Aqueous oxytetracycline degradation and the toxicity change of degradation compounds in photoirradiation process

JIAO Shaojun1, ZHENG Shourong1, YIN Daqiang2*, WANG Lianhong1, CHEN Liangyan1

1. State Key Laboratory of Pollution Control and Resource Reuse, School of the Environment, Nanjing University, Nanjing 210093, China.
E-mail: jwoodward@163.com
2. Key Laboratory of Yangtze Water Environment of Ministry of the State Education, Tongji University, Shanghai 200092, China

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Abstract

The extensive use of antibiotics has been a worldwide environmental issue. In this study the fate of oxytetracycline (OTC), under photoirradiation, was investigated. The results showed that OTC photolysis followed first order model kinetics. Direct photolysis rate was found to be dependent on the initial OTC concentration, with k value ranging from 0.0075 to 0.0141 min⁻¹, in the OTC concentration from 40 to 10 mg/L. OTC photolysis was highly pH-dependent and strongly enhanced at a high pH value, with a k value of 0.0629 min⁻¹ at pH 9. Enhanced OTC photolysis has also been observed in the presence of nitrate and low concentration of dissolved organic matter. Upon irradiation for 240 min, only 13.5% reduction of TOC occured in spite of a rapid consumption of 90% of OTC. The byproducts from OTC photolysis have been analyzed using high-performance liquid chromatography electrospray ionization mass spectrometry (LC-ESI-MS), and the degradation pathway of OTC in the photo process was proposed. By employing luminescent bacterium to assess the adversity of the degradation compounds, an increased effect of toxicity occured in spite of the great consumption of OTC in the photoirradiation process. After irradiation for 240 min, the inhibition rate was 47%, significantly higher than the initial rate of 21% (p < 0.05), revealing a potentially higher adversity risk on the microorganism upon OTC photolysis.

Key words: antibiotics; oxytetracycline; photodegradation; LC-ESI-MS; toxicity

Introduction

Increasing worldwide concerns about antibiotics used in pharmaceutical therapies and agriculture, were mentioned in recent literature (Jagobsen and Berglind, 1988; Michael, 2001). A great amount of administered antibiotics were released daily into the environment through excretion or direct runoff (Halling-Sorensen et al., 1998; Hirsch et al., 1999). A lot of recent literature reported the introduction of antibiotics to wide environmental matrixes, including sewage treatment plants, surface water, and soils (Stackelberg et al., 2004; Zilles et al., 2005; Andreozzi et al., 2006). Furthermore, antibiotics would be more stable at a considerable depth in soil and sediment (Zhang et al., 2006), and then could re-enter the aqueous environment by surface runoff, leaching, and desorption (Herve et al., 1993; Kay et al., 2005). Eventually, antibiotics residues can enter drinking water because of their high stability against conventional treatment of biodegradation and chlorination (Andreozzi et al., 2006; Westerhoff et al., 2005). Frequent occurrences of those cumulated antibiotics in the environment could induce an irreversible adversity by directly threatening the function of the ecosystem (Costanzo et al., 2005; Schmitt et al., 2004; Davison, 1999), increasing the resistance of bacteria to drugs, and spreading the antibiotic resistance genes into the environment (Sengelow et al., 2003; Graslund and Bengtsson, 2001; Pei et al., 2007; Auerbach et al., 2007; Macauley et al., 2006), consequently jeopardizing human health (Kummerer, 2001; Rhodes et al., 2000).

Oxytetracycline (OTC), one of the most widely prescribed antibiotics, has three ionization equilibriums with pKₐ values of 3.57, 7.49, and 9.88 (Figueroa et al., 2004), and the four protonation states can be represented by H₃OTC⁺, H₂OTC, HOTC⁻, and OTC⁻². Scheme 1 shows the structure of the OTC, compilation of the four states of OTC, and the ionization equilibriums.

Photodegradation under solar irradiation is considered as an important method of antibiotic degradation in natural aquatic environment (Andreozzi et al., 2003). In addition, the wide presence of dissolved organic matter (DOM) and nitrate in the natural aquatic environments may influence the photolysis behavior of antibiotics (Squillace et al., 2002; Zhan et al., 2006; Andreozzi et al., 2003). The removal of antibiotics by various methods has been frequently reported in recent literature (Polubesova et al., 2006; Huber et al., 2005), whereas, OTC photolysis in the...
aQUATIC ENVIRONMENT is seldom addressed. IN THIS STUDY, THE EFFECT OF ENVIRONMENTAL MATRIXES ON THE PHOTODEGRADATION OF AQUEOUS OTC AND ON THE DETERMINATION OF BYPRODUCTS, AS WELL AS THE TOXICITY OF OTC AND ITS DEGRADATION PRODUCTS WAS INVESTIGATED.

1 EXPERIMENTAL SECTION

1.1 MATERIALS AND CHEMICALS

OXYTETRACYCLINE (OTC) AND HUMIC ACID WERE PURCHASED FROM FLUKA Co., Ltd. (USA). ACETONITRILE AND FORMIC ACID OBTAINED FROM TEDIA COMPANY Inc. (USA) WERE OF HPLC GRADE. SODIUM NITRATE AND OTHER REAGENTS WERE OF ANALYTICAL GRADE. ULTRA PURE WATER WAS PREPARED BY AN ULTRA-FILTER FROM LABCONCO Co. (USA). THE BACTERIA Photobacterium phosphoreum T3 (P. phosphoreum) WAS PROVIDED AS FREEZE-DRIED POWDER (0.5 g EACH BOTTLE) BY THE INSTITUTE OF SOIL SCIENCE, CHINESE ACADEMY SCIENCES, NANJING, CHINA. THE WORKING SOLUTIONS WERE FRESHLY PREPARED.

1.2 PHOTOCHEMICAL EXPERIMENTS

THE PHOTOLYSIS SYSTEM CONSISTED OF A 500-W MEDIUM MERCURY LAMP, COOLED BY TAP WATER, QUARTZ REACTOR PLACED AT A FIXED DISTANCE. LIGHT BELOW 290 nm WAS WELL FILTRATED BY THE BOROSILICATE GLASS. THE LIGHT INTENSITY AT 365 nm WAS DETERMINED TO BE 5.25 × 10⁻⁴ W/cm². FOR OTC PHOTOLYSIS, 50 ml OF OTC SOLUTION WAS ADDED INTO THE QUARTZ REACTOR. SAMPLES WERE TAKEN AT SELECTED INTERVALS AND THE RESIDUAL CONCENTRATION OF OTC WAS ANALYZED USING HPLC (1200, Agilent, USA). EACH PHOTOLYSIS EXPERIMENT WAS CONDUCTED IN TRIPlicate.

1.3 ANALYTICAL METHODS

THE CONCENTRATION OF OTC WAS MEASURED BY HPLC COUPLED WITH A C18 REVERSED PHASE COLUMN (250 mm × 4.5 mm I.D., 5 μm, Agilent, USA) AT 30°C. THE MOBILE PHASE HAD ACETONITRILE-WATER (Vacetonitrile/Vwater = 80/20, pHwater = 2) AT A FLOW RATE 1.0 ml/min. DETECTION WAS PERFORMED WITH AN ULTRAVIOLET (UV) DETECTOR (1200, Agilent, USA) AT A WAVELENGTH OF 355 nm. THE SPECTRUM OF THE TOC SOLUTION WAS SCANNED BY A UV-Vis SPECTROPHOTOMETER (UV2550, Shimadzu, Japan). DISSOLVED HUMIC ACID AND TOTAL ORGANIC CARBON (TOC) OF THE REACTION SOLUTIONS WERE DETERMINED BY A TOC ANALYZER (2200, Shimadzu, Japan) AND EXPRESSED AS TOC mg/L.

TO IDENTIFY THE DEGRADATION PRODUCTS OF OTC, THE SOLUTION SAMPLES WITH 20 mg/L OTC WERE SUBMITTED TO IRRADIATION FOR 0, 90, AND 150 min. THE DEGRADATION COMPOUNDS OF OTC WERE ANALYZED BY LC-MS (FINNIGAN LCQ Advantage MAX, USA) AT A 10-μL SOLUTION INJECTED INTO THE COLUMN. THE MOBILE PHASE WAS A MIXTURE OF 50% ACETONITRILE AND 50% FORMIC ACID (0.1% BY VOLUME) AT A FLOW RATE 0.2 ml/min. THE ELECTROSPIRAY IONIZATION MASS SPECTROMETRY ANALYSES WERE PERFORMED IN THE NEGATIVE MODE, WITH A SPRAY VOLTAGE OF 4.5 kV AND AN ION-TRANSFER CAPILLARY TEMPERATURE OF 200°C. NITROGEN WAS USED AS SHEATH GAS AT A FLOW RATE OF 20 arb units (arbitrary unit).

1.4 OTC TOXICITY AND DEGRADATION

IN THE IRRADIATION PROCESS, SAMPLES CONTAINING 20 mg/L OTC WERE SELECTED IN CERTAIN INTERVALS TO ASSESS THE TOXICITY CHANGE OF THE DEGRADATION PRODUCTS. PRIOR TO TOXICITY ASSESSMENT, THE BACTERIA WERE REACTIVATED IN 1 ml 2.5% NaCl SOLUTION AND STORED IN AN ICE WATER BATH. Subsequently, 0.2 ml OF EACH TREATED SAMPLE AND 10 μL REACTIVATED BACTERIA WERE ADDED TO 2 ml OF 3% NaCl SOLUTION. THE DECREASE IN BIOLUMINESCENCE INDICATED A TOXIC EFFECT BY DEGRADATION COMPOUNDS AND WAS MEASURED BY THE MODEL TOXICITY ANALYZER (DXY-2, INSTITUTE OF SOIL SCIENCE, CHINESE ACADEMY SCIENCES, NANJING, CHINA) AFTER A 10-min EXPOSURE AT 15 ± 1°C. LUMINESCENCE INHIBITION PERCENTAGE WAS USED TO EXPRESS THE EFFECT DEGREE OF OTC AND DEGRADATION COMPOUNDS ON P. phosphoreum.

2 RESULTS AND DISCUSSION

2.1 OTC PHOTOLYSIS

2.1.1 PHOTOLYSIS KINETICS

THE DEGRADATION OF OTC WITH INITIAL CONCENTRATION VARYING FROM 10 TO 40 mg/L WERE INVESTIGATED, THE RESULTS ARE COMPILED IN FIG.1. THE OTC PHOTOLYSIS KINETICS WAS SIMULATED AND WAS FOUND TO BE A WELL-FITTED FIRST ORDER MODEL:

\[ C = C_0 \times e^{-kt} \]  

WHERE, \( C_0 \) IS THE INITIAL OTC CONCENTRATION, \( C \) IS THE RESIDUAL OTC CONCENTRATION AT IRRADIATION TIME \( t \), AND \( k \) WAS THE RATE CONSTANT.

IRRADIATION OF AQUEOUS OTC WITH THE INITIAL CONCENTRATION OF 10 TO 40 mg/L FOR 240 min RESULTED IN DEGRADATION OF
95% to 73%. The degradation kinetics rate constants were found to be dependent on the OTC initial concentration (Table 1). Increasing OTC initial concentration from 10 to 40 mg/L led to the decrease of the rate constants from 0.0141 to 0.0075 min\(^{-1}\), indicative of an enhanced photolysis of OTC, with an increase in the solution of pH.

OTC is a zwitterionic molecule and the fraction of certain protonation states can be calculated by the following equations:

\[
\alpha^+ = \frac{1}{1 + 10^{\text{pH}-pK_{a1}} + 10^{2\text{pH}-pK_{a1}-pK_{a2}} + 10^{3\text{pH}-pK_{a1}-pK_{a2}-pK_{a3}}}
\]

\[
\alpha^0 = \frac{1}{1 + 10^{pK_{a1}} + 10^{2\text{pH}-pK_{a1}-pK_{a2}} + 10^{3\text{pH}-pK_{a1}-pK_{a2}-pK_{a3}}}
\]

\[
\alpha^- = \frac{1}{1 + 10^{pK_{a1}+pK_{a2}}+pK_{a2}+pK_{a3}-2\text{pH} + 10^{pK_{a2}-pH} + 10^{pK_{a2}-pH}}
\]

\[
\alpha^{2-} = \frac{1}{1 + 10^{pK_{a1}+pK_{a2}+pK_{a3}-2\text{pH} + 10^{pK_{a2}-pH}} + 10^{pK_{a3}-2\text{pH}} + 10^{2pK_{a3}-pH}}
\]

where, \(\alpha\) is the fraction of certain species.

Its existing form, depending on aqueous pH, is depicted in Fig.3. The OTC molecules exist in a neutral and positively charged form at pH 4; in contrast, negatively charged OTC molecules are dominant in a solution of pH 9. The degradation results show that the photolysis of OTC is markedly inhibited in OTC\(^+\) form and promoted in the OTC\(^-\) form. Zertal et al. (2001) has also observed an enhanced photochemical degradation of 4-chloro-2-methylphenoxyacetic acid in the anionic form, compared to its neutral form. In general, the pH influences photolysis by altering the protonation state of organic matters as well as the absorbance spectrum (Boreen et al., 2004). Fig.4 compares the UV-Vis spectra of OTC in the range of 290 to 500 nm, at different pH. The absorbance of OTC at pH 4 is lower than that of OTC at pH 6, evidenced by the fact that the photolysis rate constant is 0.0089 min\(^{-1}\) at pH 6, higher than that at pH 4 (\(k = 0.0068\) min\(^{-1}\)). Increasing solution pH leads to the increase in the photolysis rate.

![Fig. 1](image1.png)

**Fig. 1** Effect of initial OTC concentration on photodegradation.

![Fig. 2](image2.png)

**Fig. 2** Effect of solution pH on OTC photodegradation.

![Fig. 3](image3.png)

**Fig. 3** OTC ionization states according to solution pH.

**Table 1** Calculated \(k\) values of oxytetracycline (OTC) photodegradation

<table>
<thead>
<tr>
<th>OTC initial conc. (mg/L)</th>
<th>(k) (min(^{-1}))</th>
<th>pH</th>
<th>(k) (min(^{-1}))</th>
<th>DOM* (mg/L)</th>
<th>(k) (min(^{-1}))</th>
<th>NO(_3)−* (mg/L)</th>
<th>(k) (min(^{-1}))</th>
</tr>
</thead>
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<tr>
<td>10</td>
<td>0.0141</td>
<td>4</td>
<td>0.0068</td>
<td>0</td>
<td>0.0089</td>
<td>0</td>
<td>0.0089</td>
</tr>
<tr>
<td>20</td>
<td>0.0089</td>
<td>6</td>
<td>0.0089</td>
<td>3.75</td>
<td>0.0139</td>
<td>5.0</td>
<td>0.0099</td>
</tr>
<tr>
<td>30</td>
<td>0.0085</td>
<td>7</td>
<td>0.0186</td>
<td>7.50</td>
<td>0.0161</td>
<td>10.0</td>
<td>0.0139</td>
</tr>
<tr>
<td>40</td>
<td>0.0075</td>
<td>9</td>
<td>0.0692</td>
<td>15.0</td>
<td>0.0133</td>
<td>50.0</td>
<td>0.0150</td>
</tr>
</tbody>
</table>

* At the condition of OTC 20 mg/L. DOM: dissolved organic matter.
constant from 0.0089 min\(^{-1}\) at pH 6 to 0.0692 min\(^{-1}\) at pH 9, indicating low stability of OTC at high pH. It can be noted that a red shift of the OTC absorbance spectrum occurs with the increasing of pH from 6 to 7. It is attributed to the formation OTC molecules from OTC\(^0\) to OTC\(^-\) anion, concomitant with the transition of \(\pi\) to \(\pi^*\) state of OTC chromophore (Dos Santos et al., 2000). In addition, negatively charged OTC molecules, with a high electrical density on the ring system, tend to attract reactive species such as \(\cdot\)OH, which consequently facilitate OTC photolysis.

2.1.3 Influence of DOM and NO\(_3^-\)

DOM and nitrate are ubiquitously present in natural aquatic environment (Squillace et al., 2002; Canonica et al., 1995). They play important roles in the transformation of a variety of aqueous organic pollutants (Brezonik and Fulkerson-Brekken, 1998). Typically in a natural aqueous environment, humic acid (HA) is the majority component of DOM. In this study, the photolysis of OTC, with initial concentration of 20 mg/L, in the presence of different concentrations of aqueous nitrate and humic acid, have been investigated.

OTC photolysis in the presence of HA, with concentration varying from 0 to 15 mg/L, was conducted to evaluate the influence of HA on OTC photolysis, the results are described in Fig.5a. The OTC photolysis rate constants fitting the experimental data using first order kinetic model are listed in Table 1. The presence of HA enhanced the photolysis of OTC, compared to photolysis in the absence of HA. As a natural photosensitizer, HA can induce either direct degradation of organic compounds, via energy transferring (Zepp et al., 1985; Canonica et al., 1995), or the formation of the reactive species such as \(\cdot\)OH (Scully et al., 1996).

It was noteworthy that increasing HA concentration from 7.5 to 15 mg/L resulted in a decrease in the OTC photolysis rate constant from 0.0161 to 0.0133 min\(^{-1}\). It indicated the inhibiting effect of HA, with a relatively high concentration for OTC degradation. Le-Clech et al. (2006) also observed a similar trend in the presence of a high concentration of DOM. In principle, HA could service as a \(\cdot\)OH source and enhance OTC photolysis. However, high concentration of HA might act as an irradiation filter and a \(\cdot\)OH sink, more than a source (Brezonik and Fulkerson-Brekken, 1998).

The OTC photolysis in the presence of nitrate with the concentration ranging from 0 to 50 mg/L is compiled in Fig.5b. The degradation of OTC obeyed the first order kinetic model and the rate constants are listed in Table 1. OTC photolysis rate constants are found to be 0.0089, 0.0099, 0.0119, and 0.0150 min\(^{-1}\) at nitrate concentrations of 0, 5, 10, and 50 mg/L, respectively. This demonstrated an elevated OTC photolysis efficiency at high nitrate concentration. Upon irradiation, reactive species can be generated in nitrate solution (Lam et al., 2003), and Brezonik and Fulkerson-Brekken (1998) have found that nitrate-induced \(\cdot\)OH is positive concentration dependant. Consequently, the presence of nitrate gives a pronounced impact on the rise of OTC decay rate.

2.2 Identification of OTC photodegradation products

2.2.1 Changes of TOC content in the irradiation process

Typically, the photolysis of organic pollutants could lead to the structural decomposition and eventually to the mineralization to CO\(_2\) (Wolters and Steffens, 2005). For most antibiotics, complete mineralization is usually
difficult because of the structural stability (Westerhoff et al., 2005). The changes in TOC during OTC photolysis are described in Fig.6. After irradiation for 240 min, approximate 90% OTC is degraded, in comparison with 13.5% of TOC reduction. Dalmázio et al. (2007) has also observed a low degree of mineralization in spite of the rapid consumption of tetracycline under ozonation. The low degree of OTC mineralization implies that consumed OTC mainly transforms to byproducts and further identification of the intermediates is desirable.

2.2.2 Proposed OTC photolysis pathway

On the basis of the experimental result, determination of the degradation product was conducted with LC-ESI-MS, and the obtained MS spectra, taken at 0, 90, and 150 min, are compiled in Fig.7. Upon irradiation, a new anion (M-H)$^-$ peak of compounds are observed, and further irradiation leads to an increase in the abundance of this peak, which includes most of the main degradation products. While, in the second peak, OTC molecular with $m/z$ of 459.0 is found to be dominant. On the basis of LC-MS results, the degradation pathway of OTC arising from the cleavage and its substitution is proposed in Scheme 2. Product P$_3$ comes from the OTC degradation by the loss of N-methyl and amino groups because of the low bond energy of N–C (Raphael et al., 2000), with the intermediate of P$_1$ and P$_2$. Further degradation of P$_3$ leads to the generation of P$_4$ via loss of hydroxyl groups (Dalmázio et al., 2007), with subsequent formation of P$_5$ by a further loss of N-methyl. Product P$_6$ stems from OTC by the substitution of -OH in an aromatic ring and undergoes further scission to P$_7$ (Zhan et al., 2006). It is worthy to note that the naphthol ring of OTC remains intact during the degradation process.

2.3 Changes of toxicity in photolysis process

The toxicity of OTC and its degradation compounds was evaluated using luminescent bacterium. The toxic-
Ities were expressed in luminescence inhibition rate, as shown in Fig. 8. The inhibition rate of the initial solution containing 20 mg/L OTC was about 21%, and trended to increase during the degradation process, with an inhibition rate of 47% after irradiation for 240 min. Formation of segmented products by structure fragmentation or increasing mineralization of the organic pollutants would result in the decrease of toxicity (Andreozzi et al., 2006; Chaabane et al., 2007). In contrast, the degraded OTC mainly transformed to new products instead of structure fragmentation and mineralization. Halling-Sorensen et al. (2002) also observed that the toxicity of certain degradation products of tetracycline was similar to that of tetracycline. It is possible that the combined toxicity of OTC and the byproducts led to the increase in toxicity during the irradiation process. These effects of toxicity increase in the presence of photolysis products were also observed with other kinds of phenolic pollutants (Zertal et al., 2001; Bonnemoy et al., 2004). Therefore, the degradation compounds, upon photoirradiation, would lead a higher adversity to the bacteria and the environmental ecosystem function, and deserved further treatment.

3 Conclusions

In this study, the fate of OTC under irradiation was presented. The contribution of nitrate and low content of HA in the natural environment to the OTC photolysis is apparent. The pH has a great effect on the photodegradation of OTC in an aqueous environment. Cationic form of OTC$^+$ with low pH value can augment the chemical stability of OTC under photolysis, whereas, anion OTC$^−$ with high pH, results in a stronger activity than the neutral form. Upon irradiation, the degraded OTC mainly transforms into intermediate byproducts and the degradation pathway was proposed. It is noteworthy that the main byproducts remain in the structure of the naphthol ring.

The increasing toxicity of degradation compounds would probably lead to higher adversity with the bacteria in the environment, eventually influencing the health of human. Further toxicity study might focus on the clarity of the toxicity effect of single byproducts, and the extended detailed adversity to other organisms, such as plants, algae, and other animals in the aquatic environment.

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References


