Photocatalytic degradation of tetracycline in aqueous solution by nanosized TiO$_2$

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Highlights

- NH$_4^+$ ions were the end-product of photocatalytic degradation of TC by TiO$_2$.
- The photocatalysis eliminated 95% of TC after 60 min irradiation.
- Photocatalytic pathway of TC was proposed based on the identified intermediates.
- The toxicities of reaction solution and free radical were discerned.

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Abstract

Tetracyclines are widely-used antibiotics in the world. Due to their poor absorption by human beings, or poultry and livestock, most of them are excreted into the environment, causing growing concern about their potential impact, while photodegradation has been found to dominate their sequestration and bioavailability. Coupling with high-performance liquid chromatography–mass spectroscopy (HPLC–MS), gas chromatography–mass spectroscopy (GC–MS) and electron spin resonance (ESR), the mechanism of photocatalytic degradation of TC in aqueous solution by nanosized TiO$_2$ (P25) under UV irradiation was investigated. The photocatalysis eliminated 95% of TC and 60% of total organic carbon (TOC) after 60 min irradiation, and NH$_4^+$ ion was found to be one of the end-products. Bioluminescence assay showed that the toxicity of TC solution reached the maximum after 20 min irradiation and then gradually decreased. The degradation of TC included electron transfer, hydroxylation, open-ring reactions and cleavage of the central carbon. A possible photocatalytic degradation pathway of TC was proposed on the basis of the identified intermediates. Overall, the TiO$_2$ photocatalysis was found to be a promising process for removing TC and its intermediates.

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1. Introduction

Tetracyclines (TCs) are one of the most widely-used antibiotics in aquaculture and veterinary medicines (Palominos et al., 2009). Due to their poor absorption, most of them are excreted through feces and urine as un-metabolized parent compound. The most dangerous effect of antibiotics in the environment is the development of multi-resistant bacterial strains that can no longer be treated with the presently known drugs (Addamo et al., 2005). Hence, the occurrence, fate and behavior of antibiotics in the environment have been the subject of growing concern and scientific interest (Verma et al., 2007; Gómez-Pacheco et al., 2011; Yuan et al., 2011). Significant amount of TCs have been detected in superficial, potable water and sludge due to their ineffective removal by the conventional water treatment methods. It seems that traditional biological methods could not effectively eliminate antibiotics (Bautitz and Nogueira, 2007; Palominos et al., 2009; Wang et al., 2011a). Therefore, to develop effective techniques for rapid degradation of TCs is of environmental interest.

TiO$_2$ photocatalysis is a promising method in water treatment for removal and mineralization of organic pollutants (So et al., 2002; Zhang et al., 2005; Pelaez et al., 2011; Yahiat et al., 2011; Wang et al., 2011a). Photocatalytic degradation of organic pollutants could lead to produce end-products, but they are usually unclear. In addition to that, the intermediates possibly induce negative effects to the environmental ecosystem function (Jiao et al., 2008), which suggested that the variations of toxicity of the solution after photocatalytic reaction should be examined. The intermediates of TC photolysis and ozonation have been identified (Dalmázio et al., 2007; Jiao et al., 2008; Wang et al., 2011b), however, to our best knowledge, intermediates and possible pathway of TC photocatalytic degradation were seldom addressed.

In this study, the photocatalytic degradation of TC in aqueous solution was investigated using nanosized TiO$_2$ (P25) as the photocatalyst under UV irradiation, and the effects of solution pH and...
dissolved oxygen on TC degradation were evaluated. We attempted to use flow injection analysis to explore the evolution of some inorganic ions. The principal intermediates of TC photocatalytic degradation were determined by HPLC–MS and GC–MS techniques. In addition, the ESR technique was employed to verify the role of inhibitors. The reaction pathways were also proposed based on the experimental results.

2. Materials and methods

2.1. Materials and chemicals

Hydrochloride salt of TC (96% purity), trimethylchlorosilane (TMCS), N,O-bis(trimethylsilyl) trifluoroacetamide (BSTFA), and 5,5-dimethyl-pyrolone-N-oxide (DMPO) were purchased from Sigma Company. Nanosized titanium dioxide (TiO2, named P25, ca. 70% anatase and 30% rutile, size 20–30 nm, surface area 50 m² g⁻¹) was purchased from Degussa Company (Germany). Acetonitrile, isopropanol (HPLC grade) and phosphoric acid (85%, HPLC grade) were purchased from TEDA Company (USA). Dichloromethane (HPLC grade) was from Tianjin Kemiu Chemical Reagent Company. All other chemicals were of reagents grade.

2.2. Photocatalytic reactions

The photocatalytic reactions were performed in 40 mL aqueous dispersions containing 1000 mg L⁻¹ TiO₂ and 40 mg L⁻¹ TC (the preliminary experiments showed the optimized TiO₂ concentration was 1000 mg L⁻¹, Fig. S2). Before each reaction, TC solution was kept in dark for 30 min with constant stirring to reach the adsorption equilibrium in the photocatalytic system (preliminary results indicated that adsorption equilibrium could be quickly reached less than 30 min). The initial pH was adjusted with NaOH or HNO₃ (2.0 M). The suspension was magnetically stirred before and during irradiation. Samples were withdrawn at different intervals, and then the samples were subjected to filtration through a 0.22-μm membrane filter (Nylon, Anpel Scientific Instrument Co., Ltd., Shanghai, China). All experiments were performed in triplicate.

For examining the effect of dissolved oxygen on the photocatalytic degradation of TC, the suspension was always purged by nitrogen or air before and during irradiation. In order to confirm the role of holes and hydroxyl radical in the photocatalytic degradation of TC, EDTA (3.0 mM) or isopropanol (100 mM) as radical scavengers was added to the TC solution, respectively.

2.3. Analytical methods

The concentration of TC was determined by an Agilent 1100 HPLC with a 4.6 × 150 mm Zorbax ODS column at 25 °C. The wavelength of ultraviolet (UV) detector was set at 260 nm. Mobile phase was 0.05 M phosphate buffer at pH 2.3 (80%) and acetonitrile (20%) with a flow rate at 1.0 mL min⁻¹ (Jia et al., 2008). Standard calibration showed good linearity ($R² > 0.999$) between the concentrations of TC and the peak area responses. The removal efficiency of TC was expressed using following equation:

$$\text{Removal efficiency} = \frac{C_1 - C_2}{C_1} \times 100\%$$

where $C_1$ and $C_2$ are the concentrations of TC before and after photocatalytic reaction (Wong et al., 2004). Solution pH was measured by a pH electrode (Leichi Instruments, Shanghai, China). TC solutions were scanned by a UV–Vis spectrophotometer (UV-1600, Ruili Inc., China). The total organic carbon (TOC) of reaction solution was determined by the TOC analyzer (multi N/C 3000, Analytik Jena, Germany). The concentration of NH₄⁻–N in the reaction solution was measured by the flow injection analysis (san+++, Skalar, Holland), and the method followed the standard method (EN ISO 11732-2005).

The toxicity of TC solution after UV irradiation for different time was determined by bioluminescence assay. Prior to toxicity assessment, the bacteria was reactivated in 1.0 mL 3% NaCl solution and stored in the ice water bath. A 200 μL of sample and 20 μL reactivated bacteria were added to 2.0 mL 3% NaCl solution and the decrease in bioluminescence was measured by Model Toxicity Analyzer (DXV-3, Institute of Soil Science, Chinese Academy of Sciences, Nanjing, China) after 15 min exposure at 20 ± 1 °C. Luminescence inhibition percentage was used to reflect the effect of both TC and degradation compounds on V. fischeri, and the inhibition rate was addressed as following equation (L means luminescence) (Jiao et al., 2008). The higher the inhibition rate, the more toxic the sample was (Yuan et al., 2011).

Inhibition rate = \frac{L_{\text{blank}} - L_{\text{sample}}}{L_{\text{blank}}} \times 100\%

Electron spin resonance (ESR) experiments were performed by a spectrometer (Brucker EMX 10/12, Germany) equipped with a 180 W high pressure mercury lamp. Sample in borosilicate glass capillary placed into the quartz tube was irradiated in the cavity of ESR spectrometer. The ESR tests were conducted under the X-band.

The intermediates analysis was performed by a HPLC–MS system (Agilent 1290/6460, Triple Quad MS) equipped with a Zorbax XDB-C18 column (150 × 2.1 mm, 3.5 μm). Mobile phase was a mixture of 15% acetonitrile and 85% formic acid (1%) with a flow rate of 0.2 mL min⁻¹. The injection volume was 5.0 μL, and the column temperature was 30 °C. MS was performed by operating in the positive ion mode using ESI under the following conditions: capillary voltage, 2800 V; fragment voltage 135 V; nebulization pressure, 45 psi; temperature of drying gas, 340 °C; drying gas flow, 8 L min⁻¹; temperature of sheath gas, 370 °C; sheath gas flow, 11 L min⁻¹. MS was scanned by mass range from 50 to 800 m/z.

The identification of intermediates was also conducted by a GC–MS system (CP 3800 coupled with Sursum2200 MS detector) equipped with a CP-8 MS capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness, Varian Company). The column temperature was programmed to increase from 50 (hold for 3 min) to 300 °C (hold for 2 min) at 5 °C min⁻¹. The injector temperature was set at 250 °C. All injections were performed in a splitless mode, and the injection volume was 1.0 μL. For the pre-treatment, the pH of 40 mL filtered solution was adjusted to around 2.5 with 10% HCl, and then the solution was extracted with 15 mL dichloromethane for three times. The extracted solution was concentrated to 1.0 mL by rotary evaporation. Before injection, trimethylsilylation was carried out using 0.5 mL of BSTFA-TMCS (99:1) at 50 °C for 30 min (He et al., 2009).
3. Results and discussion

3.1. Effect of solution pH and dissolved oxygen on the removal efficiency of TC

Solution pH was the most important factor in affecting photocatalytic reactions in general (Jiao et al., 2008). It is well known that both TC species and generation of \( \text{OH} \) depended on solution pH. Fig. 1a shows the removal kinetics of TC in solution at different pH values. After 10 min of irradiation, there were 70%, 75%, and 88% of TC removed at pH 3.0, 7.0, and 9.0, respectively. It was evident that TC was degraded more quickly in basic solution than in acidic one. Similar trends were reported for its photolysis and ozone oxidation (Dalmázio et al., 2007; Jiao et al., 2008). And in these two cases, the solution pH decreased from 9.0 to 7.1 and from 7.0 to 5.9, respectively (Fig. 1a insert).

Several reasons could account for the pH effect (Konstantinou and Albanis, 2004). First, the adsorption behavior of TC influenced its removal efficiency in the UV–TiO\(_2\) system at different pH values. The pH point of zero charge (pzc) of TiO\(_2\) (P25) was 6.8 (Konstantinou and Albanis, 2004). It means that the TiO\(_2\) surface is positively charged in acidic solution (pH < 6.8) while negatively charged in basic solution (pH > 6.8). The following equations clearly illustrated this acid–base chemistry of TiO\(_2\) surface.

\[
\text{TiOH} + H^+ \rightarrow \text{TiOH}_2^+ \\
\text{TiOH} + \text{OH}^- \rightarrow \text{TiO}^- + H_2O
\]

TiOH\(^+\), TiO\(^-\), and TiO\(^2-\) were the positive, neutral, and negative surface hydroxyl groups on the TiO\(_2\) surface, respectively (Chiou et al., 2008). TC was an amphoteric molecule with \( pK_a \) values of 3.3, 7.7, 9.7 and 12 (Jiao et al., 2008), therefore it predominantly existed as \( H_2\text{TC}^+, H_3\text{TC}, H_2\text{TC}^- \) at pH 3.0, 7.0, and 9.0 (Wang et al., 2011a), respectively. Because both the solution properties of TiO\(_2\) surface and speciation of TC are dependent on solution pH, the interaction and affinity between TiO\(_2\) and TC molecule would vary with solution pH. In addition, TiO\(_2\) particles would agglomerate under acidic solution, and the photo-absorption would be reduced (Konstantinou and Albanis, 2004). Second, the concentration of TiO\(_2\)-generated \( \text{OH} \) also affected the photocatalytic degradation of substrate. More \( \text{OH} \) could be produced with increasing solution pH (Wang et al., 2011a). In addition, negatively charged TC molecules at high pH tended to attract reactive groups (such as \( \text{OH} \)) due to their high electrical density on ring system (Jiao et al., 2008), which would result in the enhancement of removal efficiency of TC in basic solution.

To evaluate the effect of dissolved oxygen on the removal efficiency of TC, nitrogen (N\(_2\)) and air were purged to the suspension, respectively, as shown in Fig. 1b. TC is steady in the solution purged by air/N\(_2\) without UV irradiation (Fig. 1b insert), while the degradation of TC was substantial under UV irradiation. Without air/N\(_2\) purging (stirred), 70% and up to 90% of TC were removed after 10 and 40 min of irradiation, respectively. With air purging, the removal efficiency of TC was highest, whereas the TC removal was suppressed by N\(_2\) purging. This indicated that the removal efficiency of TC increased with increasing concentration of dissolved oxygen. The reason was attributed to the following factors: the recombination of photogenerated electron–hole pairs was inhibited because conduction band electrons could be trapped by dissolved oxygen molecular, and the generation rate of reactive oxygen species increased with the increasing concentration of dissolved oxygen that could subsequently lead to the formation of hydroxyl radical and singlet oxygen (Liang et al., 2008; Pelaez et al., 2011).

3.2. Effects of radical and holes inhibitors on the removal efficiency of TC and ESR signal analysis

EDTA and isopropanol were used to demonstrate the roles of \( h^+ \) and \( \text{OH} \) in the photocatalytic process, because EDTA was a holes (\( h^+ \)) scavenger (Minero et al., 2000; Zhang et al., 2008) while isopropanol was proposed to discern the role that the hydroxyl radical (\( \text{OH} \)) played in the oxidation mechanism (Chen et al., 2005; Palomino et al., 2009). As suggested in Fig. 1c, the removal efficiency of TC was significantly inhibited in the presence of EDTA. This indicated that \( h^+ \) participated in the photocatalytic degradation. In addition, EDTA can suppress the removal efficiency of TC due to competitive adsorption as compared to the removal efficiency of TC before UV irradiation (0 min). It is well known that the main steps of the photocatalytic process took place on the surface of photocatalyst (Chang et al., 2010). Therefore, the significant inhibition effect by
EDTA could be attributed to both competitive adsorption and consumption of h\(^{+}\). Apparently, isopropanol also inhibited the removal efficiency of TC. Since isopropanol is a well-known \(\cdot \)OH scavenger due to its high-rate constant with the radical (1.9 \(\times\) 10\(^{9}\) L mol\(^{-1}\) s\(^{-1}\)) (Chen et al., 2005), we suggested that \(\cdot \)OH also played an important role in the photocatalytic degradation of TC.

The ESR spin-trap technique was applied to probe the reactive oxygen species generated in this study (Yang et al., 2009), and DMPO was used as the spin trap to detect \(\cdot \)OH and \(\cdot \)O\(_{2}^{-}\) (Zhan et al., 2000). Fig. 2 showed the ESR signals with different inhibitors at different irradiation time. ESR signals (Fig. 2a) showed 1:2:2:1 quartet with hyperfine splitting constants \(a_H = a_{hf} = 1.497\) mT and \(g\)-factor = 2.0064, which was the typical structure of DMPO–OH adduct. And no ESR signal was observed when the reaction was performed without UV irradiation (0 min). The intensity of the ESR signals rapidly increased with the irradiation time and basically reached maximum after 1 min of irradiation and then decreased. This decrease was probably caused by the repeated attack of \(\cdot \)OH on DMPO which destructed the spin trap (Blažková et al., 2000).

Furthermore, ESR analyses were carried out in the presence of either EDTA or isopropanol in order to verify the role of \(h^{+}\) and \(\cdot \)OH. A lower formation rate of DMPO–OH adduct was observed in the presence of EDTA because of competitive consumption of \(h^{+}\) (Fig. 2b). In addition, the ESR signal of DMPO–OH adduct was also greatly suppressed by the isopropanol, because \(\cdot \)OH could be converted to \(\cdot \)O\(_{2}^{-}\) in the presence of isopropanol, accordingly, the ESR signal of DMPO–O\(_{2}^{-}\) adduct (\(a_H = 1.569\) mT, \(a_{hf} = 2.361\) mT and \(g\)-factor = 2.0063) was observed (Fig. 2c). DMPO–O\(_{2}^{-}\) adduct was still found after 7 min of irradiation, suggesting that DMPO–O\(_{2}^{-}\) adduct was more stable than DMPO–OH adduct in the solution. Based on these ESR results, we may conclude that the order of the formation rate of DMPO–OH adduct was as follows: without inhibitor > isopropanol > EDTA. This trend well explained the different removal efficiency of TC in the presence of EDTA and isopropanol as suggested in Fig. 1c.

### 3.3. Changes of TOC, NH\(_{4}^{+}\) and UV–Vis spectra in the irradiation process

After 60 min of irradiation, more than 95% of TC was removed, whereas only approximate 40% of TOC was detected. Although the removal efficiency of TC was high, the mineralization percentage of TC was still low. Longer irradiation time was required to obtain high mineralization percentage. Dalmázio et al. (2007) and Jiao et al. (2008) also observed that the mineralization percentages of TC photolysis and ozonation were only 15% and 5%, respectively, in spite of rapid removal of TC. Hence, the mineralization percentage of TC photocatalytic degradation was relatively high compared with TC photolysis and ozonation.

In order to investigate the structural changes of TC, the UV–Vis spectra obtained before and after UV irradiation are shown in Fig. 3a. The absorption band of TC was 360 nm, which originated from aromatic rings B–D, comprising the extended chromophores (Wang et al., 2011b,c). This absorption band gradually decreased with the irradiation time, which suggested that the fragmentation of phenolic groups connected to aromatic ring B (Wang et al., 2011c).

The concentration of NH\(_{4}^{+}\) ions was determined by flow injection analysis. The theoretical maximum concentration of NH\(_{4}^{+}\)–N was 2.5 mg L\(^{-1}\) when mineralization percentage of TC was 100%. After 60 min of irradiation, the percentage of TC mineralization to NH\(_{4}^{+}\) reached 28.8% (Fig. 3c), which means that TC was not completely decomposed to harmless inorganic ions.

### 3.4. Toxicity of reaction solution in the irradiation process

The toxicity of TC solution during its photocatalytic degradation was evaluated using the inhibition rate of luminescent bacterium (Jiao et al., 2008). As shown in Fig. 3b, the inhibition rate was greatly increased and reached the maximum value of around 80% after 15 min of reaction, and then gradually reduced to around 40% after reaction for 180 min. In contrast, the concentration of TC and TOC decreased all the way over the reaction. From the above results, we can propose two stages over this process. In the first stage of the initial 30 min, the characteristic structures of reduction products were not totally destructed although almost 80% of TC were degraded (Yuan et al., 2011). The major intermediates of TC degradation lead to a lower steric resistance and an easier penetration into the cell of luminescent bacteria (Lu et al., 2002;
Jiao et al., 2008), which led to the increasing toxicity of reaction solution. In the second stage (after 30 min), these more toxic compounds were further degraded to produce low toxic byproducts such as organic acids (Yuan et al., 2011), which accounted for the decreasing toxicity of reaction solution.

3.5. Identification of intermediates and the proposed degradation pathway

In the process of TiO$_2$ photocatalysis of TC, numerous intermediates were produced. HPLC–MS was used to identify polar intermediates with higher molecule weight (Fig. 4 - the first row), while GC–MS was used to identify non-polar and polar intermediates with lower molecule weight (Fig. 4 – the second and third row). The total ion chromatograms of HPLC–MS of the TC solution are represented in Fig. S3, and the TC peak as well as the peaks of four main intermediates were observed. The retention time of three kinds of intermediates were shorter than that of TC, which indicated that the more polar intermediates were formed during the photocatalytic process (Wang et al., 2011c). Fig. S4 describes the changes of normalized peak area of TC and intermediates. The intensity of TC peak decreased with reaction time, whereas the intensity of intermediates peaks increased at the early stage and then decreased thereafter. This indicated that these intermediates were mineralized in the later stages of the reaction. Based on the total ion chromatograms, the overall MS characteristics of these intermediates are shown in Fig. S5. For the peak with the retention time of 3.33 min, m/z of 445 was observed which was corresponding to the deprotonated TC molecular ion (Wang et al., 2011b).

Fig. 3. Changes of UV–Vis spectra as a function of time (a), toxicity change of TC solution during irradiation process (b) and change of the concentration of NH$_4^+$ as a function of time (c) (C$_0$: 40.0 mg L$^{-1}$, TiO$_2$: 1000 mg L$^{-1}$, UV: 300 W, pH: 4.2).
the more hydroxyl group was introduced, the shorter retention time was observed. These intermediates have larger m/z and were obviously derived from the hydroxylation process.

As the TC structure was wrecked, the reaction mixture became more complex, which was attributed to the formation of some small molecular intermediates. GC–MS technique was used to obtain more intermediates, and 19 kinds of compounds were identified. The total ion chromatograms of GC–MS are presented in Fig. S6, and the retention time (Rt), spectrum characteristics and possible structures of intermediates are listed in Table S1. All the intermediates were mainly produced through two reaction routes: the open-ring reactions and the cleavage of the central carbon (Ju et al., 2008; Yu et al., 2009). The intermediates (the second row in Fig. 4) were produced through the open-ring reactions, and these intermediates could be further oxidized into small organic molecules such as organic acids and alcohols through the cleavage of the carbon reaction (the third row in Fig. 4). Fig. S7 showed the UV spectra of intermediates with retention time 1.78 and 2.22 min,

Fig. 4. Proposed photocatalytic degradation pathway of TC.
and TC itself from HPLC (DAD detection) techniques. The two intermediates had the similar UV adsorption, but different from TC.

During the photocatalytic degradation of TC, TiO₂ absorbed UV light to generate electron–hole pairs (Hong et al., 1998). After addition of OH to the aromatic group, the ring was broken, and CO₂, H₂O, and inorganic ions were ultimately evolved via oxidation steps. Based on the experimental results, the possible degradation pathway of TC is proposed in Fig. 4.

4. Conclusions

More than 95% of TC was removed within 40 min (40 mg L⁻¹ of TC, 1000 mg L⁻¹ of TiO₂) under UV irradiation. The removal efficiency of TC was facilitated in the presence of dissolved oxygen. The mineralization percentage of TC was significantly lower than the removal efficiency of TC, and only 60% of TOC was eliminated after 60 min of UV irradiation. The toxicity of TC solution increased at first, and then gradually decreased due to the subsequent mineralization of intermediates. ESR signal indicated that the formation rate of DMPO–OH adduct was in accordance with the removal efficiency of TC under different inhibitors. At last, the possible degradation pathway of TC is proposed based on the intermediates identified by HPLC–MS and GC–MS analysis.

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Appendix A. Supplementary material

Schematic diagram of photochemical reactor (Fig. S1), effect of TiO₂ concentration on the removal efficiency of TC (Fig. S2, C₀: 40.0 mg L⁻¹, UV: 300 W, pH: 4.2), total ion chromatograms of TC solution after 80 min UV irradiation (Fig. S3), spectrum characteristics (trialkylsilyl derivatives) and possible structures from photocatalytic degradation of TC (Table S1) are included. Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.chemosphere.2012.02.066.

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