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Occurrence and fate of quinolone and fluoroquinolone antibiotics in a municipal sewage treatment plant

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ABSTRACT

This study developed a method for analysis of nineteen quinolone and fluoroquinolone antibiotics (FQs) in sludge samples, and investigated the occurrence and fate of the FQs in a municipal sewage treatment plant (STP) with anaerobic, anoxic, and aerobic treatment processes. Eleven compounds, including pipemidic acid, fleroxacin, ofloxacin, norfloxacin, ciprofloxacin, enrofloxacin, lomefloxacin, sparfloxacin, gatifloxacin, moxifloxacin, and sarafloxacin (only in sludge), were detected in the STP. The predominance of ofloxacin and norfloxacin, followed by lomefloxacin, ciprofloxacin, gatifloxacin, and moxifloxacin, were found in wastewater, suspended solids, and sludge. The total concentrations of FQs were 2573 ± 241 ng/L, 1013 ± 218 ng/L, and 18.4 ± 0.9 mg/kg in raw sewage, secondary effluent, and sludge, respectively. Extremely low mass change percentages were observed for FQs in anaerobic, anoxic, and aerobic treatment units, suggesting biodegradation to be of minor importance in the removal of FQs in STPs. 50–87% of the initial FQs loadings (except for pipemidic acid (36%)) were ultimately found in the dewatered sludge. Mean removal efficiencies of FQs in the STP were 56–75%, except for new generation drugs such as moxifloxacin (40 ± 5%) and gatifloxacin (43 ± 13%). A significant positive correlation was found between removal efficiencies and K_d of FQs. The major factor in the removal of FQs in the STP was sorption to sludge, which was not governed by hydrophobic interactions. The long-term cycling and persistence of FQs in the STP has made activated sludge as a huge reservoir of FQ antibiotics.

1. Introduction

Antibiotics are commonly used in human and veterinary medicine, and the presence of these compounds in the environment is of concern due to their role in the development of antimicrobial resistance among microorganisms (Daughton and Ternes, 1999). Quinolones and fluoroquinolones (FQs) are a group of antibiotics widely used to treat a broad variety of Gram (+) and Gram (−) bacterial infections since their derivation from nalidixic acid in 1962 (Ball, 2000). The FQs are among the five classes of antibiotics (β-lactam, macrolides, fluoroquinolones, sulfonamides, and tetracyclines) frequently detected in the environment in relatively high concentrations (Diaz-Cruz and Barcelo, 2006; Khetan and Collins, 2007), and their ubiquitous presence has been reported in wastewater (Gros et al., 2007), surface water (Kolpin et al., 2002), ground water, and even in drinking water (Barnes et al., 2008; Ye et al., 2007). Extensive usage and wide occurrences have led to increasing bacterial resistances to FQs in wastewater from hospitals and livestock feedlots, effluent and sewage sludge from municipal sewage treatment plants (STPs), and rivers (Hu et al., 2008; Polk et al., 2004; Reinthaler et al., 2003; Taylor

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et al., 2008), making human and animals more susceptible to these microbes.

A broad number of FQ antibiotics (more than twenty chemicals cross four generations) have been developed and are commercially available around the world (Martinez et al., 2006). Except for the most concerning FQs (e.g. ciprofloxacin) in previous studies (Castiglioni et al., 2005, 2006; Ghosh et al., 2009; Golet et al., 2001; Miao et al., 2004; Renew and Huang, 2004; Shi et al., 2009; Vieno et al., 2007, 2006; Xu et al., 2007), a new generation FQs such as gatifloxacin and moxifloxacin has been introduced to provide better therapeutic effects and counteract bacterial resistance (Kowalski et al., 2003). The significant disadvantages of the new generation, however, have resulted in their restricted use (Faich et al., 2004) due to serious side effects (e.g. hyperglycemia (Yip and Lee, 2006) and hallucination (Adams and Tavakoli, 2006)), and the genotoxic potentials of these compounds have been reported to be higher than those of other FQs based on *in vitro* bioassay (Hartmann et al., 1998; Hu et al., 2007). Therefore, the occurrence of a broad range of FQs is important for understanding their environmental risks. In most previous investigations, of all the FQs only ciprofloxacin, norfloxacin, and ofloxacin have been included as major target compounds possibly due to a lack in analytical methods (Castiglioni et al., 2005, 2006; Ghosh et al., 2009; Golet et al., 2001; Miao et al., 2004; Renew and Huang, 2004; Shi et al., 2009; Vieno et al., 2007, 2006; Xu et al., 2007). Although sensitive liquid chromatography–electrospray tandem mass spectrometry (LC-MS/MS) has been established for 20 quinolone and fluoroquinolone antibiotics in various water matrices (Xiao et al., 2008), there is still no method available for these compounds in solid samples as it is difficult to develop a method with high extraction efficiencies and low matrix effects for so many antibiotics in solid samples.

Removal of FQ antibiotics in STPs plays a crucial role in their pollution control, since most FQs used to treat humans or livestock are ultimately discharged into the aquatic environment via STPs. While the presence of some FQs in STP influents and effluents have been widely investigated (Castiglioni et al., 2005, 2006; Ghosh et al., 2009; Golet et al., 2001; Miao et al., 2004; Renew and Huang, 2004; Shi et al., 2009; Vieno et al., 2007, 2006; Xu et al., 2007), little is known about the fates and major removal units of these antibiotics. Recently, the fates of norfloxacin and ciprofloxacin in two European STPs (Sweden and Switzerland) with mechanical and anaerobic treatment processes were reported (Golet et al., 2003; Lindberg et al., 2006). It is well known that anoxic and aerobic treatment units are very common in STP systems, and Wu et al. (2008) reported that some antibiotics are more persistent under anaerobic conditions than aerobic conditions. However, the fate of norfloxacin and ciprofloxacin in anoxic and aerobic units remains unclear, and no research has been conducted on the fates of other FQs. Thus, assessing the removal efficiencies of all FQs in anoxic or aerobic treatment units could provide basic information for the improvement of STP performance.

In this study, we developed a single solid-phase extraction (SPE) method that allowed the simultaneous analysis of nineteen FQ antibiotics (cinoxacin, lomefloxacin, pipemidic acid, ofloxacin, danofloxacin, enrofloxacin, ciprofloxacin, sarafloxacin, difloxacin, sparfloxacin, moxifloxacin, fleroxacin, norfloxacin, oxolinic acid, pefloxacin, flumequine, nalidixic acid, piromidic acid, and gatifloxacin, shown in Supplementary Data Fig. S1) in sewage sludge. The method was then applied to investigate the fate of each compound during STP treatment with complete mechanical, anaerobic, anoxic, and aerobic treatment processes. The removal efficiencies of detected FQs were evaluated, and their potential removal mechanisms in the STP were explored based on the mass balance analysis in anaerobic, anoxic, and aerobic treatment units.

2. Materials and methods

2.1. Sample collection

Qinghe STP (Beijing, China) serves a residential population of about 814,000, and its incoming raw sewage consists mainly of domestic wastewater at a rate of 400,000 m^3/day. The sewage is firstly treated with a screen and an aerated grit chamber as the primary clarification. The primary sludge is pumped into the dewater room, while the primary effluent flows directly through the activated sludge system, which is comprised of anaerobic, anoxic, and aerobic units. After a final secondary clarification step, the effluent of the activated sludge reactor is discharged into the receiving river. The scheme of the STP and sampling locations are shown in Supplementary Data (Fig. S2). The hydraulic retention times in the aerated grit chamber, anaerobic tank, anoxic tank, and aerobic tank were 15, 2, 3, and 11 h, respectively, and total solid retention time was 20–25 d. To investigate the fates of quinolones and fluoroquinolones in the Qinghe STP, three-day sampling was carried out in dry weather at the outlet of each treatment step on July 7, 8, and 11, 2008. During this period, the sewage for treatment was around 200,000 m^3/d, and water temperatures were 23–25 °C. Several regularly measured parameters (e.g., BOD_5, COD, and DO, NH_4–N, and NO_3–N) are displayed in Table S3 (Supplementary Data). Raw sewage and primary and secondary effluents were taken as 24 h flow-proportional composite samples, and the suspended solids were collected by filtering these water samples. All water samples were collected in 10-L amber glass bottles, which were washed with 0.5 g/L ethylenediamine tetraacetic acid disodium (Na_2EDTA) water solution, followed by methanol and purified water before use. The sludge samples were generally taken at the outlet of every treatment step. Both the water and sludge samples were prepared for FQ analysis to assess the mass balance of the compounds in the STP. Water samples were extracted on the same day after being filtered by a glass microfiber filter GF/C 1.2 μm (Whatman, Maidstone, UK), and sludge samples were centrifuged and stored at −20 °C until analysis.

2.2. Sample preparation

Reagents and materials used in the analysis were shown in the Supplementary Data. The methods used to quantify 19 FQs in wastewater samples have been published previously (Xiao et al., 2008). Briefly, after filtration, wastewater samples

were added with Na_2EDTA (0.5% w/v) and adjusted to pH 3 with hydrochloric acid (HCl). The HLB cartridge (Waters Corporation, Milford, MA, USA) was preconditioned by 6 mL of methylene chloride, 6 mL of methanol, and 6 mL of purified water (0.5% Na_2EDTA w/v, pH = 3). Approximately 250 mL of water spiked with 40 ng of surrogate (norfloxacin-d_5) was extracted with HLB cartridge at a flow rate of 5–10 mL/min. The cartridges were rinsed with 10 mL of distilled water, and were then dried under a flow of nitrogen. All target compounds were eluted with 6 mL of methanol containing 0.1% formic acid (v/v). The elute was evaporated to dryness under a gentle stream of nitrogen, and dissolved in 500 μL of a mixture of methanol and pure water containing 0.1% formic acid (1:9, v/v). Analyses of all target compounds were carried out using ultra performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS, Waters Acquity UPLC™, Milford, MA, USA).

After freeze-dried in a freeze dryer (FDU-200, EYELA, Japan), about 0.1 g of sludge was spiked with 40 ng of norfloxacin-d_5 in a 50 mL centrifuge tube and stayed overnight before extraction. A total of 10 mL of triethylamine/methanol/purified water (5/25/75, v/v/v) was added to the tube, and the mixture was shaken for 20 min at 300 rpm. After 20 min sonication, the sample was centrifuged for 10 min at 3000 rpm. Following centrifugation, the supernatant was transferred to an amber glass bottle. The extraction was repeated twice and all three extracts were combined. The extract was diluted with 1 L of pure water, and then extracted by HLB cartridge with the same procedures of water samples described above. The detail of UPLC-MS/MS analysis was provided in the Supplementary Data.

2.3. Quantitation and quality control

Identification of the target quinolone and fluoroquinolone antibiotics was accomplished by comparing the retention time (within 2%) and the ratio (within 20%) of the two selected multiple-reaction monitoring (MRM) ion transitions with those of standards. Laboratory blanks made every day from purified water were analyzed to assess potential sample contamination. In this study, concentrations of laboratory blank samples were all lower than detection limits. To compensate for the loss of target compounds during the extraction process and correct the variation of instrument response and matrix effect, norfloxacin-d_5 surrogate was spiked to samples prior to extraction. The same instrumental method which has been reported to determinate FQs in wastewater samples (Xiao et al., 2008) was used for quantifying 19 FQs in sludge samples. Namely, 11 FQs (pipemidic acid, fleroxacin, ofloxacin, pefloxacin, enoxacin, norfloxacin, ciprofloxacin, danofloxacin, enrofloxacin, lomefloxacin and difloxacin) were quantified relative to norfloxacin-d_5 surrogate, and the method of external calibration was applied for quantification of the other nine target antibiotics. Recovery experiments were conducted to further assess method accuracy and precision. Samples collected from raw sewage, secondary effluent, and excess sludge samples were spiked with standard solutions of at least three times the original concentration, which was determined prior to the fortification experiment. Recoveries for spiked samples were 79–106%, 75–97% and 67–98% for raw sewage, secondary effluent, and excess sludge samples, respectively. Method detection limits (MDLs) were based on the peak-to-peak noise of the baseline near the analyte peak obtained by analyzing field samples and on a minimum value of 3 for signal-to-noise. For those non-detected chemicals, samples were spiked using a mixture of standard solution. The MDLs for the target antibiotics ranged from 0.0085 to 0.085 mg/kg in the sludge samples, and were 0.5–37 ng/L in wastewater samples.

2.4. Data analysis

To assess the contribution of sorption and degradation of FQ antibiotics in the STP, we took initial raw sewage loading (including dissolved and suspended solid phases) as the system input (100%), while the system output consisted of (i) secondary effluent, and (ii) dewatered sludge (Fan et al., 2011). The third part was expressed as (iii) lost, due to the total effect of degradation or transformation mechanism in each treatment unit within the STP, and was calculated as

$$W_{\text{Lost}} = W_{\text{Influent}} - W_{\text{Effluent}} - W_{\text{Sludge}} \quad (1)$$

where the "W" was the mass of total FQs within the STP. The mass change percentage (%) in each treatment unit was calculated using $(W_{\text{Inflow}} - W_{\text{Outflow}})/W_{\text{Inflow}} \times 100\%$ to assess the mass variations of FQs under different treatment processes, where W_{Inflow} and W_{Outflow} respectively represent the total mass flow (aqueous phase and sorbed phase) of the detected compound in the inflow and outflow of the individual treatment unit. The calculations of mass flow and solid–water partition coefficient (K_d) in STP treatment units were provided in the Supplementary Data.

3. Results and discussion

3.1. Method performance

To explore the fates of FQ antibiotics within STPs, several studies have developed analytical methods for norfloxacin, ciprofloxacin, and ofloxacin in sludge samples (Golet et al., 2002, 2003; Lindberg et al., 2006, 2005). Extraction efficiencies and matrix effects were reported to greatly influence the analysis of antibiotics in sludge samples (Gobel et al., 2002, 2005). In the current study, different extraction solvents were optimized for simultaneously analyzing 19 quinolone and fluoroquinolone antibiotics from sludge samples with high extraction efficiencies and low matrix effects. Of the nineteen target compounds, seven FQs had very poor recoveries (<40%) when extracted with methanol/citrate buffer (1/1, v/v, pH 2.6) reported in previous studies (Jacobsen et al., 2004; Kim and Carlson, 2007), and even low recoveries were observed with acetonitrile/phosphate buffer (1/1, v/v, pH 2.2) used previously for FQs analysis (Golet et al., 2002, 2003) (Table S2 in Supplementary Data). Following methods reported for norfloxacin, ciprofloxacin, and ofloxacin in STPs (extracted with phosphate buffer (pH 6.0) and triethylamine/methanol/purified water (5/25/75, v/v/v, pH 11.0)) (Lindberg et al., 2005), relatively high recoveries in sludge samples (61–80%) were observed; however, recoveries of

pipemidic acid, pefloxacin, and norfloxacin-d_5, which have not been analyzed previously, were limited to 49–55%. Since norfloxacin-d_5 was used as a surrogate in the current study, its good recovery was important in the quantification of all target compounds. Since FQs were positively-charged compounds, relatively high recoveries of FQs were obtained by basic extraction solutions compared to acidified solutions (Golet et al., 2002, 2003; Lindberg et al., 2005). Thus, we extracted sludge samples three times with triethylamine/methanol/purified water (5/25/75, v/v/v, pH 11.0). The recoveries of all target compounds including pipemidic acid, pefloxacin, norfloxacin, ciprofloxacin, enrofloxacin, and norfloxacin-d_5 improved greatly (67–98%) (Table S2). To assess matrix effects of the extraction solvent and purification steps, the percentages of signal intensity in the extracted sludge sample versus the signal of the same concentration in pure solvent (methanol) were calculated. The matrix effects for all target antibiotics ranged from −13 to 26%, and the MDLs of the current method in sludge samples ranged from 0.0085 to 0.085 mg/kg (Table S2 in Supplementary Data). MDLs of ofloxacin (0.028 mg/kg), norfloxacin (0.036 mg/kg) and ciprofloxacin (0.028 mg/kg) were improved compared with those (0.1 mg/kg) in previous studies (Lindberg et al., 2006, 2005).

3.2. *Occurrence*

Of the 19 target antibiotics, 10 compounds, including pipemidic acid, fleroxacin, ofloxacin, norfloxacin, ciprofloxacin, enrofloxacin, lomefloxacin, sparfloxacin, gatifloxacin, and moxifloxacin, were detected in wastewater samples from Qinghe STP (Table 1). Ofloxacin (1287 ± 97 ng/L) and norfloxacin (775 ± 87 ng/L) were the dominant FQs in the raw sewage, accounting for 50 ± 1% and 30 ± 0.6% of total concentrations, respectively (Table 1). Relatively low concentrations were detected in the raw sewage for lomefloxacin (162 ± 4 ng/L), ciprofloxacin (99 ± 21 ng/L), pipemidic acid (86 ± 17 ng/L), moxifloxacin (72 ± 34 ng/L), gatifloxacin (66 ± 7 ng/L), fleroxacin (14 ± 1 ng/L), enrofloxacin (8.3 ± 3.2 ng/L), and sparfloxacin (4.4 ± 0.3 ng/L). The profile of detected FQs was constant throughout the STP treatment units, and concentrations of individual compounds ranged from 1.1 ng/L (sparfloxacin) to 528 ng/L (ofloxacin) in secondary effluent (Table 1). It should be noted that concentrations of most FQs in secondary effluent were higher than those in the aerobic effluent sample, which were possibly due to desorption of the antibiotics from sludge in the secondary clarifier. Of all the target compounds in the current study, three FQs (ofloxacin, norfloxacin, and ciprofloxacin) have been extensively investigated by previous studies, and the predominance of ofloxacin and norfloxacin were also observed in STP wastewater in most developed countries (Castiglioni et al., 2005; Ghosh et al., 2009; Golet et al., 2001, 2003; Lindberg et al., 2006, 2005; Miao et al., 2004; Renew and Huang, 2004; Vieno et al., 2007, 2006). In the present study, raw sewage concentrations of ofloxacin and norfloxacin were higher than those in most developed countries (norfloxacin: 155–486 ng/L in Japan (Ghosh et al., 2009), ofloxacin: 19–287 ng/L and norfloxacin: 72–174 ng/L in Sweden (Lindberg et al., 2005), ofloxacin: 20–350 ng/L and norfloxacin: 13–960 ng/L in Finland (Vieno et al., 2007)). However, ciprofloxacin, generally reported with similar or higher levels compared to norfloxacin in the USA, Finland, Sweden, and Switzerland (Golet et al., 2003; Lindberg et al., 2006, 2005; Renew and Huang, 2004; Vieno et al., 2007, 2006), was detected at concentrations of only about 13% of norfloxacin in raw sewage from the Qinghe STP. Similar concentration ratios between ciprofloxacin and norfloxacin were also reported in five STPs in Shanghai, China (Shi et al., 2009). Such a profile of FQs in Chinese wastewater is in accordance with the low production and consumption of ciprofloxacin compared with other countries. It has been documented that the production of ciprofloxacin was about 40% that of norfloxacin in China in 1999 (Chemical and Industry Association of China, 2000), and consumption of ciprofloxacin was about 20% that of norfloxacin in Beijing hospitals between 2003 and 2008 (Liu et al., 2010), while similar or higher production and consumption for ciprofloxacin compared with norfloxacin has been reported in Europe and North America (Golet et al., 2001, 2002; Vieno et al., 2007). It should be noted that fourth generation FQs, including gatifloxacin and moxifloxacin, were detected in raw sewage (66 ± 7 and 72 ± 34 ng/L) and secondary effluents (40 ± 8 and 40 ± 20 ng/L), suggesting that these compounds should not be neglected in future studies.

In the suspended solids and sludge samples, ten antibiotics in wastewater and sarafloxacin (only in sludge) were

Table 1 – Concentrations of quinolone and fluoroquinolone antibiotics in sewage water (ng/L).

Chemicals	Raw sewage	Primary effluent	Anaerobic	Anoxic	Aerobic	Secondary effluent	Return sludge	Excess sludge	Supernatant of excess sludge
Pipemidic acid	86 ± 17	78 ± 12	28 ± 3	22 ± 6	22 ± 8	33 ± 12	18 ± 8	52 ± 7	33 ± 12
Fleroxacin	14 ± 1	15 ± 1	7.2 ± 0.7	4.9 ± 0.4	5.2 ± 1.0	5.2 ± 0.3	3.6 ± 0.5	11 ± 1	5.2 ± 0.3
Ofloxacin	1287 ± 97	1575 ± 101	546 ± 165	384 ± 22	336 ± 71	528 ± 89	238 ± 83	1139 ± 58	528 ± 89
Norfloxacin	775 ± 87	831 ± 98	249 ± 29	207 ± 38	190 ± 52	256 ± 64	155 ± 85	610 ± 14	256 ± 64
Ciprofloxacin	99 ± 21	105 ± 13	37 ± 5	30 ± 5	28 ± 7	37 ± 16	23 ± 12	88 ± 5	37 ± 16
Enrofloxacin	8.3 ± 3.2	6.6 ± 3.2	2.6 ± 1.1	2.5 ± 0.7	2.6 ± 1.1	2.1 ± 0.6	0.7 ± 0.4	4.4 ± 1.4	2.1 ± 0.6
Lomefloxacin	162 ± 4	186 ± 26	85 ± 21	71 ± 16	62 ± 17	71 ± 14	52 ± 24	295 ± 2	71 ± 14
Sarafloxacin	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
Gatifloxacin	66 ± 7	72 ± 9	37 ± 11	29 ± 4	25 ± 5	40 ± 8	20 ± 6	111 ± 1	40 ± 8
Sparfloxacin	4.4 ± 0.3	3.9 ± 0.3	1.0 ± 0.2	0.7 ± 0.1	0.7 ± 0.2	1.1 ± 0.2	0.6 ± 0.3	5.5 ± 0.3	1.1 ± 0.2
Moxifloxacin	72 ± 34	98 ± 45	36 ± 3	31 ± 4	33 ± 7	40 ± 20	21 ± 11	85 ± 24	40 ± 20

Table 2 – Concentrations of Quinolone and Fluoroquinolone Antibiotics in Sewage Sludge (mg/kg).

Chemicals	Raw sludge	Primary sludge	Anaerobic	Anoxic	Aerobic	Return sludge	Excess sludge	Dewatered sludge
Pipemidic acid	0.02 ± 0.01	0.04 ± 0.05	0.29 ± 0.01	0.27 ± 0.01	0.30 ± 0.05	0.32 ± 0.10	0.27 ± 0.04	0.27 ± 0.03
Fleroxacin	0.02 ± 0.01	0.02 ± 0.01	0.08 ± 0.01	0.08 ± 0.01	0.10 ± 0.01	0.09 ± 0.01	0.09 ± 0.02	0.08 ± 0.01
Ofloxacin	0.35 ± 0.07	0.33 ± 0.07	6.36 ± 0.36	7.24 ± 0.65	7.35 ± 0.79	7.57 ± 0.61	7.40 ± 0.57	7.79 ± 0.55
Norfloxacin	0.86 ± 0.40	1.06 ± 0.36	6.39 ± 0.33	6.67 ± 0.15	7.55 ± 0.70	7.25 ± 1.05	6.40 ± 0.46	7.23 ± 0.22
Ciprofloxacin	0.20 ± 0.05	0.22 ± 0.08	0.93 ± 0.02	0.99 ± 0.07	1.05 ± 0.13	1.15 ± 0.14	0.96 ± 0.07	1.04 ± 0.14
Enrofloxacin	0.02 ± 0.01	0.02 ± 0.01	0.10 ± 0.01	0.08 ± 0.01	0.09 ± 0.04	0.10 ± 0.01	0.07 ± 0.01	0.07 ± 0.03
Lomefloxacin	0.06 ± 0.01	0.06 ± 0.03	1.01 ± 0.05	0.97 ± 0.01	1.06 ± 0.12	1.18 ± 0.23	0.98 ± 0.05	1.00 ± 0.08
Sarafloxacin	<MDL	<MDL	0.22 ± 0.30	0.49 ± 0.05	0.36 ± 0.27	0.62 ± 0.11	0.39 ± 0.30	0.53 ± 0.03
Gatifloxacin	0.07 ± 0.03	0.09 ± 0.04	0.39 ± 0.03	0.40 ± 0.01	0.48 ± 0.03	0.48 ± 0.12	0.42 ± 0.05	0.49 ± 0.03
Sparfloxacin	0.01 ± 0.01	0.01 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.04 ± 0.01	0.03 ± 0.01	0.04 ± 0.01
Moxifloxacin	0.17 ± 0.03	0.17 ± 0.04	0.51 ± 0.02	0.46 ± 0.02	0.56 ± 0.06	0.57 ± 0.13	0.56 ± 0.01	0.53 ± 0.10

detected (Table 2). Ofloxacin and norfloxacin were also dominant in the suspended solids and sludge samples. Relatively high concentrations of ofloxacin (7.29 ± 0.45 mg/kg) and norfloxacin (7.01 ± 0.51 mg/kg) in the sludge samples were found in the current study compared with other countries (0.1–4.2 mg/kg), but concentrations of ciprofloxacin (0.20 ± 0.05 mg/kg) were lower than those reported previously (0.5–7.7 mg/kg) (Golet et al., 2003; Lindberg et al., 2006, 2005). The profile of FQs in sludge samples was consistent with that in wastewater. In all the treatment units, concentrations of detected FQs in excess sludge were 3–9 folds higher than those in return sludge (Table 1), which were possibly due to the fact that excess sludge stored at least three days for thickening before further treatment, but return sludge was rapidly pump back to the active sludge treatment units.

Mean removal efficiencies, calculated by comparing concentrations in the raw sewage and secondary effluent, ranged from 40% to 75% for the ten detected FQ antibiotics (Fig. 1). The removal efficiencies of some FQs in the current study were in the range of those previously reported for ciprofloxacin (37–86%), ofloxacin (33–66%), norfloxacin (58–87%), and lomefloxacin (21–72%) (Castiglioni et al., 2005; Lindberg et al., 2005; Shi et al., 2009; Xu et al., 2007). Compound-specific removal efficiencies were firstly observed for the detected FQs, and it should be noted that the lowest removal efficiencies were found for the new generation drugs, moxifloxacin (40 ± 5%) and gatifloxacin (43 ± 13%) (Fig. 1). Since moxifloxacin and gatifloxacin have been withdrawn or classified for restricted use in North American due to their serious side effects (Adams and Tavakoli, 2006; Faich et al., 2004; Yip and Lee, 2006), the presence and low removal efficiencies of these compounds in STPs indicates the importance of strict control of these antibiotics in China.

3.3. *Mass flow and mass balance*

Mass flows and mass balance of individual FQs were determined to assess their potential removal mechanisms in the STP (Fig. 2 and Supplementary Data Table S4). In raw sewage, the combined aqueous and solid phase mass flows of all detected FQs were about 612 g/d in the Qinghe STP, and mass flows entering the STP were 515 ± 48 g/d and 97 ± 14 g/d in the dissolved and sorbed fraction, respectively. The proportion of FQs sorbed to particles in the raw sewage ranged from 7% (pipemidic acid, ofloxacin) to 39% (moxifloxacin), and those of norfloxacin (23%) and ciprofloxacin (39%) were similar to that reported in an STP in Switzerland (33%) (Golet et al., 2003), but lower than that in Sweden (80%) (Lindberg et al., 2006). The different sorption proportions were possibly due to the different characterizations of the sewage in the various cities. Total FQs mass flow entering the activated sludge system was about 595 ± 38 g/d, with individual chemicals varying from 0.8 (sparfloxacin) to 315 g/d (ofloxacin). In the activated sludge system, mass change percentages were −13 to 16%, −4 to 22%, and −33 to 2% in anaerobic, anoxic, and aerobic treatment units, respectively. The negative mass change percentages were due to the potential variations between the three sampling days and the analytical RSD. The low mass change percentages and variability in the three treatment units

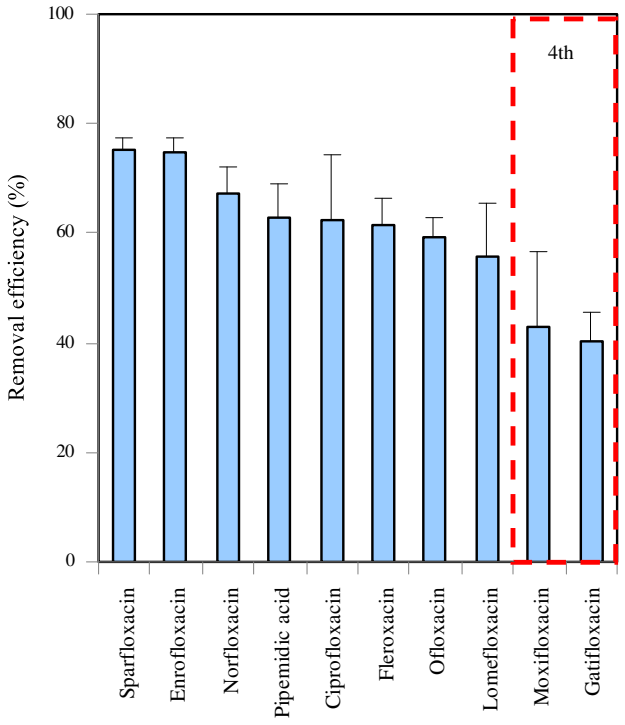

Fig. 1 – Removal efficiencies (%) of detected FQ antibiotics in Qinghe STP. Numbers indicate the new generation of the FQ antibiotics used in human and veterinary medicine.

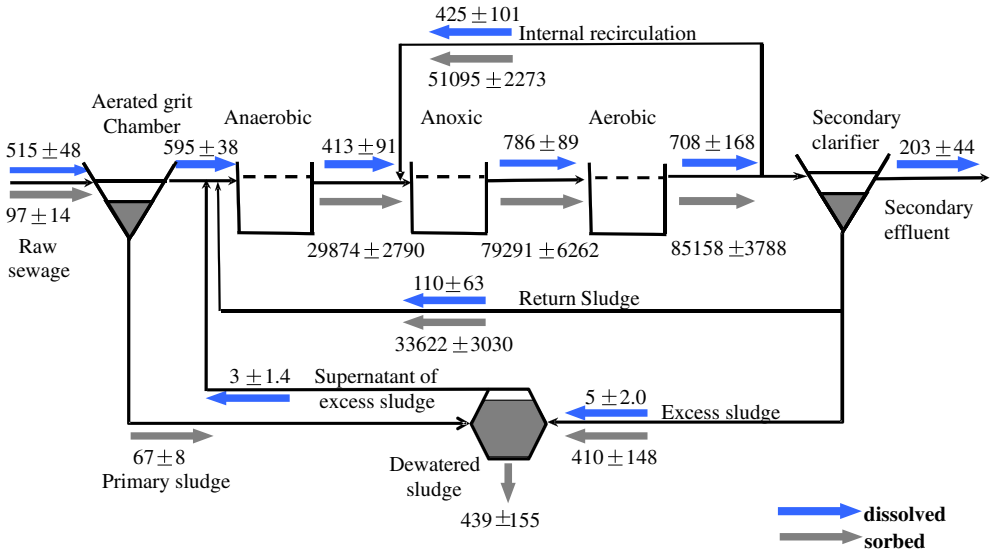

Fig. 2 – Total mass flows (g/d) of detected quinolone and fluoroquinolone antibiotics in STP processes.

suggested that biodegradation in anaerobic, anoxic, and aerobic treatment units was of minor importance in the removal of FQs. For filtered secondary effluents, the mass flows of FQs were 203 ± 44 g/d in the aqueous phase and varied from 0.2 g/d (sparfloxacin) to 106 g/d (ofloxacin), while the sorbed amount was ignored due to the low concentrations of suspended solids in the effluent. The excess sludge was then moved through a dewaterer without further digestion, and the total FQs mass in the dewatered sludge in the Qinghe STP was 439 ± 155 g/d, varied from 263 to 552 g/d during the three sampling days.

The mass balance of FQs in the Qinghe STP were expressed in chemical mass fractions (%) detected in (i) secondary effluent, (ii) dewatered sludge, and (iii) lost, relative to the calculated initial loading (100%) (Fig. 3). We observed that the calculated fraction of most FQs in the dewatered sludge accounted for 50–87% (except for pipemidic acid, 36%) of the initial loadings, while less than 47% of the total amount of FQs was found in the effluents. Mass balance of ciprofloxacin and norfloxacin in anaerobic treatment process has only been reported in Switzerland and Sweden (Golet et al., 2003; Lindberg et al., 2006). In those studies, the mass fractions for ciprofloxacin and norfloxacin were 72–83% in dewatered sludge (Golet et al., 2003; Lindberg et al., 2006), which are comparable to those in the present study (85–87%). These results further confirmed that sorption via sludge was the major removal mechanism of FQs in the STP.

To further understand compound-specific removal efficiencies, pK_a, octanol-water coefficient (log P_{ow}, calculated by ACD/log Pow ver. 1.0 (Advanced Chemistry Development, Inc.)), and the apparent solid–water partition coefficients (K_d) were calculated (Table S5 in Supplementary Data). The average K_d values of target FQs in current study (12,300–37,700, Table S5) were consistent with those of ciprofloxacin, norfloxacin, trovafloxacin, gemifloxacin, sarafloxacin and enrofloxacin in a previous study (12,600–39,800) (Golet et al., 2003). The K_d values of the FQs in sludge obtained in this study were slightly higher in aerobic units than in the anoxic and anaerobic units. Since it has been reported that adsorption of chemicals was correlated with the cation exchange capacity (CEC) (Hang and Brindley, 1970; Jaynes and Boyd, 1991), CEC values of sludge samples from the three treatment units were measured to assess the variation of K_d. The CEC values were 149 ± 3.7, 211 ± 1.3, and 164 ± 7.1 cmolc/kg dw for sludge samples collected from aerobic, anoxic and anaerobic units, respectively, and no significant correlation was found between CEC and K_d of all detected FQs. On the other hand, it was found that the hydraulic retention times in aerobic (11 h), anoxic (3 h) and

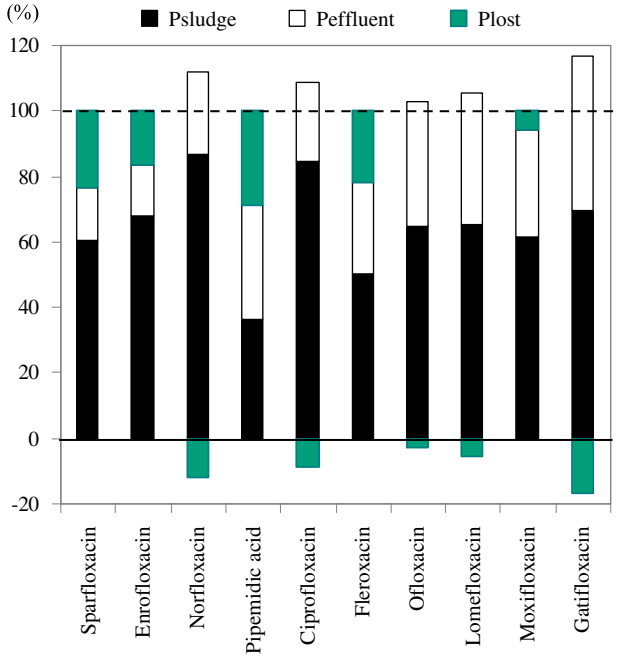

Fig. 3 – Mass proportions of detected quinolone and fluoroquinolone antibiotics in (i) secondary effluent (Peffluent), (ii) dewatered sludge (Psludge) and (iii) total lost (Plost) relative to the calculated initial loading (100%) in Qinghe STP.

anaerobic (2 h) units were consistent with the K_d variations within the three treatment units, thus would influence the K_d values of FQs in the STP. The relatively low log P_{ow} values (<2.5) of the detected FQs except for sarafloxacin indicated that these compounds have a weak sorption potential by hydrophobic interactions (Golet et al., 2003; Hu et al., 2007). A recent study suggested that electrostatic interactions is involved and, in some cases, may be dominating for positively charged compounds absorbed to sludge solid surfaces (Stevens-Garmon et al., 2011). Given the fact that all the target FQs contained nitrogen as positively-charged moiety (Fig. S1), the high sorption potentials of FQs were possible due to sorption through electrostatic interactions involved with the positively charged locations of these compounds (Golet et al., 2003; Stevens-Garmon et al., 2011). And the positively charged moieties for each chemical should be occurred at the nitrogen atoms, of which the atom partial charges were calculated by MOPAC (version 6, CAChe Scientific Inc., Oxford, U.K.) and showed in Fig. S1. The K_d of moxifloxacin and gatifloxacin were notably lower than the compounds with high removal efficiencies, especially for sparfloxacin, enrofloxacin, norfloxacin, and ciprofloxacin (about 2–3 times lower). The K_d of detected FQs also showed a significant positive correlation with their removal efficiencies ($r^2 = 0.54$, $p = 0.02$), suggesting that high absorption to the activated sludge resulted in the high removal efficiencies of these compounds in the STP (Fig. 4). This further confirmed that sorption associated with sludge was a major removal mechanism of FQs in raw sewage (Golet et al., 2003; Lindberg et al., 2006).

It should be noted that the mass load of FQs in the raw sewage was less than 1% the recycling sludge in the activated sludge treatment (Fig. 2). The high mass load of FQs in sludge in the Qinghe STP could result from the long-term cycling and accumulation of FQs in the STP. This also indicated that FQs were quite persistent in activated sludge, which is supported by previous research on the long-term persistence of FQs in sludge-treated soils (Golet et al., 2003). Therefore, the long-term cycling and persistence of FQs in STPs indicated that activated sludge was a huge reservoir of FQ antibiotics, emphasizing the importance of sludge management.

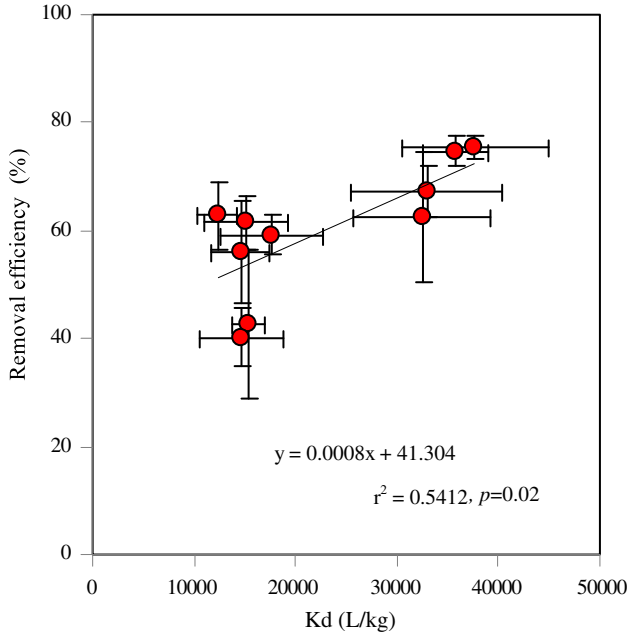

Fig. 4 – Relationships between removal efficiencies (%) and K_d (L/kg) of detected quinolone and fluoroquinolone antibiotics in Qinghe STP.

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Appendix. Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.watres.2011.10.055.

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