



Research Article

Oxytetracycline Mineralization inside a UV/H₂O₂ System of Advanced Oxidation Processes: Inorganic By-Product

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Abstract

Oxytetracycline (OTC) was widely used antibiotic in agricultural industry. However, most of them were secreted from the body and entered the water stream, due to low absorption. The occurrence of the antibiotics in water stream may lead to serious health hazards. Hence, finding the effective method that capable to achieve total mineralization of antibiotic-contaminated wastewater, followed by the production of benign inorganic and organic by-product, was necessarily deemed. Photochemical degradation method, such as: UV/H₂O₂ system, was capable to achieve total mineralization of OTC at its optimized condition. In this paper, inorganic by-products of OTC mineralization inside a UV/H₂O₂ system at its optimum condition were analyzed. The presence of nitrate, ammonium, chloride ions, and chlorine were detected at the sample solution after mineralization. The presence of these inorganic by-product has proven that the experimental setup chosen was capable to achieve total mineralization. In addition, possible routes of the inorganic by-products detachment from the OTC's structure, were also presented.

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Keywords: Oxytetracycline; UV/H₂O₂; inorganic by-product; Oxidation Processes; Photochemical degradation

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1. Introduction

In livestock production, beside acting as a microbial agent, antibiotics are also used as a growth promoter, steroid, insecticides, and tranquilizing agent. Living body of organism only

absorbs minimal amount of consumed antibiotics. Hence, most of the consumed antibiotics are secreted from the living body and ultimately entered the water stream. Continuous consumption of antibiotic-contaminated water could cause antibiotic resistance and harm the internal organ of the aquatic animals AP [1]. The presence of the antibiotics has been detected in surface and groundwater, drinking water, tap water, ocean water, sediments, and soil [2].

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One distinct character of antibiotics that it is biologically active, hence it could cause the antibiotics to be resistant against biological degradation process. This is caused by the capability of antibiotics to reduce or even eliminate the microbial growth in the biodegradation tank of conventional treatment. Therefore, there is a need to remove antibiotic traces effectively from the water stream. In this regard, a non-biological method, such as Advanced Oxidation Processes (AOPs) could become alternatives. Generally, to identify the efficient process for removing certain type of organic contaminant, the term of organic degradation and mineralization are used. These two terminologies can be found in numerous studies and measured with different instruments [3–5]. Typically, to evaluate the degradation efficiency, the concentration of the parent compound of the organic contaminant is directly measured using chromatography or spectroscopy technique. Therefore, once the organic parent compound peak is not detected inside the chromatogram or spectrum, the organic degradation is considered completed. About 100% reduction of oxytetracycline concentration was achieved, after 20 min of ozonation treatment. However, its toxicity by products (after 5-30 min of treatment) were highly increased [5]. Similar trend was also observed for degradation of tetracycline using UV/H₂O₂/Fenton system, where tetracycline concentration was removed after 1 min of treatment [4]. Whereas, 72% Total Organic Carbon (TOC) removal was achieved after 60 min of treatment. Liu *et al.* studied the tetracycline decomposition by using photo electron Fenton [6]. Based on their study, after 120 min of treatment, 98.3% of tetracycline was removed, whilst 84.3% of total organic carbon reduction was observed after 10 h of treatment. Lower degree of mineralization compared to degradation of parent compound also shown for UV photolysis of Oxytetracycline (OTC) [7]. About 100% of OTC was removed after 7.5 h, while only 5.37% of OTC was mineralized. In addition, 80% increment of inhibition rate was observed after 2 h of UV photolysis, which indicates toxicity increment in the partially degraded solution.

Mineralization is the transformation of the organic parent compound into final product of inorganic compound, such as: CO₂, H₂O, NH₃, or even diatomic gases like Cl₂ and N₂. Whereas the mineralization progress is commonly measured by evaluating the TOC value. Meanwhile, an UV/H₂O₂ process could destruct the antibiotics mainly by non-selective attack of hydroxyl radical. Hence, complex reaction path-

ways may be triggered and various by-products along with the organic intermediates were formed [8]. These by-products also can be categorized as low molecular weight organic acids and inorganic ions. Hence, reduction of the TOC value implies the progress of the mineralization process. To date, most published works were mainly investigating the OTC parent compound removal and TOC reduction. However, no works have been published on investigating the inorganic by-product of OTC mineralization, especially inside a UV/H₂O₂ system.

In this paper, mineralization of OTC inside a UV/H₂O₂ system were conducted under the optimum condition. The inorganic by-products were analyzed to prove the complete mineralization of the optimized parameter. Possible released routes of inorganic by-product from the main structure of OTC were also presented. From this by-product analysis, possible pathways for OTC transformations can be established. Hence, additional insight on the OTC's transformation pathways could contribute for evaluating the treatment efficiency and possible changes and its interactions in the environment.

2. Materials and Methods

2.1 Materials

OTC hydrochloride, C₂₂H₂₄N₂O₉HCl (Merck) and hydrogen peroxide (30%, Merck) were used as the source of organic contaminant and the source of hydroxyl radical, respectively. H₂SO₄ (98%, Merck) and NaOH (Merck) were used for pH adjustment. All chemicals were used without further purification.

2.2 Methods

2.2.1 UV/H₂O₂ system

In this study, the optimized experimental parameters were applied, as described elsewhere [9]. All the present experiments were carried out in a glass reactor (400 mL working volume) with a provision for irradiation using a low-pressure Hg lamp (8 W power GPH295T5L; Serial no. EC90277, USA) to produce UV light at 254 nm. The schematic diagram of the reactor is shown in Figure 1. During irradiation, the solution was stirred using magnetic stirrer at which speed. The temperature was maintained using water circulation through the reactor jacket. After adjusting the OTC solution to the optimum pH, desired amount of H₂O₂ were then added into the reactor. To monitor the progress of mineralization, 3 mL of liquid samples were drawn from the

reactor at scheduled time intervals. Since the total amount of samples withdrawn are less than 5% (<5%) of the total volume, for calculation purpose the total sample volume was neglected. About 180 min of irradiation time were applied for all experiments. All the experiments were conducted at the same UV lamp power which emits the light intensity of 12.06 mW/cm².

2.2.2 Ion Chromatography

The concentrations of nitrate, nitrite, and chloride ions were analyzed using Ion Chromatography (Metro Ohm-761, Switzerland), equipped with Metrosep-A Supp 5-150 columns, and eluted using 3.2 mM Na₂CO₃/1 mM NaHCO₃ as the mobile phase. The ion chromatography was using a conductivity meter as the ion detector and deionized water as the blank solution. Three sets of standard solutions of chloride, nitrate, and nitrite ions were used to construct the standard calibration curve as listed in Table 1.

2.2.3 Nessler Ammonia

Concentration of ammonia in the solution sample was estimated by using Nessler reagent (K₂HgI₄) and method No. 8038 (HACH, USA). The reaction occurs in alkaline condition produce complex compound with yellow colour. The intensities were proportional to ammonia concentration in the solution. In addition, Ness-

ler's reagent, mineral stabilizer and polyvinyl alcohol were also added into the sample. These additions were carried out to minimize the interference from the water matrix. The ammonia concentration was measured using spectrophotometer DR 2000 (Hach, USA).

2.2.4 Total Chlorine

This method measures the value of total chlorine, which is equal to the summation of free chlorine and combined chlorine. The measurement was carried out based on method No. 10070 (HACH, USA) which is in accordance with USEPA DPD (N,N-diethyl-p-phenylenediamine) method. The present total chlorine in the solution will oxidize iodide in the reagent to iodine. Then the iodine reacts with DPD along with the free chlorine present in the sample to form a pink color which is proportional to the total chlorine concentration. The absorbance value of pink solution was measured at 530 nm using spectrophotometer 2000 (HACH, USA).

2.2.5 Concentration of CO₂

To monitor mineralization progress of OTC, the TOC measurements were used. The values of TOC were measured using a TOC-VCSH analyzer (Shimadzu, Japan). About 2 mL of samples were drawn from the sample port and diluted with distilled water until the final volume of 20 mL. As shown in Equation (1), the value of TOC was determined from the difference between the total carbon (TC) with total inorganic carbon (TIC). During TOC analysis, the sample solution was acidified using H₃PO₄ to ensure that all the carbonate and bicarbonate were transformed into CO₂. In water solution, CO₂ could transform into carbonate and bicarbonate ions which their transformation depends on the solution's pH. In this research work, after UV/H₂O₂ treatment, the pH final solution is expected to be in the range below the initial pH, and most probably lower than the pK_a of carbonate (pK_a = 10.3) and bicarbonate (pK_a = 6.3). This is due to the concentration of organic acids and other acidic by-

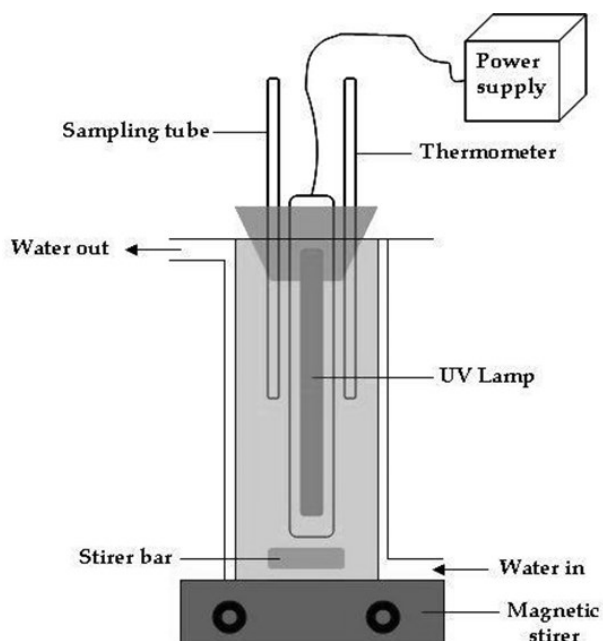


Figure 1. Experimental setup of UH/H₂O₂ system.

Table 1. The correlation constants for the calibration curves for chloride, nitrate and nitrite.

Ion	Calibration curve	R ²
Chloride	00036x + 0.0974	0.9996
Nitrate	0.0065x - 0.0703	0.9996
Nitrite	0.0057x + 0.1068	0.9996

products will be higher compared to the basic by-product. Therefore, the concentration of CO₂ can be approximated into the concentration of inorganic carbon as measured by the TOC analyzer.

$$TOC = TC - IC \quad (1)$$

where TC is total carbon and IC is inorganic carbon.

3. Results and Discussion

In the present study, OTC hydrochloride was used as the model component. Therefore, the OTC solution contains C, H, O and N elements and Cl ions. Inorganic compounds such as NO₂⁻, NO₃⁻, and Cl⁻ were determined using ion chromatography. The nitrogen containing compound such as ammonia was identified using Nessler reagent, in the form of ammonium ion. Whereas the by-product analyses as well as the degradation mechanism development were carried out at the optimum condition. During the optimization, total mineralization was chosen to be as the optimized response. Therefore, at the end of the reaction, CO₂, H₂O, NH₃, NH₄⁺, NO₂⁻, NO₃⁻, N₂, Cl, Cl₂, and fractions of organic acid are expected to be present in the solution.

As it mentioned earlier, the ratio of inorganic by-product containing nitrogen moieties was depend on the character of N-containing structure [10]. Based on Figure 2, OTC structure has two types of nitrogen bond configuration, namely the tertiary and primary amine. Both structures contain hydrogen extractable character. As shown in Figure 3, there were changes in concentration by time for the NO₃⁻ ion and NH₄⁺ ions throughout the mineralization process. By introducing the UV/H₂O₂ into the OTC solution, instead of by ionization, the re-

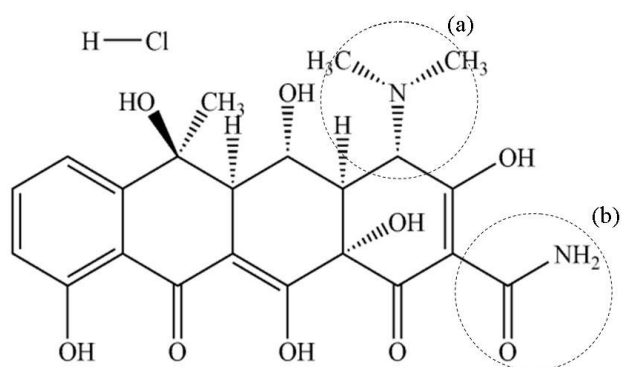


Figure 2. Nitrogen bond configuration of OTC molecule with (a) tertiary amine and (b) primary.

lease of ammonia group was mediated by indirect oxidation of hydroxyl radical [11]. Therefore, there was an increase in NH₄⁺ ions concentration at *t* = 10 min of irradiation which was followed by the formation of NO₃⁻ and observed similarly at *t* = 10 min. During this first 10 min, the hydroxyl radical generation was initiated by the photon from the UV lamp and started to attack the OTC compound. The generations of NO₃⁻ and NH₄⁺ occurred until *t* = 180 min. Moreover, starting at *t* = 10 min, there was a sharp decline in the NH₄⁺ ions concentrations indicating their conversion into NO₃. However, at the end of the reaction, not all NH₄⁺ was transformed into NO₃⁻. This was due to the reason that no H₂O₂ remained in the solution thus stopping the generation of hydroxyl radical. The presence of NO₃⁻ and NH₄⁺ were also detected for mineralization of levofloxacin which resulted from the released of nitrogen content from the antibiotic main structure [12]. Mineralization of tetracycline using electro Fenton process also produced NO₃⁻ and NH₄⁺ ions [13].

Basically, there are two steps of NO₃⁻ formation [14]. In the first step, the conversion of nitro group into NO₂⁻ ion and followed by the oxidation into the NO₃⁻ ion. It is then followed by the oxidation of NH₄⁺ into hydroxylamine and followed by the formation of NO₃⁻. However, in the present study, the most probable mechanism of OTC mineralization would be the second step, since no NO₂⁻ ion was observed throughout the experiment. Moreover, the nitrogen attached to the heterocyclic aromatic rings could be transformed into NH₄⁺ and NO₃⁻, whereas the secondary, tertiary, and quaternary nitrogen atom will be transformed into NH₄⁺ ions [10]. Ammonium ions were formed directly from the release of nitrogen as ammonia when the oxidation state of the

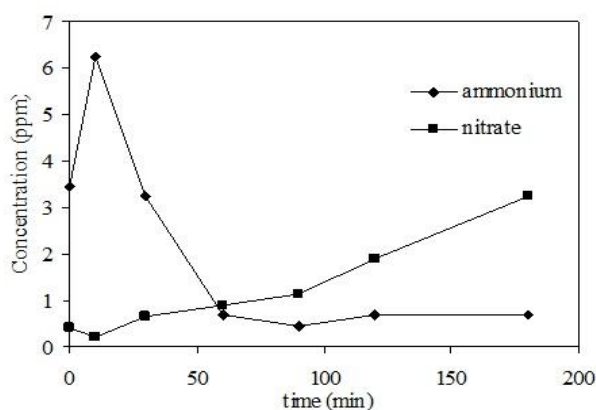


Figure 3. Nitrate and ammonium ion profile for OTC removal at optimum condition.

bound nitrogen is -3 . In addition, an organic compound with a weak C-H bond at amine nitrogen position also often experiences fragmentations via hydrogen transfer or electron-proton transfer. The possible transformation pathways of nitrogen atom of OTC molecule are presented in Figure 4, which is derived based on Pelizetti *et al.* proposed pathways for formamide [15]. Pelizetti *et al.* [15] have studied the photocatalytic degradation of formamide which has similar primary amine position of with the of OTC (position b, Figure 2). They postulated that the degradation mechanism was affected by the carbon atom oxidation state rather than the nitrogen atom. In other word, the final product of degradation is determined by the amount of the carbon atom substituent (*i.e.* 1 = primary carbon, 2 = secondary carbon, *etc.*) rather than the nitrogen atom substituent. Moreover, the hydroxyl radical attack on nitrogen atom was also occurred with bicarbonate and nitrate ions as their final product, with lesser extent. The proposed OTC intermediate structure after the primary amine release is presented in Figure 5. Similar mechanism was also presented by Jeong *et al.* [16] that describe the primary amine fragmentation of OTC structure which was initiated through ketonol tautomerization.

Beside the primary nitrogen, OTC molecule also has another nitrogen atom which belong to the tertiary amine group (position a, Figure 2).

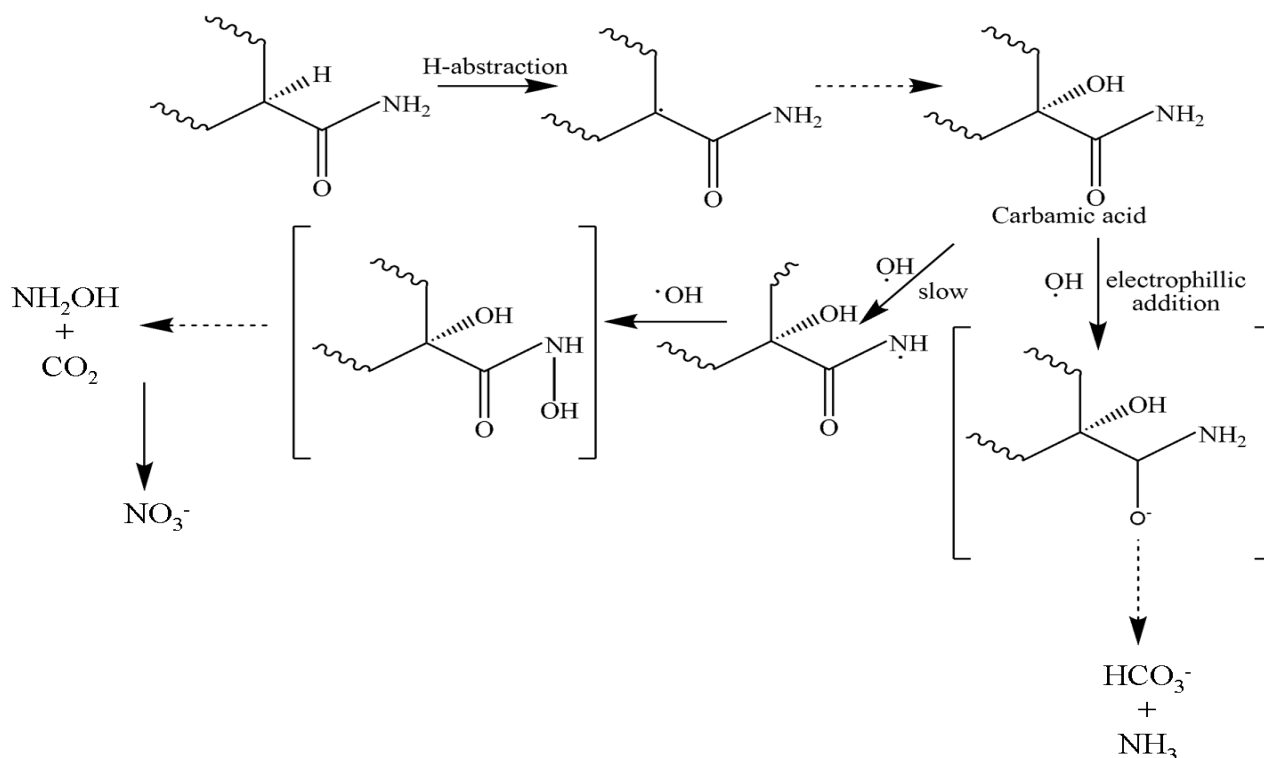


Figure 4. Proposed transformation pathways of primary amine functional group of OTC.

Figure 6 shows the proposed transformation pathways of tertiary amine of OTC molecule which was proposed based on the study performed by Klare *et al.* [17]. The radical attack on α -CH was carried out based on hydrogen abstraction mechanism. Further hydroxyl radical attack would drive the occurrence of hydroxyl radical addition and followed by the C-C bond cleavage, producing carbonylic compound and an amine. In accordance with Figure 4 and Figure 6, at the earlier stage the amide and tertiary amine of OTC molecules may contribute to the formation of ammonium. Meanwhile, the generation of nitrate ion come from the transformation of amide group of OTC. Hence nitrate ion was detected in smaller concentration, compared to ammonium. Moreo-

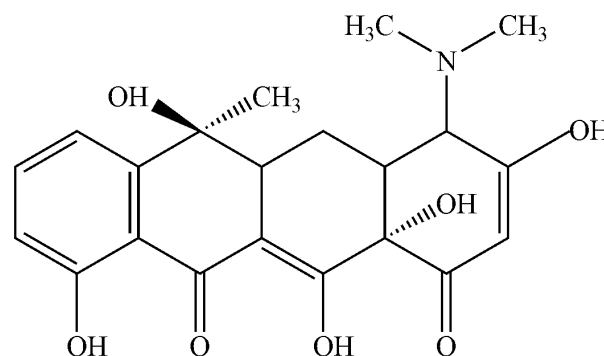


Figure 5. Proposed OTC intermediate structure after primary nitrogen release.

ver, longer irradiation time would cause the reduction of ammonium to nitrate ions, hence lead to increased nitrate ion concentration. Similar results were also observed for the photocatalytic degradation of n-pentylamine [18].

Figure 7 shows the pH profile during OTC mineralization. After 10 min of irradiation, the solution pH decreased from 6.5 to 3.8 which continues until 90 min of irradiation. This pH reduction most probably due to the higher concentration of acidic intermediates (organic acid) and CO_2 formed, rather than ammonia. Similar results were also obtained for photocatalytic degradation of piperidine, after 10 min of irradiation, the piperidine solution pH decreased rapidly from 7.1 to 5.9 [18]. However, after 120 mins irradiation, pH of the solution started to increase until 180 min of irradiation. This increase was due to the higher concentration of amine intermediate (*i.e.* ammonia) in the solution compared to acidic intermediate (*i.e.* organic acid and CO_2). In contrast with OTC, n-pentylamine oxidation experienced acidic pH at the end of irradiation time due to the excess of CO_2 being produced compared to ammonia [18].

During TOC analysis, the sample solution was acidified using H_3PO_4 to ensure that all the carbonate and bicarbonate were transformed into CO_2 . However, since the partially-degraded OTC sample had pH below the pK_a

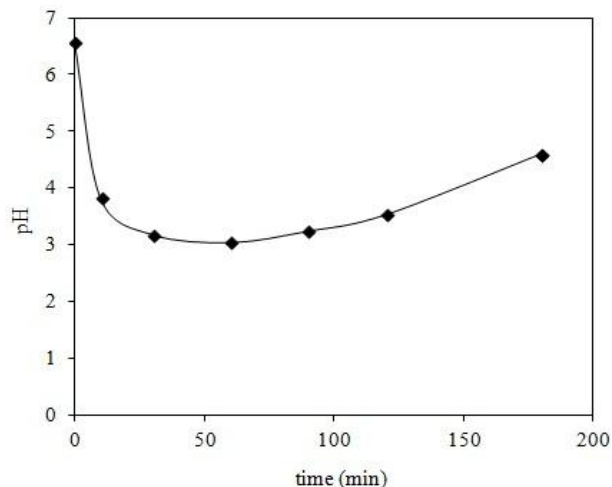


Figure 7. pH profile of OTC removal at optimum condition.

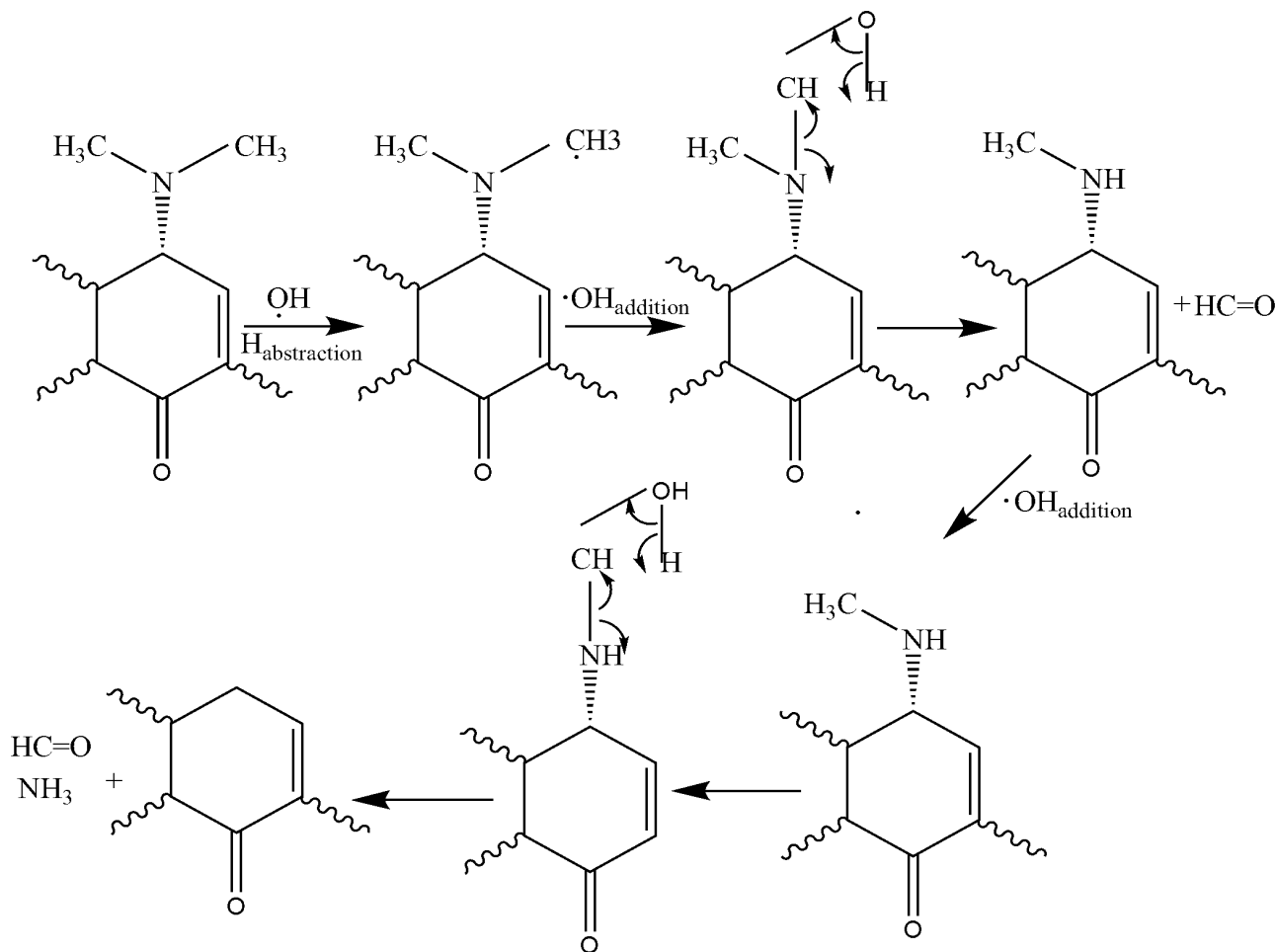
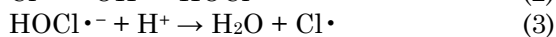
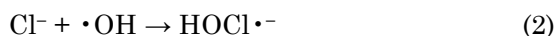


Figure 6. Proposed transformation pathways for tertiary amine of OTC molecule.

of carbonate ($pK_a = 10.3$) and bicarbonate ($pK_a = 6.3$), the value of inorganic carbon could be approximated as the concentration of CO_2 . Based on Figure 8, the CO_2 concentration profile increased along the irradiation time which proved the occurrence of the OTC mineralization. Similar CO_2 profiles were also shown during the piperidine and chloramphenicol removal [17–18].

Since the OTC hydrochloride was used as the antibiotic sample, the chloride ion was detected inside the solution sample due to the ionization of OTC compound. Inorganic chloride ion was also observed during the photocatalytic oxidation of cyclophosphamide and atrazine [10]. After irradiation took place, the hydroxyl radicals were started to form. Since the reduction potential of hydroxyl radical is higher ($E^\circ = +2.8$ V) compared to chloride ion ($E^\circ = +2.2$ V), the hydroxyl radical is capable to oxidize the chloride ions (Equation 2). The $HOCl^{\bullet-}$ radical could decompose into radical (Equation 3) that could recombine into to form chlorine atom [19].



The oxidation of chloride ions into Cl_2 was progressing as the mineralization of OTC took place. In the present study, the presence of chlorine atom was represented by the value of total chlorine total chlorine is equal to total amount of free chlorine plus chlorine attached to other components. Their profiles are shown in Figure 9. As the reaction proceed ($t = 30$ min), a certain amount of chloride ion was started to dissociate from the OTC parent structure hence increased in chloride ion concentration was observed. At the same time in-

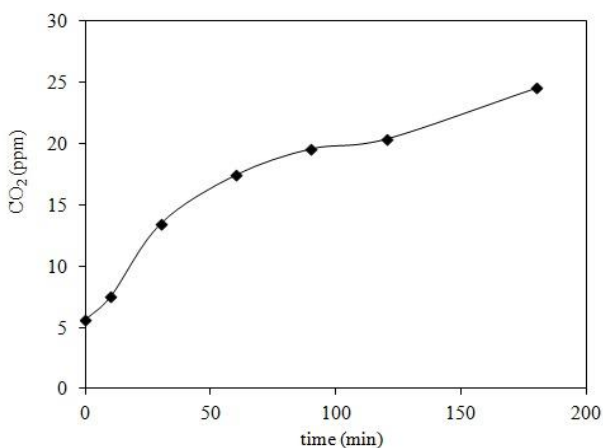


Figure 8. CO_2 concentration profile of OTC removal at optimum condition.

terval ($t = 30$ min), some of chloride ion were oxidized which led to the formation of chlorine atom. Thus, an increasing concentration of chlorine was observed. However, after 180 min of irradiation, the chloride ions were not oxidized completely into chlorine.

4. Conclusions

Mineralization of OTC have been conducted at its optimum TOC mineralization. Several inorganic by-products were detected, namely, nitrate, ammonium, chloride ion, chlorine. The detected by-products have proven the efficacy of the mineralization process. Based on the generated by-products, several reaction sequences have been proposed.

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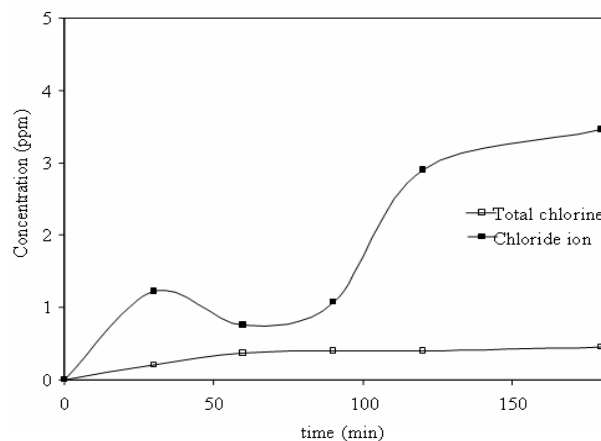


Figure 9. Concentration profile of total chlorine and chloride ion at the optimum condition.

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