Chemosphere 90 (2013) 1427–1434

Contents lists available at SciVerse ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

Transformation of tetracycline during chloramination: Kinetics, products and pathways

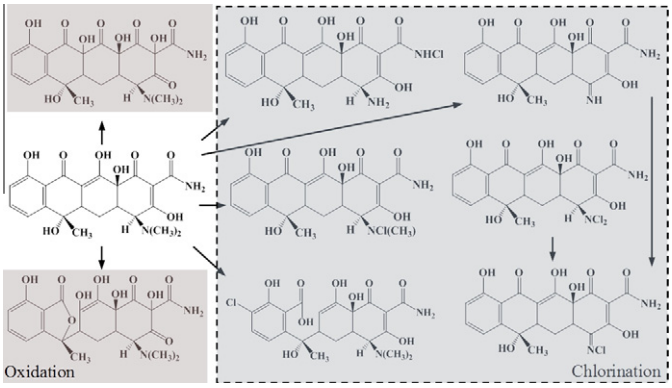
Yi Wan, Ai Jia, Zhou Zhu, Jianying Hu *

MOE Laboratory for Earth Surface Processes, College of Urban and Environmental Sciences, Peking University, Beijing 100871, China

HIGHLIGHTS

- Chloramination of TC exhibited pseudo-first-order kinetics.
- Chloramination of TC generated at least 13 discernible products.
- Two main pathways were proposed: chlorination and oxidization.
- TC inhibitory effects were observed after chloramination towards sludge bacteria.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 4 May 2012
Received in revised form 1 September 2012
Accepted 4 September 2012
Available online 29 September 2012

Keywords:
Tetracycline
Chloramine
Chlorination
Growth inhibition toxicity
Sludge bacteria

ABSTRACT

To assess the potential adverse effects stemming from tetracycline (TC) in drinking water or disinfected wastewater, the kinetics of the chloramination of TC was investigated at room temperature, the transformation products and pathways of their generation were elucidated, and their growth inhibiting properties towards sludge bacteria were assessed. The chloramination of TC exhibited pseudo-first-order kinetics with the rate constants (k_{obs}) ranging from 0.0082 to 0.041 min^{-1} at pH of 6–8. Chloramination of TC generated at least 13 discernible products, and the structures of 12 products, including five chlorinated compounds, were identified using LC–ESI–MS. Two main pathways for the generation of these products were proposed: (1) chlorine substitution reactions followed by dehydration; and (2) oxidization by chloramine. The chlorinated products were proposed to be further degraded to small molecules via the scission of benzene rings of TC, and two oxidization products (2,11a-dihydroxy-keto-TC and 6,11-epoxy-2,11a-dihydroxy-TC) were the final products obtained under the experimental conditions. The chlorinated solution, even without detection of TC, exhibited greater than 80% of TC inhibitory effects towards sludge bacteria, suggesting potential effects on microorganisms in aquatic environment.

1. Introduction

Antibiotics are commonly used in human and veterinary medicine, and the presence of antibiotics in the aquatic environment has raised great concerns due to their adverse effects on exposed organisms and the development of antimicrobial resistance among native bacterial populations (Daughton and Ternes, 1999). There is growing evidence that these compounds are only partially eliminated in sewage treatment plants (STPs), and they are frequently detected in wastewater, surface water, groundwater, seawater and even drinking water (Kümmerer 2009). Although a direct link between exposure to residual antibiotics and induction of antibacterial resistance has not been proven (Zhang et al., 2009), antibacterial resistant bacteria have been detected in various aquatic

* Corresponding author. Tel./fax: +86 10 62765520.
E-mail address: hujy@urban.pku.edu.cn (J. Hu).

0045-6535/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.chemosphere.2012.09.001

environmental samples, constituting a potential risk for human health (Kim et al., 2004; Pruden et al., 2006; Hu et al., 2008).

Tetracycline (TC) antibiotics, as one of the most important antibiotic families, are extensively used in clinical treatment and the livestock industry. The estimated consumption of TCs was approximately 5500 t y^{-1} in the United States and Europe in the mid-1990s (Chopra and Roberts, 2001), and the annual usage of TCs in China was more than 9000 t in 1999 (Hu et al., 2008). As one of the most frequently occurring antibacterial resistances, tetracycline resistances have been described with more than 40 classes of resistance genes in different aquatic environments (Chopra and Roberts, 2001; Kim et al., 2004; Smith et al., 2004; Hu et al., 2008; Brown et al., 2008; Wu et al., 2010). In addition to the potential resistance selection, TCs were reported to be especially toxic toward both aerobic sludge bacteria and microalgae compared to other antibiotics (Halling-Sørensen 2000, 2001, 2002), and elicited significant phytotoxicity on the aquatic higher plant, *Lemna gibba* (Brain et al., 2004).

The occurrence of TCs in the aquatic environment is due to effluent discharge from STPs, agricultural runoff, and the disposal of unused drugs (Khetan and Collins, 2007). Approximately 50–80% of the administered TCs are excreted in urine as the parent compound (Halling-Sørensen et al., 2002). In the statewide survey of STPs in Wisconsin, USA, TCs were the most frequently detected antibiotic, being present in 80% of the wastewater influent and effluent samples (Kolpin et al., 2002;). Due to their persistence in the aquatic environment, TCs have also been widely detected in surface water, groundwater, soil and lagoon samples (Lindsey et al., 2001; Hamscher et al., 2002; Kolpin et al., 2002; Miao et al., 2004; Kim and Carlson, 2007; Jia et al., 2009). While many studies have highlighted the occurrence of TCs in the environment, little attention has been paid to their behaviors in water treatment systems. It is well-known that disinfection by chlorination is an essential step for disinfecting wastewater and drinking water, and chlorine is capable of transforming pharmaceuticals with reactive functional groups, such as phenol (Sedlak and von Gunten, 2011). Concerns have arisen over the production of toxic disinfection by-products (DBPs) in chlorination or chloramination processes (Sedlak and von Gunten, 2011). For example, in previous studies, it was shown that compounds with significantly high toxicity and mutagenicity were generated from chlorination of acetaminophen (Bedner and Maccrehan, 2006), and the estrogen receptor binding affinity of chlorinated bisphenol A was 24-fold greater than the parent compound (Hu et al., 2002). Chlorotetracycline (CTC), a monochloro-TC, is a tetracycline antibiotic that was reported to exhibit 2-fold increased potency towards sludge and soil bacteria compared to TC (Halling-Sørensen et al., 2002). Therefore, knowledge of the fate of TCs after chlorination in sewage treatment plants or water supply plants is needed in order to allow effective assessment of residual TCs in the aquatic environment. A recent study explored the potential reactions of TCs with chlorine dioxide and free available chlorine, and oxidations of TCs were found to be very rapid (Wang et al., 2011). However, to date, no study has described the transformation of TC after reaction with chloramine in water treatment systems while chloramine is a popular alternative disinfectant to free available chlorine and can be formed in wastewater and receiving river water in the presence of ammonia (Qiang and Adams, 2004).

In this study, the first-order reaction kinetics of TC with chloramines was quantified. The aqueous DBPs in chloraminated TC solution were identified by a HPLC–ESI–MS method. Based on the results of these studies reaction pathways related to the transformation of TC by chloramination were proposed. Finally, the growth inhibition toxicity of the products of TC chloramination were tested on sludge bacteria to determine the possible effects of these products in the aqueous environment.

2. Materials and methods

2.1. Chemicals and standards

TC (purity: 97.3%) and anhydro-chlortetracycline (ACTC) were purchased from Sigma–Aldrich (Germany). H_2SO_4, NaOH, NaClO, NH_4Cl, $Na_2S_2O_3$, L-ascorbic acid, n,n-diethyl-p-phenylenediamine sulfate (DPD), ethylenediaminetetraacetic acid disodium (Na_2-EDTA) and formic acid were analytical grade and were purchased from Sinopharm Chemical Reagent Beijing (Beijing, China). Acetonitrile, dichloromethane (DCM) and methanol were HPLC grade and obtained from Fisher Scientific (New Jersey, USA). All reagent solutions were prepared with Milli-Q pure water. The stock solution of TC (1000 mg L^{-1}) was prepared in methanol, and the solutions were replaced after 2 months of storage at −80 °C.

2.2. Preparation and analysis of chloramines

Chloramine stock solutions were prepared daily by slowly adding NaOCl into a rapidly stirred NH_4Cl solution in phosphate buffer (0.1 M) adjusted to pH = 8 with NaOH solution (2 M). The chlorine-to-ammonia weight ratio was 4:1 in the resulting solutions to avoid breakpoint chlorination. The solution was agitated for 1 h at 25 °C (pH = 8) on an orbital shaker to assure the complete residual formation. The chloramine stocks were freshly prepared prior to each experiment. Concentrations and forms of chloramines were determined by using n,n-diethyl-p-phenylenediamine sulfate (DPD) spectral photometric method (GAQS, 2006).

2.3. Kinetics experiments

Tested aqueous solutions (200 mL) were buffered using phosphate salts (10 mM, pH: 6–8). The initial concentration of TC was 1.125 μM, and the initial concentration of total chlorine ranged from 20.6 to 22.8 μM in the reaction mixtures. Variation of chloramines in the reaction was less than 5% under these conditions.

Kinetic runs were initiated by adding, under rapid mixing, an aliquot of chloramine stock solution. At different reaction times, 3 mL of solution was rapidly transferred into a vial containing 50 μL of L-ascorbic acid solution (100 g L^{-1}), which was used to quench the residual chloramine and stop the reaction reported previously (Caro and Gallego, 2007). Samples were then analyzed using HPLC-UV (Waters 600E-Waters 2487 dual λ absorbance detector) to determine the remaining TC concentration. When the TC started disappearing, the kinetic experiments were pursued until at least 85% TC consumption was achieved.

Each sample was analyzed by HPLC-UV with a SymmetryShield RP18 column (4.6 mm × 250 mm × 5 μm, Waters, USA) at 25 °C. The mobile phase consisted of acetonitrile and water containing 0.1% formic acid. Gradient conditions were initiated with 5% acetonitrile followed by a linear increase to 50% acetonitrile in 25 min, and acetonitrile was increased to 100% in 5 min. The flow rate, UV-detection wavelength, and sample injection volume were 1.0 mL min^{-1}, 355 nm, and 100 μL, respectively.

2.4. Identification and analysis of byproducts

The experiments were carried out in a glass reactor which was placed in a water bath to maintain the reaction temperature at 25 °C. Synthetic raw water was prepared by dissolving 105 mg of a standard TC into 2.1 L of Milli-Q pure water of which the pH was adjusted to 7.0 by adding phosphate buffer (10 mM). A 113-mL aliquot was first removed before 13 mL of chloramine solution was added to the remaining solution, producing an initial concentration of total chlorine in the reaction solution of 2 mM. Next,

113 mL aliquots were periodically taken up to 720 min. The 110 mL of each aliquot were used for determination of growth inhibition toxicity. The 3 mL of the collected samples were added into a vial containing 200 μL of L-ascorbic acid solution (100 g L^{-1}), and the samples were analyzed directly by HPLC–ESI–MS (Alliance 2490 HPLC connected to ZMD single quadrupole MS, Waters, USA) for identification and analysis of byproducts.

Identification and analysis of byproducts were conducted using an Alliance 2490 HPLC connected to a platform ZMD single quadrupole mass spectrometer (Waters, USA). A Symmetry® C18 column (2.1 mm × 150 mm × 5 μm, Waters) was used for chromatographic separation. The column was maintained at 30 °C at a flow rate of 0.20 mL min^{-1} and the injection volume was 50 μL. Methanol and water containing 0.1% formic acid were used as the mobile phase. Gradient conditions were initiated with 10% methanol followed by a linear increase to 80% methanol in 15 min. Then methanol was increased to 100% in 10 min and held for 5 min. TC and its chlorination byproducts were detected in the positive ESI mode; typical ion source parameters used were as follows: ESI capillary voltage at 2.0 kV; extractor voltage at 4 V; source block temperature at 100 °C; desolvation temperature at 400 °C; multiplier voltage at 750 V. Nitrogen was used as the desolvation gas with a flow of 400 L h^{-1} and the cone gas at a flow of 100 L h^{-1}. The cone voltage was ramped from 30 to 70 V and the full scan mass ranged from 100 to 600.

The identification of target compounds was conducted in a full-scan mode and quantitative analysis was performed using selected ion monitoring to achieve maximum sensitivity. ACTC was added to the samples as internal standard before LC–ESI–MS analysis for correction of variations in instrumental response from injection to injection. Masslynx 3.4 work station software was used for data processing.

2.5. Growth inhibition of sludge bacteria

After the addition of 10 mL $Na_2S_2O_3$ (1 M), the 110-mL collected samples were shaken for 3 min for decomposition of the residual sodium hypochlorite, and the samples collected at 0 min was used as a positive control. Next, 0.06 g of Na_2EDTA was added and samples were concentrated by solid phase extraction (HLB, Waters, USA), which has been developed to simultaneously determine TCs and their degradation products in environmental waters (Jia et al., 2009). The cartridge was conditioned with 6 mL DCM, 6 mL methanol and 6 mL Na_2EDTA solution (0.5 g L^{-1}, pH = 3). Samples were passed through the cartridge at a flow rate of 10-15 mL min^{-1}, and the residual water was removed by passing a gentle nitrogen stream through the cartridge for approximately 30 min. Finally, 6 mL methanol was percolated at 4–5 mL min^{-1} through the sorbent bed to mobilize TC and its chlorinated products. The eluate was dried under a gentle nitrogen stream and redissolved in 4.4 mL of DMSO for growth inhibition toxicity assays.

The growth inhibition toxicity of the products of TC during aqueous chlorination was tested by viable plate counting of aerobic sludge bacteria as described previously (ISO, 1999; Halling-Sørensen et al., 2002). Activated sludge used in the analysis was collected in activated sludge treatment units from a STP in Beijing. The suspension of activated sludge samples were serial diluted (1, 10, 100, 1000×) and 1 mL of each dilution was added to separate agar plates with or without eluate of TC and its chlorinated products. After incubation at 21 °C for 48 h, all plates were counted and the Inhibitory Ability (IA) was calculated as

$$IA(\%) = \frac{(A - B)}{A} \times 100,$$

where A is the mean number of counted colonies on the non-exposed agar plates, and B is the mean number of counted colonies on the chlorination extract-treated agar plates.

3. Results and discussion

3.1. Reaction kinetics with combined chlorine

Since chloramine is a popular alternative disinfectant to free available chlorine due to the minimal formation of chlorine-containing by-products (Richardson et al., 2000) and because it is also formed in wastewater and receiving river in the presence of ammonia (Qiang and Adams, 2004), the chloramination of TC was explored in the present study. As shown in Supplementary Material (SM), Fig. SM-1, the reactions of TC with combined chlorine exhibited pseudo-first-order kinetics with pH varying from 6 to 8 (r^2 = 0.989–0.996). Relatively high k_{obs} (0.012) at pH 6were possibly due to appreciable amount of $NHCl_2$, and high k_{obs} (0.0041) at pH 8 were due to the high abundance of TC anion at the pH. The pseudo-first-order constants (k_{obs}) ranged from 0.0082 to 0.041 min^{-1} with half-life of 17–85 min ($[CC]_0$:$[TC]_0$ = 18, pH: 6–8), which were much longer than that between TCs and water disinfection oxidants (chlorine dioxide/free available chlorine) (Wang et al., 2011).

3.2. Characterization of products

The reaction of TC with chloramine resulted in the formation of 13 products (Fig. 1). Eleven products (A-K) were generated within the initial 30 min of the reaction (Fig. 1b). While the eleven products (A-K) predominated from 30 to 180 min, two transformation products (L and M) were measured from 300 to 720 min (Fig. 1c). The mass spectra of TC before chloramination is shown in Fig. SM-2. As illustrated, the molecular ions and fragmentation upon collision-induced dissociation yielded the following sequence: $[M + H]^+$ (m/z 445) → $[M + H—NH_3]^+$ (m/z 428) → $[M + H—H_2O—NH_3]^+$ (m/z 410). Similar fragment pattern was also

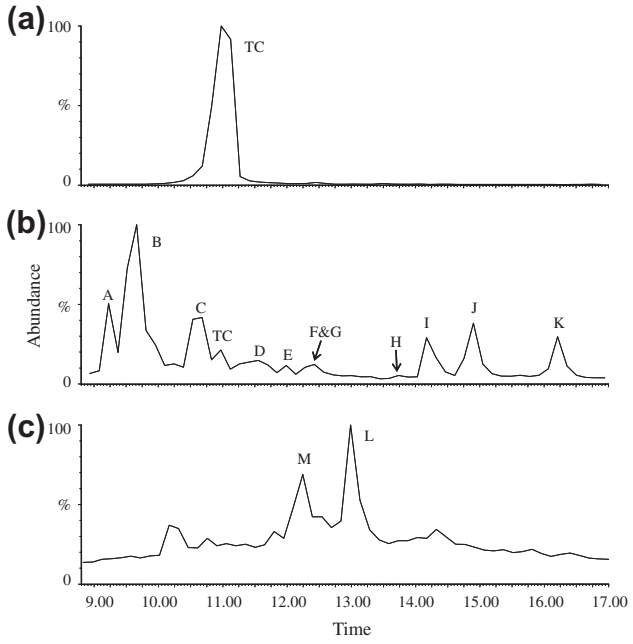


Fig. 1. LC–ESI–MS chromatogram of TC during chloramination: (a) before chloramination; (b) 30 min after chloramination, unknown products A-K; and (c) 720 min after chloramination, unknown products M and L.

observed for products E, K and F, suggesting that these products would have similar structures as TC. Compared to TC, the molecular ion of product A (*m/z* 415, Fig. 2a) was generated via the loss of $(CH_3)_2$, and chlorine atom was not contained in the compound based on the isotope abundances of molecular ions. With increasing cone voltage, more fragmentation ions of product A were produced in sequence: $[M + H—NH_3]^+$ (*m/z* 398) → $[M + H—NH_3—CH_3]^+$ (*m/z* 383) → $[M + H—NH_3—CH_2O]^+$ (*m/z* 368) (Fig. SM-3). On the basis of the above structural information from the mass spectrum, one possible structure (N-didesmethyl-tetracycline) can be proposed for product A (Fig. 2a). The different fragment pattern between product A and TC was possibly due to the hydrogen bonds between the —OH at position C6 and the =NH at position C4. The structure of product A is also consistent with the previously proposed degradation pathway in solution of demethylation of the amino groups bound at position C4 in TC (Fig. SM-2) (Halling-Sørensen et al., 2002). The mass spectra of product D suggested that this compound contained one chlorine atom (Fig. 2b), and the difference in molecular ion between product D (*m/z* 449) and A (*m/z* 415) was 34 Da (chlorine atom – a proton). When the cone voltage was increased to 90 V, similar profiles of fragmentation ions were observed for products A and D ($[M + H—NH_3]^+$, $[M + H—NH_3—CH_3]^+$, and $[M + H—NH_3—CH_2O]^+$ showed in Figs. SM-3 and SM-4) suggesting similar structures of these two compounds. Thus product D was proposed to be chlorinated product A, with the chlorine substitution reaction having occurred at nitrogen position C4, which has been shown to be easily attacked during the abiotical degradation of TC in solution (Halling-Sørensen et al., 2002) (Fig. 2b). The molecular ions of products E and H were 465 (*m/z*) and 451 (*m/z*), respectively, and both of these compounds were monochlorinated products of TC based on the isotope abundances (Fig. 2c and d). However, because the abundances of peaks E and H were very low (Fig. 1), it is difficult to obtain accurate fragmentation ions for the two compounds at high cone voltage due to the background interference. Since molecular ions of products E and H had a mass difference of 16 Da (CH_4) and 2 Da (2H), respectively, from that of product D, the structure of product E was proposed to be N-chlorinated-tetracycline (Fig. 2c) and that of product H was loss of $—CH_3$ from product E (Fig. 2d). Considering that the fragment pattern in the mass spectrum of product H ($[M + H]^+$, $[M + H—H_2O]^{+\cdot}$ $[M + H—H_2O—NH_3]^+$) was largely different from D, E and K ($[M + H]^+$, $[M + H—NH_3]^{+\cdot}$ $[M + H—H_2O—NH_3]^+$), the chlorination position of product H was different from product E. As shown in Fig. 1, product H shows a longer retention time than product E, suggesting the low polarity of product H. And when calculating the log Pow values, log Pow of product H was higher than that of product E only when the chlorine was substituted on NH2 at position C2 (Fig. 2d), which would have the fragment ion ($[M + H—H_2O]^+$) as observed in the spectrum of product H. Fig. 2e and Fig. SM-5 show the mass spectra of product K, which indicates that this compound contain two chlorines. With the increasing cone voltage, molecular and fragmentation ions of product K were produced in following sequence: $[M + H—NH_3]^+$ (*m/z* 468) → $[M + H—NH_3—H_2O]^+$ (*m/z* 450) → $[M + H—NH_3—CO]^+$ (*m/z* 440) → $[M + H—NH_3—H_2O—CO]^+$ (*m/z* 422) → $[M + H—NH_3—CO—COO]^+$ (*m/z* 396) (Fig. 2e and Fig. SM-5). Considering the similar fragment pattern between product K and product E, the structure of product K was proposed to be N-dichlorinated-tetracycline according to the same chlorination mechanism discussed above ((Halling-Sørensen et al., 2002) (Fig. 2e).

Fig. 2f and Fig. SM-6 show the mass spectra of product F, suggesting that chorine was not contained in the compound. The molecular ion of product F was 463 (*m/z*), and fragmentation ions produced with increasing cone voltage were $[M + H—NH_3]^+$ (*m/z* 446) → $[M + H—NH_3—H_2O]^+$ (*m/z* 428) → $[M + H—NH_3—CO]^+$ (*m/z* 400). Since the difference in the molecular ion between product F

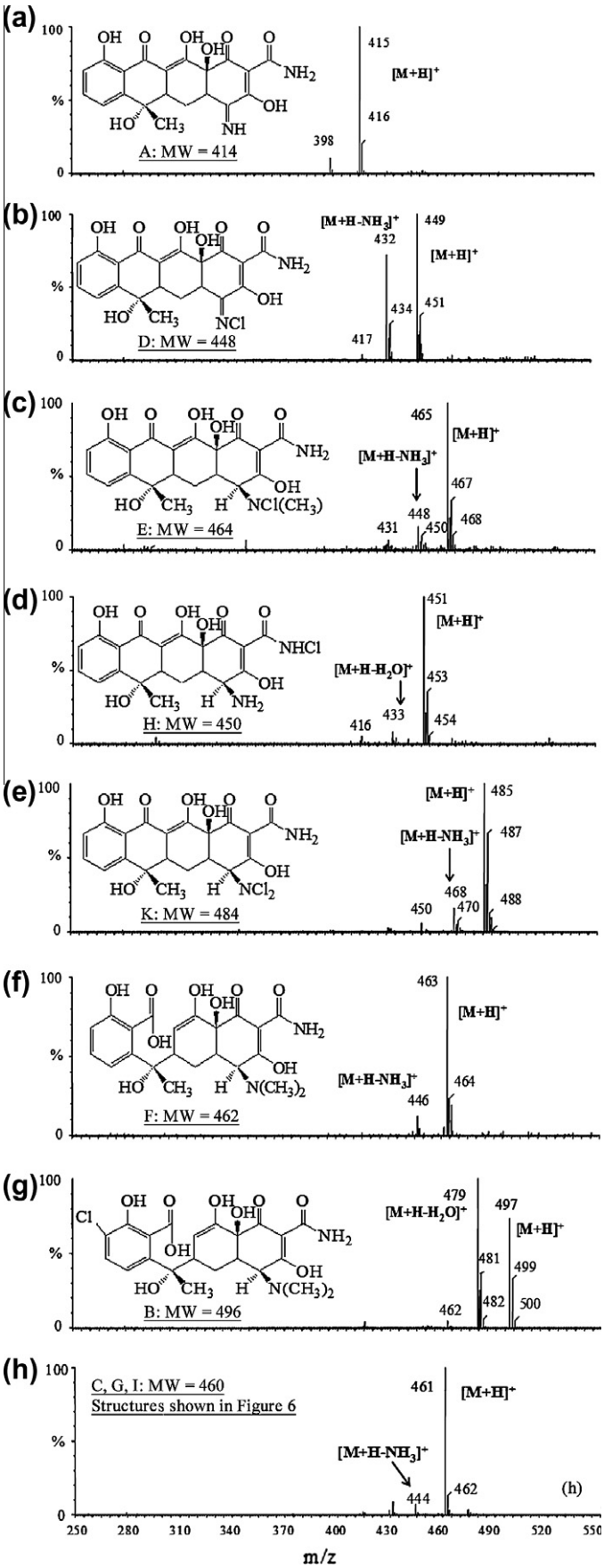


Fig. 2. LC–MS mass spectra of products A (a), D (b), E (c), H (d), K (e), F (f), B (g), C (h), G (i), and I (j) at a cone voltage of 30 V.

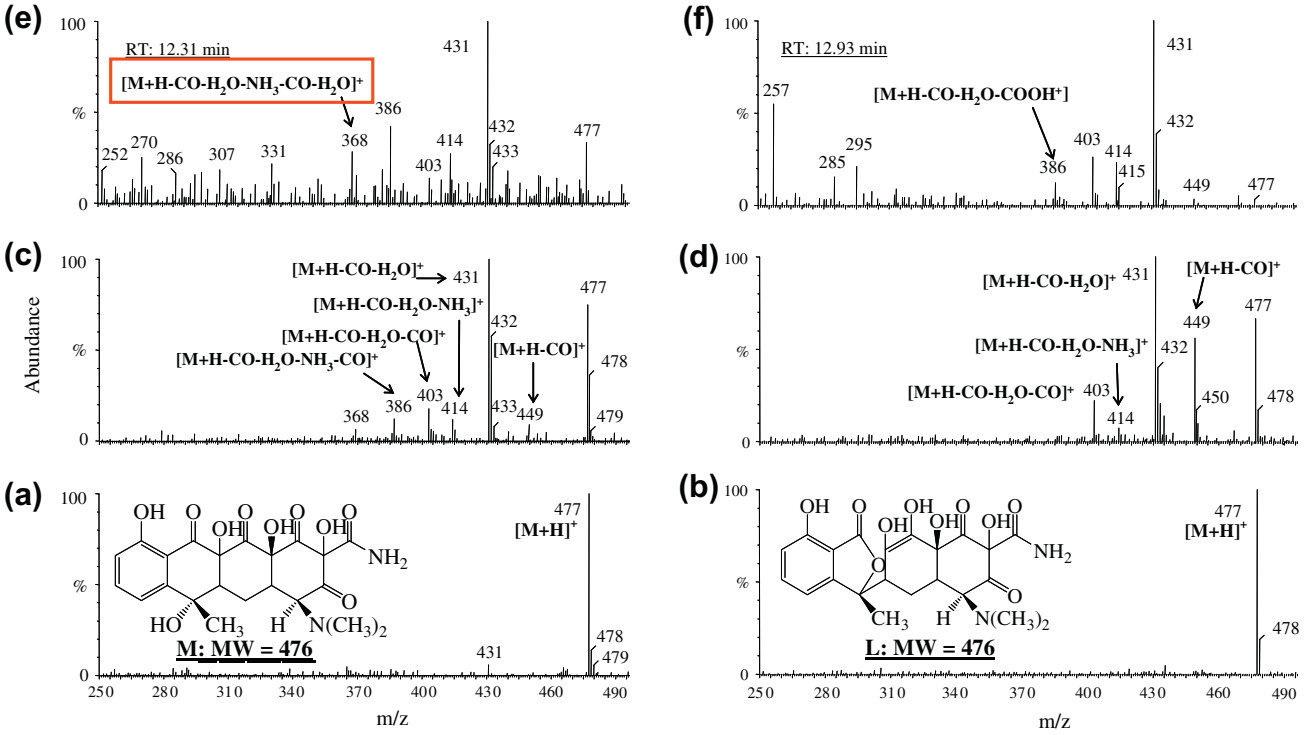


Fig. 3. LC–MS mass spectra of product M (cone voltage: 30 (a), 60 (c), and 80 (e)) and L (cone voltage: 30 (b), 60 (d), and 80 (f)).

(*m/z* 463) and TC (*m/z* 445) was mainly due to addition of H_2O, product F was generated through hydration of TC. The most likely hydration position was proposed to be position C12a (Fig. 2f) based on previous studies regarding theoretical formation of degradation products of TC in solutions (Halling-Sørensen et al., 2002). The mass spectra of product B indicated that this compound contains one chlorine (Figs. 2g and SM-7). The difference in the molecular ion between products B (*m/z* 497) and F (*m/z* 463) was 34 Da (a chlorine atom), thus product B was proposed to be chlorinated product F. The chlorine substitution reaction likely occurred at position C9, according to the chlorine-substitution mechanism of phenolic compounds discussed in our previous studies (Fig. 2g) (Hu et al., 2002). And the *p*Ka values of products B and F were calculated to be 2.5 and 3.05 for the —COOH at C11 site (ACD/ChemSketch), respectively, thus product B was mainly in anion form at the pH (3.1) of the mobile phase (0.1% formic acid in water) which possibly leading to relatively short retention time.

The mass spectra of products C, G, and I indicated that these compounds have the same molecular ion (*m/z* 461) and do not contain chlorine (Fig. 2h). When the cone voltage was increased to 60 V, products C, G, and I all generated the fragmentation ions $[M + H—NH_3]^+$ (*m/z* 444) and $[M + H—NH_3—COO]^+$ (*m/z* 400). The fragmentation ions ($[M + H—NH_3—H_2O]^+$ (*m/z* 426) and $[M + H—NH_3—H_2O—COOH]^+$ (*m/z* 381)) were only generated by product C and ($[M + H—NH_3—COO—H_2O]^+$ (*m/z* 382)) was only generated by product I (Fig. SM-8). While no additional fragmentation ions were detected for product I when the cone voltage was increased to 80 V, the abundance of $[M + H—NH_3—COO—H_2O]^+$ (*m/z* 382) of product G increased and $[M + H—NH_3—H_2O—COOH—CH_3]^+$ (*m/z* 366) was generated for product C (Fig. SM-8). In a previous oxidation study it was determined that TC could react with ozone in aqueous solution to form an oxidized and derivative via an initial 1,3-dipolar cycloaddition of ozone at the C11a–C12 double bond of TC, which has the same molecular ion (*m/z* 461) as products C, G, and I (Dalmázio et al., 2007). Considering the suggested oxidation and degradation pathways of TC in previous studies (Halling-Sørensen et al., 2002; Dalmázio et al., 2007), the structures and reaction pathways of products C, G and I were proposed (Fig. SM-9) based on the mass information and retention times of the three compounds. The molecular ion of product J was 433 (*m/z*) and the fragmentation ions obtained at high cone voltage were 426, 398, and 382 (Fig. SM-10). However, it is impossible to identify the structure of this product based on the current information.

Products L and M had the same molecular ion (*m/z* 477), and detected even after a reaction time of 720 min. The mass spectrum indicated that these compounds were not chlorinated products (Fig. 3a and b). During the reaction of TC with ozone, an oxidation product with molecular ion (*m/z* 477) was reported to be formed via net insertion of oxygen atoms at C11a–C12 and the C2–C3 double bond (Dalmázio et al., 2007). The fragmentation ions of product M with increasing cone voltage were $[M + H\text{-}CO]^+$ (*m/z* 449), $[M + H—CO—H_2O]^+$ (*m/z* 431), $[M + H—CO—H_2O—NH_3]^+$ (*m/z* 414), $[M + H—CO—H_2O—CO]^+$ (*m/z* 403), $[M + H—CO—H_2O—NH_3—CO]^+$ (*m/z* 386), and $[M + H—CO—H_2O—NH_3—CO—H_2O]^+$ (*m/z* 368), confirming that this compound has the same structure as the oxidation product reported previously (Dalmázio et al., 2007) (Fig. 3c and e). Compared to product M, product L also had the fragmentation ions 449, 431, 414, 403 and 386 (*m/z*). However, the fragmentation ion 386 only appeared at cone voltage of 80 V and fragmentation ion 368 was not observed even under high cone voltage (Fig. 3d and f). Therefore, the structure of product L was proposed to contain a epoxide between position C6 and C11, which yielded fragmentation ions of 386 ($[M + H—CO—H_2O—COOH]^+$) through decarboxylation at position C11, but could not form fragmentation ions of 368 due to the loss of OH at position C6.

3.3. Chloramination pathways

Fig. 4 shows the variation in the level of TC and its products and the corresponding IA over the time course of the chloramination reaction. TC reacted rapidly with chloramine, and only 5.3% of

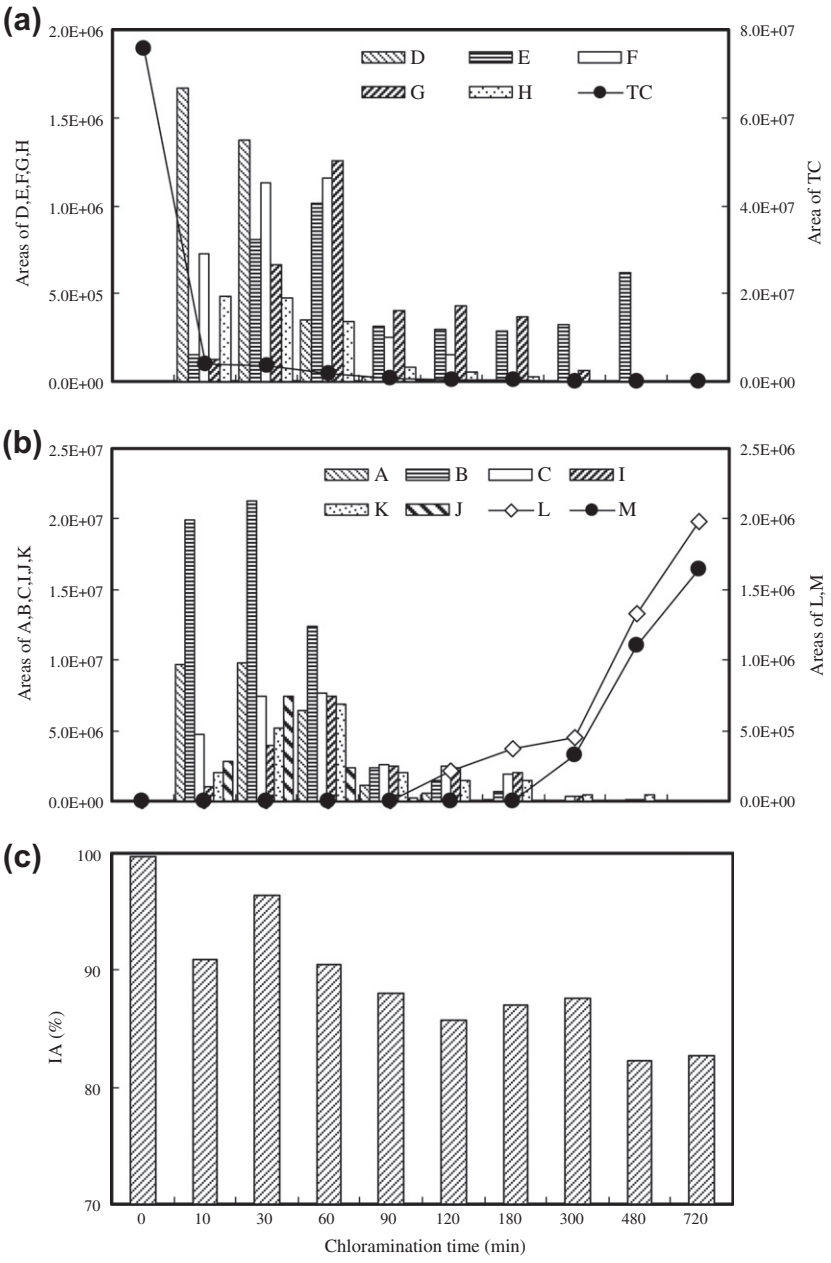


Fig. 4. Abundance variation of TC and its products with chloramination time. (a) TC and products D, E, F, G, H; and (b) products A, B, C, I, K, J, L, and M.

the initial level of TC was detected 10 min after the reaction was initiated. Eleven products were detected from 10 to 90 min post-reaction. While levels of products D and H decreased with reaction time, levels of products A, B and J increased to maximum at 30 min. The levels of products C, E, F, G, I and J increased to maximum at 60 min, when the concentration of TC was 2.3% of the initial concentration (1.13 mg L^{-1}). Product L was detected at 120 min, when the concentration of TC was 0.6% of the initial concentration (320 μg L^{-1}). At 300 min, the products E and H were no longer detected, and one new product (M) was detected. Levels of products L and M both increased with reaction time, even after 480 min, when products A, B, D and G all were no longer detected, and the concentration of TC was only <0.2% of the initial concentration (71 μg L^{-1}). After 720 min, only products L and M were detected in the solution, and TC and products A-K were not detected, suggesting that products L and M were the final products formed during the chloramination processes.

On the basis of the above results, four pathways for product generation from chloramination of TC were proposed (Fig. 5). These pathways include either chlorine substitution reactions followed by dehydration (pathways I and II) or oxidation (pathways III and IV). During the initial reaction stages, product B was the primary measured chloramination product resulting from C9-chlorination of product F, which is formed via dehydration of TC at C11 (pathway I). Pathway II first involved the demethylation of the dimethylamino group at position C4 of TC under the oxidation of chloramine, which is also proposed in the degradation of TC in aqueous solutions (Halling-Sørensen et al., 2002). Then mono- (E and H) and di-chlorinated products (K) were then generated via chlorination of amino group at the demethylated intermediates. The formation of products A and D is proposed to occur via the loss of HCl, of which the mechanism (Eq. (1)) has been widely reported in the transformation of organic compounds containing amino group reacted with chlorine (Conyers and Scully, 1997; Fox et al.,

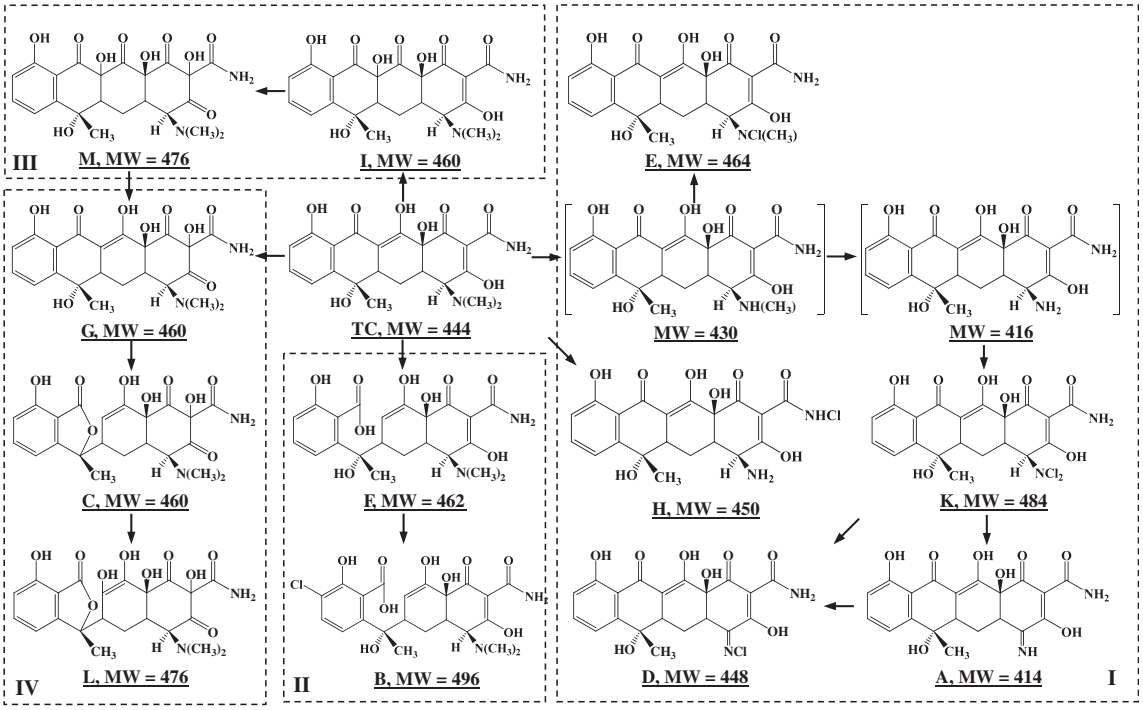
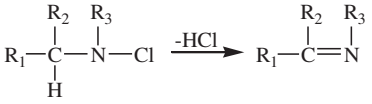


Fig. 5. Chloramination pathways of TC. A-M: detected in this experiment; []: possible but not detected intermediates.

1997; Heasley et al., 2004; Mitch and Sedlak, 2004; Na and Olson, 2006).



(1)

Pathway III and IV were proposed to involve an initial cycloaddition of oxygen from oxidation of chloramine toward C2–C3 and C11a–C12 double-bonds to yield an unstable epoxide intermediate, which finally undergoes a rearrangement to generate a hydroxyl group at position C2 (product G) and C11a (product I). Next, product G was further degraded via scission of the benzene ring at C11, and the hydroxyl group at position C6 was connected to C11 to form product C. Products C and I finally undergo an oxygen addition reaction to yield products L and M, respectively.

In the current study, the products formed via pathways I and II (e.g. product A, B, D, E, H, and K) possibly undergo the transformation pathway III and IV, or were proposed to be further degraded to small molecules via the scission of benzene rings of TC, which could not be detected in the present study, and products L and M were the final products obtained under the experimental chloramination conditions.

3.4. Environmental implications

The results of reactions between TC and chloramine suggest that TC will be significantly transformed in wastewaters with high ammonia and organic nitrogen content that will consume free chlorine. The persistence of products L and M in the pure water experiments suggested that attention should be paid to these compounds. The structures of all transformation products, except for products B, F, C, M and J (unidentified), still contained the pharmacophore of TC (Mitscher, 1978), and products B, E, H, K and D all contained chlorine(s). These compounds could cause greater antimicrobial activity than the parent compound in sludge or soil in the environment (Miao et al., 2004). To assess the antibacterial activities of the transformation products, the IA of the product mixtures at different reaction times were studied using the extracts from the HLB cartridge. And the concentration processes eliminate all possible chemicals that could contribute to background interference (e.g. residual chloramine, phosphate buffers and $Na_2S_2O_3$, etc.) in the IA assay (Fig. 5c). The IA of TC was 99.7% before chloramination and showed a decreasing trend with reaction time. More than 80% of the sludge bacteria were inhibited even after 720 min of the reaction. IA was constant from 90 to 300 min (86–88%), and decreased slightly following 300 min (88–83%). Since only products L and M were detected at 720 min as shown in Fig. 4c and the chloraminated solution still showed IA of 83%, products L and M would also elicit antibacterial activities, and further studies will be necessary to fully evaluate the environmental occurrence and impact(s) of these compounds.

Acknowledgments

Financial support from the National Nature Science Foundation of China (20837003) and the Education Committee of Beijing (YB20081000103) are gratefully acknowledged.

Appendix A. Supplementary material

Pseudo-first-order kinetics, LC–ESI–MS mass spectra of TC and its chloramination products, and proposed structures and reaction pathways among products C, G, I and TC. Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.chemosphere.2012.09.001.

References

Bedner, M., Maccrehan, W.A., 2006. Transformation of acetaminophen by chlorination produces the toxicants 1,4-benzoquinone and N-acetyl-p-benzoquinonelmine. Environ. Sci. Technol. 40, 516–522.

Brain, R.A., Johnson, D.J., Richards, S.M., Sanderson, H., Sibley, P.K., Solomon, K.R., 2004. Effects of 25 pharmaceutical compounds to *Lemna gibba* using a 7-day static-renewal test. Environ. Toxicol. Chem. 23, 371–382.

Brown, M.G., Mitchell, E.H., Balkwill, D.L., 2008. Tet 42, a novel tetracycline resistance determinant isolated from deep terrestrial subsurface bacteria. Antimicrob. Agents Chemother. 52, 4518–4521.

Caro, J., Gallego, M., 2007. Assessment of exposure of workers and swimmers to trihalomethanes in an indoor swimming pool. Environ. Sci. Technol. 41, 4793–4798.

Chopra, I., Roberts, M., 2001. Tetracycline antibiotics: mode of action, applications, molecular biology, and epidemiology of bacterial resistance. Microbiol. Mol. Biol. Rev. 65, 232–233.

Conyers, B., Scully, F.E., 1997. Chloramines V: products and implications of the chlorination of lysine in municipal wastewaters. Environ. Sci. Technol. 31, 1680–1685.

Dalmázio, I., Almeida, M.O., Augusti, R., 2007. Monitoring the degradation of tetracycline by ozone in aqueous medium via atmospheric pressure ionization mass spectrometry. J. Am. Soc. Mass Spectrom. 18, 679–687.

Daughton, C.G., Ternes, T.A., 1999. Pharmaceuticals and personal care products in the environment: agents of subtle change? Environ. Health Perspect. 107 (Suppl. 6), 907–938.

Fox, T.C., Keefe, D.J., Scully, F.E., 1997. Chloramines VII: chlorination of alanylphenylalanine in model solutions and in a wastewater. Environ. Sci. Technol. 31, 1979–1984.

General Administration of Quality Supervision, Inspection and Quarantine of the People's Republic of China. 2006. GB/T 5750.11. Standard determination methods for drinking water, National Standards, China.

Halling-Sørensen, B., 2000. Algal toxicity of antibacterial agents used in intensive farming. Chemosphere 40, 731–739.

Halling-Sørensen, B., 2001. Sludge toxicity of antibacterial agents as inhibited nitrification and aerobic sludge growth. Arch. Environ. Contam. Toxicol. 40, 451–460.

Halling-Sørensen, B., Sengeløv, G., Tjørnelund, J., 2002. Toxicity of tetracyclines and tetracycline degradation products to environmentally relevant bacteria, including selected tetracycline-resistant bacteria. Arch. Environ. Contam. Toxicol. 42, 263–271.

Hamscher, G., Sczesny, S., Hoeper, H., Nau, H., 2002. Determination of persistent tetracycline residues in soil fertilized with liquid manure by high-performance liquid chromatography with electrospray ionization tandem mass spectrometry. Anal. Chem. 74, 1509–1518.

Heasley, V.L., Fisher, A.M., Herman, E.E., Jacobsen, F.E., Miller, E.W., Ramirez, A.M., Royer, N.R., Whisenand, J.M., Zoetewey, D.L., Shellhamer, D.F., 2004. Investigations of the reactions of monochloramine and dichloramine with selected phenols: examination of humic acid models and water contaminants. Environ. Sci. Technol. 38, 5022–5029.

Hu, J.Y., Aizawa, T., Ookubo, S., 2002. Products of aqueous chlorination of bisphenol A and their estrogenic activities. Environ. Sci. Technol. 36, 1980–1987.

Hu, J.Y., Shi, J.C., Chang, H., Li, D., Yang, M., Kamagata, Y., 2008. Phenotyping and Genotyping of antibiotic-resistant *Escherichia coli* isolated from a natural river basin. Environ. Sci. Technol. 42, 3415–3420.

International Organization for Standardisation, 1999. ISO 15522. Determination of the Inhibitory Effect of Water Constituents on the Growth of Activated Sludge Micro-organisms, Switzerland.

Jia, A., Xiao, Y., Hu, J.Y., Asami, M., Kunikane, S., 2009. Simultaneous determination of tetracyclines and their degradation products in environmental waters by liquid chromatography–electrospray tandem mass spectrometry. J. Chromatogr. A 1216, 4655–4662.

Khetan, S.K., Collins, T.J., 2007. Human pharmaceuticals in the aquatic environment: a challenge to green chemistry. Chem. Rev. 107, 2319–2364.

Kim, S.C., Carlson, K., 2007. Temporal and spatial trends in the occurrence of human and veterinary antibiotics in aqueous and river sediment matrices. Environ. Sci. Technol. 41, 50–57.

Kim, S.R., Nonaka, L., Suzuki, S., 2004. Occurrence of tetracycline resistance genes tet(M) and tet(S) in bacteria from marine aquaculture sites. FEMS Microbiol. Lett. 237, 147–156.

Kolpin, D.W., Furlong, E.T., Meyer, M.T., Thurman, E.M., Zaugg, S.D., Barber, L.B., Buxton, H.T., 2002. Pharmaceuticals, hormones, and other organic wastewater contaminants in US streams, 1999–2000: a national reconnaissance. Environ. Sci. Technol. 36, 1202–1211.

Kümmerer, K., 2009. Antibiotics in the aquatic environment – a review – Part I. Chemosphere 75, 417–434.

Lindsey, M.E., Meyer, M., Thurman, E.M., 2001. Analysis of trace levels of sulfonamide and tetracycline antimicrobials in ground-water and surface water using solid-phase extraction and liquid chromatography/mass spectrometry. Anal. Chem. 73, 4640–4646.

Miao, X.H., Bishay, F., Chen, M., Metcalfe, C.D., 2004. Occurrence of antimicrobials in the final effluents of wastewater treatment plants in Canada. Environ. Sci. Technol. 38, 3533–3541.

Mitch, W.A., Sedlak, D.L., 2004. Characterization and fate of N-nitrosodimethylamine precursors in municipal wastewater treatment plants. Environ. Sci. Technol. 38, 1445–1454.

Mitscher, L.A., 1978. The Chemistry of the Tetracycline Antibiotics. Marcel Dekker, New York.

Na, C.Z., Olson, T.M., 2006. Mechanism and kinetics of cyanogen chloride formation from the chlorination of glycine. Environ. Sci. Technol. 40, 1469–1477.

Pruden, A., Pei, R., Storteboom, H., Carlson, K.H., 2006. Antibiotic resistance genes as emerging contaminants: studies in northern Colorado. Environ. Sci. Technol. 40, 7445–7450.

Qiang, Z.M., Adams, C.D., 2004. Determination of monochloramine formation rate constants with stopped-flow spectrophotometry. Environ. Sci. Technol. 38, 1435–1444.

Richardson, S.D., Thruston, J.R.A.D., Gaughran, T.V., Chen, P.H., Collette, T.W., Schenck, K.M., Lykins, J.R.B.W., Rav-Acha, C., Glezer, V., 2000. Identification of new drinking water disinfection by products from ozone, chlorine dioxide, chloramine, and chlorine. Water Air Soil Pollut. 123, 95–102.

Sedlak, D.L., von Gunten, U., 2011. The chlorine dilemma. Science 331, 42–43.

Smith, M.S., Yang, R.K., Knapp, C.W., Niu, Y., Peak, N., Hanfelt, M.M., Galland, J.C., Graham, D.W., 2004. Quantification of tetracycline resistance genes in feedlot lagoons by real-time PCR. Appl. Environ. Microbiol. 70, 7372–7377.

Wang, P., He, Y.L., Huang, C.H., 2011. Reactions of tetracycline antibiotics with chlorine dioxide and free chlorine. Water Res. 45, 1838–1846.

Wu, N., Qiao, M., Zhang, B., Cheng, W.D., Zhu, Y.D., 2010. Abundance and diversity of tetracycline resistance genes in soils adjacent to representative swine feedlots in China. Environ. Sci. Technol. 44, 6933–6939.

Zhang, X.X., Zhang, T., Fang, H.H.P., 2009. Antibiotic resistance genes in water environment. Appl. Microbiol. Biotechnol. 82, 397–414.