

*Environmental Research in China*DETERMINATION AND FATE OF OXYTETRACYCLINE AND RELATED COMPOUNDS
IN OXYTETRACYCLINE PRODUCTION WASTEWATER AND THE RECEIVING RIVERDONG LI,[†] MIN YANG,^{*†} JIANYING HU,[‡] LIREN REN,[§] YU ZHANG,[†]
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Abstract—This study investigated the occurrence and fate of oxytetracycline (OTC) and its related substances, 4-epi-oxytetracycline (EOTC), α -apo-oxytetracycline (α -apo-OTC), and β -apo-oxytetracycline (β -apo-OTC), in a wastewater treatment plant (WWTP) treating OTC production wastewater and a river receiving the effluent from the WWTP using liquid chromatography electrospray ionization mass spectrometry (LC-ESI-MS). The percent removal of OTC in the WWTP was $38.0 \pm 10.5\%$, and the concentration of OTC was still up to 19.5 ± 2.9 mg/L in the treated outflow. The concentration slightly decreased along the river, from 641 ± 118 μ g/L at site R2 (discharging point) to 377 ± 142 μ g/L at site R4 (~20 km from site R2), which was still higher than the minimal inhibition concentration of OTC reported (~250 μ g/L). On the other hand, the total amount of its related substances in the treated effluent was less than 5% of OTC. Concentrations of α -apo-OTC and β -apo-OTC increased along the river, from 5.76 ± 0.63 and 2.08 ± 0.30 μ g/L at site R2 to 11.9 ± 4.9 and 12.0 ± 4.6 μ g/L at R4, respectively, although EOTC decreased from 31.5 ± 3.8 to 12.9 ± 1.1 μ g/L, respectively. The mean concentration of β -apo-OTC in river sediments was 20.8 ± 7.8 mg/kg, and its ratio to OTC was approximately 0.11, nearly twice the ratio of α -apo-OTC and EOTC to OTC (0.058 ± 0.014 and 0.061 ± 0.015 , respectively).

Keywords—Oxytetracycline Antibiotics Related substances Production wastewater

INTRODUCTION

Oxytetracycline (OTC), which belongs to tetracyclines, is a broad-spectrum antibiotic mainly used as a growth promoter for livestock and aquaculture [1]. A high percent of OTC administered to livestock is excreted in the active form, and the excrement of livestock often is used as manure on farmland, with OTC reported to persist in soils for more than 40 d [2]. In aquaculture practices, large amounts of OTC are even poured directly on water bodies. It has been reported that OTC could highly sorb to soil by cation exchange [3,4]. However, the adsorption of OTC has been found reversible in certain conditions, and OTC could again be released to aquatic systems still displaying antibiotic ability [5]. The environmental influence of OTC has aroused a great deal of concern. For this reason, the environmental behavior of OTC has been investigated in different environments such as sewage treatment plants [6], soils [7], and marine sediments [8]. It has been found that several bacteria, including pathogenic, display antibiotic resistance. This resistance partially might be due to the residues of OTC in the environment [9,10].

The degradation of OTC has been found to be mainly dependent on pH, redox, and light [11,12]. 4-Epi-oxytetracycline (EOTC), α -apo-oxytetracycline (α -apo-OTC), and β -apo-oxytetracycline (β -apo-OTC, Fig. 1), are reported to be the main related substances of OTC [11,13,14]. Oxytetracycline could be epimerized reversibly to form EOTC, with the normal epimerization ratio of EOTC versus OTC ranging from 0.4 to 0.6

[11]. The main degradation product of OTC in alkaline solutions is terranoic acid, although in acidic solutions OTC irreversibly transforms to α -apo-OTC and β -apo-OTC through the unstable intermediates of anhydrooxytetracycline and 4-epi-anhydrooxytetracycline. In soil, interstitial water or manure containing anaerobic slurry systems, EOTC, α -apo-OTC, or β -apo-OTC are also the main degradation product of OTC [14–16]. These main related substances still process antibiotic properties, with the minimal inhibition concentration required to inhibit the growth of 50% of organisms reported to be 1.0, 32, and more than 32 mg/L for EOTC, α -apo-OTC and β -apo-OTC, respectively [15]. More investigations thus should be performed focusing on the environmental behaviors of OTC-related substances [17,18].

Oxytetracycline production wastewater from production facilities is also an important source of OTC and its related substances. In 1988, Qiting and Xiheng [19] found the concentration of OTC above 50 mg/L in the outflow of an OTC production facility in China. This amount was four to six orders higher than those reported in the secondary effluents of municipal wastewater treatment plants [6,20]. However, to our knowledge, the environmental behaviors of OTC and its related substances discharged from OTC production facilities have not yet been investigated. On the other hand, elucidation of the fate of antibiotics during wastewater treatment is important for improving the removal efficiency of antibiotics in wastewater treatment plants (WWTPs) [21,22].

In the present study we investigated the occurrence and fate of OTC and its main related substances, EOTC, α -apo-OTC, and β -apo-OTC in the WWTP of an OTC production facility of North China Pharmaceutical Group Corporation of China

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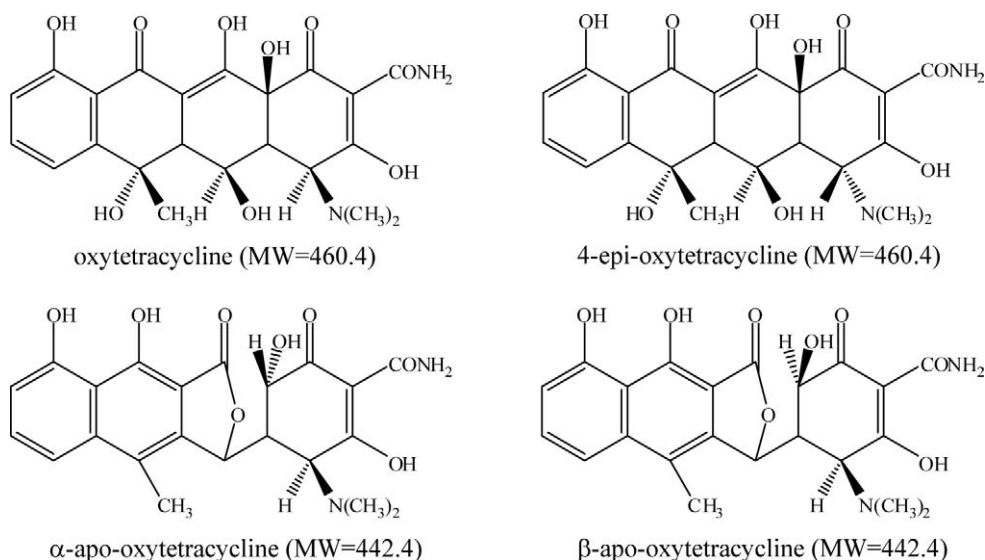


Fig. 1. Chemical structures and molecular weights (MW) of oxytetracycline (OTC), 4-epi-oxytetracycline (EOTC), α -apo-oxytetracycline (α -apo-OTC), and β -apo-oxytetracycline (β -apo-OTC).

and the receiving surface water. The concentrations of the target analytes in the wastewater and sludge at different treatment units and in the receiving surface water and its sediments were determined using liquid chromatography electrospray ionization mass spectrometry (LC-ESI-MS). The results of the present study also might be helpful to estimate the fate of other antibiotics of tetracycline type in the production wastewater.

MATERIALS AND METHODS

Chemicals and materials

Oxytetracycline dihydrate was purchased from Sigma (St. Louis, MO, USA). 4-Epi-oxytetracycline, α -apo-OTC, and β -apo-OTC were bought from Acros Organics (Geel, Belgium). Stock solutions of all standards were prepared monthly by dissolving 1 mg of each compound in 1 ml of methanol and stored in the dark at 4°C. Working dilutions were prepared freshly on the day of use. Formic acid and citric acid (Merck, Darmstadt, Germany) were of pro analysis grade. The high-performance liquid chromatography-grade ammonium acetate was purchased from Fluka (Steinheim, Germany). Ultrapure water (18.2 M Ω -cm) was prepared by passing water through a PURIC-MX (Organo, Tokyo, Japan). Methanol and acetonitrile, both from Fisher Scientific (Fair Lawn, NJ, USA), and ethyl acetate (J. T. Baker, Phillipsburg, NJ, USA) were of high-performance liquid chromatography grade.

Sample collection

China, one of the largest producers of OTC worldwide, had an annual OTC output of 10,000 tons in 2004. Approximately 800 tons of OTC is produced in North China Pharmaceutical Group Corporation in Hebei Province every year. The production wastewater following dilution is treated in the WWTP, which includes a sequence batch reactor (SBR) and a continuous-flow activated sludge reactor (Fig. 2), with the average hydraulic residence time of approximately 14 h for each unit. The total production of excess sludge from the WWTP is approximately 125 tons (dry wt) per year. The effluent from the WWTP is discharged into the receiving surface water, the Xiao River (Fig. 2).

In December 2004, April 2005, and August 2005, samples

from the WWTP (W1–W4) and the receiving river (R1–R4) were collected during three successive days for each sampling campaign. No rain event was registered either during the previous week or on the sampling days. Raw wastewater and effluents from each treatment process were collected, and river water samples were taken from four sites distributed along the river (R1 located at the upstream as the control). Sludge samples were taken from the two reactors and the sediments were obtained at each sampling site of the river. The detailed characteristics of water samples are summarized in Table 1.

All glassware used in the present study was heated at 450°C for 2 h, rinsed with 2.5 ml of a saturated methanolic ethylenediaminetetraacetic acid solution, and air-dried prior to analysis. Water samples were collected in 4-L brown bottles that were rinsed with water samples three times before final sampling was performed. The samples were stored at 4°C in the dark for a maximum of 2 d. For activated sludge, 4-L samples were taken from each reactor. After centrifugation, the solid was separated and stored at 4°C in the dark for a maximum of 3 d. For sediment samples, 1.5 kg of sediment at each sampling river site was collected.

Sample preparation

Before analysis, water samples were added with the same volume of a 1 M citric acid buffer (pH 4.7) and mixed for 1 min to release OTC and its degradation products [7]. Water samples then were filtered through 0.2- μ m polyethersulfone syringe filters (Whatman, Puradisc, 25 Aqueous Solution, Maidstone, UK). Because the concentrations of the analytes were all high enough, no extraction was performed and they were analyzed directly through LC-ESI/MS. When analyte concentrations exceeded the higher limits of the calibration curves (1 mg/L), the water samples were diluted properly using ultrapure water. The extraction of the analytes from sludge and sediments was performed as reported by Hamscher et al. [7]. Briefly, 1-g solid sample was vortexed intensively with 1.2 ml of 1 M citrate buffer (pH 4.7) for 1 min. Later, 6 ml of ethyl acetate was added and samples were vortexed again for 1 min. After centrifugation, the organic phase was separated. The same procedure was repeated with another 6 ml of ethyl ac-

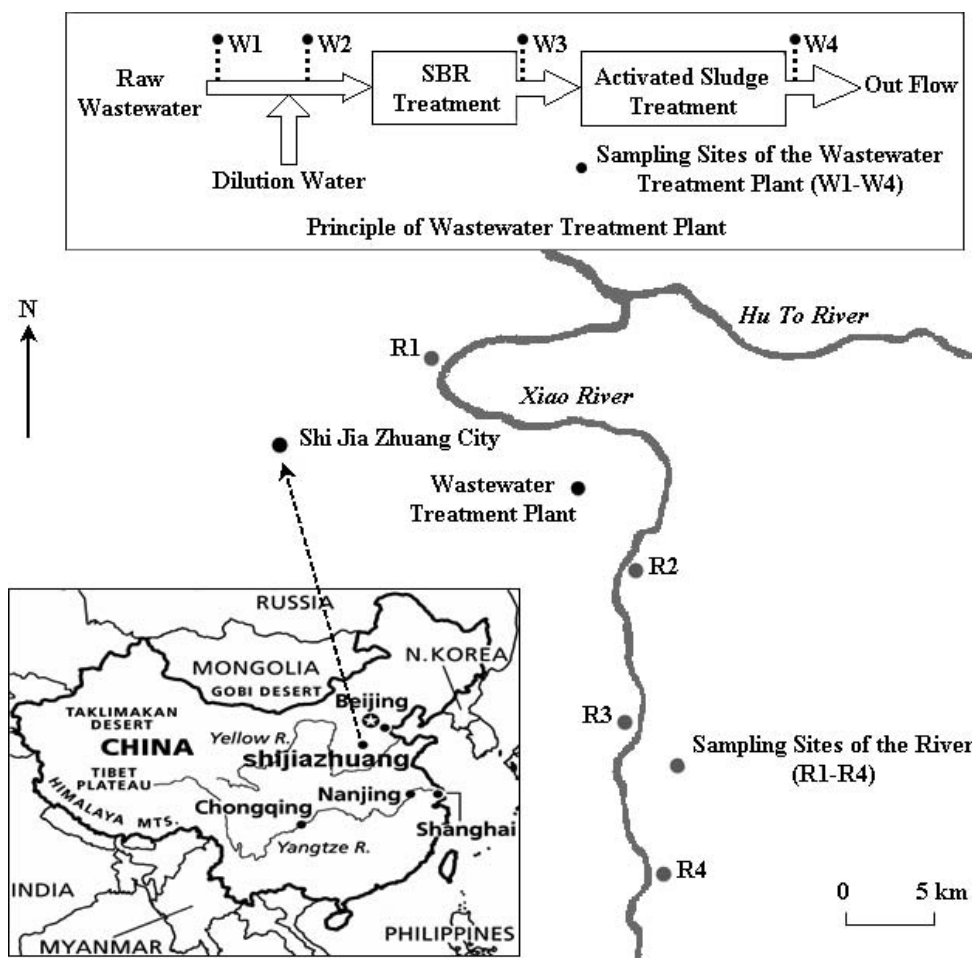


Fig. 2. Sampling sites of the Xiao River, China with schematic diagram of the wastewater treatment plant (WWTP). Sites W1 to 4 were in the WWTP for the oxytetracycline production wastewater treatment with W3 in the sequence batch reactor (SBR), and R1 to 4 were distributed along the Xiao River, with R1 located at the upstream of the discharge point of the WWTP.

etate. The organic phases then were combined and evaporated to dryness under nitrogen gas. The residue was dissolved in a 1-ml solution containing 90% acetonitrile and 10% ammonium acetate (100 mM in water). Samples were passed through 0.2- μm filters and analyzed with LC-ESI/MS. The dry weight of sludge and sediments was obtained by incubating 1 g of sample at 105°C for at least 48 h until constant weight was

Table 1. Characteristics of water samples from the wastewater treatment plant and the Xiao River, China

Sampling site	Flow rate (m ³ /d $\times 10^4$) ^a	COD ^b (mg/L)	pH	Temperature (°C) ^c
Site W1	0.048	14,400 \pm 2,400	4.9 \pm 0.3	33
Site W2	1.4	1,500 \pm 1,200	6.1 \pm 0.2	32
Site W3	1.4	400 \pm 100	6.8 \pm 0.1	28–30
Site W4	1.4	300 \pm 40	7.0 \pm 0.2	25–28
Site R1	43	40 \pm 5	7.1 \pm 0.1	15–23
Site R2	43	70 \pm 8	7.1 \pm 0.1	16–22
Site R3	43	60 \pm 10	7.0 \pm 0.2	16–21
Site R4	43	60 \pm 6	7.1 \pm 0.2	14–22

^a Calculated from yearly flow rate. The variations of the river flow between seasons were not considered.

^b COD = chemical oxygen demand.

^c Variations of the temperatures mainly were due to different seasons of three sampling times (December 2004, April 2005, and August 2005).

reached. The concentrations of the analytes in solid samples then were modified with dry weight.

Analytical method

The high-performance liquid chromatography system consisted of an Alliance liquid chromatograph 2695 (Waters, Milford, MA, USA) and a Waters SymmetryShield[®] RP18 column (150 \times 2.1-mm inside diameter, particle size 5 μm) operated at 25°C, and the flow rate was 0.2 ml/min. The separation conditions were very similar to those reported in a previous study [7]. The injection volume was 20 μl for each sample.

Mass spectrometry was carried out with a single-quadrupole mass spectrometer ZQ 4000 (Micromass, Manchester, UK). All instrumental parameters for monitoring compounds were optimized by infusing mixed standard solutions of the analytes at a concentration of 10 $\mu\text{g/L}$ by a syringe pump with water/acetonitrile (1:1, v:v) as the mobile phase. The electrospray ionization was operated in the positive ion mode. The capillary and the cone voltages were 3.5 kV and 60 V, respectively. Source temperature was 110°C, and desolvation temperature was 300°C. Nitrogen gas was used as the desolvation gas with a flow rate of 300 L/h and the cone gas of 60 L/h.

Qualification and quantification

Analytes were identified only when the retention time was within 2% of the relative standard deviation of the average

time for the corresponding analyte in calibration standards and mass spectrometry fragmentation patterns of the samples were consistent with the standards. The confirmation ratio of the peak areas of molecular ion and one or two fragment ions was evaluated for each analyte in all samples and standards, and the variability of the ratio between samples and standards all was within 15% (Table 2). Selected ion recording mode was chosen with $[M+H]^+$ ion of each analyte for the analytes' quantification. A five-point external calibration curve was established for each analyte. Quantification was performed by comparing the peak area of the selected ion recording signal trace with the calibration curves. The limit of quantification was calculated based on the concentration corresponding to the signal at the y-intercept plus 10 times its standard deviation. Linear ranges were obtained from the limit of quantification to 1 mg/L for all the analytes, and the regression coefficients all were above 0.99. The retention times, molecular and fragment ions obtained at 60 V of the cone voltage, and the limit of quantifications for all the analytes are listed in Table 2.

Recoveries for the extraction of the analytes from solid samples were evaluated by spiking the control samples with a mixture of the analytes' stock solutions at the concentrations of 0.01, 0.1, 0.5, 1.0, and 10.0 mg/kg. The control sludge sample was taken from another WWTP, and the control sediment sample was obtained at point R1. Both control samples were examined with LC-ESI-MS in order to confirm they did not contain detectable analytes. Six replicates for each sample were performed to calculate the mean recovery and the relative standard deviations (Table 2). The concentrations of the analytes in solid samples were corrected for extraction recoveries.

Matrix effects, which have been found in complex samples and lead to ion signal suppression or enhancement especially in electrospray ionization mode [23,24], were investigated for water samples of the present study by spiking OTC and related substance standards in raw wastewater, the effluent of the WWTP, and river water from site R4, which first were diluted properly with ultrapure water if needed. The analytes were spiked at the concentrations more than three times those already in the samples, and determined with LC-ESI/MS before and after the fortification. The matrix effects were measured by determining the ratio of the selected ion recording trace area for each analyte added in water samples versus that for standards in ultrapure water at the same concentration, with each sample injected six times. The signal ratios were found to be 82 ± 7 to $95 \pm 4\%$ for the objective compounds in all of the water samples, indicating that matrix effect was not significant.

RESULTS AND DISCUSSION

Determination of the analytes in the WWTP

Oxytetracycline and its degradation products were detected in all wastewater samples. Because the wastewater was already diluted at site W2, the load of each analyte was obtained by multiplying the concentration with the flow rate at each sampling site. The role of each wastewater treatment process in the removal of the analytes from wastewater was evaluated by calculating the percent removal (Fig. 3). This was obtained as the ratio of the load of each analyte in the effluent versus that in the influent of each treatment process. The average results with standard deviations for three sampling campaigns are shown in Table 3.

In raw wastewater, the load of OTC was 442 ± 9.6 kg/d,

Table 2. Retention times (RT), molecular ions $[M+H]^+$ and fragment ions, ion ratios, limits of quantification (LOQ), and recoveries of oxytetracycline (OTC), 4-epi-oxytetracycline (EOTC), α -apo-oxytetracycline (α -apo-OTC), and β -apo-oxytetracycline (β -apo-OTC)

Analyte	RT (min)	Molecular ions and fragment ions m/z^a	Ion pair I		Ion ratio I		Ion pair II		Ion ratio II		Recoveries and RSDs ^b (%)	
			Ion pair I	Standard	Sample	Standard	Sample	Standard	Sample	Standard	Sample	Sludge
OTC	9.28	461.5, 444, 426	426/461.5	8.33 \pm 0.13	8.17 \pm 0.32	444/461.5	4.18 \pm 0.07	4.31 \pm 0.08	1.0	88.4 \pm 18.8	76.7 \pm 15.9	
EOTC	9.01	461.5, 444, 426	426/461.5	9.01 \pm 0.17	9.25 \pm 0.25	444/461.5	2.31 \pm 0.05	2.38 \pm 0.23	1.2	71.2 \pm 13.9	65.6 \pm 21.3	
α -apo-OTC	10.14	443.5, 426	426/443.5	3.70 \pm 0.06	3.63 \pm 0.19	—	—	—	1.3	67.5 \pm 13.6	72.3 \pm 21.9	
β -apo-OTC	13.03	443.5, 426	426/443.5	7.15 \pm 0.09	7.18 \pm 0.14	—	—	—	0.6	84.5 \pm 19.0	77.6 \pm 13.9	

^a Ions with relative abundance lower than 10% were not listed in this table. The molecular ions are italicized.

^b The recoveries and the relative standard deviations (RSDs) were obtained at 1.0 mg/kg of the analytes spiked in the control.

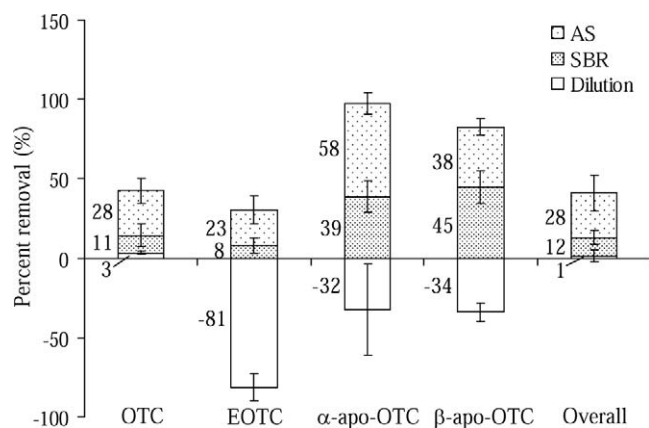


Fig. 3. The percent removal of oxytetracycline (OTC), 4-epi-oxytetracycline (EOTC), α -apo-oxytetracycline (α -apo-OTC), and β -apo-oxytetracycline (β -apo-OTC) during each step in the wastewater treatment plant including dilution, sequence batch reactor (SBR), and activated sludge (AS) reactor, and the overall removal based on the molar concentrations of the analytes. The detailed values of the percent removal have been labeled in the figure.

accounting for 96.9% of the total load of all the analytes, although the loads of the degradation products were 8.69 ± 0.19 , 4.52 ± 0.03 , and 1.00 ± 0.02 kg/d for EOTC, α -apo-OTC, and β -apo-OTC, respectively. As shown in Table 3, an increase of the loads of EOTC, α -apo-OTC, and β -apo-OTC was observed in the water samples at site W2, which might be related to the pH shift during wastewater dilution (from 4.9 ± 0.3 to 6.1 ± 0.2). The loads of OTC and its related compounds then decreased at site W3 (after SBR) and site W4 (after continuous flow reactor). As a result, the total percent removals in the WWTP were 38.0 ± 10.5 , -29.5 ± 22.2 , 67.0 ± 8.4 , and $54.3 \pm 7.2\%$ for OTC, EOTC, α -apo-OTC, and β -apo-OTC, respectively, demonstrating that the WWTP could remove OTC, α -apo-OTC, and β -apo-OTC to some extent. The continuous-flow reactor was found comparably more effective in removing most of the analytes (from 22.7 ± 8.9 to $59.4 \pm 4.2\%$) than SBR (from 7.7 ± 4.8 to $44.9 \pm 10.3\%$), possibly due to the longer hydraulic residence time of the continuous flow (~ 14 h) compared to SBR (~ 8 h).

During the whole wastewater treatment process, the ratio of EOTC to OTC ranged from 0.020 to 0.041, which was much lower than the epimerization ratio of 0.4 to 0.6 reported previously [11]. This result was accordant with that obtained by Loke et al. [16] in manure-containing anaerobic systems spiked with OTC, in which EOTC could not be detected in general, and this might be accounted for by the existence of calcium or magnesium in the wastewater, which has been reported to inhibit markedly epimerisation of OTC [11]. In addition, the ratios of α -apo-OTC and β -apo-OTC to OTC were only 0.0056

to 0.014 and 0.0017 to 0.0031, respectively, which also were accordant with the result previously reported (<0.02) [13].

As shown in Table 3, the concentrations of OTC in activated sludge samples were as high as $4,363 \pm 520$ and $3,763 \pm 353$ mg/kg in SBR and the continuous flow reactor, respectively. For its related substances, 176 to 214 mg/kg of EOTC, 45.2 to 102 mg/kg of α -apo-OTC, and 21.8 to 32.0 mg/kg of β -apo-OTC also were detected in the sludge samples. The distribution factors (ratio of the concentration of the analyte in sludge samples vs. that in the corresponding water samples) were 161 to 193, 206 to 220, 392 to 411, and 604 to 661 L/kg for OTC, EOTC, α -apo-OTC, and β -apo-OTC, respectively. It has been reported that the distribution coefficient K_d of OTC varied from 290 L/kg in marine sediment to 1,030 L/kg in sandy loam [4], which was higher than the distribution factor of OTC obtained in the present study. This might be due to the extremely high concentrations of OTC in the samples of the present study.

According to the loads of the compounds in the effluent, the amounts of the target analytes discharged to the aquatic environment from the WWTP was calculated to be 99.6, 4.1, 0.56, and 0.17 tons per year for OTC, EOTC, α -apo-OTC, and β -apo-OTC, respectively, the total of which accounted for approximately 62.1% of that in the raw wastewater, indicating that large amounts of OTC and its related substances were discharged into aquatic environment from the effluent, and their environmental impacts should not be neglected. On the other hand, it has been reported that leaching of antibiotics from excess sludge was also an important pathway into water bodies when the sludge is used as fertilizer on farmlands [25]; however, in this study, the target compounds adsorbed by activated sludge were less than 1% of those discharged into aquatic environment, individually approximately 550, 24, 9.2, and 3.4 kg per year based on the average concentrations of the analytes in sludge samples and the dry weight of the excess sludge (~ 125 tons per year), indicating that the removal of these compounds due to the sludge adsorption was not very significant. It also has been reported that biodegradation does not play an important role in the removal of tetracyclines [22]. Thus, transformation of OTC to some hydrolytic degradation products, which were not the target analytes in the present study, might be the main reason for the partial removal of OTC in the WWTP [14]. Although photodegradation is known as one of the main transformation reactions of tetracyclines in the environment [12], it should not be the dominant reaction in the WWTP due to the low light transmission efficiency under high concentrations of sludge in the system.

Determination of analytes in receiving river

The Xiao River received the treated wastewater from the WWTP, and the typical SIR LC-ESI/MS chromatograms for

Table 3. Concentrations, loads, and percent removal of oxytetracycline (OTC), 4-epi-oxytetracycline (EOTC), α -apo-oxytetracycline (α -apo-OTC), and β -apo-oxytetracycline (β -apo-OTC) in the wastewater and sludge samples taken from the wastewater treatment plant at Shijiazhuang City, China

Analyte	Wastewater concn. (mg/L)		Load (kg/day)				Sludge concn. (mg/kg)	
	Site W1	Site W4	Site W1	Site W2	Site W3	Site W4	Site W3	Site W4
OTC	920 ± 20	19.5 ± 2.9	442 ± 9.6	427 ± 15	379 ± 18	273 ± 40.6	$4,363 \pm 520$	$3,763 \pm 353$
EOTC	18.1 ± 0.4	0.80 ± 0.13	8.69 ± 0.19	15.7 ± 0.3	14.5 ± 0.8	11.2 ± 1.8	214 ± 31	176 ± 14
α -apo-OTC	9.42 ± 0.06	0.11 ± 0.03	4.52 ± 0.03	5.97 ± 1.4	3.64 ± 0.56	1.54 ± 0.42	102 ± 29	45.2 ± 7.0
β -apo-OTC	2.09 ± 0.05	0.033 ± 0.005	1.00 ± 0.02	1.34 ± 0.04	0.74 ± 0.14	0.46 ± 0.07	32.0 ± 2.3	21.8 ± 3.8

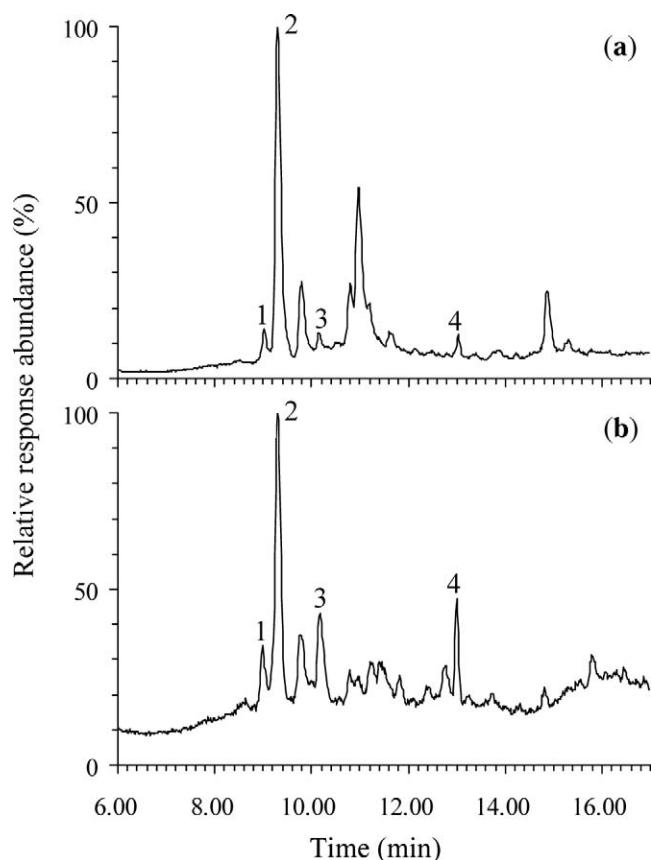


Fig. 4. Time-scheduled selected ion recording chromatograms of all the analytes in environmental samples collected in December 2004. (a) Surface water and (b) sediments. 1 = 4-epi-oxytetracycline (EOTC); 2 = oxytetracycline (OTC); 3 = α -apo-oxytetracycline (α -apo-OTC); 4 = β -apo-oxytetracycline (β -apo-OTC).

the surface water and sediments are shown in Figure 4a and b. From Figure 4, it is clear that OTC and its related compounds (EOTC, α -apo-OTC, and β -apo-OTC) all were detected in both water and sedimentary samples. The average concentrations of analytes in water samples at site R2 were 641 ± 118 , 31.5 ± 3.8 , 5.76 ± 0.63 , and 2.08 ± 0.30 $\mu\text{g/L}$ for OTC, EOTC, α -apo-OTC, and β -apo-OTC, respectively (Table 4). Concentration of OTC slightly decreased along the river. However, it was still 377 ± 142 $\mu\text{g/L}$ at the last site R4, approximately

20 km from the discharging point. 4-Epi-oxytetracycline also decreased as distance from the discharging point increased. Thus, 12.9 ± 1.1 $\mu\text{g/L}$ of EOTC was detected at site R4. The ratio of the concentration of EOTC to OTC ranged from 0.034 to 0.049 in surface water samples, which was similar to those obtained in the WWTP (0.020–0.041). It is interesting that concentrations of α -apo-OTC and β -apo-OTC significantly increased along the river reaching 11.9 ± 4.9 and 12.0 ± 4.6 $\mu\text{g/L}$, respectively at site R4. On the other hand, as shown in Table 4, relatively low concentrations of OTC and EOTC have been detected at almost all of the sampling sites in August compared to those in December and April. This possibly could be explained by higher temperature, stronger illumination, and larger surface water flow in August.

Mean concentrations of OTC, EOTC, α -apo-OTC, and β -apo-OTC in sediments were 201 ± 19 , 12.5 ± 4.2 , 11.4 ± 1.7 , 20.8 ± 7.8 mg/kg, respectively, and relative standard deviations for concentrations of analytes in sediment samples taken from each site were generally less than 30%. Comparing to EOTC and α -apo-OTC, relatively high concentrations of β -apo-OTC were detected in sediment samples, and the mean ratio of β -apo-OTC concentration versus OTC in sediments was approximately 0.11, about twice the ratio of α -apo-OTC and EOTC to OTC (0.058 ± 0.014 and 0.061 ± 0.015 , respectively), indicating that β -apo-OTC was possibly the main degradation product in sediments under anaerobic conditions. This was in accordance with the result obtained by Halling-Sørensen et al. [14] in an OTC-spiked soil interstitial water system under the anaerobic condition, but different than that obtained by Loke et al. [16] in manure-containing anaerobic systems spiked with OTC. Distribution factors also were calculated to be 322 to 571, 480 to 739, 851 to 1,684, and 2,383 to 7,019 L/kg for OTC, EOTC, α -apo-OTC, and β -apo-OTC, respectively, which were much higher than those in the activated sludge (161–193, 206–220, 392–411, and 604–661 L/kg, respectively).

CONCLUSION

In conclusion, concentrations of OTC and related compounds found in the present study were much higher than those normally detected in surface and groundwater [26,27]. Large amounts of OTC and its related compounds were discharged into environment due to the low removals by the WWTP. Such compounds remained in the receiving surface water over a

Table 4. Concentrations of oxytetracycline (OTC), 4-epi-oxytetracycline (EOTC), α -apo-oxytetracycline (α -apo-OTC), and β -apo-oxytetracycline (β -apo-OTC) in surface water and sediment samples taken from the Xiao River, China in December 2004 and April and August 2005

Analyte	Month	Concentrations					
		Surface water ($\mu\text{g/L}$)			Sediments (mg/kg)		
		Site R2	Site R3	Site R4	Site R2	Site R3	Site R4
OTC	December	712 ± 95	612 ± 88	484 ± 50	236 ± 54	197 ± 58	212 ± 60
	April	689 ± 71	531 ± 46	411 ± 97	262 ± 71	240 ± 63	201 ± 62
	August	523 ± 74	371 ± 34	235 ± 55	171 ± 53	166 ± 45	139 ± 45
EOTC	December	34.2 ± 4.6	23.3 ± 3.6	13.7 ± 1.5	16.4 ± 4.8	13.2 ± 3.4	9.88 ± 3.5
	April	32.6 ± 6.7	22.8 ± 2.7	13.3 ± 2.4	19.4 ± 6.1	13.8 ± 4.2	9.66 ± 3.2
	August	27.7 ± 4.2	16.6 ± 2.1	11.8 ± 2.1	14.2 ± 4.0	8.28 ± 1.8	8.72 ± 2.7
α -apo-OTC	December	6.31 ± 0.85	9.57 ± 0.97	12.9 ± 1.07	8.3 ± 2.4	8.8 ± 2.5	9.43 ± 2.4
	April	5.84 ± 0.71	8.12 ± 1.48	11.1 ± 1.58	10.1 ± 4.8	14.2 ± 3.7	12.0 ± 3.7
	August	5.13 ± 0.83	10.1 ± 1.05	11.8 ± 1.33	9.1 ± 2.9	15.0 ± 4.3	13.6 ± 4.0
β -apo-OTC	December	2.10 ± 0.34	5.48 ± 2.2	15.1 ± 2.3	16.1 ± 3.2	18.1 ± 4.9	27.4 ± 5.4
	April	2.35 ± 0.18	4.68 ± 1.0	13.4 ± 4.5	14.0 ± 3.9	21.2 ± 6.4	34.6 ± 10.2
	August	1.78 ± 0.35	4.74 ± 1.6	7.42 ± 2.1	12.8 ± 4.1	18.7 ± 3.2	23.8 ± 6.4

long distance. The minimal inhibition concentration of OTC has been reported to be approximately 250 $\mu\text{g/L}$ for sensitive strains, and the minimal inhibition concentration required to inhibit the growth of 50% of organisms of EOTC, α -apo-OTC, and β -apo-OTC were 1.0, 32, and more than 32 mg/L , respectively [15]. Considering that the antibiotic resistance of bacteria could be induced and maintained even at a level of the antibiotics significantly lower than the minimal inhibition concentration for some bacteria species [28,29], more efficient methods other than activated sludge treatment, such as ozonation, should be introduced for the removal of OTC and its related substances from the production wastewater [21].

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