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# Degradation of tetracycline in aqueous media by ozonation in an internal loop-lift reactor

Yan Wang, Hui Zhang*, Jianhua Zhang, Chen Lu, Qianqian Huang, Jie Wu, Fang Liu

*Department of Environmental Engineering, Wuhan University, P.O. Box C319, Luoyu Road 129#, Wuhan 430079, China*

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ABSTRACT

The degradation of tetracycline by ozone was investigated in this paper. In the laboratory scale experiments, the effect of major parameters, including pH, gas flow rate, gaseous ozone concentration, hydrogen peroxide concentration and hydroxyl radical scavenger (*tert*-butyl alcohol) on the degradation of tetracycline was studied. A pseudo-first order kinetic model was used to simulate the experimental results. The results indicated that the tetracycline degradation rate increased with pH, gaseous ozone concentration and gas flow rate. The addition of hydrogen peroxide or hydroxyl radical scavenger had little effect on tetracycline removal, indicating that the direct oxidation of tetracycline by ozone was dominant process and the radical contribution to the tetracycline oxidation could be neglected. The main intermediates were separated and identified as well as the simple degradation pathway of tetracycline was proposed. The COD removal reached to 35% after 90 min reaction. The acute toxicity experiments illustrated that the *Daphnia magna* mortality reached the maximum after 25 min ozonation and then decreased to zero after 90 min ozonation.



## 1. Introduction

In recent years, an increasing concern focuses on the presence of pharmaceuticals in the aquatic environment. Because there are approximately 3000 different pharmaceuticals used commonly in Europe, including antibiotics and painkillers [1]. According to the report of Union of Concerned Scientists, it is known that the estimated 16,000 tons of antimicrobial compounds are used in US, nearly 93 tons of antibiotics used in New Zealand and 14,600 tons of active antimicrobials used in Kenya [2]. However, these pharmaceuticals are very difficult to be metabolized completely in the body of animals or people. Inappropriate disposal of several drugs continuously contaminated urban wastewaters and effluents from sewage treatment plants (STP) [3], which would result in a widely contaminated range of environmental matrixes, including surface, ground and drinking water, as well as soils [4,5].

Among a variety of pharmaceuticals, antibiotics are most frequently detected in the environment that are difficult to be removed through conventional biological treatment methods [6]. In this work, tetracycline hydrochloride, a well-known class of antibiotics, was chosen as the target contaminant due to its large global consumption in animal food industry to treat, control and prevent infectious diseases [5,7]. Tetracycline is also used as a food additive to improve growth rate of animals at lower cost [1]. Therefore, tetracycline had many different pathways entering the environment, including emissions during the manufacture, formulation, disposal of unused or expired compounds, even transport from field to aquatic environment while agricultural waste had been applied [1,2]. Particularly, in mainland China and Hong Kong the enormous quantities of tetracyclines are produced, imported, and used [8], which are invasive to not just aquatic environment, but also soils and air. Hence, various technologies are employed to degrade tetracycline [9].

Among these technologies, the growing interest has been focused on the application of advanced oxidation process for the treatment of antibiotics in water, such as UV/$H_2O_2$ process [10,11], electrochemical method [12], electro-Fenton process [13], photocatalysis [14] and photo-Fenton-like oxidation [15]. In particular, ozonation is capable of oxidizing organic compounds to simpler and more easily biodegradable compounds [15–17], such as procaine penicillin G [17], amoxicillin [18,19], ceftriaxone sodium [20], macrolide [21], sulfonamide [21,22], penicillin [23] and tetracycline [7,24–27]. However, almost all the researches were performed in conventional bubble column reactors.

Ozonation of tetracycline is a gas–liquid reaction, and mass transfer rate of ozone from gas phase to liquid phase is very critical to the degradation of tetracycline. The internal loop-lift reactor has been regarded as a promising type of gas–liquid reactor due

* Corresponding author. Tel.: +86 27 68775837; fax: +86 27 68778893.
*E-mail address:* eeng@whu.edu.cn (H. Zhang).

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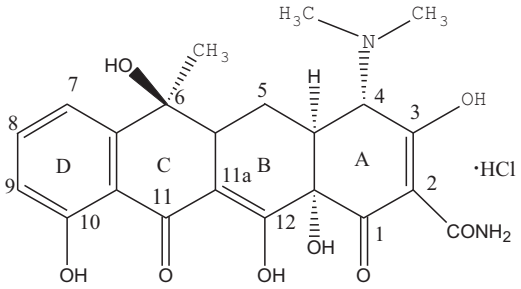
**Table 1**
Chemical structures and relevant data for tetracycline hydrochloride.

| Name | Tetracycline hydrochloride |
|---|---|
| Formula | $C_{22}H_{25}N_2O_8Cl$ |
| Structure |  |
| FW ($g\,mol^{-1}$) | 480.9 |
| $\lambda_{max}$ (nm) | 365 |

to its good mixing with low shear stress and energy consumption as well as its advantages of high gas–liquid mass transfer rate [28]. The aim of this work was to present the experimental results concerning the degradation of tetracycline by ozone in an internal loop-lift reactor. The effect of operating conditions such as pH, gas flow rate, ozone concentration, hydrogen peroxide concentration and hydroxyl radical scavenger on the degradation of tetracycline was investigated. The main intermediate products were identified by liquid chromatography–mass spectrometry with an APCI source operating in the positive ion mode (LC–APCI(+)–MS) technique and a simple degradation pathway of tetracycline was proposed. The biodegradability and the acute toxicity of the tetracycline solution were also investigated during the ozonation process.

## 2. Materials and methods

### 2.1. Materials

The commercial tetracycline hydrochloride (AR Grade, 99%) was used without further purification. The structure and relevant data for tetracycline were shown in Table 1. Acetonitrile and methanol (HPLC Grade) were obtained from Shanghai Sinopharm Chemical Reagent Co. Ltd. (China). Oxalic acid and potassium iodide (AR Grade, 99%) were obtained from Shanghai Zhanyun Chemical Co. Ltd. (China). Hydrogen peroxide (AR Grade, 30%, v/v) was obtained from Shanghai Yuanda Peroxide Co. Ltd. (China). Potassium dichromate (AR Grade, 99.8%) was obtained from Beijing Hongxing Chemical Plant (China). *tert*-Butyl alcohol (AR Grade, 99.9%) was obtained from Chengdu Institute of the Joint Chemical Reagent (China).

### 2.2. Experimental reactor and procedure

Ozone was generated by electric discharge using 99.9% oxygen in a laboratory ozone generator (XFZ-5BI, China). Ozone concentrations in the gas phase and the liquid phase were monitored by the iodometric method with potassium iodide solution [29] and indigo method [30], respectively.

To determine the reaction stoichiometric ratio $z$ between ozone and tetracycline, the experiments were conducted in a 0.1 L flask. Excess tetracycline and ozone solution were mixed quickly and ozone was completely consumed at an instantaneous rate. The ozone solution was prepared by an ozone–oxygen mixture stream bubbling into deionized water until saturation. The reaction ratio is calculated by the following equation:

$$z = \frac{[O_3]_0}{[TC]_0 - [TC]f} \quad (1)$$

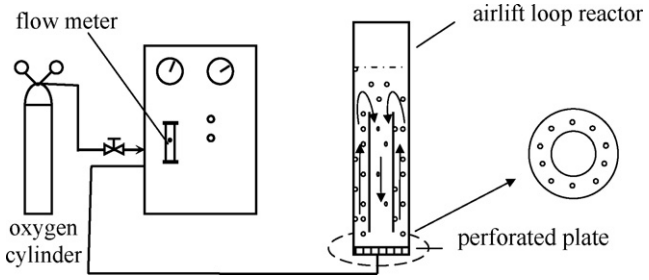



**Fig. 1.** Experimental set up.

where $[O_3]_0$ is the initial concentration of ozone, $[TC]_0$ is the initial concentration of tetracycline and $[TC]f$ is the final concentration of tetracycline.

In the ozonation experiments, a stock solution of tetracycline was prepared fresh in buffer solution to keep the pH stable during the reaction process. The buffer solution was composed of 0.025 mol $L^{-1}$ $Na_2HPO_4$ and $KH_2PO_4$. The initial tetracycline concentration was set at 2.08 mmol $L^{-1}$. The solution pH was measured with a Mettler Toledo FE20 pH-meter. Semi-batch experiments were performed in an internal loop-lift reactor containing 500 mL solution (Fig. 1). This reactor consists of coaxial cylinder with inner and outer diameters of 56 and 100 mm and the heights of these two cylinders were 250 and 340 mm, respectively. The inner cylinder was located at a distance of 5 mm from the bottom of the reactor. A perforated plate was designed and located at the bottom of the riser. During the experiments, the ozone–oxygen mixture was continuously bubbled into the solution throughout the perforated plate.

### 2.3. Analysis methods

The samples were taken by syringe and filtered through 0.45 μm membranes at pre-selected time intervals, and then measured using high-performance liquid chromatography (HPLC). HPLC was consisted of a LC-20AB pump, a Shimadzu HPLC system manager program and a SPD-10A UV–vis detector. The UV–vis detector was set at the maximum absorption wavelength for 365 nm determined using a Shimadzu UV-1700 spectrophotometer to scan from 200 to 800 nm. Aliquots of 20 μL were injected manually using a model Rheodyne 7725i injection valve (Rheodyne, Berkeley, CA, USA). A shim-pack VP-ODS C18 (4.6 mm × 250 mm) packed with 5 μm spherical particles was used for separation. An acetonitrile/0.01 mol $L^{-1}$ aqueous oxalic acid (31:69, v/v) mixture was used as mobile phase at room temperature with a constant flow rate of 1.0 mL $min^{-1}$. Chemical oxygen demand (COD) was determined using closed reflux titrimetric method based on the standard methods [31]. The five-day biochemical oxygen demand ($BOD_5$) was measured by the respirometric method (WTW Oxitop®IS6, Germany).

The acute toxicities were determined with *Daphnia magna* immobilization essays. These tests were performed in accordance with testing conditions prescribed by OECD Guideline 202 [32]. *D. magna* was cultured in laboratory for more than three generations. The acute toxicity experiments were carried out in 50-mL-capacity test beakers using 25 24-h-old *D. magna* which were divided to five groups. Four groups were performed as test group while one group as the blank group. They were set in the incubator along with testing samples. The incubator was set at 20 °C in a 16 h light–8 h dark cycle. No foods were given during the acute toxic test. Surviving and mobile animals were counted after 48 h.

The intermediates during the reaction were detected using an Agilent 1100 instrument connected with an APCI source operating in the positive ion mode. The mass spectra were obtained

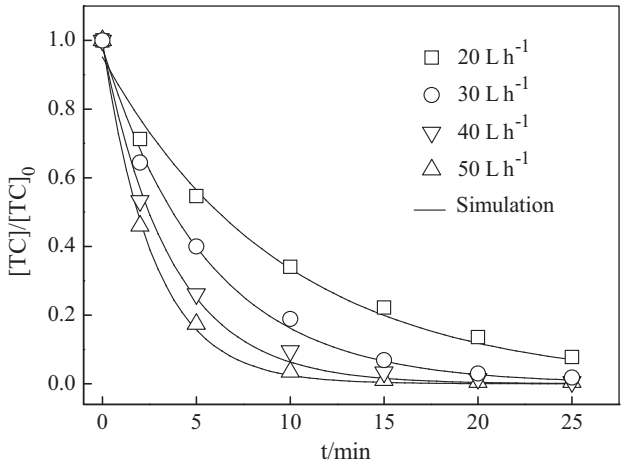



**Fig. 2.** Effect of ozone gas flow rate on the degradation of tetracycline ($[TC]_0$ = 2.08 mmol $L^{-1}$, $[O_3]_g$ = 1.13 mmol $L^{-1}$, pH = 7.8).

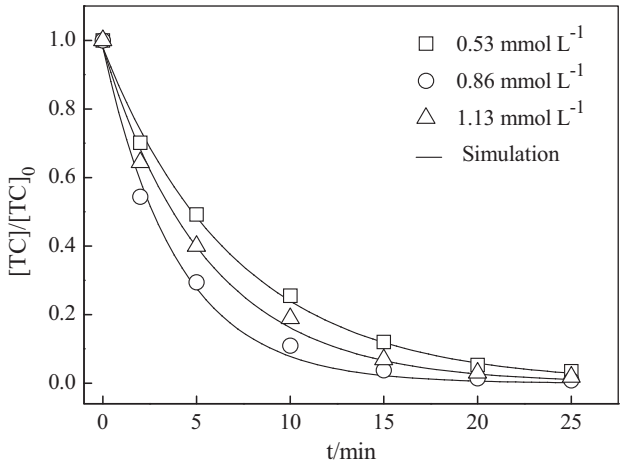



**Fig. 3.** Effect of tetracycline degradation at different ozone concentrations ($[TC]_0$ = 2.08 mmol $L^{-1}$, $Q$ = 30 L $h^{-1}$, pH = 7.8).

continuously when chromatographic running, each scan requiring 0.02 s. The half was injected to the APCI source from the LC system fitted with an auto-sampler and coupled with a ZORBAX Eclipse XDB-C18 column (4.6 mm × 150 mm, 5 μm particle size). An acetonitrile/0.01 mol $L^{-1}$ aqueous oxalic acid mixture was used as the mobile phase with a flow rate of 1 mL $min^{-1}$. Depending on intermediates, gradient elution was used by varying the eluent ratio. The initial mobile phase composition was 10/90 (acetonitrile/0.01 mol $L^{-1}$ aqueous oxalic acid), then this composition linearly changed to 35/65 in 10 min and the composition remained constant for 10 min, injection volume of 20 μL, and UV detector set up at 365 nm. APCI source conditions were as follows: HV capillary and capillary voltage 3500 V and 4 V, discharge current 3.5 μA, vaporizer and capillary temperatures 400 °C and 150 °C, sheath gas ($N_2$) flow rate 60 units and total compounds mass spectra were scanned from 113 *m*/*z* to 800 *m*/*z*.

## 3. Results and discussion

### 3.1. Effect of gas flow rate

To investigate the effect of gas flow rate on the degradation of tetracycline, the experiments were carried out at the gas flow rates from 20 to 50 L $h^{-1}$ when the tetracycline concentration was 2.08 mmol $L^{-1}$, the ozone concentration was 1.13 mmol $L^{-1}$, and the pH was 7.8.

As can be seen in Fig. 2, the tetracycline removal follows apparent pseudo-first-order kinetics according to the following rate equation:

$$-\frac{d[TC]}{dt} = k[TC] \quad (2)$$

where [TC] is tetracycline concentration at time $t$ and $k$ is the pseudo-first-order rate constant. The high correlation coefficients $R^2$ ranged from 0.984 to 0.999 verified that the apparent pseudo-first-order kinetics could fit the experimental results well.

As shown in Fig. 2, the degradation rate increased with the increasing gas flow rate. The pseudo-first-order rate constants calculated were 0.111, 0.185, 0.279, 0.371 $min^{-1}$, when the gas flow rates were 20, 30, 40 and 50 L $h^{-1}$, respectively. Degradation of pharmaceuticals such as tetracycline by ozonation can be regarded as a mass transfer process coupled with chemical reactions. Gaseous ozone was absorbed into the aqueous phase and then reacted with pharmaceutical compound. It is generally well-accepted that the gas–liquid reaction can occur in different regimes depending upon the relative rates of gas–liquid mass transfer and chemical reaction involved [33]. Since no ozone was detected in the bulk solution, it was confirmed that the reaction between the dissolved ozone and the tetracycline was fast and occurred in the liquid film, which falls in the fast or instantaneous kinetic regime according to the film theory [34]. Thus, the tetracycline removal rate is considered to be equal to the ozone absorption rate [35]:

$$-\frac{d[TC]}{dt} = zEk_La[O_3]^* \quad (3)$$

where $E$ is the enhancement factor, $[O_3]^*$ is the equilibrium ozone concentration in water, $k_La$ is the volumetric mass transfer coefficient and the stoichiometric ratio of ozone/tetracycline $z$ is determined to be 2 according to Eq. (1).

The increasing gas flow rate corresponded to a larger net surface area for mass transfer of ozone to the aqueous phase, and hence increased the volumetric mass transfer coefficient of ozone according to Eq. (3) [36]. Therefore, the tetracycline removal increased with the increasing gas flow rate.

### 3.2. Effect of gaseous ozone concentration

The degradation of tetracycline by ozone was investigated at different gaseous ozone concentrations when the tetracycline concentration was 2.08 mmol $L^{-1}$, the ozone gas flow rate was 30 L $h^{-1}$ and the pH was 7.8. When oxygen was bubbled into the reactor at the same flow rate, little tetracycline was removed (data not shown). This indicated that oxygen could not oxidize tetracycline. Ozone could effectively remove tetracycline, and higher ozone concentration would lead to higher degradation rate, as illustrated in Fig. 3. The removal rate constants of tetracycline were 0.144, 0.185, and 0.259 $min^{-1}$ when gaseous ozone concentrations were 0.53, 0.86, and 1.13 mmol $L^{-1}$, respectively. The increasing gaseous ozone concentration would increase the equilibrium ozone concentration in the aqueous phase according to according to Henry's law [37]:

$$[O_3]^* = \frac{[O_3]_g}{H} \quad (4)$$

where $H$ is the Henry law constant, $[O_3]_g$ is the gaseous ozone concentration (mol $L^{-1}$).

The increasing equilibrium ozone concentration could result in the increase of mass transfer driving force, which would lead to the increasing volumetric mass transfer coefficient of ozone from gas phase to liquid phase [38]. Thus, the degradation rate increased with the increasing gaseous ozone concentration.

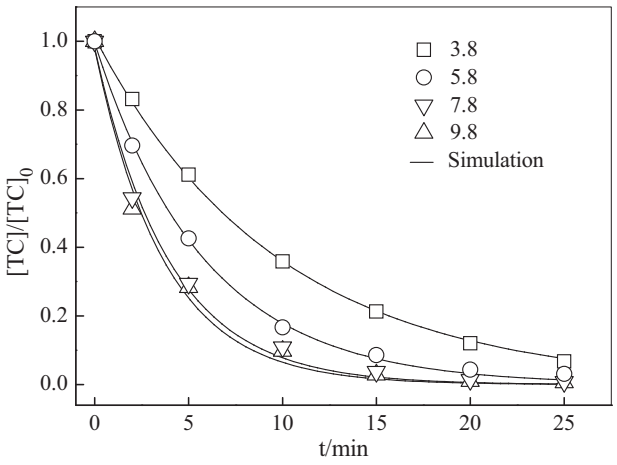



**Fig. 4.** Effect of tetracycline degradation during its ozonation at different pHs ($[TC]_0$ = 2.08 mmol $L^{-1}$, $[O_3]_g$ = 1.13 mmol $L^{-1}$, $Q$ = 30 L $h^{-1}$).

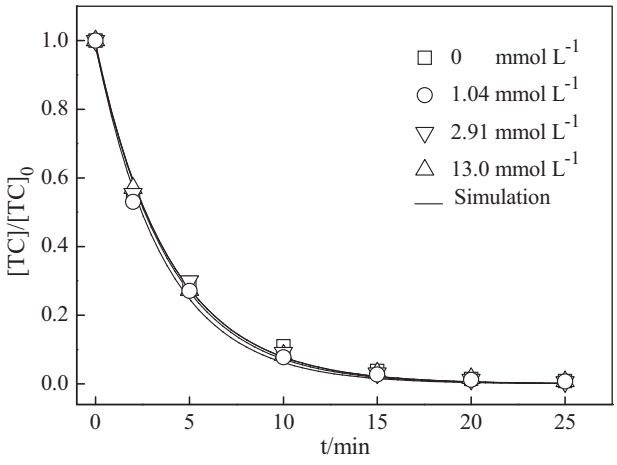



**Fig. 5.** Degradation efficiency of tetracycline in the presence of hydrogen peroxide ($[TC]_0$ = 2.08 mmol $L^{-1}$, $[O_3]_g$ = 1.13 mmol $L^{-1}$, $Q$ = 30 L $h^{-1}$, pH = 7.8).

### 3.3. Effect of pH

The pH is usually considered as one of the most important parameters for ozonation. To elucidate the effect of pH on the degradation rate of tetracycline by the ozonation process in buffered solutions, the investigated pH values were 3.8, 5.8, 7.8 and 9.8, respectively when the tetracycline concentration was 2.08 mmol $L^{-1}$, the gaseous ozone concentration was 1.13 mmol $L^{-1}$ and the gas flow rate was 30 L $h^{-1}$. As shown in Fig. 4, the tetracycline removal rate increased significantly with increasing pH before the pH reached 7.8, but the influence of pH became insignificant thereafter. Generally, ozone reacts with organic pollutants via either direct ozone attack or indirect free radical attack [39]. When organic pollutants were directly attacked by ozone molecules, the oxidation reaction depended strongly on the nature of organic molecules. It should be recognized that tetracycline has four possible molecular species which could engage in protonation or deprotonation depending on the solution pH [40]. At low pH, tetracycline (TC) is fully protonated to be TCH3+. With the increase of pH, deprotonation reactions take place in three steps, which give rise to $TCH_2$, $TCH^-$ and $TC^{2-}$, respectively [40,41]. Moreover, the deprotonated tetracycline with a positively charged group would be more easily attacked by ozone molecules than tetracycline itself when the solution pH is less than 7.8 [26]. With the increase of pH, the degree of deprotonation is higher, and the removal rate increases accordingly. However, the fraction of $TCH^-$ and $TC^{2-}$ becomes predominant while pH > 7.8 and their effect on the tetracycline removal rate may be less than that of $TCH_3^+$ or $TCH_2$ at pH below 7.8. Thus, the degradation rate of tetracycline increased from 0.102 to 0.259 $min^{-1}$ when pH values increased from 3.8 to 7.8, while it increased only to 0.279 $min^{-1}$ when pH reached to 9.8.

On the other hand, the increasing pH improves the decomposition of ozone to generate hydroxyl radicals [3,42]. Under acidic conditions, direct ozone attack occurs primarily. In contrast, the free radical reaction may become significant with the increase of pH due to hydroxyl radicals generated from the reaction of ozone with $OH^-$ [39]: [39]:

$$O_3 + OH^- \rightarrow {}^{\bullet}OH + O_2{}^- + O_2 \quad (5)$$

Accordingly, the degradation rate of tetracycline increased with the increasing generation rate of hydroxyl radicals.

### 3.4. Effect of $H_2O_2$ concentration

It has been observed that the capability of ozonation is enhanced by hydrogen peroxide [17,18,35] through initiating ozone decomposition to generate more reactive hydroxyl radicals than ozone alone [3,17] via the following pathways:

$$O_3 + H_2O_2 \rightarrow {}^{\bullet}OH + HO_2{}^{\bullet} + O_2 \quad (6)$$

$$O_3 + HO_2{}^{\bullet} \rightarrow {}^{\bullet}OH + 2O_2 \quad (7)$$

The experiments were carried out with the hydrogen peroxide concentration ranged from 0 to 13 mmol $L^{-1}$ when the initial tetracycline concentration was 2.08 mmol $L^{-1}$, the ozone concentration was 1.13 mmol $L^{-1}$, the gas flow rate was 30 L $h^{-1}$, and pH was 7.8. It can be seen in Fig. 5 that the effect of hydrogen peroxide was not significant on the degradation rate of tetracycline by ozone, which suggested that the reaction of tetracycline with ozone was principally a direct molecular reaction in this study. A similar result was reported by Ternes et al. [43] when iomeprol, diatrizoate, and iopamidol were decomposed by the combination of ozone and hydrogen peroxide.

### 3.5. Effect of tert-butyl alcohol concentration

To further investigate whether the direct ozone reaction was a major process in the degradation of tetracycline, the ozonation experiments were conducted in the presence of *tert*-butyl alcohol, a strong hydroxyl radical scavenger, due to its high bimolecular reaction rate constant ($4.56 \times 10^{10}$ L $mol^{-1}$ $min^{-1}$ with hydroxyl radical as compared to 0.18 L $mol^{-1}$ $min^{-1}$ with ozone) [18,44]. When the tetracycline concentration was 2.08 mmol $L^{-1}$, the ozone concentration was 1.13 mmol $L^{-1}$, the gas flow rate was 30 L $h^{-1}$, and the pH was 7.8, the *tert*-butyl alcohol concentrations investigated were 0, 1, 5 and 10 mmol $L^{-1}$, respectively.

As can be seen in Fig. 6, the degradation rates of tetracycline were 0.259, 0.234, 0.203 and 0.201 when the *tert*-butyl alcohol concentrations were 0, 5, 10 and 15 mmol $L^{-1}$, respectively. It illustrated no conspicuous effect of *tert*-butyl alcohol concentration on the degradation rates of tetracycline. With increasing dosages of scavenger, the tetracycline degradation rate decreased by 22.3%, indicating that the tetracycline removal was mainly contributed by direct ozone oxidation. Therefore, the primary mechanism of the tetracycline degradation was not the reaction between tetracycline and hydroxyl radicals.

### 3.6. The degradation mechanism of tetracycline

The representative UV–visible (vis) spectra changes of the tetracycline solution as a function of reaction time were shown in Fig. 7 in order to clarify the changes of molecular and structural

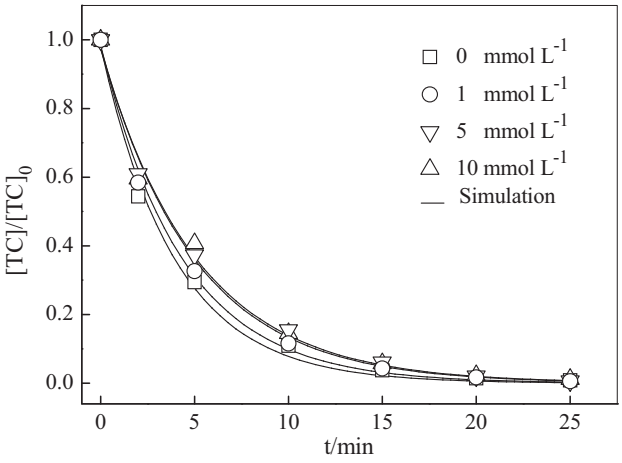



**Fig. 6.** The effect of hydroxyl radical scavenger (*tert*-butyl alcohol) on the degradation of tetracycline ($[TC]_0 = 2.08\ mmol\ L^{-1}$, $[O_3]_g = 1.13\ mmol\ L^{-1}$, $Q = 30\ L\ h^{-1}$, pH = 7.8).

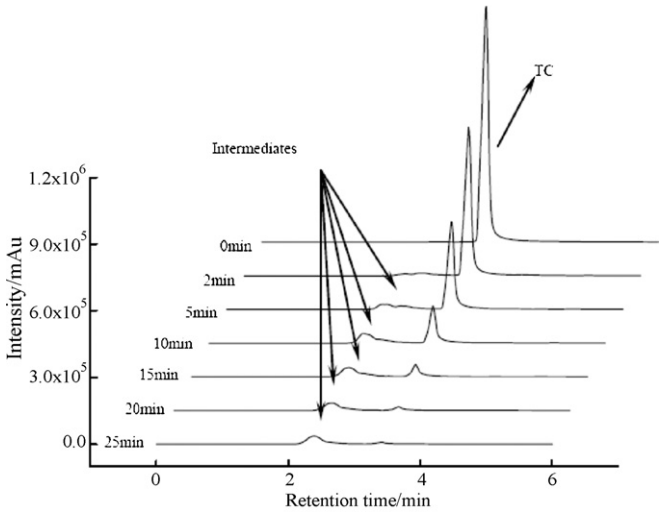



**Fig. 8.** HPLC-UV (365 nm) chromatograms of aliquots taken at different times of reaction with ozone.

characteristics of tetracycline as a result of ozonation. As could be seen from these spectra, the absorption spectrum of tetracycline in water was characterized by two main bands before the ozonation. Its one maximum absorption in the visible region located at 365 nm as another band in the ultraviolet region located at 275 nm. The peak at 275 nm was associated with aromatic ring A structure including acylamino and hydroxyl in the molecule (Table 1), and that at 365 nm was originated from aromatic rings B–D (Table 1), comprising the extended chromophores [45]. The disappearance of the visible band with the reaction time might be due to the fragmentation of enolic groups connected to aromatic ring B by direct ozone attack. In addition, the decay of the absorbance at 275 nm was considered as evidence of acylamino and enolic groups connected to the aromatic ring A degradation in tetracycline molecule and its intermediates.

The direct detection of reactive intermediates was important for explaining and understanding the mechanisms of ozonation reactions in solution. To further identify the intermediate products during the oxidation, atmospheric pressure chemical ionization (APCI) methods were employed. Fig. 8 illustrated the HPLC-UV chromatograms obtained from aliquots collected at different reaction times. It demonstrated that the intensity of the tetracycline peak decreased with the process of the reaction. At the same time the new peaks were detected and their intensities increased with the reaction time. Moreover, their retention times were shorter than that of tetracycline. It indicated that the more polar intermediate compounds were formed in solution during the oxidation processes. Fig. 9 represented the total ion chromatograms of tetracycline at 0, 15 and 25 min ozonation, respectively. As can be seen from Fig. 9, the tetracycline peak as well as the peaks of three main by-products have been observed. Based on the total ion chromatograms in Fig. 9 and the further analysis of the mass spectrum, the overall MS and MS/MS characteristics corresponding to the peaks are shown in Table 2.

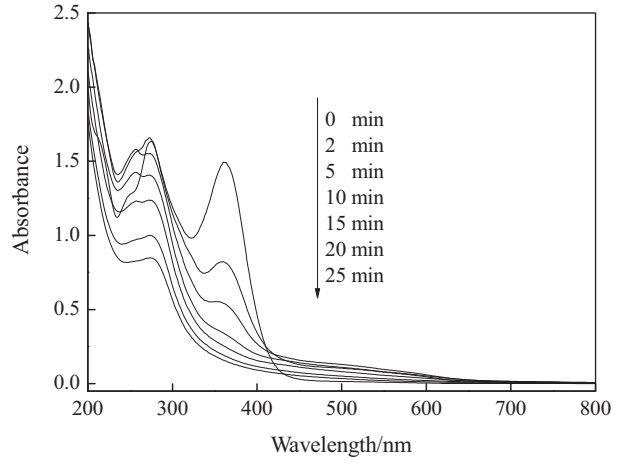



**Fig. 7.** UV–vis spectral changes with reaction time ($[TC]_0 = 1.13\ mmol\ L^{-1}$, $[O_3]_g = 1.16\ mmol\ L^{-1}$, $Q = 30\ L\ h^{-1}$, pH = 7.8).

As shown in Fig. 9, an intense prominent ion with the retention time of 6.9 min and *m*/*z* 445 was observed and it was corresponding to the deprotonated TC molecular ion. The following sequence was yielded by successive mass-selection of the main precursor ions and their fragmentation upon collision-induced dissociation: *m*/*z* 445 → *m*/*z* 427 (by loss of $H_2O$) → *m*/*z* 410 (then by loss of $NH_3$) (APCI(+)–$MS^2$). In this work, the fragmentation pathways proposed are consistent with the results reported by Dalmázio [5], Dessalces [46], Kamel [47] and Vartanian [48]. The $NH_3$ was lost and followed by the water loss including the hydroxyl group bound at the C6 atom.

As indicated in Fig. 9, the other peak of *m*/*z* 461 assumed to be P1 was observed and the retention time was 5.7 min. According to the resonance hybrid structure of ozone, it generally reacted with double bonds by electrophilic substitution or 1,3-dipolar cycloaddition because of its electrophilic or nucleophilic character [5]. Moreover, there were four primary ozone target sites for the oxidative decomposition of TC [49]. The C11a–C12 double-bond of TC was the only one carbonyl group and much more susceptible to be attacked by ozone or free radical attack through electron-withdrawing substituent compared with another available double-bond C2–C3 of TC [28]. Thus, it could be proposed that an initial 1,3-dipolar

**Table 2**
Main fragment ions obtained from MS and MS/MS analyses of tetracycline (TC) and transformation products.

| Compounds | Observed fragments ions at *m*/*z* vale | |
|---|---|---|
| | MS | MS/MS |
| TC | 445 | 427, 410 |
| P1 | 461 | 444 |
| P2 | 465 | 448 |
| P3 | 469 | 452 |

P: the by-product generated from the TC ozonation process.

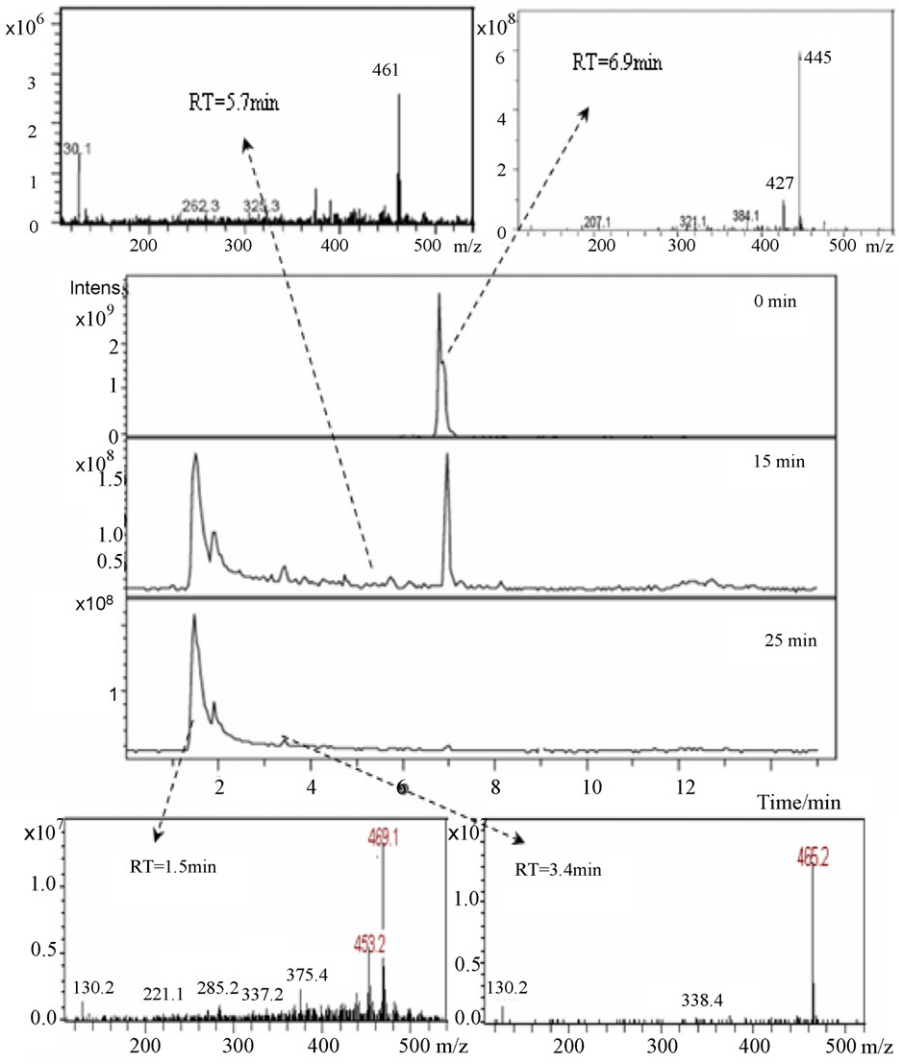



**Fig. 9.** LC–APCI(+)–MS total ion chromatograms for monitoring of the degradation of tetracycline by ozone in aqueous medium after reaction times of 0, 15 and 25 min. The insets show the mass spectra of the products eluted at 1.5 min, 3.4 min, 5.7 min and 6.9 min.

cycloaddition towards the C11a–C12 double-bond [5] and a rearrangement with the hydroxyl at the position C12 would lead to the generation of P1. With the results obtained in this work and previously identified by several authors [5,26], the major fragments were corresponding to the fragmentation of by-products through the loss of $NH_3$. Therefore, the structure of the possible by-product and their major fragments could be deduced as shown in Fig. 10.

The structures of P2 and P3 could be determined in similar way. The peak of P2 at *m*/*z* 465 certainly was formed through the loss of methyl in C6 and chloro-substituent in C7 by $OCl^-$, $ClO_2^-$ and/or $Cl_2$ species generated from ozonated free chloride ions in the solution [9,41,49]. Then P2 was further oxidized to P3 at *m*/*z* 469. P2 and P3 were different from the results reported by Dalmázio [5] and Khan [26]. Maybe it is attributed to the different target compounds used. Thus, based on the above experimental results and previous study [5,25,26], the possible degradation pathways of TC were proposed as shown in Fig. 10.

### 3.7. The changes of COD, biodegradability and active toxicity with reaction time

It is known that the complete removal of the refractory organic does not mean that they are completely oxidized to small inorganic molecules [13], so the degradation of tetracycline in terms of COD removal was investigated. As can be seen in Fig. 11, less than 20% COD was removed after 25 min reaction compared with 99.2% of tetracycline removal efficiency. After the reaction time was extended to 90 min, 35% of COD removal efficiency could be achieved. The gentle increase of COD removal efficiency during the reaction process may result from the intermediates formed which were more stable than tetracycline under the experimental conditions. The similar phenomenon was reported by Wu et al. [27] when tetracycline was degraded by ozone. They found that only 15% COD of tetracycline was removed by the first 5 min and the COD removal was not increased by extending the reaction time to 20 min. Thus, the biodegradability and toxicity of the parent compound and its oxidation intermediates need to be assessed.

In this study, $BOD_5$ and the acute toxic tests were determined before and after ozonation at different time intervals. The evolution of the $BOD_5$/COD ratio with the reaction time was shown in Fig. 11. As can be seen, the tetracycline solution itself exhibited the low biodegradability with $BOD_5$/COD ratio of 0.013, which illustrated that the tetracycline was very difficult to be biodegraded and this was in agreement with the result reported by Wen et al. [6]. After ozonation, the $BOD_5$/COD ratio increased and reached the maximum at 15 min. Then the $BOD_5$/COD ratio decreased

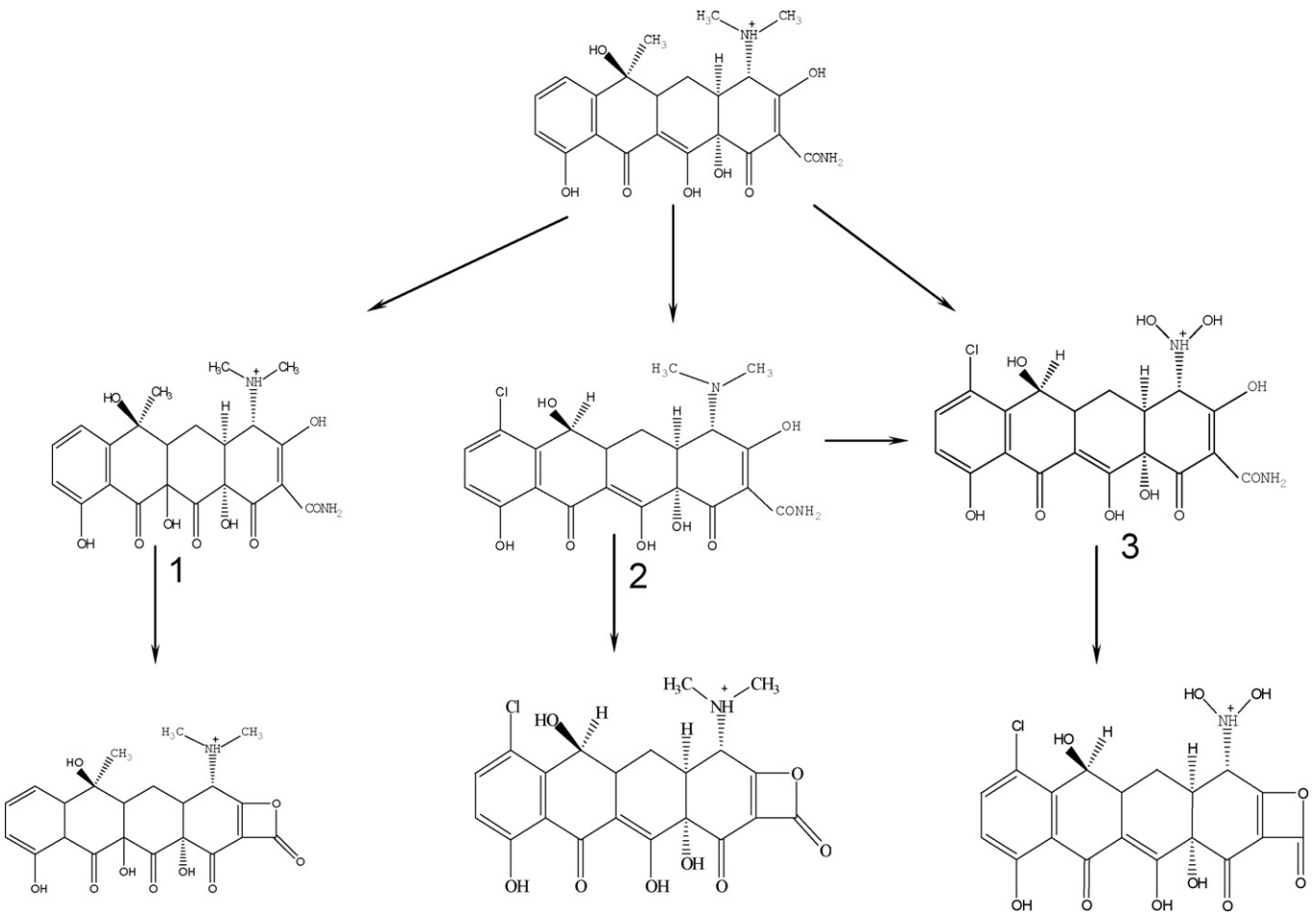



**Fig. 10.** Proposed degradation pathways of tetracycline in aqueous solution by ozone. The chemical structures of intermediates were estimated from the progress of degradation pathways.

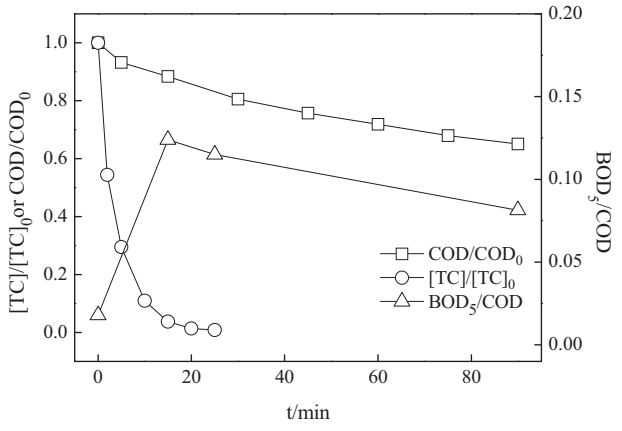



**Fig. 11.** The ratios of $[TC]/[TC]_0$, $COD/COD_0$ and $BOD_5/COD$ versus the reaction time.

slightly afterwards. This indicated that tetracycline was firstly oxidized to the intermediates with higher biodegradability than tetracycline itself [27], but further degradation of the intermediates could not improve the biodegradability. Moreover, the $BOD_5/COD$ ratio during the whole reaction time was higher than that of the tetracycline.

The acute toxicity of the tetracycline degradation effluents during ozonation process was evaluated using 48-h immobilization test with *D. magna*. The results showed that the inhibition values of the tetracycline solution on the mortality of *D. magna* were 60, 60, 95 and 0 percent when the ozonation time were 0, 15, 25 and 90 min, respectively. After 15 min ozonation, nearly no tetracycline was detected, and the intermediates with the similar toxicity were formed. The intermediates were further oxidized with the progress of ozonation, which led to the increasing *D. magna* mortality [50,51] and the inhibition ratio reached 95%. The similar tendency was reported by Wu [27] and Beltrán [52] when tetracycline and sulfamethoxazole were oxidized by ozone, respectively. They found the more toxic by-products were formed during ozonation process compared with the target pollutants. However, it was drastically reduced after the toxicity reached the maximum at 25 min. This indicated that the toxic structures were further oxidized by ozone, which resulted in the decrease of the toxicity. The extent of toxicity reduction was higher than that of biodegradability, improvement though they showed the similar tendency. The similar phenomenon was reported by Arslan-Alaton [53]. *D. magna* belongs to crustaceans which are different from microorganism. The part of toxic materials was prevented from entering the body of *D. magna* by its shell, which decreased the effective absorption of toxic materials into the body of *D. magna*.

### 3.8. Cost evaluation

The economics is an important parameter for selecting any water/wastewater treatment process. Ozone consumption is an electric-energy-intensive process, and the electric energy can represent a major fraction of the operating costs. Thus, the electrical

energy per order of pollutant removal (EE/O) was determined according to the following equation [54]:

$$\mathrm{EE/O} = \frac{P \times t \times 1000}{V \times 60 \times \log([\mathrm{TC}]_0/[\mathrm{TC}]_f)} \quad (8)$$

where $P$ is the rated power (kW) of the ozonation system, $t$ is the irradiation time (min), $V$ is the volume of liquid treated (L), $[\mathrm{TC}]_0$ and $[\mathrm{TC}]_f$ are the initial and final pollutant concentrations (mmol $L^{-1}$).

It could be calculated that the tetracycline removal reached 99% with 15 min ozonation. Thus, the electrical energy was 6.05 kWh $m^{-3}$ $order^{-1}$ when the tetracycline concentration was 2.08 mmol $L^{-1}$, the ozone concentration was 1.13 mmol $L^{-1}$, gas flow rate was 50 L $h^{-1}$, and the pH was 7.8. This indicated that ozonation is suitable to degrade tetracycline.

## 4. Conclusion

In this study, the tetracycline could be degraded effectively by ozonation and the degradation of tetracycline fitted the pseudo-first-order kinetic model for various experimental results. The effect of pH, gas flow rate, gaseous ozone concentration, hydrogen peroxide concentration, and hydroxyl radical scavenger (*tert*-butyl alcohol) on the tetracycline removal was investigated. The result proved that the degradation rate increased with the pH values, gas flow rate and gaseous ozone concentration. But the reaction rate did not increase significantly with the increasing $H_2O_2$ concentration and the reaction rate did not decrease significantly with the increasing hydroxyl radical scavenger concentration. This conformed that the direct ozonation of tetracycline was the dominant process. A 35% COD removal obtained after 90 min ozonation, indicating that tetracycline could not be mineralized completely by ozone.

The transformation products during the ozonation of tetracycline were detected and the simple degradation pathways of tetracycline were proposed. As clearly proved by LC–APCI(+)–MS analysis, in combination with HPLC, UV–vis, and COD data, ozone reacted with tetracycline via 1,3-dipolar cycloaddition and electrophilic reactions to form by-products. Further studies showed the biodegradability of treated tetracycline was improved and almost no acute toxicity of the tetracycline solution to *D. magna* was observed after 90 min ozonation. The results could be used as the risk assessment of the intermediates from the ozonated tetracycline in the environment. The electrical energy evaluation showed that ozonation was an appropriate method to degrade the tetracycline solution.

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