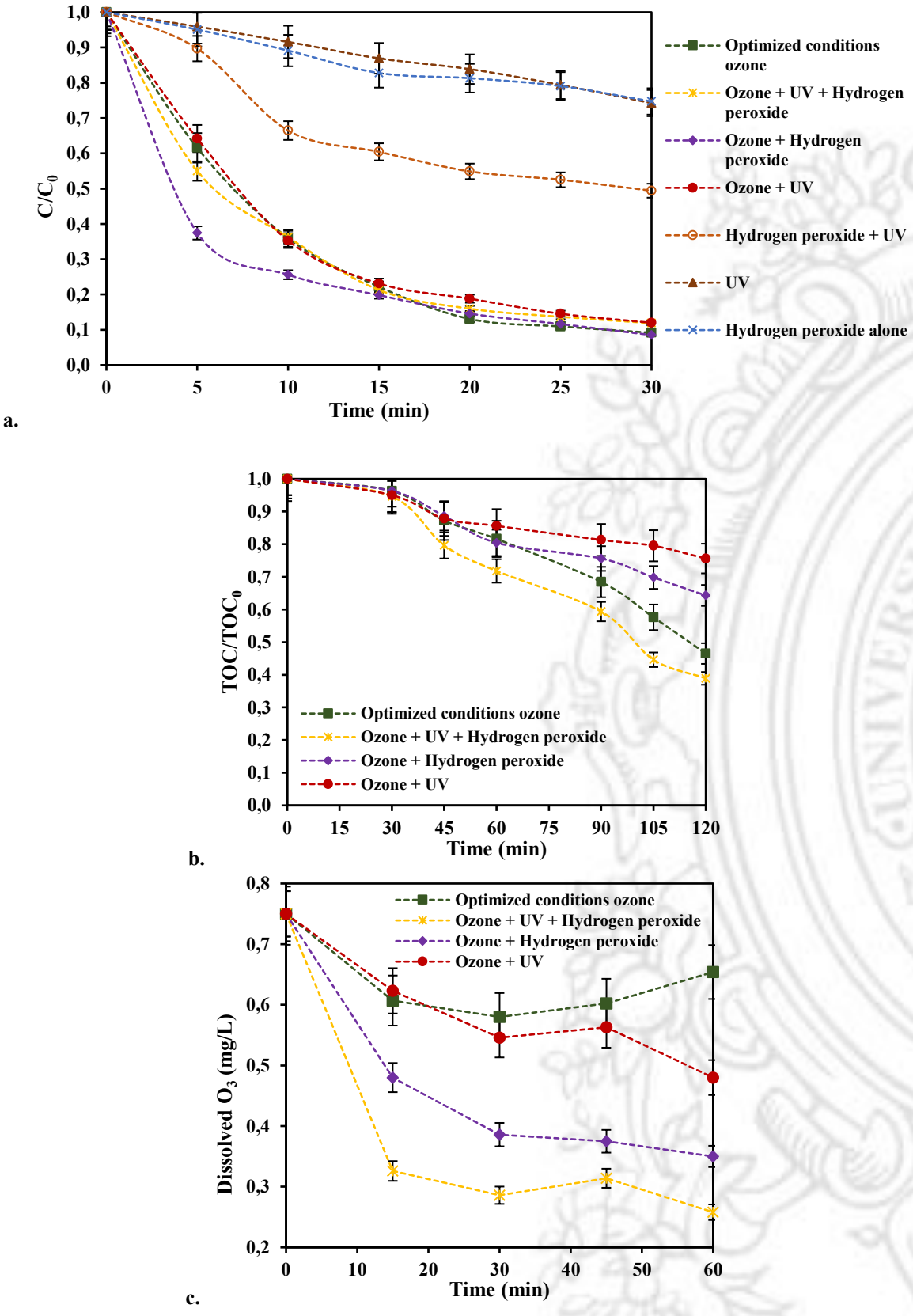


Figure 9. a. Antibiotics, b. TOC evolution, and c. Dissolved O_3 during AZT, CPX, and DOX removal using O_3-based technologies (pollutants initial concentration 3.0 mg/L (1.0 mg/L per antibiotic), O_3 initial concentration 0.75 mg/L, H_2O_2 initial concentration 0.13 mg/L, solution pH 8.0, and temperature 25.0°C).

Figure 9c. illustrates the evolution of the concentration of O_3 dissolved in water during the different treatments. It is clear from this that the concentration of O_3 decreases significantly under the O_3/H_2O_2/UV process, because of the decomposition of O_3 by UV and the reaction with peroxide. This trend is also observed in the O_3/H_2O_2 and O_3/UV cases.

In ozonation, a marked decrease in O_3 is noted during the first 15 min, followed by a stable concentration and a subsequent rise. While it seems that O_3 is the primary agent responsible for eliminating AZT, CPX, and DOX, the treatment effectiveness is limited due to the introduction of other agents that consume available O_3. However, a more comprehensive view suggests that the O_3/H_2O_2/UV process, which would promote a more significant generation of HO• radicals, could lead to greater mineralization, counteracting the lower elimination of the antibiotics.

5.6. Mechanism and reaction kinetics analysis.

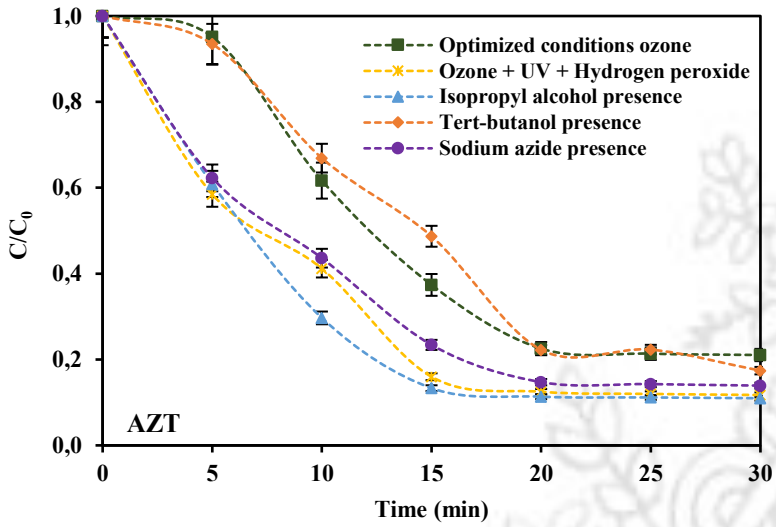
Removing organic contaminants using ozone-based technologies could involve different reaction mechanisms, including direct oxidation by O_3 and the generation of different species, such as HO• radicals. The decomposition of O_3 by the UV light action also promotes the generation of oxygen singlet ($^1O_2$), which has a higher selectivity to electron-rich organic compounds and could participate in the removal of AZT, CPX, and DOX [64].

Analysis of Figure 9 indicated that AZT, CPX, and DOX removal depends on the amount of ozone dissolved in the solution, which is reduced in the presence of UV light and peroxide. This would imply that O_3 plays a determining role in removing the contaminants. However, in terms of mineralization, the O_3/H_2O_2/UV treatment has a better performance. In addition, Figure 9a. shows that photolysis with UV light and oxidation with H_2O_2 cannot completely remove contaminants.

Considering the above, to clarify the role of HO• and $^1O_2$ in the AZT, CPX, and DOX removal, O_3/H_2O_2/UV tests were conducted in the presence of isopropanol (iPrOH), tert-butanol (TBA), and NaN_3. iPrOH and TBA are HO• scavengers, while NaN_3 is for $^1O_2$ [53,65,66]. The initial concentration of each scavenger was at least 100 times higher than the pollutants. Thus, Figure 10 indicates that, in general, the presence of iPrOH, TBA, and NaN_3 does not significantly inhibit the removal of the antibiotics. Only in the case of DOX, a significant decrease in the extent of pollutant removal was observed during the first 10 min of reaction under the iPrOH presence, indicating that HO• could contribute to the compound oxidation. Also, it was previously reported that at pH 8.0, antibiotics are mainly in their ionic form, which may favor their reaction with ozone. Furthermore, it has been reported that O_3 is selective in reacting with organic molecules with electron-rich functional groups. In this case, all three antibiotics have tertiary amines in their structure, along with aromatic rings, cycles, and unsaturations, which would favor their reaction with O_3.

This discussion allows to infer that the reaction mechanism involved in eliminating the antibiotics is linked to their oxidation via molecular O_3 under basic pH conditions (pH 8.0). However, the by-products generated in the first stages of the treatment could be removed by HO• radicals, which is evidenced by a greater extent of

mineralization under the $O_3/H_2O_2/UV$ treatment (Figure 9b.), that favors a higher generation of radicals compared to ozonation.



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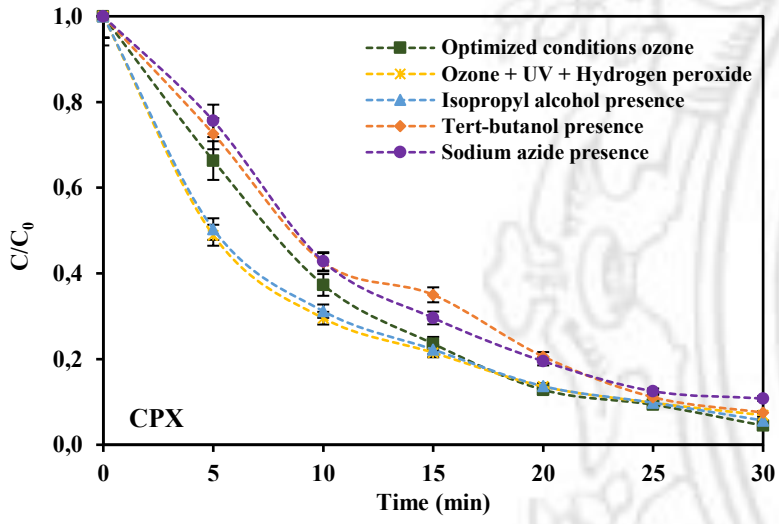

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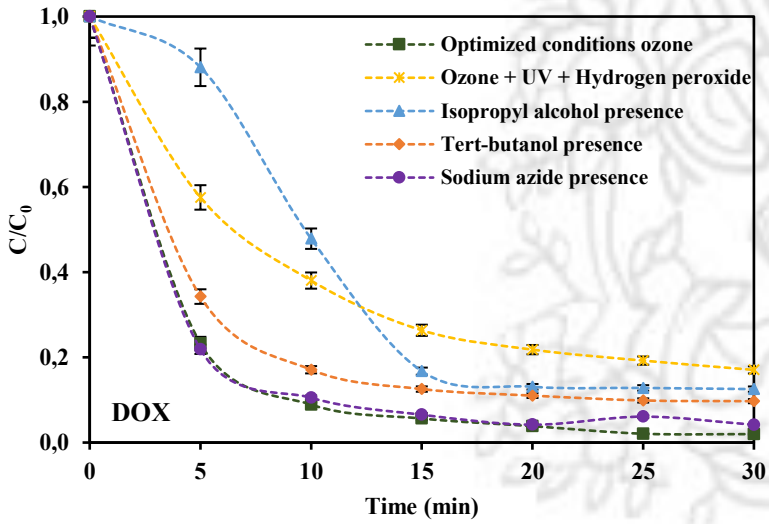

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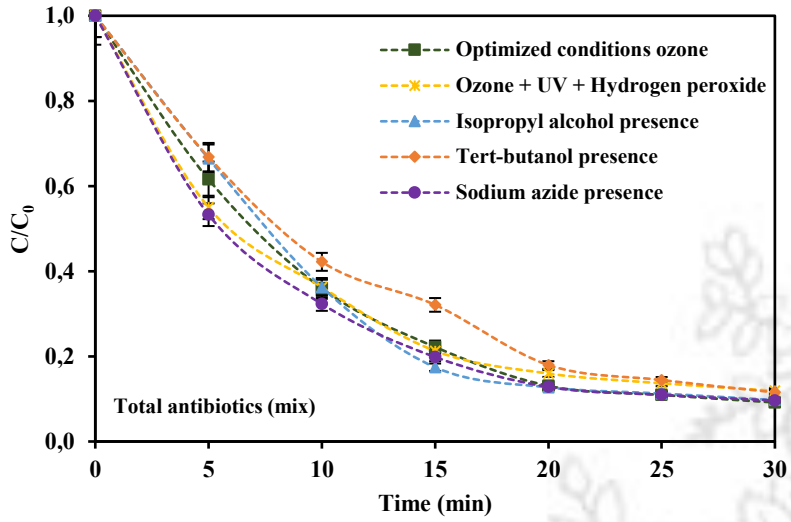


d.

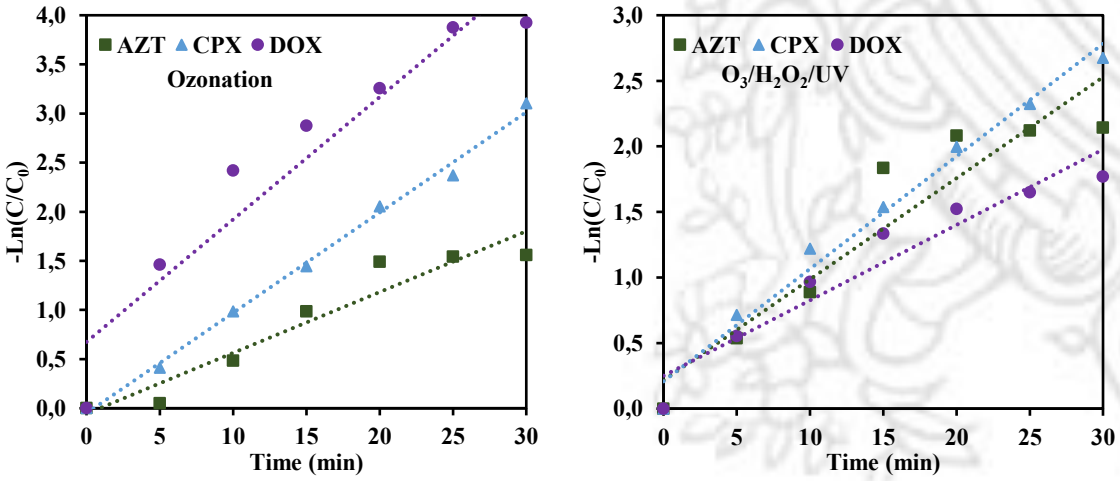
Figure 10. AZT, CPX, and DOX removal using O_3 and $O_3/H_2O_2/UV$ (pollutants initial concentration 3.0 mg/L (1.0 mg/L per antibiotic), O_3 initial concentration 0.75 mg/L, H_2O_2 initial concentration 0.13 mg/L, solution pH 8.0, and temperature 25.0°C).

Assuming that O_3 is the main responsible for the removal of the pollutants, and that it has been reported that pseudo-first and second order kinetic models (equations 12 and 13, respectively) could describe the removal of organic contaminants using ozonation [67,68], Figure 11 presents the analysis of the experimental data obtained for the removal of AZT, CPX, and DOX using O_3 and $O_3/H_2O_2/UV$. The figure shows that both models fit the data.

$$\mathrm{Ln}\left(\frac{C}{C_0}\right) = -kt \tag{12}$$

$$\frac{1}{C} = Kt + \frac{1}{C_0} \tag{13}$$

C is the pollutant concentration in time t; $\mathbf{C_0}$ is the pollutant initial concentration; **k** is the pseudo-first order kinetic constant; and **K** is the pseudo-second order kinetic constant.



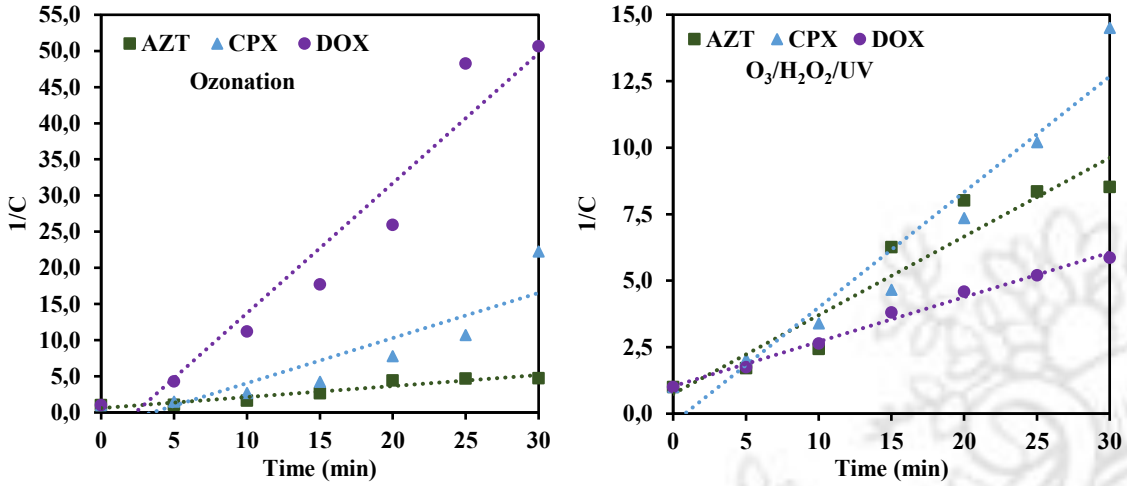


Figure 11. AZT, CPX, and DOX removal kinetics using O_3 and $O_3/H_2O_2/UV$ (pollutants initial concentration 3.0 mg/L (1.0 mg/L per antibiotic), O_3 initial concentration 0.75 mg/L, H_2O_2 initial concentration 0.13 mg/L, solution pH 8.0, and temperature 25.0°C).

Table 5 indicates that, based on the correlation coefficients (R^2), the removal of AZT and DOX can be described with a pseudo-second order model. At the same time, CPX would obey pseudo-first-order kinetics. However, it is important to keep in mind that this type of models should be assessed in depth since in the oxidation of contaminants using O_3, there are factors associated with mass transfer, O_3 diffusivity in water, the generation of oxidation intermediates, and the presence of various oxidizing agents that condition the reaction kinetics and will affect the removal of the pollutants [67,68].

Table 5. Reaction kinetics constants for AZT, CPX, and DOX removal using ozone (pollutants initial concentration 3.0 mg/L (1.0 mg/L per antibiotic), O_3 initial concentration 0.75 mg/L, H_2O_2 initial concentration 0.13 mg/L, solution pH 8.0, and temperature 25.0°C).

Antibiotic	Constant for pseudo-first order kinetics (k, 1/min)		Constant for pseudo-second order kinetics (K, L/mg min)	
	Ozonation	O_3 + UV + H_2O_2	Ozonation	O_3 + UV + H_2O_2
AZT	0.0619 (R^2 0.9243)	0.0771 (R^2 0.8875)	**0.1524 (R^2 0.9193)**	**0.2958 (R^2 0.9085)**
CPX	**0.1021 (R^2 0.9951)**	**0.0858 (R^2 0.9824)**	0.6238 (R^2 0.8013)	0.4340 (R^2 0.9369)
DOX	0.1246 (R^2 0.9087)	0.0575 (R^2 0.9444)	**1.7967 (R^2 0.9377)**	**0.1676 (R^2 0.9916)**

5.7. AZT, CPX, and DOX removal using $O_3/H_2O_2/UV$ in hospital wastewater.

Experiments ($O_3/H_2O_2/UV$) were conducted using hospital wastewater from a medium-category healthcare institution of the city of Medellín (Colombia). Table 6 presents some of the physicochemical properties of the water, highlighting its chloride, sulfate, turbidity, and organic matter content. The AZT, CPX, and DOX initial concentration in the matrix was less than 50.0 µg/L and was adjusted to 1.0 mg/L for each antibiotic. The analysis was executed under optimized conditions: O_3 initial concentration 0.75 mg/L, H_2O_2 initial concentration 0.13 mg/L, and solution pH 8.0 for 60 minutes. Figure 12 shows the obtained results, which indicate that the removal

of the antibiotics was lower in hospital water, reaching extents of removal between ~70.0 and 87.0% in 60 min of reaction.

These results would be associated with the nature of the treated matrix as follows:

i. The presence of ions such as Cl^-, SO_4^{2-}, F^-, and NO_3^- could be related to inhibitory effects given their ability to react with different radicals, including HO•, generating species with less oxidative capacity [51,69,70].

ii. The turbidity of the water can prevent UV light rays from penetrating the solution, decreasing the photolytic processes that take place and favor the elimination of the contaminants [52].

iii. The organic matter content can reduce the removal of the antibiotics since competitive effects can occur by reacting with ozone and other present oxidizing species. Likewise, more intermediaries can be generated, limiting the possibility of reacting with AZT, CPX, and DOX [52].

Table 6. Physicochemical properties of hospital wastewater used in the AZT, CPX, and DOX removal using ozone-based technologies.

Parameter	Value
Chloride (Cl^-)	33.800 mg/L
Nitrate (NO_3^-)	1.015 mg/L
Sulfate (SO_4^{2-})	13.181 mg/L
Nitrite (NO_2^-)	<0.08 mg/L
Fluoride (F^-)	0.038 mg/L
Total Organic Carbon (TOC)	6.973 mg/L
pH	7.14
Apparent color	48.03 CU
Turbidity	14.00 NTU
Chemical Oxygen Demand (COD)	28.499 mgO_2/L
AZT	<50.0 µg/L
CPX	<50.0 µg/L
DOX	<50.0 µg/L

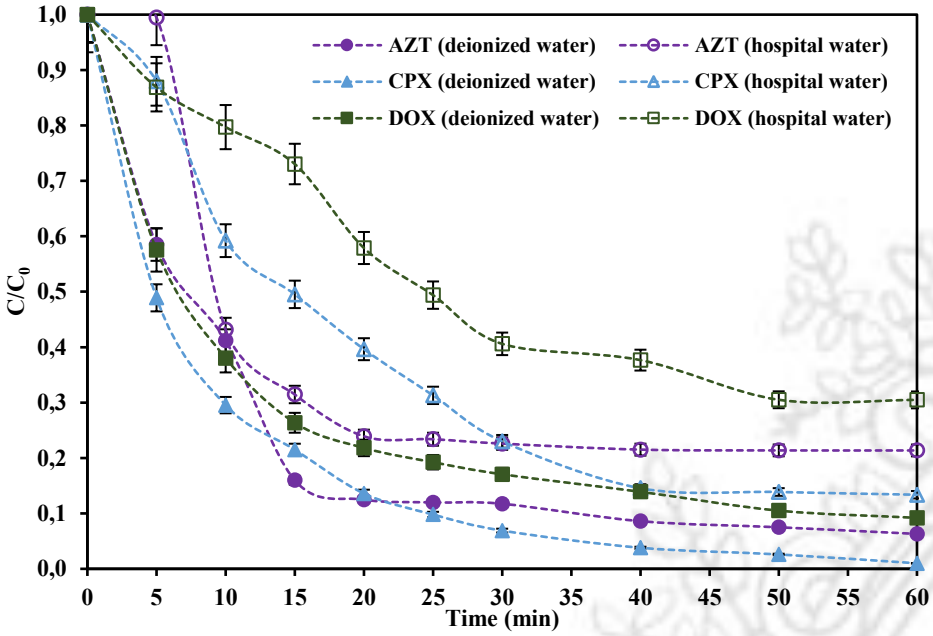


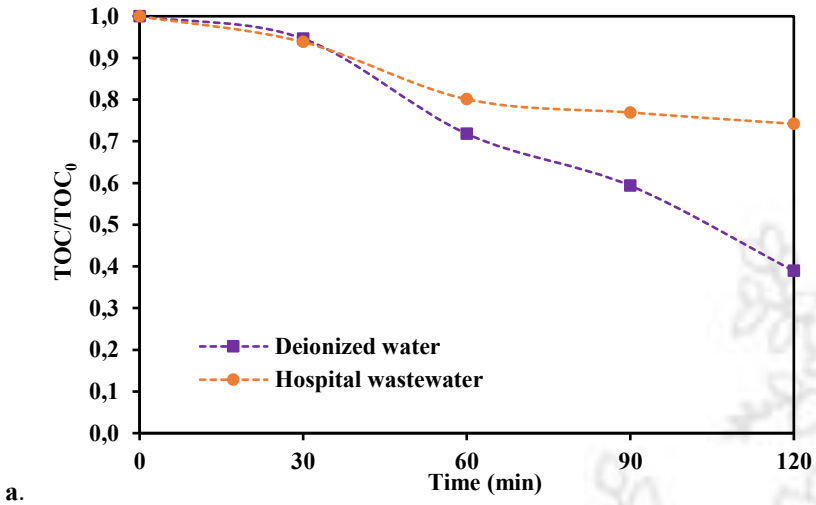
Figure 12. AZT, CPX, and DOX removal using O_3/H_2O_2/UV in hospital wastewater (pollutants initial concentration 3.0 mg/L (1.0 mg/L per antibiotic), O_3 initial concentration 0.75 mg/L, H_2O_2 initial concentration 0.13 mg/L, solution pH 8.0, and temperature 25.0°C).

5.8. Mineralization analysis.

The variation of total organic carbon and the presence of NO_3^- and SO_4^{2-} in the treated samples was evaluated considering the reaction conditions under the O_3/H_2O_2/UV treatment. Figure 13 presents the results obtained in deionized water and hospital wastewater. First, Figure 13a. indicates, as expected, that the removal of TOC in wastewater was lower. On the one hand, because the initial value of this parameter in that matrix is higher, the decrease should be more significant to reach higher percentages of mineralization, and, as previously discussed, some of the properties of water (presence of ions and turbidity) can limit the performance of the treatment.

Regarding the NO_3^- and SO_4^{2-} content in the solutions, the experiments in deionized water showed a higher increase, reaching concentrations equivalent to 26.94 and 41.95% of the maximum possible values of nitrates and sulfates (according to the mass balance). In the case of the hospital wastewater, an increase in the concentration of NO_3^- and SO_4^{2-} was also observed. This indicates that the applied treatment eliminates AZT, CPX, DOX, and some of the organic matter present in the water.

Greater mineralization is achieved in deionized water because there is less interference from other compounds than in hospital water.



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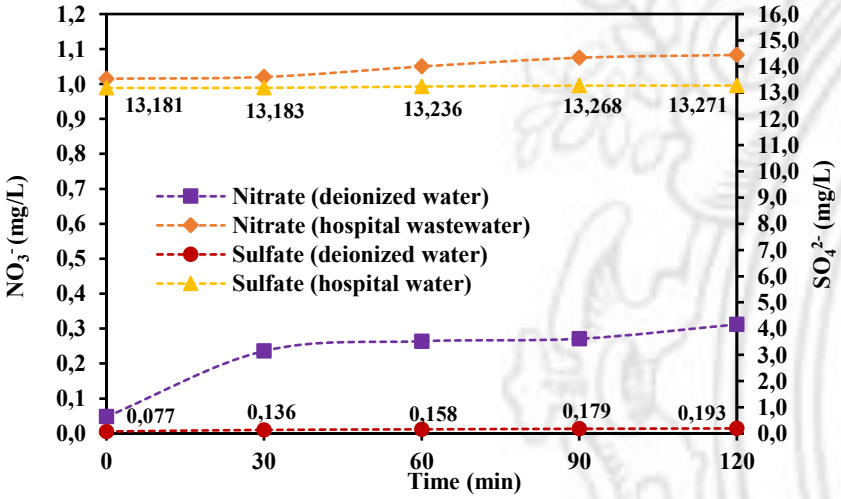

b.

Figure 13. a. Total organic carbon and b. Nitrate and sulfate content during AZT, CPX, and DOX removal using $O_3/H_2O_2/UV$ (pollutants initial concentration 3.0 mg/L (1.0 mg/L per antibiotic), O_3 initial concentration 0.75 mg/L, H_2O_2 initial concentration 0.13 mg/L, solution pH 8.0, and temperature 25.0°C).

6. CONCLUSIONS

The O_3, O_3/H_2O_2, O_3/UV, and O_3/H_2O_2/UV treatments were evaluated for removing the antibiotics AZT, CPX, and DOX. Ozonation and the O_3/H_2O_2/UV system allowed to obtain higher extents of pollutants removal and mineralization, respectively. The initial concentration of dissolved O_3 and the solution pH are parameters that condition the removal of the contaminants. An excess of O_3 can be associated with a scavenger effect on the HO• radicals present in the solution and with the generation of turbulence that limits mass transfer. Ozonation could remove organic pollutants through direct oxidation by molecular O_3 at acid pH. At higher pH, organic pollutants are oxidized by molecular ozone and hydroxyl radicals.

The predominant reaction mechanism in removing AZT, CPX, and DOX is direct oxidation by O3. The removal of antibiotics can be described using pseudo-first- and second-order kinetic models. However, factors such as the diffusivity of O_3 in water, the reaction time, and the presence of organic matter in the solution must be considered.

The percentages of AZT, CPX, and DOX removal and mineralization depend on the type of treated matrix. In hospital wastewater, the presence of inorganic ions, turbidity, and organic matter determines the performance of the O_3/H_2O_2/UV treatment.

Finally, **this research demonstrates that ozonation is a viable alternative for treating deionized water and hospital wastewater containing AZT, CPX, and DOX**.

7. RECOMMENDATIONS & FUTURE RESEARCH

This research focused on evaluating the potential use of ozone-based technologies for antibiotics removal. The research hypothesis was verified, and the effects of different operational parameters, some reaction mechanisms, and the removal of organic matter were evaluated. However, some aspects remain to be addressed in future research:

- **Identify reaction byproducts.** The results obtained show that antibiotics did not completely mineralize. This implies that part of the organic matter is transformed into organic byproducts. Identifying these compounds would allow for the design of post-treatment stages or the modification of the reaction conditions.

- **Evaluate samples toxicity.** In many cases, complete removal of organic matter in the water is not achieved, and some reaction byproducts can be more harmful than the target pollutant. In this sense, including toxicity analysis of the treated water samples is important, ensuring minimal risk of ecosystem impacts and bacterial resistance proliferation.

- **Compare different reactor configurations.** Some aspects related to the transfer of O_3 in water and its solubility can limit the removal of organic contaminants; for that, it is appropriate to evaluate different reactors or diffuser configurations that favor longer contact times between the oxidant and the contaminants.

- **Scaling up the process.** This type of research aims to optimize reaction conditions at the laboratory scale and subsequently evaluate their application at the pilot plant or full-scale level. The treatment efficiency in hospital wastewater was evaluated. Thus, one of the following steps should be implementing the treatments on a larger scale and under complex water matrices.

8. ASSOCIATED PRODUCTS

- **Scientific papers:**

  M. Vásquez-Vásquez, M. Araque-González, J. E. Escobar-Zuluaga, H. Zúñiga-Benítez, G. Peñuela (2025). **Assessment of the use of ozone-based technologies in the removal of azithromycin, cephalexin, and doxycycline in deionized water and hospital wastewater**. Journal of Water Process Engineering 71, 107303. DOI: 10.1016/j.jwpe.2025.107303.

- **Participation in academic events:**

  1. M. Vásquez-Vásquez, H. Zúñiga-Benítez, G. Peñuela (2023). **Removal of antibiotics from aqueous solutions using ozone-based oxidation technologies (oral presentation)**. 11th World Congress of Chemical Engineering (WCCE11), Buenos Aires - Argentina.
  2. M. Vásquez-Vásquez, R. Mira Betancur, H. Zúñiga-Benítez (2022). **Efecto de la aplicación de ozono como preoxidante en el proceso de potabilización en la planta San Nicolás (oral presentation)**. II Congreso Internacional Objetivos de Desarrollo Sostenible, Medellín - Colombia.

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