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Enhanced Degradation of Tetracycline by the O_3/ H_2O_2 Process: Efficiency, Biodegradability, and Toxicity Assessment

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Abstract

Introduction: Antibiotics are among the most persistent pollutants in aquatic environments, posing ecological and health risks. This study evaluated the degradation of tetracycline in aqueous solutions using ozone-based advanced oxidation processes (AOPs).

Methods: Ozone (O_3), O_3/UV, and O_3/H_2O_2 systems were compared under varying operational conditions in a batch reactor. The effects of pH, oxidant dosage, and reaction time on degradation efficiency were assessed. Biotoxicity and biodegradability changes were monitored using *E. coli* inhibition and the BOD_5/COD ratio.

Results: The O_3/H_2O_2 system achieved the highest degradation efficiency (99.2%) and improved wastewater biodegradability (BOD_5/COD = 0.67). In contrast, single ozonation achieved 69.2% removal. The combination of ozone and hydrogen peroxide significantly reduced effluent toxicity, lowering *E. coli* inhibition by 6.34%.

Conclusion: The synergistic O_3/H_2O_2 process demonstrated superior performance in tetracycline removal and detoxification, highlighting its potential as a sustainable and effective pre-treatment for pharmaceutical wastewater.

Keywords: Ozone, Tetracycline, Hydrogen peroxide, Oxidation-reduction

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Introduction

The limited efficacy of conventional wastewater treatment plants (WWTPs) in removing emerging contaminants, such as pharmaceutical compounds, represents a major challenge for water reuse. (1). Some pharmaceutical chemicals have the potential to disrupt the endocrine systems of organisms, leading to adverse effects like decreased fertility, feminization, organ abnormalities, and a higher probability of cancer (2). Antibiotics are widely used worldwide. Wastewater from both homes and factories may include antibiotics, increasing the prevalence of drug-resistant bacteria and rendering antibiotic treatments ineffective (3,4). Tetracycline (TC) is one of the most commonly used antibiotics. Generally, conventional methods such as coagulation, gelation, filtration, and precipitation exhibit limited efficacy in removing tetracycline (5-8). Hence, the significance of developing a proficient method for the degradation and detoxification of tetracycline in an aqueous environment is growing. Many antibiotic chemicals may be eliminated via advanced oxidation processes (AOPs), characterized by forming radical species such as hydroxyl radicals. Diverse techniques are utilized in these technologies to produce hydroxyl radicals (9). Although many emerging oxidation technologies remain in early developmental stages or face considerable practical challenges, ozone-based processes are comparatively more established and have demonstrated higher energy-efficiency and effectiveness in wastewater treatment (10,11). In wastewater containing antibiotics, ozone-based processes can increase the BOD_5/COD ratio, degrading non-biodegradable wastewater. Dissolved O_3 reacts with the pollutant either directly or by the creation of radicals (12). In the aquatic environment, molecular O_3 may directly oxidize organic materials, and indirect oxidation by free radicals created during O_3 breakdown, such as hydroxyl radicals (·OH) with reduction of potentials around 1.8–2.8 V, superoxide radicals ($O_2^{-\bullet}$) with 0.7 V, and hydroperoxide radicals ($HO_2^{\bullet}$) with 0.93 V, is also possible (13). Nevertheless, the production of hydroxyl radicals (·OH) in a single ozone (O_3) process was sometimes limited, especially in acidic conditions, due to the strong influence of pH on the O_3 decomposition

(14). Complete mineralization is rarely achieved with ozonation alone, as reactions between ozone and organic molecules typically produce ketones and carboxylic acids, which are less reactive toward ozone (15). Hence, to tackle this challenging problem, it has been recommended to integrate and combine O_3 with an activated technique like UV or H_2O_2. H_2O_2 can significantly enhance ozonation by generating HO_2^- ions, which are more effective than OH^- ions in initiating and accelerating ozone decomposition (16). Nevertheless, concerns have been raised regarding the potential formation of toxic transformation products (TPs) during AOPs, which in some cases may be more harmful than the parent tetracycline (17-19). These studies have primarily focused on UV-assisted AOPs, and there is a paucity of data on the TPs generated during ozone-based processes and their toxicity. Up to now, various studies have been conducted on tetracycline removal by AOPs processes such as Fenton-like process using ZnO_2 (20), photodegradation process in the presence metal oxide catalyst and H_2O_2 (21), sulfate radical-advanced oxidation process (22), ultraviolet/persulfate advanced oxidation process (23), and HA/Fe-Al_2O_3/O_3 process (24). To our knowledge, there is a lack of data evaluating both the biotoxicity and biodegradability of the O_3/H_2O_2 (Peroxone) process for treating tetracycline-contaminated wastewater. The combination of ozone and hydrogen peroxide (O_3/H_2O_2), known as the Peroxone process, enhances hydroxyl radical ($^{\bullet}OH$) generation and achieves higher oxidation efficiency than ozonation alone, making it effective for removing antibiotics from wastewater (25,26). Accordingly, the present study aimed to identify the optimal operating conditions for an ozone-based process to effectively eliminate the target pollutant, to conduct a comparative evaluation of various ozone-based techniques for the removal of tetracycline, to investigate variations in biotoxicity throughout the treatment process using the *Escherichia coli* susceptibility test, and to assess the enhancement of biodegradability based on the BOD_5/COD ratio.

Materials and Methods

Reagents and microorganism

The complete set of experimental chemicals including tetracycline ($C_{22}H_{24}N_2O_8$), Hydrogen peroxide (H_2O_2, 30%), Sulfuric acid (H_2SO_4), Potassium sulfate (K_2SO_4), Calcium phosphate ($Ca_3(PO_4)_2$), Oxalic acid ($C_2H_2O_4$), Phenol (C_6H_6O), Potassium iodate (KIO_3), Humic acid ($C_9H_9NO_6$), Para-chlorobenzoic acid ($C_7H_5ClO_2$), Sodium hydroxide (NaOH) Starch ($(C_6H_{10}O_5)_n$), Ammonium Nitrate (NH_4NO_3), and Sodium Nitrite ($NaNO_2$) with high purity analytic grade were purchased from Sigma-Aldrich (Steinhein, Germany) and Merck & Co. Moreover, for biotoxicity tests, *E. coli* (ATCC 25922) was grown in LB-broth (25 g/L) at 37°C. Milli-Q® equipment (Millipore) was utilized to produce ultrapure water.

Experimental set-up and procedure

A 1.2 L batch, cylindrical glass reactor with a height of 70 cm and an inside diameter of 4.7 cm was employed. Ozone was supplied by an ozone generator (Tonglin 3S-A3, China) with a pure oxygen source (capacity of 7 mg min^{-1}), and a rotameter was used to monitor and regulate the ozone flow rate. The cylindrical O_3/UV reactor was fitted with a vertically mounted 15 W low-pressure mercury UV lamp, with an average irradiation distance of only 3 cm. The UV lamp emits UV light with a wavelength of 254 nm and has a size of 20 × 430 mm with an output of 5WUV. In both reactors, ozone gas was continually injected into the reaction solution from bottom at a flow rate of 0.3 L min^{-1}, and sodium thiosulfate solution was used to eliminate any remaining ozone in the tail gas. To determine the efficiency of the O_3/UV and O_3/H_2O_2 process the influence of different parameters such as reaction time (5, 10, 15,20, 25, 30 min), pH (3, 5,7,9), initial concentrations of tetracycline (100, 200, 300, 400, 500 mg L^{-1}), Hydrogen peroxide concentration (0, 2.5, 5, 7.5, 10, 15, 20, 25, 30 mg L^{-1}), and concentration of soluble ozone (0.04, 0.22, 1, 1.14, 1.24 mg min^{-1}) were evaluated. All experiments were performed in triplicate, and the results are reported as mean ± standard deviation (SD). Error bars in the figures represent SD values obtained from three independent measurements, calculated using Microsoft Excel (version 2021). Tetracycline was dissolved in deionized water to produce the samples. Using a pH meter (Philips PW 9422), the starting solution pH of 200 mL in each run was adjusted to the desired value (3, 5, 7, or 9) by adding diluted 0.1 M NaOH or 1 M HCl. At predetermined intervals, a specific volume of samples was taken from the reactor and filtered through a 0.22 µm filter for further examination. The iodide technique (technique 2350 E) and the Indigo method (Method 4500) were used to calculate the O_3 content in the gaseous form and reaction solution, respectively (27). The experiments were performed at a stable temperature (20 ± 2°C). The ·OH radical concentration was determined using the following equation (Eq (1)), where $pCBA_0$ (Para-chlorobenzoic acid) and $pCBA_t$ are the initial and terminal pCBA concentrations (mg L^{-1}), respectively, and t is the reaction time ($k_{HO\bullet, pCBA} = 5.0 \times 10^9\ M^{-1}\ s^{-1}$) (28).

$$HO^{\bullet} = \frac{\ln\left(\frac{pCBA_t}{pCBA_0}\right)}{K_{HO}.t} \qquad (1)$$

Analytical methods

To prevent any additional reaction, materials were analyzed as soon as the reaction time for each step had elapsed. Tetracycline concentrations were measured using an HPLC system (Agilent 1260 Infinity) equipped with a ZORBAX Eclipse Plus C18 column (4.6 × 100 mm, 3.5 µm) and a diode array detector set at 359 nm. The mobile phase comprised 75% oxalic acid (0.01 mol L^{-1}) and 25% acetonitrile; injection volumes and flow rate were 20 µL and 1 mL min^{-1}, respectively. High-performance liquid chromatography (HPLC) was employed to measure the concentration of Para-chlorobenzoic acid (pCBA) in solution. *Kinetex*® C18 (2.6 m particle size; 4.6 150 mm) was utilized as the chromatographic column. In isocratic mode at 0.60 mL min^{-1}, the mobile phase consisted of 70% acid ultrapure water solution (0.1% formic acid) and 30% acid acetonitrile solution (0.1% formic acid); the detector wavelength was set to 240 nm; and the injection volume was 100 µL. Moreover, titanium (IV) oxysulfate at 410 nm was employed in a spectrophotometer by the DIN 38402H15 standard to calculate the concentration of residual H_2O_2 (29). Using a Portable Data Logging Spectrophotometer (DR/2000) Hach (USA) and an Oximeter model 538, the samples were examined for soluble COD and BOD_5. Thiourea was employed to prevent nitrification in the phases displaying nitrification for the BOD_5 tests. The samples were stored at 4°C prior to analysis, and the HPLC column temperature was maintained at 30°C. The Standard Methods for the Analysis of Water and Wastewater were used for all analyses.

Results

Effect of pH

Since the hydroxyl radical acts as a catalyst in O_3 breakdown, the pH of the solution plays a crucial role in determining the reaction kinetics of organic molecules with O_3. To prevent hydroxyl radicals from being decomposed into other radical species that might contribute to oxidation (peroxyl radicals), the pH range was selected to be 3–9, and other parameters were kept constant (TC = 200 mg L^{-1}, H_2O_2 = 10 mg L^{-1}, reaction times = 10 min, and ozone dose = 0.22 mg min^{-1}). The results are shown in Figure 1. With increasing pH from 3 to 9, the removal efficiency of TC increased from 20 to 77% for O_3 and from 22 to 83.05% for the O_3/H_2O_2 process. TC removal was not significantly affected by H_2O_2 alone at different pH levels; however, its combination with O_3 enhanced the overall system performance. At higher pH values, the formation of HO_2^- may contribute to TC removal, slightly enhancing the process efficiency, as further quantified and discussed in Section 4.

Effect of H_2O_2 concentration

As shown in Figure 2, adding varying levels of H_2O_2 before the beginning of ozonation allowed us to investigate the efficacy of the H_2O_2-boosted ozonation process (i.e., O_3/H_2O_2) for the TC degradation. Based on the results, the tetracycline removal efficiency increased from 72% at a concentration of 2.5 mg L^{-1} to 83% at 10 mg L^{-1}. However, removal efficiency did not change significantly when the H_2O_2 concentration increased from 10 to 15 mg L^{-1}. Notably, increasing the concentration to more than 15 mg L^{-1} not only did not change the efficiency but also decreased the removal rate.

Effect of O_3 dosage on the O_3/H_2O_2 process

The flow rates of O_3 was increased at pH 9 to assess the impact of ozone dosage on TC degradation by the O_3/H_2O_2 process. As shown in Figure 3, TC removal increased from 20% to 97% as the ozone dosage increased from 0.04 to 1 mg min^{-1}. At O_3 = 1.24 mg min^{-1}, TC removal reached 98.2%, and further increases in ozone dosage resulted in minimal improvement, indicating that the system approaches saturation under these conditions. Ozone decomposition and consumption rates were also measured during the experiments.

Kinetics of O_3/H_2O_2 and O_3 processes and ·OH concentration

The investigation of the kinetics of tetracycline degradation in the O_3/H_2O_2 process was done under the experimental circumstances adopted (tetracycline concentration (100-500 mg L^{-1}), H_2O_2 = 10 mg L^{-1}, O_3 dose = 1 mg min^{-1}, reaction time = 0-60 min), and the results are shown in Figure 4 and Table 1. The results indicated that the time needed for complete deterioration increased as the initial TC content increased. In this case, at starting tetracycline concentrations of 200, 300, 400, and 500 mg L^{-1}, the elimination efficiencies were 100, 92, 76.3, and 54% at the end of the first 10 min. As a result, the TC removal efficiency may be enhanced at lower TC starting concentrations.

The $^{\bullet}OH$ radical concentration was calculated using Eq. (1) as described in Section 2.2. To provide clarity, Table 2 presents the time-dependent $^{\bullet}OH$ concentrations measured during the O_3/H_2O_2 process. The experiments were conducted at pH 9, with an initial TC concentration of 200 mg L^{-1}, H_2O_2 dose of 10 mg L^{-1}, and O_3 dose of 1 mg min^{-1}. As shown, the $^{\bullet}OH$ concentration gradually decreased from 1.20×10^{-9} to 1.12×10^{-9} mol L^{-1} over

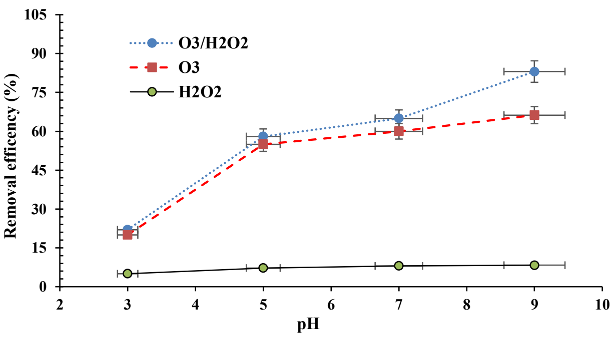

Figure 1. Effect of pH value on O_3/H_2O_2 process in tetracycline removal (time = 15 min, O_3 = 0.2 mg min^{-1}, H_2O_2 = 10 mg L^{-1}, and TC = 200 mg L^{-1})

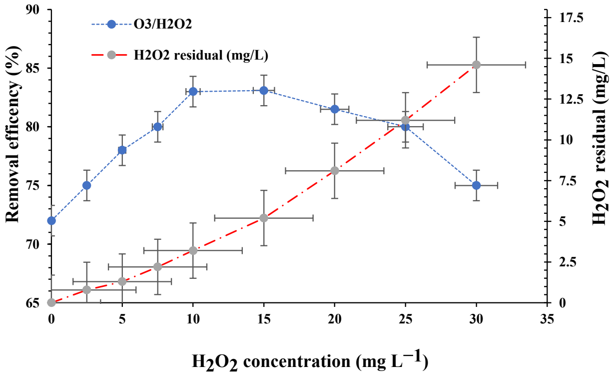

Figure 2. Effect of H_2O_2 dosage on O_3/H_2O_2 process in tetracycline removal (time = 15 min, O_3 = 0.2 mg min^{-1} and TC = 200 mg L^{-1})

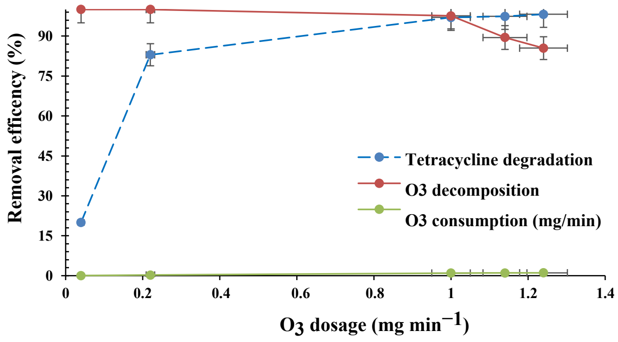

Figure 3. Effect of O_3 dosage on O_3/H_2O_2 process in tetracycline removal (time = 15 min, H_2O_2 = 10 mg L^{-1}, and TC = 200 mg L^{-1})

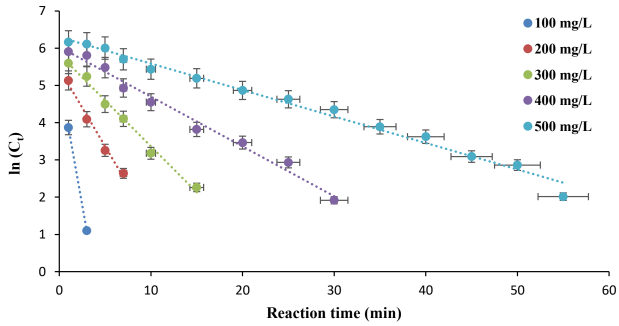

Figure 4. Pseudo-first-order kinetic model for O_3/H_2O_2 process with diverse tetracycline concentrations (pH = 9, H_2O_2 = 10 mg L^{-1}, and O_3 = 1 mg min^{-1})

30 min, reflecting the consumption of radicals during TC degradation, while new radicals were continuously generated, maintaining a nearly steady-state concentration.

Effect of anions on O_3/H_2O_2 process

Inorganic anions, including CO_3^{2-}, SO_4^{2-}, PO_4^{3-}, Cl^-, and NO_3^-, are abundant in natural water and wastewater. Therefore, the effect of the main anions on tetracycline (TC) degradation by the O_3/H_2O_2 process was evaluated using a constant concentration of 8 mM of their corresponding salts (Na_2CO_3, Na_2SO_4, Na_3PO_4, NaCl, and $NaNO_3$) at pH = 9. According to Figure 5, the removal efficiency of TC by the O_3/H_2O_2 process was 99.2% in the absence of anions but decreased to 30.8, 55, 81.8, 83.0, and 86.3% in the presence of NaCl, $NaNO_3$, Na_2SO_4, Na_2CO_3, and Na_3PO_4, respectively. The decrease in TC removal in the presence of anions is likely due to radical scavenging effects, as further discussed in Section 4. Before adding anion, all reaction solutions were adjusted to pH 9. Although the post-addition pH was not directly measured, the concentration of added salts (8 mM) was relatively low, and the reaction medium exhibited sufficient buffering capacity under alkaline conditions. Therefore, significant pH variations were not expected. Moreover, minor fluctuations (±0.1–0.2 pH units) would have negligible influence on $^\bullet$OH formation and TC degradation efficiency in the O_3/H_2O_2 system. Accordingly, the observed inhibition effects are mainly attributed to the intrinsic chemical reactivity and $^\bullet$OH scavenging behavior of the respective anions rather than to pH changes.

Table 1. Pseudo-first-order kinetic model parameters

Initial tetracycline Concentration (mg L^{-1})	R^2	K_{app}
100	0.999	1.36
200	0.984	0.41
300	0.989	0.24
400	0.985	0.133
500	0.987	0.071

Table 2. Time-dependent $^\bullet$OH radical concentrations during O_3/H_2O_2 process

Time (min)	[$^\bullet$OH] (moL L^{-1})
0	1.20×10^{-9}
5	1.15×10^{-9}
10	1.18×10^{-9}
15	1.16×10^{-9}
20	1.14×10^{-9}
25	1.13×10^{-9}
30	1.12×10^{-9}

Biodegradability assessment during O_3/H_2O_2 process

The biological oxygen demand (BOD_5)/chemical oxygen demand (COD) ratio characterizes the biological degradability of organic material. The BOD_5 test was conducted according to standard procedures, which include 5-day incubation. Samples were collected immediately after the O_3/H_2O_2 treatment, and then, incubated for 5 days to measure BOD_5. The treated tetracycline (TC) solution exhibited a significantly higher BOD_5/COD ratio (0.67), indicating improved biodegradability. These results confirm that the O_3/H_2O_2 process effectively converts recalcitrant compounds into more biodegradable intermediates.

Toxicity assessment

During AOP treatment, partially oxidized intermediates can influence both toxicity and biodegradability. Therefore, the toxicity of treated samples was assessed using the inhibition rate of *E. coli*. The inhibition percentage was calculated using the following equation (30):

$$\text{Inhibition }(\%) = \frac{\textit{Control luminescence} - \textit{Sample luminescence}}{\textit{Control luminescence}} \times 100$$

The assay was conducted with an initial tetracycline (TC) concentration of 200 mg L^{-1}.

Comparison various AOPs in TC degradation

Figure 6 presents the TC degradation achieved by different AOP combinations over a 30-minute reaction period. The O_3/H_2O_2 process exhibited the highest removal efficiency (99.2%), followed by O_3/UV (94%) and O_3 alone (69.2%). All measurements were recorded at 5-minute intervals,

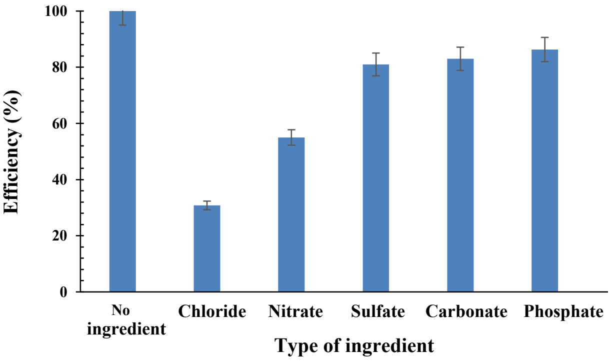

Figure 5. Effect of different anions on tetracycline (TC) removal by the O_3/H_2O_2 process (pH = 9, H_2O_2 = 10 mg L⁻¹, O_3 = 1 mg min⁻¹, TC = 200 mg L⁻¹, anion salts = 8 mM, and reaction time = 15 min)

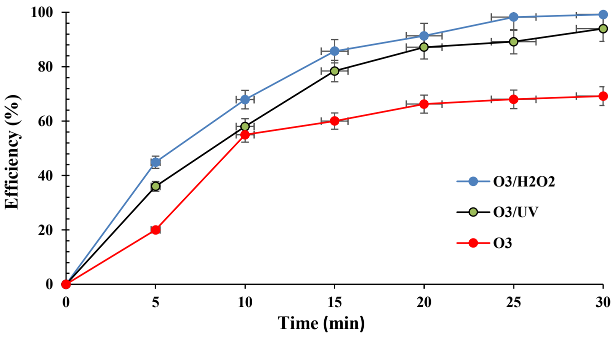

Figure 6. Comparison of various AOPs for tetracycline degradation (TC = 200 mg L⁻¹, pH = 9, O_3 = 1 mg min⁻¹, H_2O_2 = 10 mg L⁻¹ for O_3/H_2O_2, UV irradiation for O₃/UV: 15 W low-pressure mercury lamp, λ = 254 nm, 20 × 430 mm, output = 5 W, 3 cm distance, and reaction time = 30 min)

and the error bars represent the standard deviation from duplicate experiments. These results indicate that combining O_3 with H_2O_2 enhances the degradation efficiency of TC compared to the other processes.

Discussion

Effect of pH

According to the Figure 1, the O_3/H_2O_2 process is more effective in an alkaline solution, as shown by Eq. 2 to 7. The generation of O_3^- and HO^- is the rate-limiting phase in reactions linked to the O_3/H_2O_2 pathway, particularly in acidic conditions.

$$H_2O_2 \rightleftarrows HO_2^- + H^+ \quad k_a = 1/6\times10^{-12} a^+ 25^\circ C \tag{2}$$

$$O_3 + HO_2 \overset{k_1}{\rightarrow} O_3^{-0} + H\dot{O}_2 \quad k_1 = 2/8\times10^6 M^{-1}s^{-1} \tag{3}$$

$$H\dot{O}_2 \rightleftarrows O_2^{-0} + H^+ \quad k_a = 1/6\times10^{-5} \tag{4}$$

$$O_2^{-0} + O_3 \rightarrow O2 + O_3^{-0} \quad k_1 = 1/6\times10^9 M^{-1}s^{-1} \tag{5}$$

$$O_3^{-0} + H^+ \rightarrow H\dot{O}_3 \quad k = 5/2\times10^{10} M^{-1}s^{-1} \tag{6}$$

$$H\dot{O}_3 \rightarrow O_2 + \dot{O}H \quad k = 1/1\times10^5 s^{-1} \tag{7}$$

Depending on the water's pH and chemical structure, ozone may react with different chemicals in water either directly (through molecular ozone) or indirectly (by radical species generated during ozone's decomposition in water). The ozone molecule is a strong oxidant at low pH because it interacts selectively with molecules based on their functional groups, using mechanisms including electrophilic, nucleophilic, and dipolar addition (31). In contrast, OH− enhanced ozone breakdown occurs at pH > 7 due to alkaline circumstances, making radical oxidation (mostly hydroxyl radical) the dominating process at that pH. Since the hydroxyl radical has a higher oxidation potential (E_0 = 2.8 V) than the ozone molecule (E_0 = 2.08 V), direct oxidation is less efficient than radical oxidation. It leads to incomplete oxidation of organic molecules. Additionally, the rise in pH makes it easier for hydrogen peroxide to dissociate, facilitating the interaction between HO_2^- and O_3 (32). Moreover, according to the TC pK_a values (pKa_1, 3.32, pKa_2, 7.78, and pKa_3, 9.58), high TC degradation at solution pH levels above seven can be attributed to the species' increased reactivity with increasing deprotonation, which results in a boost in negative charge density and, consequently, a higher potential for reaction with O_3 (33).

Effect of H_2O_2 concentration

Hydrogen peroxide has been shown to accelerate O_3 decomposition by promoting the formation of oxidizing radicals (34). However, when added in excess (more than 15 mg L⁻¹ in our study), H_2O_2 can accumulate in the reactor and undergo auto decomposition, generating reactive species such as hydroxyl (•OH) and hydroperoxyl ($HO_2^\bullet$) radicals. Excess H_2O_2 may also scavenge •OH radicals (Eqs. 8 and 9) and potentially hinder O_3 decomposition or the production of hydroperoxyl and other less reactive radicals:

$$H_2O_2 + \cdot OH \rightarrow HO_2^\bullet + H_2O \tag{8}$$

$$HO_2^\bullet + \cdot OH \rightarrow O_2 + H_2O \tag{9}$$

Moreover, recombination of •OH radicals can occur under high H_2O_2 concentrations, decreasing the effective concentration of radicals available for pollutant degradation. Studies have shown that although H_2O_2 can enhance •OH production and improve contaminant removal, excessive H_2O_2 can act as a radical scavenger, reducing the overall oxidation efficiency (35,36).

Effect of O_3 dosage on the O_3/H_2O_2 process

As shown in Figure 1, Ozone's oxidation efficacy drops precipitously at low doses, producing unwanted byproducts in large quantities that counteract the treatment's intended purpose. The reaction of TC with hydroxyl radicals produced in solution led to its oxidation. Increasing the ozone flow rate increased the ozone/water contact, which raised the ozone and free radical concentration in the reaction solution and sped up the deterioration of TC. Nonetheless, the reaction rate and TC degradation were unaffected by the amount of ozone flow rate when the ozone concentration in the liquid phase was close to its maximum value (based on the solubility of ozone) (37). During the initial stage of ozonation, when the concentration of pollutants is relatively high and ozone intake is rapid, the quantity of dissolved ozone plays a crucial role in the degradation of TC. Conversely, once the ozone concentration reaches a level where it is either too high compared to the contaminant concentration or the pollutant content has decreased substantially, excess ozone remains. This surplus ozone leads to a decrease in elimination percentage and ozone utilization ratio. At this point, the response is controlled by the concentration of pollutants, and increasing ozone levels further does not enhance degradation. These observations are consistent with those of previous studies on tetracycline degradation in O_3/H_2O_2 systems, which also reported that degradation efficiency initially increases with ozone dosage but reaches a plateau once dissolved ozone exceeds the pollutant concentration (38-42).

Kinetics of O_3/H_2O_2 and O_3 processes and ·OH concentration

The elimination efficiency decreases when the initial concentration of tetracycline in the solution increases due to increased tetracycline molecules that react with the designated hydroxyl radicals (43). The linear correlation (R^2) and K_{app} values for a pseudo-first-order kinetic model of the O_3/H_2O_2 process are shown in Table 1. R^2 values as high as 0.999 showed that the apparent pseudo-first-order model fit the experimental data well. Based on the results, the K_{app} values of TC degradation in the O_3/H_2O_2 process range from 1.36 to 0.071 min^{-1} with increasing starting TC concentrations from 100 to 500 mg L^{-1}. During the process, accelerating competitive interactions between ·OH radicals and the recently generated oxidant by-products of TC causes a drop in k_{app} as TC concentration increases (44). Time-dependent UV-vis spectra of TC solution during O_3/H_2O_2 were generated to visualize the changes in the TC molecule. Before the O_3/H_2O_2 process, as shown in Figure 4, the TC had a dominant band at the typical max = 359 nm and a second, weaker band in the middle of the visible spectrum at 410 nm. Moreover, the strength of the absorption peaks gradually decreased with O_3/H_2O_2 time until they disappeared and became nearly negligible at 10 min. Still, corresponding maximum wavelengths and the formation of new absorption bands remained unchanged. Overall, these results are consistent with those of previous studies on tetracycline degradation in O_3/H_2O_2 systems, which reported pseudo-first-order kinetics, decreasing Kapp with higher initial concentrations, and similar UV-Vis spectral changes (42,45).

Effect of anions on O_3/H_2O_2 process

Because anions compete for hydroxyl radicals (•OH) (reactions 10–13), the effective availability of •OH for oxidizing tetracycline is reduced. The radicals formed from these scavenging reactions (e.g., Cl•, $CO_3^{•-}$) generally have lower oxidation potential than •OH, decreasing overall oxidation efficiency (41,46).

Therefore, the decline in efficiency was most influenced by the chlorine anion with the highest reaction rate ($4.3 \times 10^9\ M^{-1}\ s^{-1}$). Bicarbonate ($HCO_3^-$) is a well-known scavenger of hydroxyl radicals: it rapidly reacts with •OH to produce carbonate radicals ($CO_3^{•-}$) with lower oxidation potential, thereby decreasing the effective •OH availability and inhibiting contaminant degradation—this explains the strong inhibitory effect of HCO_3^- observed in our experiments (47).

$$HCO_3^- + \cdot OH \rightarrow CO_3^{\cdot -} + H_2O \quad K = \frac{8}{5} \times 10^6\ M^{-1}\ s^{-1} \tag{10}$$

$$CL^- + \cdot OH \rightarrow CLOH^{\cdot -} \quad K = 4.3 \times 10^9\ M^{-1}\ s^{-1} \tag{11}$$

$$NO_3^- + \cdot OH \rightarrow NO_3^{\cdot -} + OH^{\cdot} \quad K = 5 \times 10^5\ M^{-1} s^{-1} \tag{12}$$

$$SO_4^{-2} + \cdot OH \rightarrow SO_3^{\cdot -} + O\,H^{\cdot} \quad K = 1/5 \times 10^6\ M^{-1} s^{-1} \tag{13}$$

These inorganic reactive radicals are also more capable of reacting and consuming H_2O_2 (Eqs. 14 and 15). Anion radicals, on the other hand, could not interact with the system's ozone molecules.

$$Cl^{\bullet} + H_2O_2 \rightarrow HO_2^{\cdot} + Cl^- + H^+ \tag{14}$$

$$Cl_2^{-\bullet} + H_2O_2 \rightarrow HO_2^{\cdot} + 2Cl^- + H^+ \tag{15}$$

On the one hand, varying types of anions cause hydroxyl radical reactions to proceed at different rates, and these rates are sensitive to pH fluctuations. The solution's initial pH increased when bicarbonate and phosphate were present, and it remained elevated throughout the procedure. The buffering action of bicarbonate and phosphate prevented a drastic decrease in pH. The decomposition of O_3 and the production of ·OH radicals, both generated by the relatively high and steady pH value, also improved TC removal. On the other hand, chloride and nitrate diminish system pH, affecting the ozone breakdown rate and lowering system efficiency (38,48).

Toxicity assessment

Based on the results obtained, the peroxone process effluent shows an *E. coli* inhibition rate of about 6.34%, indicating that this process produces effluents with the lowest residual toxicity among the tested systems. The reduced inhibition rate suggests that the O_3/H_2O_2 process can significantly mitigate the toxicity of tetracycline-containing wastewater (49,50). This reduction in toxicity can be attributed to the formation of hydroxyl radicals (•OH), which are highly reactive oxidants capable of degrading and mineralizing complex antibiotic molecules into smaller and less harmful intermediates (51,52). Similar findings have been reported by Gholamzadeh et al. (2025), who demonstrated that the electro-peroxone system effectively enhances antibiotic degradation and reduces microbial inhibition (53). In agreement with these studies, our results confirm that the peroxone process provides efficient detoxification under moderate pollutant concentrations, which may be due to the synergistic effect of ozone and hydrogen peroxide (54). Therefore, the O_3/H_2O_2 system can be considered a promising advanced oxidation process for reducing the ecological risk associated with antibiotic residues in wastewater.

Comparison of various AOPs in TC degradation

Figure 6 displays the TC degradation by various AOPs. During 30 min of operation, the O_3/H_2O_2 process removed 99.2% of TC, the O_3/UV process removed 92.4%, and O_3 alone removed 69.2%. The exposures to O_3 and $^{\bullet}OH$ radicals during conventional ozonation are limited after complete depletion of O_3. In contrast, the O_3/H_2O_2 process produces significantly higher $^{\bullet}OH$ exposure, resulting in greater tetracycline degradation throughout the peroxone process (55,56). Ozone alone generates insufficient hydroxyl radicals to effectively mineralize organic molecules due to the low kinetic constant of the direct ozone–pollutant reaction. However, combining O_3 with UV light rapidly produces large amounts of $\bullet OH$ radicals, enhancing mineralization efficiency. Similarly, adding a small amount of H_2O_2 during ozonation increases $^{\bullet}OH$ generation, allowing the O_3/H_2O_2 system to outperform conventional ozonation. All processes in this study were conducted under identical conditions: $TC = 200 \text{ mg L}^{-1}$, $pH = 9$, $O_3 = 1 \text{ mg min}^{-1}$, reaction time = 30 min. For the O_3/H_2O_2 process, $H_2O_2 = 10 \text{ mg L}^{-1}$ was added. For the O_3/UV process, a cylindrical reactor fitted with a vertically mounted 15 W low-pressure mercury UV lamp ($\lambda = 254$ nm, 20×430 mm, output = 5 W) with an average irradiation distance of 3 cm was used. Measurements were recorded every 5 minutes (57). To contextualize our findings, Table 3 presents representative studies on tetracycline (TC) removal using various AOPs, including their operating conditions and removal efficiencies. This comparison demonstrates that the O_3/H_2O_2 process in the present study (99.2% removal in 30 min at pH 9) achieves one of the highest reported efficiencies under similar laboratory conditions, highlighting the effectiveness of the proposed system (Table 3).

Conclusion

The efficiency of various advanced oxidation processes (AOPs) for tetracycline (TC) degradation was investigated, with particular emphasis on the O_3/H_2O_2 (Peroxone) system. The results clearly demonstrate that the O_3/H_2O_2 process performs most effectively under neutral to alkaline conditions. Under the optimum operating parameters ($pH = 9$, $H_2O_2 = 10 \text{ mg L}^{-1}$, $O_3 = 1 \text{ mg L}^{-1}$, and reaction time = 30 min), a removal efficiency of 99.2% was achieved. Under identical conditions, the comparative performance of the tested AOPs followed the order $O_3/H_2O_2 > O_3/UV > O_3$. Among the evaluated anions, chloride exhibited the strongest inhibitory effect, decreasing the removal efficiency by approximately 70%. The O_3/H_2O_2 process also enhanced wastewater biodegradability, reflected by the maximum BOD_5/COD ratio of 0.67, and reduced toxicity, with *E. coli* inhibition declining by 6.34%.

These results indicate that the Peroxone system not only enhances pollutant removal but also promotes biodegradability and detoxification of tetracycline-containing wastewater. Importantly, the findings highlight the potential of the O_3/H_2O_2 process as a practical, energy-efficient pre-treatment technology for industrial effluents containing antibiotics. Future studies should focus on operational feasibility, energy requirements, cost considerations, and performance in real water matrices to support large-scale implementation and evaluate practical applicability.

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Authors' Contribution

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Competing Interests

The authors declare no conflicts of interest.

Ethical Approval

This study was conducted with the financial support and approval of Urmia University of Medical Sciences (Ethical code: IR.UMSU.REC.1399.353).

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Table 3. Comparison of Tetracycline Removal by Various Advanced Oxidation Processes (AOPs) under Different Operating Conditions

Process	Conditions (pH, dose, time, initial TC)	Removal (%)	Reference
O_3/H_2O_2	pH 9; $H_2O_2 = 10 \text{ mg L}^{-1}$; $O_3 = 1 \text{ mg min}^{-1}$; 30 min; $TC = 200 \text{ mg L}^{-1}$	99.2	This study
Ultrafine-bubble O_3	pH 6.5–11; O_3 flow 40 mL min^{-1}; 30–60 min; $TC = 100–500 \text{ mg L}^{-1}$	up to 98.3	(58)
Catalytic ozonation (Co_3O_4/Si-ore + O_3)	pH 7.0; $O_3 \approx 1 \text{ mg L}^{-1}$; 25 min; $TC = 30 \text{ mg L}^{-1}$	93.2	(59)
UV (254 nm) and VUV photolysis	various pH; 254 nm; evaluated by fluence; TC photolysis in water	variable (high at 254 nm)	(60)
O_3 micro/nano-bubbles	micro/nano-bubble ozonation; 30 min; $TC = 10–100 \text{ mg L}^{-1}$	≈90	(61)

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