Ozonation Treatment for the Removal of Tetracycline in a Simulated Recirculating Aquaculture System

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This study examined tetracycline (TC) contamination in aquaculture and evaluated the efficiency of ozonation in removing TC from a recirculating aquaculture system (RAS). Ozonation parameters such as pH, contact time, and ozone dose were investigated in a laboratory-scale setup of a commercial RAS system. The study employed an ozone generator and ultraviolet-visible (UV-Vis) spectroscopy for analysis. The results show that the highest efficiency (84 %) was at an alkaline pH (pH 10), while a lower pH (pH 4) showed a decrease in efficiency (69 %). Concentration-dependent trends revealed reduced efficiency at higher initial TC concentrations, yet consistent degradation of at least 69 % for all concentrations after 30 min. Post-ozonation processes such as chemical oxygen demand (COD), biological oxygen demand (BOD_5) and total organic carbon (TOC) were measured at 27.2 mg/L, 3.9 mg/L and 3.9 mg/L, respectively. These findings suggest that future studies should focus on the application of catalytic ozonation or a combination of ozonation with other conventional treatments such as biodegradation or filtration, in order to achieve complete degradation of this toxic organic contaminant.

Keywords: Advanced oxidation processes, degradation, ozonation, recirculating aquaculture systems, tetracycline

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The rapid expansion of aquaculture has played a crucial role in meeting the growing global demand for protein-rich food. In the modern world, aquaculture's significance as a food, employment, and economic source has grown. Marine life offers a vital protein source for food security, particularly in developing nations, which is crucial as the global population approaches 9.7 billion by 2050 [1]. With China leading at 35 % of global fish production in 2018, the fishing sector's growth is anticipated in developing nations. Asia dominates aquaculture, representing 92 % of global production in 2020 [2]. Aquaculture's history spans freshwater, brackish water, and marine environments [3]. Malaysia initiated aquaculture in the 1920s with Chinese carp. The country's aquaculture market value reached RM 3 billion in 2017, with popular species like catfish and tilapia [4]. Marine aquaculture thrives in Sabah, Borneo, which exports seafood like tiger prawns and seaweed [5]. Modern aquaculture systems, including recirculating aquaculture systems (RAS), are designed to enhance water-use efficiency and limit environmental discharge. Nevertheless, the high stocking densities characteristic of these systems often necessitates the use of veterinary pharmaceuticals, particularly antibiotics, to prevent and manage bacterial infections. Antibiotics can kill or slow the growth of bacteria, making them effective disease management methods in aquaculture. In animal husbandry, it is used to stimulate growth; in the medical field, it is used to cure and prevent bacterial infections [6]. Antibiotics are classified by chemical composition, mechanism of action, therapeutic index, and administration methods [7, 8]. The classes of antibiotics most frequently detected in Malaysian aquaculture operations are tetracycline (TC), sulfonamides, and quinolones, indicating a widespread distribution of antibiotics [9, 10]. Among these, TC is used extensively because it offers broad-spectrum antimicrobial activity, is cost-effective, and performs well against many common pathogens found in aquatic environments [11, 12]. Figure 1 depicts the chemical structure of TC.

Nevertheless, the use of antibiotics may cause residual drugs to accumulate in the water and sediment. The development of antibiotic-resistant bacteria can adversely affect both aquatic ecosystems and human health [14, 15]. TC accumulates in the food chain and poisons microbes, creating antibiotic resistance. It affects intestinal microbial flora and

drinking and irrigation water. Residual antibiotics in aquatic environments can negatively impact aquatic life, leading to changes in microbial communities, interference with essential biological processes, and the emergence of antibiotic-resistant bacteria [11, 16]. Thus, instead of outlawing antibiotics, the aquaculture industry should promote its scientific use and suggest appropriate treatment of residual antibiotics [17]. Previous studies conducted at marine aquaculture farms in Peninsular Malaysia detected TC at concentrations ranging from below the limit of quantification (<LOQ) to 25.6 ng/L [11]. Closed-loop recirculating aquaculture systems (RAS) efficiently apply antibiotics and remove antibiotic residues. Unlike the open system that makes antibiotic waste control difficult, RAS requires less land and water than conventional fish-catching methods in which waste is recycled or turned into non-toxic products. However, the accumulation of antibiotics in RAS can have a detrimental effect on the ecology, water quality, and fish health. According to Chen et al. [18], antibiotic concentrations increased with RAS age. This shows that antibiotics gradually accumulate in the water over time. Antibiotic resistance genes (ARGs) can penetrate the biofilter and spread. Thus, it is crucial to develop long-lasting and effective techniques for removing residual antibiotics from RAS [17, 18]. Conventional wastewater treatment has employed physical, physicochemical, adsorption and biological techniques, but they are ineffective at eradicating various pollutants, including organic and inorganic compounds [19-21]. Hence, developing new strategies for effectively removing pollutants from water is vital [22, 23]. Advanced oxidation processes (AOPs) have emerged as a promising technology for the removal of antibiotics from water and wastewater, and they offer a scalable solution for wastewater treatment due to their cost-effectiveness and ease of operation.

Among the various AOPs, ozonation has attracted considerable attention due to its relatively simple operation, strong oxidizing power, applicability at ambient temperature and pressure, and formation of secondary radicals leading to the breakdown of complex molecules [24, 25]. Since the early 1900s, ozonation has been used to treat water, initially for drinking. As an alternative to traditional treatment techniques, ozonation methods generate hydroxyl radicals, which can be used as an alternative to conventional treatment methods [26]. Ozone-based oxidation processes may proceed in two ways, ozonolysis or ozonation. In the case of ozonolysis, O_3 molecules directly attack the specific functional group (mostly double bonds or aromatic rings, especially in acidic conditions). On the other hand, in ozonation hydroxyl radicals (•OH) are formed from ozone decomposition which occurs in neutral or alkaline conditions. These radicals attack the organic pollutants and convert them into their simplest forms (H_2O and CO_2); this is why ozonation is preferred over ozonolysis [27]. However, its application in removing antibiotics from recirculating aquaculture systems (RAS) remains limited and requires further optimization [28].

Therefore, the present study investigates the effectiveness of an ozonation treatment procedure in removing residual antibiotics from a laboratory-scale simulation of an RAS system and its impact on water quality. A comprehensive investigation of the influences of the independent variables, including initial TC concentration, pH and contact time, is still needed, and the present study attempts to fill this void. In addition to TC removal, changes in chemical oxygen demand (COD), total organic carbon (TOC), and biochemical oxygen demand (BOD_5) are evaluated and used to assess the organic pollutants mineralization by the ozonation treatments process. The objective of the current study is to investigate the degradation of TC by the ozonation treatment process. This study also discusses and evaluates whether ozonation alone is sufficient for its mineralization.

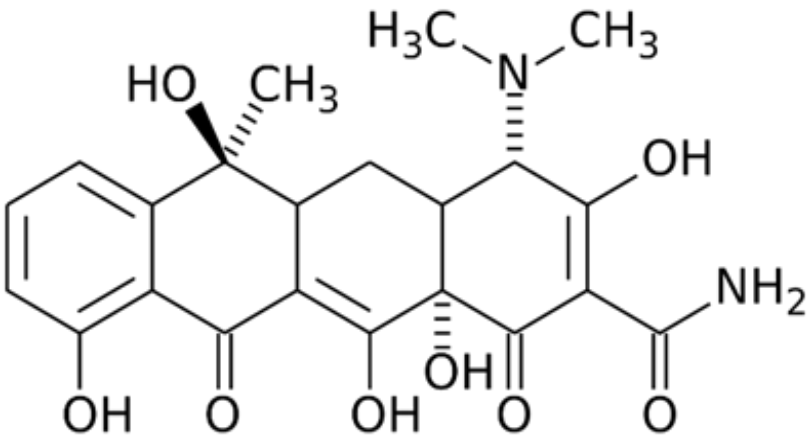

Figure 1. Chemical structure of tetracycline [13].

MATERIALS AND METHODOLOGY

Materials

TC Powder (98%) obtained from Aldrich was used without further purification. Ozone was produced using a lab-scale generator (output capacity 400 mg h^{-1}) and supplied to the reactor under ambient pressure conditions (1 atm). All experiments were carried out at a constant concentration and volume of ozone gas and at constant pressure. The experimental duration was 30 min for all parameters tested. The experiments were conducted in batches and repeated in triplicate. Experimental errors were within 2 % of the reported average value of the extent of degradation.

Preparation of Simulated Wastewater Sample

The initial concentrations of TC used in the experiments were 3 ppm, 6 ppm, 9 ppm, 12 ppm and 15 ppm, prepared by dilution from a stock standard solution of 100 ppm. To test the effects of pH, the solutions were adjusted to pH 4, pH 7 or pH 10 by adding sodium hydroxide (NaOH) or hydrochloric acid (HCl). After treatment via ozonation, the TC solutions were analyzed with an ultraviolet-visible (UV-Vis) spectrometer (Agilent Cary 3500) to determine the extent of degradation. After the optimized parameters were determined, chemical oxygen demand (COD), biological oxygen demand (BOD_5) and total organic carbon (TOC) analyses were carried out.

Experimental Setup and Parameters

The ozonation treatment process for the degradation of TC was carried out using the experimental setup shown in Figure 2. Ozone was generated by an ozone generator (Vroex Lab, China) that used ambient air to produce ozone. Ozone was introduced into the bottom of the reactor containing the sample solution of TC. A magnetic stirrer was added to the reaction chamber to ensure a better distribution of ozone in the chamber. Glass vials were prepared and labelled for the extraction of the sample. Clean syringes were used to extract the sample at pre-determined intervals.

The initial concentration of the TC antibiotic was first established to ensure consistent and reliable experimental conditions. The experiment was performed at different initial concentrations, of 3, 6, 9, 12, and 15 ppm, while keeping other parameters constant, such as the natural pH. 5 mL of each sample was extracted to be analysed at 2 min intervals for the first 10, 20, and 30 min. The treatment was carried out in triplicate, the average result was tabulated, and graphs of concentration against time and % degradation against time at different initial concentrations were plotted. The effect of contact time on the degradation of the TC was studied using various contact times of 2, 4, 6, 8, 10, 20, and 30 min, on 5 mL of a 15 ppm sample at its natural pH. The treatment was carried out in triplicate, the average result was tabulated, and graphs of concentration against time and % degradation against time were plotted.

The effect of pH on TC degradation in the solution was also investigated. The pH of simulated wastewater was altered to the appropriate level by adding drops of 0.1 M of NaOH or H_2SO_4. The effect of pH was observed at pH 4, 7 and 10. 5 mL of a 15 ppm sample was analysed at 2 min intervals for the first 10, 20 and 30 min. The treatment was carried out in triplicate, the average result was tabulated, and graphs of concentration against time and % degradation against time at different pH were plotted.

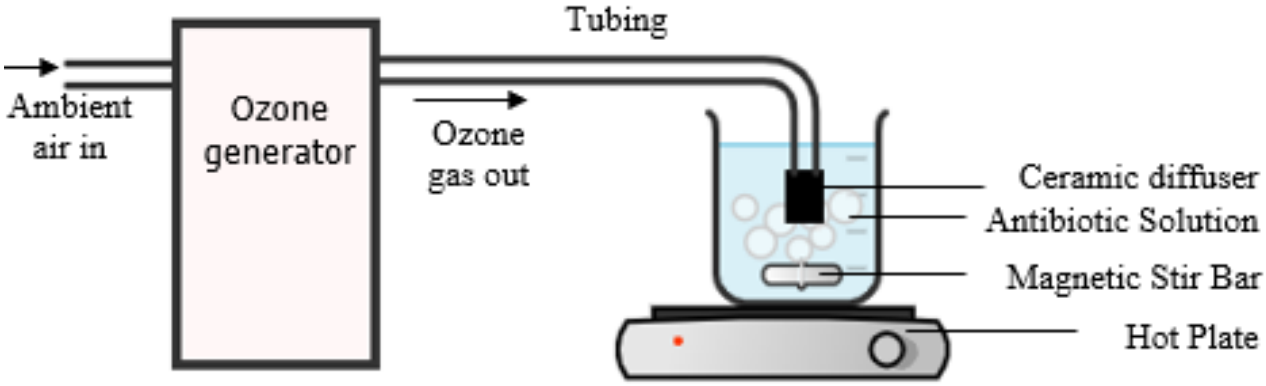

Figure 2. Experimental setup for TC degradation by ozonation treatment.

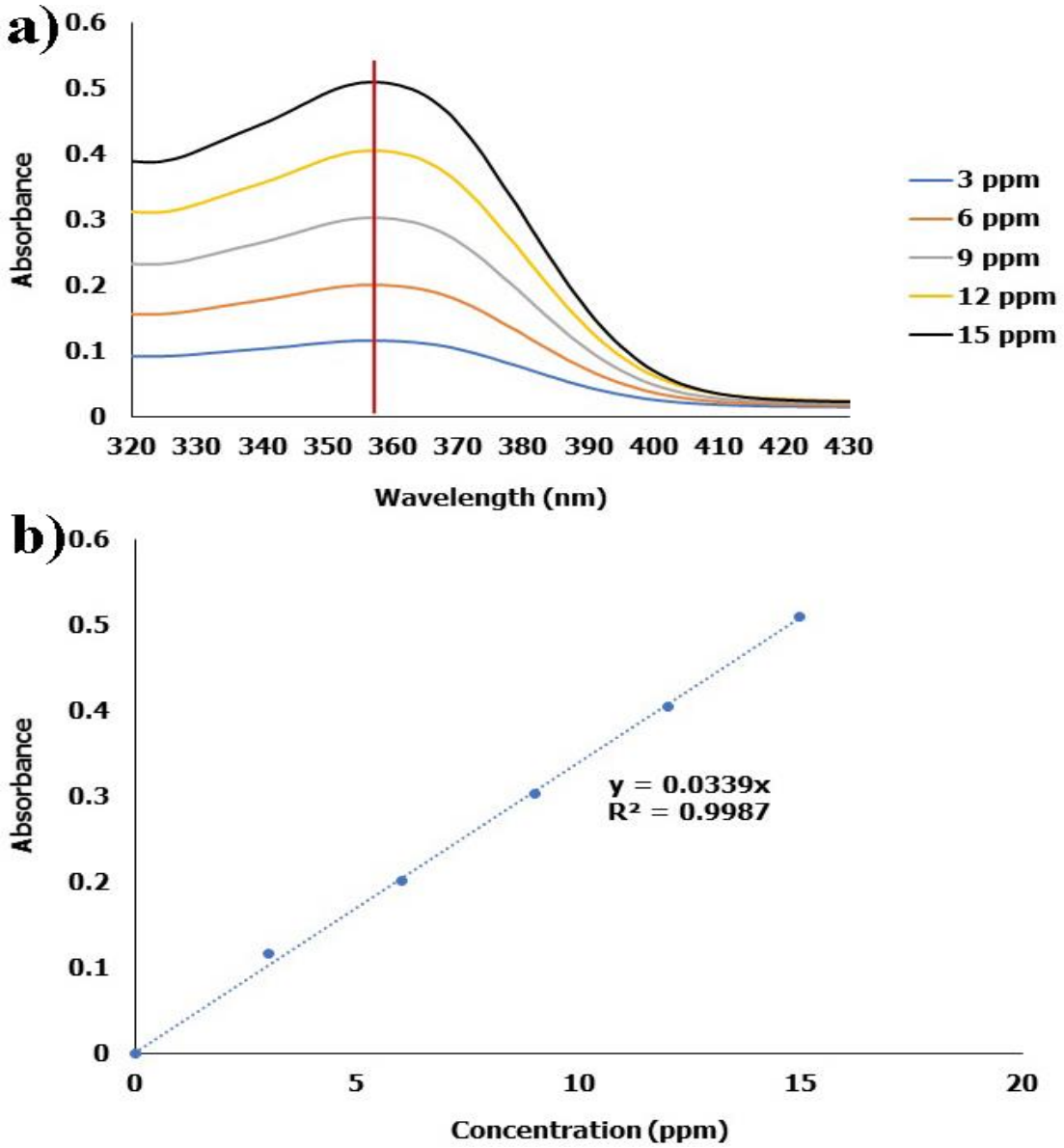

Figure 3. (a) UV–Vis absorbance spectra of TC (3-15 ppm); (b) calibration curve of TC at λ_{max} = 357 nm.

Analytical Method

Before conducting the degradation experiments, it was necessary to determine the λ_{max} values of the target pollutants. Figure 3 depicts the maximum absorbance wavelength (λ_{max}) of TC at 357 nm as determined by UV–Vis spectroscopy, together with the corresponding calibration curve. The calibration curve of TC at 357 nm showed a good regression coefficient ($R^2 = 0.9987$) indicating linearity and compliance with the Beer-Lambert law.

COD Before and After Ozonation

COD analysis was conducted using a HACH DR6000 COD Spectrophotometer and HACH DRB200 COD Digester, following the HACH 8000 COD standard procedure. This was done to measure the difference in the quantity of organic matter before and after treatment.

The sample was digested for two hours in concentrated H_2SO_4 and $K_2Cr_2O_7$, a strong oxidizing agent. The digestion was done in the COD reactor block with heating to increase oxidation of the organic component. During this process, dichromate ions ($Cr_2O_7^{2-}$) were reduced to chromic ions (Cr^{3+}). After digestion the solution was cooled and diluted for spectrophotometric analysis. For the colorimetric determination, a diluted quantity of the sample was transferred to a cuvette. The absorbance of the sample at a specific wavelength was measured using the

HACH DR6000. Subsequently, the calibration curve was used to measure the COD value, using absorbance measurements.

BOD Before and After Ozonation

BOD analysis was performed using a benchtop dissolved oxygen (DO) meter to measure the initial and final dissolved oxygen levels in the water sample. The BOD incubator provided the necessary conditions for microorganisms to consume oxygen during the incubation period. The BOD_5 test was conducted according to the APHA 5120B standard method in order to calculate the difference in dissolved oxygen content before and after incubation for 5 days. The DO concentration should have a standard of 20-30 mg/L for the BOD values to be acceptable [29].

TOC Before and After Ozonation

The total organic carbon (TOC) content was analyzed using the APHA 5310B high temperature combustion method. The sample was first combusted in the presence of an oxidative catalyst to convert all the organic carbon into CO_2 while evaporating off H_2O. The concentration of CO_2 was measured by nondispersive infrared (NDIR). The inorganic contents were removed by acidification and quantified separately. The difference was observed as TOC.

Mathematical Calculations and Statistical Analyses

Degradation efficiency provides an indication of the effectiveness of ozonation treatment in removing or breaking down TC. The degradation efficiency can be calculated using Equation 1 [30, 31].

$$\text{Degradation efficiency (\%)} = \frac{C_o - C_t}{C_o} x100 \quad (1)$$

C_o is the TC concentration at time zero, and C_t is the TC concentration at time t. The degradation rate can be displayed as a function of the ozonation reaction time. The remaining concentration during each sample interval can be calculated using the gradient value (m) of the linear equation of the straight line obtained from the plotted calibration curve.

The remaining concentration can be calculated using Equation 2:

$$\text{Concentration} = \frac{\text{Molar absorptivity}}{\text{Absorbance}} \quad (2)$$

Understanding the reaction kinetics is essential for optimizing ozonation treatment and predicting removal efficiency under different conditions. Multiple studies have demonstrated that the breakdown of antibiotics through ozonation follows a pseudo-first-order reaction [32-34]. However, this may also vary depending on factors such as the type of antibiotic, initial concentration, pH, temperature, and the presence of other compounds in the system [32, 35, 36]. The kinetic equation for the pseudo-first-order reaction was determined using Equation 4. Concentration versus time (t) data were regressed using this equation, yielding the degree of ozonation and the rate constant of the reaction [32, 37, 38].

$$\ln\left(\frac{C_t}{C_o}\right) = -kt \quad (4)$$

where C_o (mg/L) is the initial concentration at time 0, C_t (mg/L) is the concentration at time t, and the first-order rate constant, k (1/min) was computed using the slope of the best-fit line of the plot of $\ln\left(\frac{C_t}{C_o}\right)$ versus reaction time (t) [39].

Biological oxygen demand (BOD) was calculated using Equation 5 below.

$$BOD(mg) = \frac{D_1 - D_2}{P} \quad (5)$$

where D_1 is the initial DO concentration of the sample, D_2 is the DO concentration after 5 days of incubation, and P is the volumetric fraction of the sample used.

RESULTS AND DISCUSSION

Effect of Initial TC Concentration

Figure 4(a) depicts the change in antibiotic concentration over time for different initial TC concentrations. Figure 4(b) illustrates the degradation of the antibiotic over time at different initial TC concentrations. Notably, the results reveal a concentration-dependent trend, where higher initial concentrations correlated with a reduced percentage degradation at each time interval. This trend is noticeable as early as 2 min, with percentage degradation values ranging from 10.80 % to 45.69 %, highlighting the sensitivity of ozonation to initial TC concentration. After 30 min, the degradation efficiencies reinforced this trend, with the 3 ppm initial concentration achieving a higher efficiency (78 %) than the 15 ppm concentration (69 %). This finding demonstrates that a higher amount of TC was oxidised by ozone when the initial concentration was smaller. Gulnaz & Sezer [34] and Wang *et al.* [40] also observed similar findings regarding the impact of initial antibiotic concentration on its degradation by ozonation. Raising the initial concentration of TC leads to a rise in the concentration of breakdown intermediates. Consequently, additional ozone is required to remove and oxidise these by-products [33, 39].

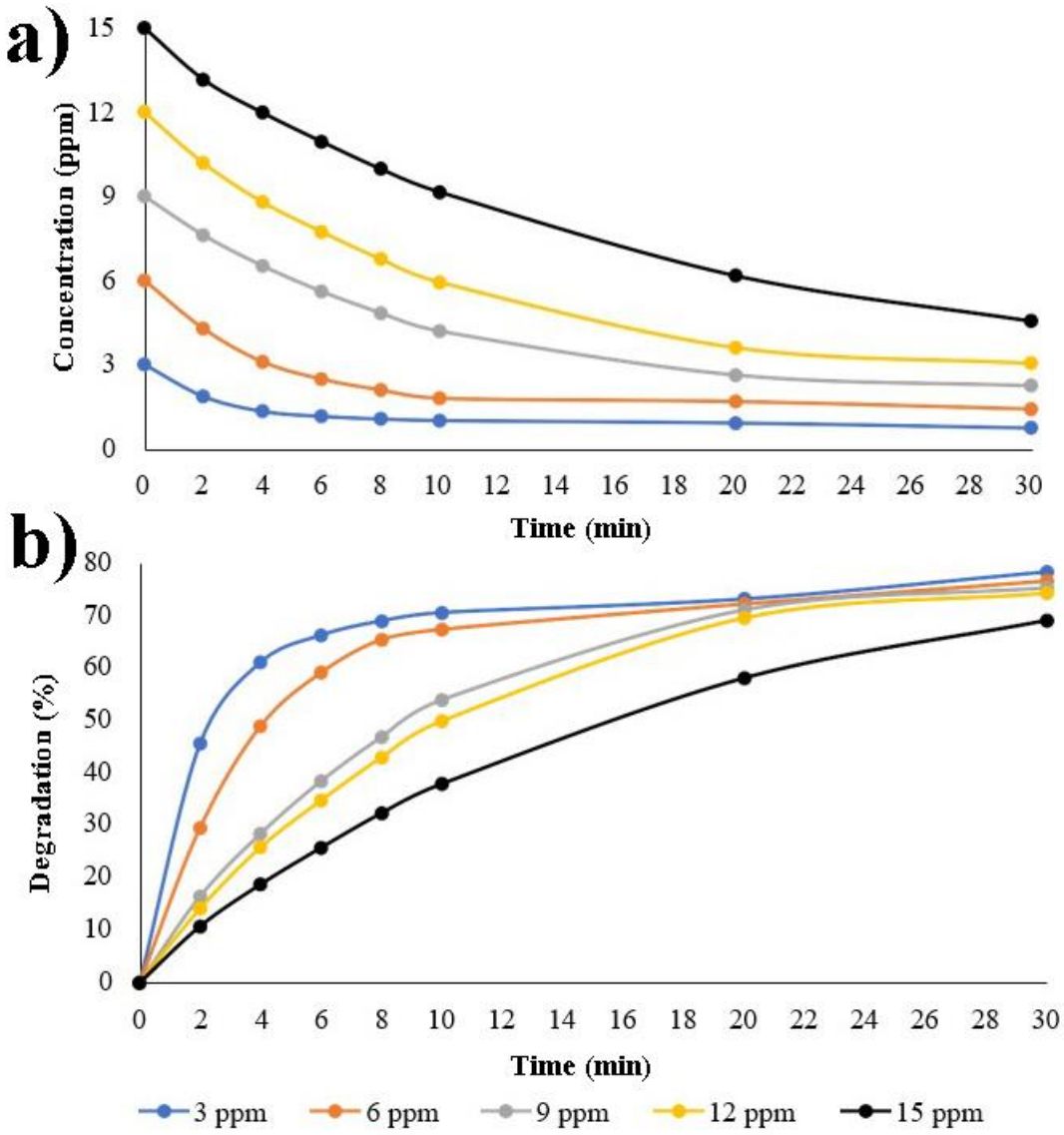

Figure 4. (a) TC concentration versus time; (b) percentage degradation of TC versus time (TC = 3-15 ppm, pH 5, ozone output = 400 mg h^{-1}, reaction time = 30 min).

The decreasing rate of initial concentrations suggests a potential saturation effect, implying a point at which the system becomes less responsive to further increases in concentration. This implies that as initial concentrations increase, the reaction kinetics may approach a plateau, suggesting that certain concentrations may exceed the optimal range for efficient ozonolytic degradation. In addition, this finding indicates that lower initial concentrations may be more efficiently treated within the studied timeframe, while higher concentrations may require extended treatment durations for optimal degradation. As Wang et al. [41] reported, the degradation of various antibiotics (ciprofloxacin, ofloxacin, trimethoprim, and norfloxacin) and some antimicrobials using an ozonation based oxidation process was dependent upon treatment time. The results showed that the highly ozone-reactive antibiotics were removed quickly, leaving the less ozone-reactive antibiotics because they require more time and rely more on hydroxyl radicals (•OH). Mehrjouei et al. [42] and Tahergorabi et al. [43] both reported similar findings, illustrating an inverse correlation between antibiotic concentration and degradation efficiency. Specifically, they found that an increase in the initial concentration of pollutants in water resulted in a rise in oxidation rate via ozonation. This could be attributed to the increased accessibility of pollutants for oxidative reactions involving active oxidizing species. The sensitivity of ozonation to initial concentration is attributed to the availability of ozone radicals and their reactivity with the target pollutants. Higher concentrations may overwhelm the oxidizing capacity of ozone, resulting in diminished degradation rates. The degradation of

antibiotics depends upon the concentration of ozone. The greater the ozone supply, more ROS are generated resulting in an increase in degradation, and vice versa. However, degradation of the antibiotic is lessened by increasing its concentration while keeping the ozone supply constant, due to the lower ozone to antibiotic ratio [39]. Despite that, TC was still effectively degraded by the treatment, as all concentrations reached at least 69 % degradation by 30 min.

At lower concentrations (3 ppm and 6 ppm), there was a big difference in the absorption spectra during the first 10 min. The results indicate that at lower TC concentrations, ozonation caused a rapid decreased in concentration, particularly in the early stages of treatment. As the TC concentration increased (9, 12, and 15 ppm), the absorbance values decreased more slowly at the beginning and then continued to decline steadily during the 30 min ozonation treatment process. This demonstrates a more gradual but consistent degradation over the entire treatment duration. This distinction in degradation kinetics between lower and higher initial concentrations provides valuable insights into the complex relationship between TC concentration and ozonolytic degradation efficiency. Faster degradation occurred at lower concentrations, but the process slowed and became more gradual at higher concentrations. These observations contribute to a comprehensive understanding of how varying initial concentrations influence the degradation kinetics of TC during ozonation.

Effect of Contact Time

Figure 5(a) depicts the effect of contact time on antibiotic concentration. Figure 5(b) illustrates the effect of contact time on the percentage degradation of the antibiotic. The results reveal a clear and progressive increase in the percentage of degradation with longer contact times. At 2 min, the degradation was 10.80 %, and this value rose steadily to 69.20 % at the 30 min mark. The results indicate that the ozonation process exhibited a time-dependent efficiency, with a substantial enhancement in the degradation of the antibiotic as the contact time was extended. Primary degradation of TC usually occurred within the first 5-10 min, but complete mineralization required 60 min.

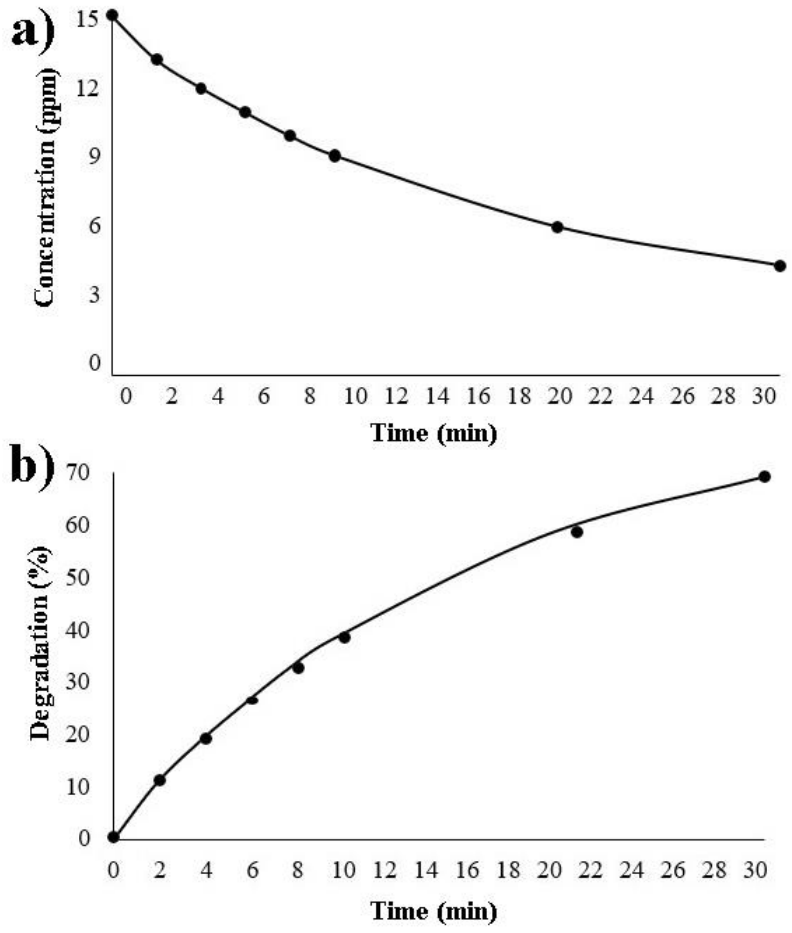

Figure 5. (a) Effect of contact time on TC concentration; (b) effect of contact time on TC degradation (%) (TC = 15 ppm, pH 5, ozone output = 400 mg h^{-1}, reaction time = 30 min).

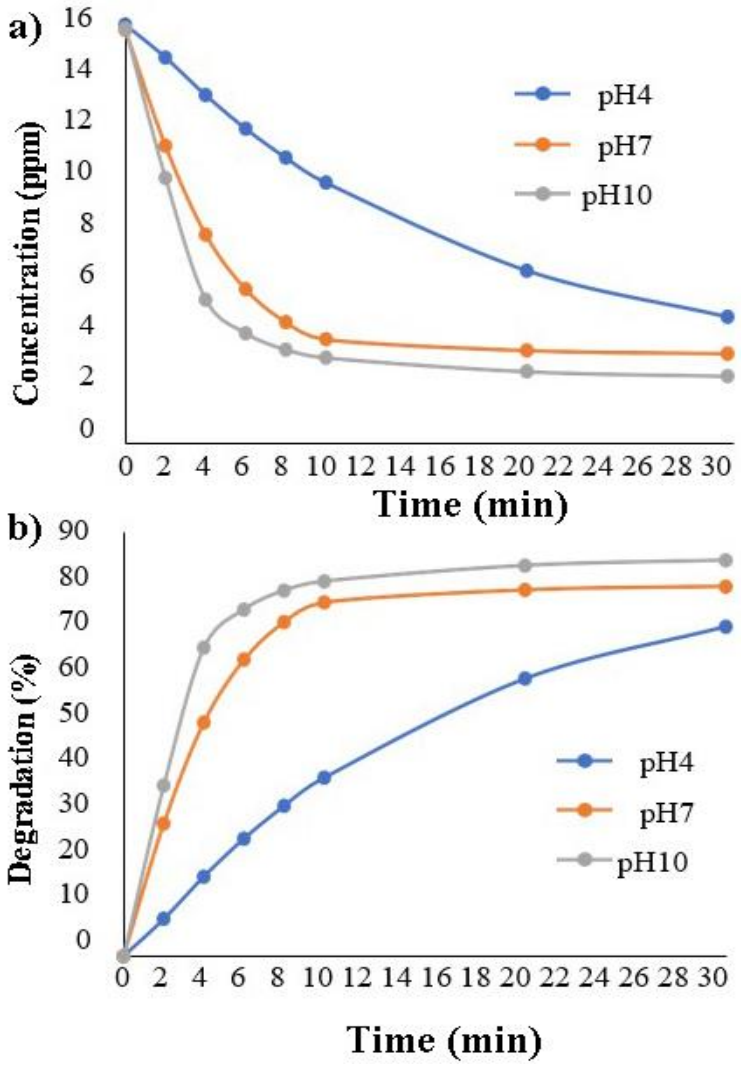

Figure 6. (a) TC concentration versus time at different pH values; (b) TC degradation (%) versus time at different pH (TC = 15 ppm, reaction time = 30 min, ozone output = 400 mg h^{-1}).

The initial stages of the treatment, reflected in the first 10 min, demonstrated a relatively moderate increase in degradation percentages. However, a notable acceleration in degradation occurred subsequently, reaching a significant 58.20 % at 20 min and further progressing to 69.20 % at 30 min. This suggests that the ozonation process had a cumulative and accelerating effect on the breakdown of the antibiotic over time. The observed trend aligns with the typical behaviour of ozone-based treatment processes, where extended contact times allow for a more thorough interaction between ozone and the antibiotic molecules, leading to increased degradation. This result has practical implications for the optimization of wastewater treatment protocols, suggesting that longer contact times can substantially improve the overall efficiency of antibiotic removal through ozonation [41].

Effect of pH

Figure 6(a) depicts the change in antibiotic concentration over time at different pH levels. Figure 6(b) illustrates the degradation of the antibiotic over time at different pH levels. The findings revealed a notable dependence on pH values, with pH 10 consistently demonstrating the highest percentage degradation across numerous intervals of time (2, 4, 6, 8, 10, 20, and 30 min). At pH 10, the antibiotic degradation steadily increased from 36.17 % at 2 min to an impressive 84.09 % at 30 min. A similar study also reported that alkaline conditions promoted more efficient degradation at higher pH levels due to the increased generation of ·OH, contributing to greater degradation compared to acidic environments [19, 36, 44]. In comparison, pH 7 exhibited substantial degradation, starting at 28.14 % and reaching 78.52 %

over the same time frame. At the lowest initial pH of 4, the degradation was notably lower, starting at 7.88 % and peaking at 69.98 % after 30 min. This trend suggests that higher initial pH values enhanced the degradation efficiency, potentially indicating an alkaline environment was more favourable [16, 32, 43, 45]. Ozone undergoes two distinct reactions with organic substances which are influenced by pH: one involves a direct attack at an acidic pH, while the other involves a free radical attack at a basic pH [46]. Ozone and hydroxyl radicals impact the breakdown of TC under neutral and alkaline environments [47]. The lower removal rates at pH 4 may be attributed to the inability of hydroxyl radicals to decompose under acidic conditions. Consequently, direct ozonolytic effects are manifested under acidic pH conditions in which ozone directly attacks the conjugated double bonds of TC [47]. Molecular ozone exhibits a highly selective electrophilic attack specifically targeting nucleophilic carbons, including nitrogen-nitrogen and carbon–carbon multivalent components [34].

Additionally, the indirect reaction pathways, specifically the formation of hydroxyl radicals, are important. The better removal rates at higher pH (pH 10) can be attributed to the increasing concentration of hydroxyl ions with pH, which causes increased reactivity of ozone and the promotion of hydroxyl radical formation. During the ozonolytic reaction, ozone reacts with a hydroxyl ion in the solution and decomposes into a hydroxyl radical; thus, the reaction involving free radicals is preferred [48]. Due to the oxidising nature of both ozone molecules and hydroxyl radicals, it is anticipated that the contaminants are eliminated at a faster rate at pH levels exceeding 7. The hydroxyl radical is a nonselective oxidant and very powerful (2.80 V) compared to regular ozone (2.07 V). It reacts and degrades the antibiotic. Thus, radical oxidation exhibits greater efficiency and speed compared to direct oxidation [34]. At higher pH (pH 7 & pH 10), there was a big difference in the absorption spectra during the first 4 min. This suggests that under these conditions TC degrades rapidly at the beginning of ozonation, showing a significant drop in concentration. In contrast, at lower pH (pH 4), the absorption spectrum showed a more even pattern throughout the entire 30 min period. This demonstrates a more gradual but consistent degradation over the entire treatment duration. This difference in degradation kinetics between lower and higher pH levels provides valuable insights into the complex relationship between pH and ozonolytic degradation efficiency. The initial rapid degradation at higher pH followed by a more constant increase at lower pH underscores the nuanced nature of the ozonation process and its sensitivity to pH. These observations contribute to a comprehensive understanding of how varying pH levels influence the degradation of TC during ozonation. The observed increase in degradation rate at pH 10.0 is consistent with the findings of Khan et al. [12] and Wu et al. [16] who attributed this to the accelerated decomposition of ozone into non-selective OH radicals. This transition from direct O_3 attack to radical-mediated oxidation facilitates the cleavage of the TC four-ring backbone, specifically targeting the C_2-C_3 and C_{11}-C_{12} diketone systems, which are known to be highly reactive sites in ozonation processes.

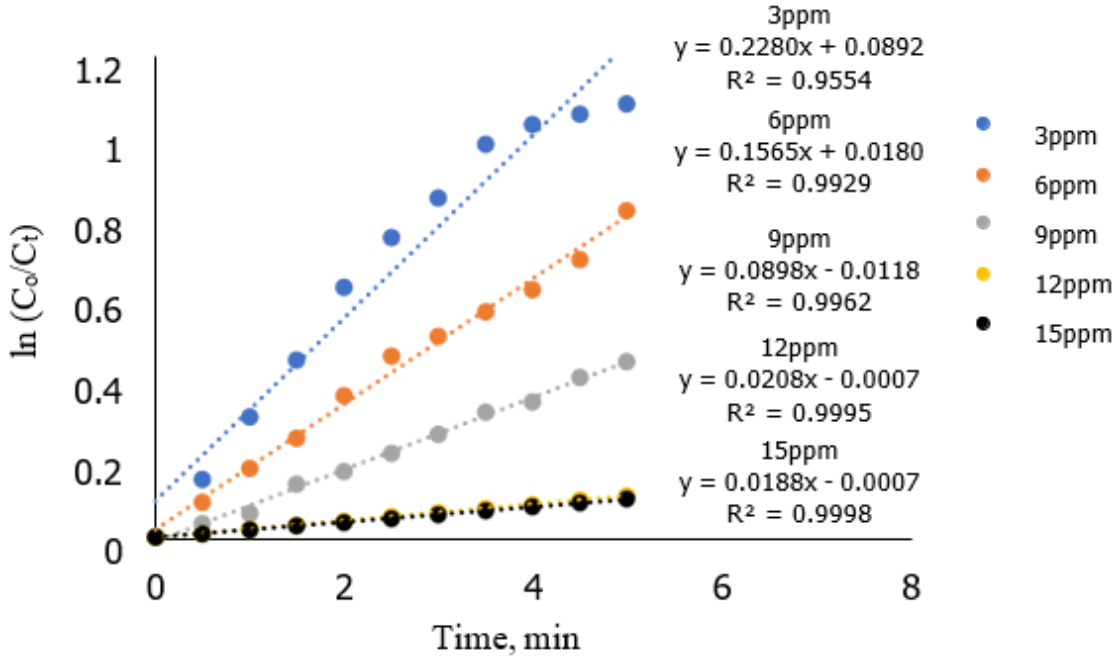

Figure 7. Pseudo-first order linear fit at various antibiotic concentrations.

Kinetic Studies

The degradation of TC was investigated by employing the pseudo first-order kinetic equation. The pseudo first-order rate constants were determined by calculating the slope of the plot of $\ln\left(\frac{C_t}{C_o}\right)$ versus time [32-34]. Figure 7 presents linearized plots of the pseudo-first-order kinetic model. Kim et al. [50] proposed that the degradation kinetics of TC could be characterised using a pseudo-first-order reaction model. Table 1 presents the pseudo-first-order rate constants and regression correlation for TC at various concentrations. Based on Table 1, the first-order rate constant exhibited a decreasing trend as the initial antibiotic concentrations increased. Greater initial concentrations of TC caused ozone to react with a greater number of molecules at lower concentrations. As a result, significantly greater amounts of ozone were needed for elevated substrate concentrations. The pseudo-first-order regression coefficient was greater than 0.9, which indicated the degradation kinetics fit well with this model under the specified test conditions [34]. The degradation kinetics in many advanced oxidation process studies are often described using pseudo-first-order models [51]. It has been previously reported that degradation of TC by ozonation followed pseudo-first-order kinetics [48]. Gulnaz and Sezer et al. reported rate constants ranging from 0.091 to 0.128 min^{-1} across different pH values [34].

Reactive Sites and Expected Pathways of TC Degradation

The susceptibility of TC to ozonation is governed by its multiple electron-rich functional groups. The tricarbonyl system in Ring A and the phenolic diketone system in Rings B and C are the primary targets for electrophilic ozone attack. At alkaline pH, the deprotonation of these groups significantly increases the electron density on the aromatic backbone, facilitating a more rapid degradation. During this process, oxidative reactions progressively target the molecule's aromatic structures and functional moieties, resulting in ring cleavage and the generation of smaller intermediate species. These intermediates subsequently undergo additional oxidation, yielding simpler compounds CO_2 and H_2O. While this study did not identify specific intermediates via MS/MS, the observed kinetics were consistent with the ring-opening and demethylation pathways established by Khan et al. [12] and Wu et al. [16], where OH radicals formed at higher pH conditions drive the breakdown of stable intermediate products that would not degrade otherwise. The proposed mechanism for the degradation of TC and the formation of possible intermediate fragments are shown in Figure 8 and Table 2, respectively.

Table 1. Rate constants and regression correlations for the ozonation of various concentrations of TC.

TC concentration (ppm)	Rate constant (K) min^{-1}	Regression correlation, (R^2)
3	0.2280	0.9554
6	0.1565	0.9929
9	0.0898	0.9962
12	0.0208	0.9995
15	0.0188	0.9998

Table 2. Reactive sites and expected pathways of TC degradation.

Functional Group	Expected Pathway/Action	pH Dependence
Tricarbonyl methane	Hydroxylation and Ring Opening	Highly reactive across all pH levels
Phenolic diketone	Electrophilic attack by O_3	Increased reactivity at pH > 7.7 due to deprotonation.
Dimethyl amino	Demethylation	Susceptible to OH radicals at alkaline pH

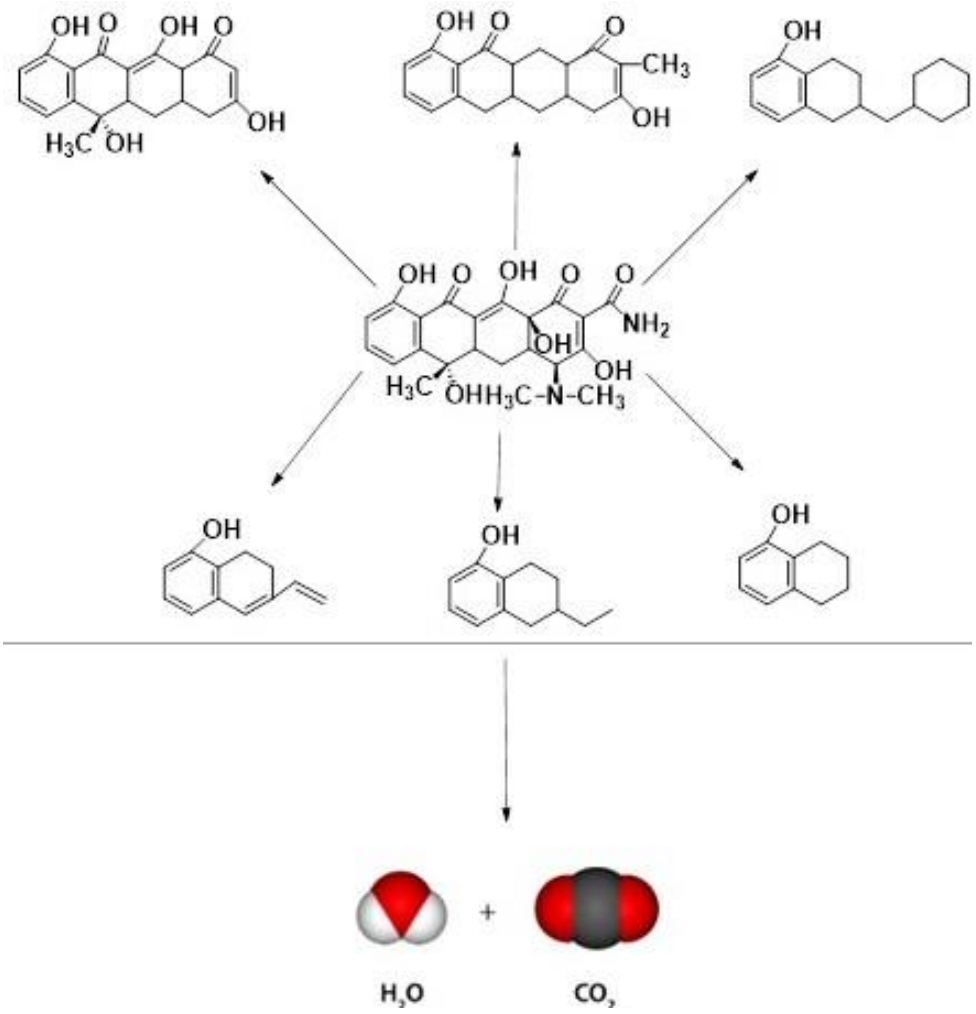

Figure 8. Proposed mechanism for the degradation of TC and formation of possible intermediate fragments.

Changes in COD, BOD_5 and TOC After Ozonation Treatment

COD, BOD_5 and TOC analyses were performed before and after ozonation treatment. Table 3 shows the water quality parameters in a TC solution before and after treatment with ozonation.

Based on the results in Table 2, the COD concentration increased after ozonation, suggesting that the complex nature of ozonation reactions might lead to the formation of by-products or partially oxidised compounds, contributing to COD. However, the rise in COD post-ozonation can be ascribed to the creation of intermediate substances that contribute to the total COD, emphasising the complex nature of ozonation reactions. The complexity of ozonation reactions and the potential for partial oxidation of organic matter underscore the challenges in achieving complete mineralisation. A study by Lei & Li [52] found that effluent still contained COD even after undergoing ozonation treatment, as there were chemicals present in the effluent that were resistant to ozone. The BOD_5 also exhibited a significant increase after ozonation, suggesting that the ozonation process may have transformed or released organic compounds that are more biodegradable. Ozonation can break down complex organic molecules, generating simpler, potentially more biodegradable by-products. These by-products could be intermediates formed during the ozonation process, and they may undergo further microbial degradation, contributing to an increase in BOD_5. Before ozonation BOD/COD<0.04 while after ozonation it was approx. 0.14, about 0.096 (nearly threefold 3.2 times) increment was observed. This is in line with the observed enhancement in the BOD_5/COD ratio from 0.09 to 0.26, of approx. 15 % as reported by [16], Similar to previous reports, TOC removal lagged behind the degradation of the parent compound; for example, TOC removal reached only 40 %, highlighting that mineralization occurred more slowly than the elimination of the parent molecule [53]. Lei & Li [52] suggested that ozone-resistant substances may be decomposed through ozonation, resulting in the formation of biodegradable compounds. To assess the biodegradability of the effluent, formal

BOD_5 measurements were conducted, with the formal BOD_5/COD ratio serving as an illustrative indicator [54]. Similarly, the treatment of pharmaceutical effluents containing antibiotics has been shown to substantially increase the BOD_5/COD ratio (0.08-0.38), further confirming that ozonation pretreatment converted organics into biodegradable intermediates [54]. The increase in acclimated microbiota concentration, demonstrated by [55], further supports higher biodegradation rates post-ozonation.

Similar to COD, the TOC concentration increased after ozonation. TOC quantifies the overall concentration of organic carbon in a water sample and helps to assess the mineralisation of target substances. The rise in TOC can be ascribed to the creation of oxidised by-products during ozonation. Ozonation aims to decompose intricate organic compounds into less complicated and more stable forms, primarily carbon dioxide and water. However, the incomplete oxidation or formation of intermediate by-products that are only partially oxidised may cause an increase in the TOC concentration. A similar study by Gulnaz & Sezer [34] observed that the decline in TOC was slower. This was attributed to the presence of various intermediate organic compounds, such as aldehydes and phenols, which were formed following the ozonolysis of TC. This also aligns with the studies by Wu et al. [16], which noted minimal reduction in the TOC of TC even after extended ozonation, indicating a challenge in mineralisation. Therefore, we can conclude that ozonation had fully destroyed TC, but the oxidation process of the pollutants to CO_2 and H_2O was not complete. The increase in the values of these water quality parameters after ozonation treatment is due to the transformation of organic contaminants, highlighting the importance of monitoring and optimizing the ozonation process for water treatment. The performance of ozonation in this study is consistent with previous reports, as shown in Table 4. These results highlight the practical applicability of ozonation for antibiotic removal. The present study represents a preliminary investigation; in future, we aim to further optimize the conditions for the ozonation treatment process by minimizing reaction times as well as maximizing degradation efficiency.

Table 3. Analysis of water quality parameters of a TC solution before and after ozonation treatment.

Parameter	Before	After	Test Method
COD (mg/L)	22.8	27.2	HACH 8000
BOD5 (mg/L)	<1	3.9	APHA 5120B
TOC (mg/L)	8.35	9.3	APHA 5310B

Table 4. Comparison of the results of the present study with previous literature.

Method	Condition	TC Removal (%)	TOC/ Mineralization	Ref
Ozonation and biodegradation	H_2O_2	100 % (10 min)	Small decrease in TOC was observed	[8]
Ozonation	At different pH (2.2 and 7.0,)	100 % (TC in 4-6 min)	40 % TOC removal	[12]
Ozonation	Micro nano bubbles, pH range (3-11)	90 % (20 min)	Resulting by-products exhibited low toxicity	[56]
Ozonation and photocatalysis	4 mg-16 mg O_3/min	95.3-99.6 % (10 min)	79.7 % & 86.6 % TOC	[57]
Ozonation vs sonolysis		89.8-92.1 % (5 min)	Resulting by- products exhibited higher mineralization	[58]
This study	pH 4-10	69-84 % (30 min)	COD: 27.2 mg/L, BOD_5: 3.9 mg/L, TOC: 3.9 mg/L	

CONCLUSION

This study demonstrated that ozonation was a highly efficient process for TC remediation, achieving 69 % removal within 30 min under optimal conditions. The results indicate a mechanistic shift from direct molecular ozonation to a more potent hydroxyl radical (OH) pathway as the pH increased, which is fundamental for maximizing degradation rates. Although higher initial TC concentrations slightly reduced efficiency, effective degradation was still achieved within a short treatment time. Kinetic modelling confirmed pseudo-first-order behaviour (R^2 >0.9), with the rate constant k = 0.0188 min^{-1} primarily governed by the ozone-to-pollutant molar ratio. The kinetic constant decreased from 0.2280 to 0.0188 min^{-1} (3-15 ppm), with strong correlations (0.9554-0.9998) under all conditions. The process showed better performance under alkaline instead of acidic conditions. The ability to achieve high degradation levels at pH 10 implies that ozonation can be integrated into existing treatment plants with minimal chemical pre-adjustment, offering a cost-effective and scalable solution for antibiotic remediation. While ozonation successfully cleaved the TC parent molecule, the observed limitations in total mineralization suggest that for complete organic carbon removal, this process should serve as a pre-treatment step or be enhanced through catalytic ozonation. These findings provide a practical framework for integrating ozone-based stages into wastewater treatment plants specifically targeting antibiotic residues. The results provide a critical benchmark for industrial wastewater treatment, suggesting that a 30 min contact time is sufficient for primary pollutant removal. The noticeable reduction in COD (27.2 mg/L), BOD_5 (3.9 mg/L), and TOC (3.9 mg/L) values after treatment confirmed an overall improvement in water quality.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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