Research article

Occurrence and removal of antibiotics in ecological and conventional wastewater treatment processes: A field study

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ABSTRACT

The occurrence and removal of 19 antibiotics (including four macrolides, eight sulfonamides, three fluoroquinolones, three tetracyclines, and trimethoprim) were investigated in two ecological (constructed wetland (CW) and stabilization pond (SP)) and two conventional wastewater treatment processes (activated sludge (AS) and micro-power biofilm (MP)) in a county of eastern China. All target antibiotics were detected in the influent and effluent samples with detection frequencies of >90%. Clarithromycin, ofloxacin, roxithromycin and erythromycin-H2O were the dominant antibiotics with maximum concentrations reaching up to 6524, 5411, 964 and 957 ng/L, respectively; while the concentrations of tiamulin, sulfamerazine, sulfathiazole, sulfamethazine, sulfamethizole and sulfisoxazole were below 10 ng/L. Although the mean effluent concentrations of target antibiotics were obviously lower than the influent ones (except ciprofloxacin), their removals were usually incomplete. Principal component analysis showed that the AS and CW outperformed the MP and SP processes and the AS performed better than the CW process in terms of antibiotics removal. Both the AS and CW processes exhibited higher removal efficiencies in summer than in winter, indicating biological degradation could play an important role in antibiotics removal. Because of the incomplete removal, the total concentration of detected antibiotics increased in the mixing and downstream sections of a local river receiving the effluent from a typical wastewater treatment facility practicing AS process. Nowadays, ecological wastewater treatment processes are being rapidly planned and constructed as the secondary and/or tertiary treatment processes for wastewater in rural areas of China; however, the discharge of residual antibiotics to the aquatic environment may highlight a necessity for optimizing or upgrading their design and operation.

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1. Introduction

Due to the extensive human and veterinary utilization and the potentials to cause the selective proliferation of antibiotic resistant bacteria, antibiotics have raised increasing concerns recently (Pontes et al., 2009; Wilkinson et al., 2016). Antibiotics, including their parent compounds and transformation products, are being continuously discharged into the environment during manufacturing, consumption and disposal (Daughton and Ternes, 1999; Lin et al., 2010; Sharma et al., 2016). Among different pathways by which antibiotics enter the aquatic environment, effluents from various wastewater treatment facilities (WWTFs) have been considered as an important pollution source (Kim et al., 2007; Massey et al., 2010). As WWTFs are designed to remove organic materials, nitrogen and phosphate from wastewater, the removal of micro-pollutants (e.g. pharmaceuticals and personal care products, estrogens, etc.) is generally incomplete (Joss et al., 2006; Sponza and Celebi, 2012). The removal of antibiotics in WWTFs is affected by many factors including the physicochemical properties of antibiotics, specific treatment process employed, sludge retention time (SRT), hydraulic retention time (HRT) and environmental temperature, which make the removal efficiencies of antibiotics vary to a large extent (Cizmas et al., 2015; Gao et al., 2012a).

Although the ecological wastewater treatment processes usually have a high surface to equivalent-inhabitant ratio, relatively low cost, simple operation and maintenance, favorable environmental appearance and other ecosystem service benefits make them being widely planned and constructed as the secondary and/or tertiary treatment processes for wastewater in rural areas of China, where wastewater collection is often difficult because of the dispersed
layout, small scale and diverse geographic situations (Hijosa-Valsero et al., 2010). Continued efforts have been made on the occurrence and removal of antibiotics in wastewater treatment processes. However, the majority of past studies focused on large-scale wastewater treatment plants (WWTPs) that usually employ an activated sludge (AS) process while the occurrence and removal of antibiotics in ecological wastewater treatment processes remain largely unknown (Andreozzi et al., 2003; Brown et al., 2006; Lindberg et al., 2005).

In this study, the occurrence and removal of 19 antibiotics were investigated in 20 WWTFs located in a county of eastern China, which adopted either an ecological (such as constructed wetland (CW) and stabilization pond (SP)) or a conventional treatment process (such as AS and micro-power biofilm (MP)). The target antibiotics comprised five groups: macrolides, including roxithromycin (ROX), clarithromycin (CLA), tiamulin (TIA) and erythromycin-H2O (ERY-H2O); sulfonamides, including sulfadiazine (SDZ), sulfamerazine (SMR), sulfathiazole (STZ), sulfamethazine (SMN), sulfamethizole (SML), sulfamethoxazole (SMX), sulfosoxazole (SFX) and sulfadimethoxine (SDM); fluoroquinolones, including ciprofloxacin (CIP), ofloxacin (OFL) and norfloxacine (NOR); tetracyclines, including tetracycline (TCN), oxytetracycline (OTC) and chlorotetracycline (CTC); and a miscellaneous one, trimethoprim (TMP). Meanwhile, the seasonal variations in antibiotics removal were compared between the AS and CW processes. To assess the impact of the effluent discharged from a typical WWTF on local water quality, the antibiotic concentrations in the influent discharged from a typical WWTF were examined. This study would provide useful information on each WWTF, such as the inhabitants served, HRT, SRT, and engineering commissioning is provided in Table 1. The CW, SP and MP processes had a treatment capacity ranging from 200 to 1200 population equivalent (PE), while the AS process had a much larger treatment capacity of 8000–60 000 PE. The 20 WWTFs were constructed between 2001 and 2008 and the HRT of ecological and conventional processes ranged from 24 to 240 h and from 10 to 24 h, respectively.

2. Sample collection

Seasonal sampling campaigns were carried out in summer and winter, each of which lasted for two weeks. Because of the discontinuity of the influent flow rate in rural areas, sampling acquisition method was adopted which spread over a certain period of time to eliminate the high variation in influent flux. The samples were collected three times per day (i.e., morning, noon and evening) to make a mixed sample. Note that during the sampling campaigns, there were no rainfalls and the daily hydraulic loading rates of the test WWTFs were nearly constant. The collected samples were stored in pre-cleaned amber glass bottles and preserved in cool boxes (ca. 4 °C). Immediately after delivery to the laboratory, the samples were filtered through prebaked glass microfiber filters (GF/C, Whatman) and analyzed within three days.

2.3. Chemicals and analytical methods

STZ, SMR, SFX, SMX, SML, SDZ, TMP, OFL, NOR, CIP, TIA and ERY, all with a purity of 99.0%, were purchased from Sigma-Aldrich (St. Louis, USA), ROX (97.0%), TCN (97.0%), OTC (96.5%) and CTC (93.0%) were purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany). CLA (98.0%), the internal standard (simatone) and SMN (99.0%) were obtained from TCI (Tokyo, Japan), Accu Standard (New Haven, USA) and Acros Organics (New Jersey, USA), respectively. The detailed structures of the investigated antibiotics are listed in Table S1 (Supplementary material, SM). The stock solutions of individual antibiotics were prepared by dissolving each compound in methanol at a concentration of 100 mg/L (Ben et al., 2008). Because the dehydration product (ERY-H2O) was the predominant form of ERY in water (Hirsch et al., 1999), ERY-H2O was prepared and determined according to the method proposed by Lindberg et al.
being rinsed with 5 mL of 5% methanol aqueous solution and 5 mL of simatone was also added to each sample before the SPE. After eluates, which were then dried under a gentle stream of N2 and re-monitoring (MRM) mode were selected for identification of target antibiotics. Two product ions under the multiple reaction sensitivity based on the chromatographic separation of the formed under time-segmented conditions to maximize the detection sensitivity were used to ensure the reliable identification and accurate quantification of target antibiotics. Qualitative analysis was accomplished on the basis of the retention times and mass spectra of target antibiotics in MRM mode. The developed UPLC-MS/MS method showed a broad linear range and a good correlation coefficient ($R^2 > 0.99$) for each antibiotic studied.

Principal component analysis (PCA) was adopted in this study to analyze the possible correlation between effluent antibiotics concentration and treatment processes in summer and winter (Guerra et al., 2014). All statistical analyses were performed using SPSS 18 Software (IBM Corporation, USA). The effluent concentrations of target antibiotics were subdivided into 8 groups according to treatment processes and seasons. To avoid problems arising from the different measurement scales and numerical ranges of the variables, the data matrix was autoscaled for each of the 8 groups separately and then joining them together to form a new data matrix to have zero mean and unit variance (correlation matrix). In this way the contribution of the different variables to the total variance is made similar, regardless of their changes in the original matrix to have zero mean and unit variance (correlation matrix). In this way the contribution of the different variables to the total variance is made similar, regardless of their changes in the original scale of concentrations and of their average (offset) contribution. UNIANOVA was also employed to analyze the effluent antibiotic concentrations in different treatment processes and $p < 0.05$ was considered as statistically significant.

The removal efficiency of a target antibiotic was generally calculated as the reduction percentage between the influent and effluent concentrations. However, if an antibiotic concentration was below its LOQ, the removal efficiency was calculated following the rules proposed by Gabet-Giraud et al. (2010). Specifically, when an antibiotic was detected at a concentration below its LOQ in both influent and effluent, the removal efficiency was not calculated; when an antibiotic was detected in the influent but not in the effluent, the removal efficiency was calculated using half of its LOQ value for the effluent. To avoid the influence of high variation in influent flux on the removal efficiency, the concentrations of mixed

A total of 168 samples, including 80 influent and 80 effluent samples from the studied 20 WWTFs as well as 8 river samples, were collected and analyzed by ultra performance liquid chromatography and tandem mass spectrometry (UPLC-MS/MS) developed by Yuan et al. (2014). Solid phase extraction (SPE) was performed to clean up the samples and enrich the target antibiotics. To each water sample 0.1 g Na2EDTA was added. Then the sample was extracted by an HLB cartridge (500 mg/6 mL, Waters) after pH adjustment to 3.0 with 40% H2SO4. The HLB cartridge was pre-conditioned by 5 mL of methanol, 5 mL of 0.5 mol/L HCl, and 5 mL of Milli-Q water sequentially. The influent (400 mL), effluent (800 mL), and river water (800 mL) samples were extracted with the HLB cartridges at a flow rate of 5 mL/min. Internal standard (simatone) was also added to each sample before the SPE. After being rinsed with 5 mL of 5% methanol aqueous solution and 5 mL of Milli-Q water in sequence, HLB cartridges were dried under vacuum and then eluted with 10 mL of methanol. 50 μL of the simatone (with final concentration of 100 μg/L) was added to the eluates, which were then dried under a gentle stream of N2 and re-dissolved in 400 μL of methanol and 600 μL of Milli-Q water. The resulting solutions were filtered through 0.2 μm polyethersulfone filters (ion chromatography acrodisc, PALL) for UPLC-MS/MS analysis.

The mass spectrometer used was an Agilent 6420 Triple Quad MS, which was operated with electrospray ionization (ESI) in the positive ion mode. The MS parameters were set as follows: capillary voltage 4.0 kV, drying gas temperature 300 °C, drying gas flow 10 L/min, and nebulizing gas pressure 35 psi. N2 was used as the nebulizer, drying and collision gases. Data acquisition was performed under time-segmented conditions to maximize the detection sensitivity based on the chromatographic separation of the target antibiotics. Two product ions under the multiple reaction monitoring (MRM) mode were selected for identification and quantification of each antibiotic selected. The limit of quantification (LOQ) was defined as a signal-to-noise ratio of 10:1, as listed in Table 3. The developed method exhibited a high sensitivity for the target antibiotics, achieving low LOQs for macrolides (0.01–0.03 ng/L), sulfonamides (0.05–0.5 ng/L), fluoroquinolones (0.03–0.35 ng/L), tetracyclines (0.09–0.3 ng/L), and TMP (0.1 ng/L). The analysis of total suspended solids (TSS) was carried out following the Standard Methods for the Examination of Water and Wastewater (APHA, 1999). The concentrations of chemical oxygen demand (COD), and ammonia nitrogen (NH3-N) were analyzed according to Hach methods 8000 and 10031 on a UV–Vis spectrophotometer (DR5000, Hach, Loveland, CO), respectively.

### Table 1

<table>
<thead>
<tr>
<th>Process WWTF Type</th>
<th>PE served (inhabitants)</th>
<th>HRT (hr)</th>
<th>SRT (day)</th>
<th>Engineering commissioning</th>
</tr>
</thead>
<tbody>
<tr>
<td>Activated sludge (AS)</td>
<td>AS-1 Conventional</td>
<td>8000</td>
<td>10</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>AS-2</td>
<td>8000</td>
<td>12</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>AS-3</td>
<td>15000</td>
<td>12</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>AS-4</td>
<td>20000</td>
<td>15</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>AS-5</td>
<td>60000</td>
<td>16</td>
<td>15</td>
</tr>
<tr>
<td>Constructed wetland (CW)</td>
<td>CW-1 Ecological</td>
<td>350</td>
<td>48</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>CW-2</td>
<td>500</td>
<td>24</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>CW-3</td>
<td>500</td>
<td>48</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>CW-4</td>
<td>600</td>
<td>48</td>
<td>–</td>
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<tr>
<td></td>
<td>CW-5</td>
<td>800</td>
<td>90</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>CW-6</td>
<td>850</td>
<td>90</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>CW-7</td>
<td>1000</td>
<td>120</td>
<td>–</td>
</tr>
<tr>
<td>Micro power biofilm (MP)</td>
<td>MP-1 Conventional</td>
<td>200</td>
<td>15</td>
<td>–</td>
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<td></td>
<td>MP-2</td>
<td>300</td>
<td>12</td>
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<td></td>
<td>MP-3</td>
<td>300</td>
<td>12</td>
<td>–</td>
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<td></td>
<td>MP-4</td>
<td>500</td>
<td>15</td>
<td>–</td>
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<tr>
<td></td>
<td>MP-5</td>
<td>900</td>
<td>24</td>
<td>–</td>
</tr>
<tr>
<td>Stabilization pond (SP)</td>
<td>SP-1 Ecological</td>
<td>500</td>
<td>120</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>SP-2</td>
<td>1000</td>
<td>160</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>SP-3</td>
<td>1200</td>
<td>240</td>
<td>–</td>
</tr>
</tbody>
</table>

* PE: population equivalent.
influent and effluent samples were adopted to calculate the removal efficiency.

Because of the discontinuity in the influent flow in rural areas, grab sampling method was adopted which spread over a certain period of time to eliminate the high variation in influent flow. The samples were collected three times per day (i.e., morning, noon and evening) to make a mixed sample.

3. Results and discussion

3.1. Detection frequency and concentration range of target antibiotics

Table 2 summarizes the LOQ, detection frequencies and concentration ranges of 19 target antibiotics in the influent and effluent samples of studied WWTFs. In general, the detection frequencies of target antibiotics in the influents were always higher than those in the effluents. The lowest detection frequency (42%) was observed for SFX in the effluents; while a detection frequency of 100% was observed for two macrolides, four sulfonamides, three fluoroquinolones, three tetracyclines and TMP, which accounts for ca. 70% of the total number of studied antibiotics and reflects the ubiquitous occurrence of antibiotics in rural wastewaters.

As for the concentration ranges, CLA, SMX, OFL and OTC were found the most abundant in the macrolide, sulfonamide, fluoroquinolone and tetracycline groups. CLA occurred at the highest concentration ranges of 19 target antibiotics in the influents and effluents. The other seven antibiotics, including SMR, STZ, SMN, SML, SFX, SDM and TIA, were detected with low concentrations, and thus were not plotted. Among the 12 antibiotics, the maximum concentrations of CLA, ROX, ERY-H2O, NOR, OFL and TMP were close to or even exceeded 1000 ng/L in the influents and 100 ng/L in the effluents; meanwhile, the influent and effluent concentrations of SDZ, SMX, CIP, TCN, OTC and CTC were generally lower by one to two orders of magnitude (see Table 2). It is noted that the majority of target antibiotics could not be completely removed in the studied WWTFs.

<table>
<thead>
<tr>
<th>Antibiotic group</th>
<th>Antibiotic name</th>
<th>LOQ* (ng/L)</th>
<th>Detection freq. (%)</th>
<th>Conc. range (ng/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Macrolides</td>
<td>ROX</td>
<td>0.01</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>CLA</td>
<td>0.02</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>TIA</td>
<td>0.03</td>
<td>100</td>
<td>90</td>
</tr>
<tr>
<td></td>
<td>ERY-H2O</td>
<td>0.01</td>
<td>100</td>
<td>74</td>
</tr>
<tr>
<td>Sulfonamides</td>
<td>SDZ</td>
<td>0.05</td>
<td>100</td>
<td>98</td>
</tr>
<tr>
<td></td>
<td>SMR</td>
<td>0.3</td>
<td>100</td>
<td>92</td>
</tr>
<tr>
<td></td>
<td>STZ</td>
<td>0.4</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>SMN</td>
<td>0.5</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>SML</td>
<td>0.3</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>SMX</td>
<td>0.1</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>SFX</td>
<td>0.05</td>
<td>61</td>
<td>42</td>
</tr>
<tr>
<td></td>
<td>SDM</td>
<td>0.01</td>
<td>100</td>
<td>94</td>
</tr>
<tr>
<td>Fluoroquinolones</td>
<td>CIP</td>
<td>0.35</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>OFL</td>
<td>0.03</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>NOR</td>
<td>0.3</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Tetracyclines</td>
<td>TCN</td>
<td>0.09</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>OTC</td>
<td>0.07</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>CTC</td>
<td>0.3</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Trimetoprim</td>
<td>TMP</td>
<td>0.1</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

* LOQ: limit of quantification.

For example, the SMX concentrations ranged from 0.4 to 611 ng/L in the influent samples, which were by far lower than those reported in the large-scale WWTPs of China (5450–7910 ng/L) (Peng et al., 2006) and Germany (820 ± 230 ng/L) (Ternes et al., 2007). In general, although the influent antibiotic concentrations were lower than the large-scale WWTPs in China, they are comparable to those reported in the large-scale WWTPs of other countries (Le–Minh et al., 2010; Dolar et al., 2012). This is not surprising because two thirds of inpatients on average use antibiotics in China, which is significant higher than WHO guideline of 30% (Hu et al., 2003). Dong et al. (1999) found out that 1.47 dose of antibiotics was prescribed to the patient after a random sample of 1320 rural outpatient visits in China, while only 15% of all outpatient visits in the US had an antibiotics prescription in 2001–2002 (Roumie et al., 2005). The high production and consumption of antibiotics would inevitably increase their concentrations in the WWTF influents.

3.2. Antibiotics removal in different treatment processes

Box-whisker plots were adopted in Fig. 1 to show the maximum, 75%, mean, 50% (median), 25%, and minimum concentrations of 12 relatively abundant antibiotics detected in the four treatment processes. The other seven antibiotics, including SMR, STZ, SMN, SML, SFX, SDM and TIA, were detected with low concentrations, and thus were not plotted. Among the 12 antibiotics, the maximum concentrations of CLA, ROX, ERY-H2O, NOR, OFL and TMP were close to or even exceeded 1000 ng/L in the influents and 100 ng/L in the effluents; meanwhile, the influent and effluent concentrations of SDZ, SMX, CIP, TCN, OTC and CTC were generally lower by one to two orders of magnitude (see Table 2). It is noted that the majority of target antibiotics could not be completely removed in the studied WWTFs.

Macrolides are among the most important antibacterial agents used in human medicine, whose logKow (octanol-water partition coefficient) values vary between 1.5 and 4.0 (Dolar et al., 2012). As shown in Fig. 1A, there was a significant portion of ROX, CLA and ERY-H2O remaining in the effluent samples, which was in agreement with previous works (Clara et al., 2005; Gobel et al., 2007). Hirsch et al. (1999) reported that the effluent concentrations of ERY-H2O and ROX in the WWTPs of Germany could reach as high as...
Fig. 1. Influent and effluent concentrations of relatively abundant antibiotics in different wastewater treatment processes: (A) macrolides, (B) TMP and sulfonamides, (C) fluoroquinolones, and (D) tetracyclines.
6 μg/L. It is particularly noted that in the SP process of this study, the mean concentration of ERY-H2O in the effluent was close to that in the influent, indicating little removal of this compound. ERY-H2O was considered to be a persistent antibiotic, with a relatively long half-life (t1/2) of 11.5 d in soil (Gulkowska et al., 2008).

Some sulfonamides appeared to be recalcitrant during the ecological and conventional wastewater treatment. In fact, SMX and SDZ were present in almost all effluent samples analyzed, although at low levels (Fig. 1B). These results were comparable with those found in the previous study showing sulfonamides’ resistance to conventional biological degradation and sufficient hydrophilicity for transport into aquatic environments (Kolpin et al., 2002). Contradictory results have been reported for sulfonamides’ removal of SMX in AS process, as some studies observed an effective removal (Choi et al., 2008) while others not (Brown et al., 2006; Le-Minh et al., 2010). Xu et al. (2007) also found that the removal efficiency of SMX in the AS process was around 50%. There was no obvious difference in the effluent SDZ concentrations among the four treatment processes, while the effluent concentrations of SMX in the AS and CW were lower than those in the MP and SP, which was also found for the TMP removal. It was noted that negative removal of SDZ occasionally occurred in MP and SP processes, probably due to influent variation, desorption from biofilm and sediment, and/or limited biological degradation.

As for fluoroquinolones, although the maximum influent concentrations of OFL and NOR were as high as 5411 and 964 ng/L, respectively, most of their effluent concentrations dropped considerably (i.e., 0.2–100 ng/L) (Fig. 1C). Previous studies showed similar removal efficiencies, i.e., 87% (OFL) and 100% (NOR) in some WWTPs of Sweden and Finland, respectively (Lindberg et al., 2005; Vieno et al., 2006). The removal differences of NOR and OFL between conventional and ecological processes were not obvious with the mean effluent concentrations in the same order of magnitude. The increases of CIP concentration were observed occasionally after AS and CW treatments. Plosz et al. (2010) have reported that the efficiency of biological removal of CIP was limited and sorption to sludge was considered as a principal removal pathway for CIP in the AS process (Giger et al., 2003; Golet et al., 2008), so its desorption from sludge and wetland substrate might cause an increased effluent concentration in the AS and CW processes.

The removals of two tetracyclines (i.e., OTC and TCN) in both ecological and conventional processes were obvious. With the influent concentrations of OTC and TCN varying from 0.1 to 326 ng/L, the mean effluent concentrations were both below 15 ng/L (Fig. 1D), which is similar to their removal efficiency in WWTPs. Sponza and Celebi (2012) found that 99% of removal could be achieved through the sequential combination of an anaerobic multi-chamber bed reactor and a completely stirred tank reactor even at an OTC influent concentration as high as 300 mg/L. Xia et al. (2012) tested the removal of antibiotics in a membrane bioreactor at different SRTs and found that the removal efficiencies of CTC and OTC could exceed 75% even at a low SRT of 3 d. The AS and CW generally exhibited lower effluent concentrations than the MP and SP (p < 0.01), with occasional negative removals of CTC (Fig. 1D). Biodegradation and adsorption by biomass are considered as two main removal pathways for tetracyclines (Gaulke et al., 2008; Kim et al., 2005). The low bioactivity and limited biomass in the MP and SP could probably account for the relatively lower removal of CTC. Roberts and Thomas (2006) found that a principal factor governing the removal of micro-pollutants in wastewater treatment processes was the extent of their interactions with solid particles. In this study, the AS and CW exhibited lower effluent concentrations than the MP and SP. The average removal efficiencies of the 19 antibiotics in the ecological and conventional processes listed in Tabel S2 (SM) also indicated the AS and CW outperformed MP and SP processes. The activated sludge in the AS and the substrate in the CW could remove the influent antibiotics through adsorption and/or biodegradation. HRT is another important factor for the removal of micro-pollutants in WWTPs. Batt et al. (2006) pointed out that a longer HRT generally resulted in a higher removal efficiency of micro-pollutants. The HRT of the CW ranged from 24 to 120 h, which was significantly higher than that of the AS (Table 1). A higher HRT in the CW process may offset its lower bioactivity, thus improving the removal of antibiotics.

3.3. Seasonal variation of antibiotics removal in different treatment processes

Among the numerous influential factors, temperature may play an important role in the removal of antibiotics in WWTPs, which was closely related to microbial activity and growth rate. However, previous studies have shown inconsistent results. Castiglioni et al. (2006) reported an enhanced removal of amoxicillin, atenolol, bezafibrate, enalapril and SMX with increasing temperature, whilst Koh et al. (2009) found a decrease in temperature from 18 to 12 °C did not affect the removal of steroid estrogens and nonylphenolic compounds in the AS process.

PCA was conducted using the effluent concentrations of the 12 antibiotics (more frequently detected in summer and winter), CODCr, TSS and NH3–N with 8 groups. As shown in Fig. 2, the first and second principal components accounted for 57.91% and 16.37% of total variance, respectively. The PCA classified the variance of effluent concentrations into three groups depending on treatment process and season. Group I included AS process in both seasons and CW process in summer, which were significantly different with other processes because of their lower effluent concentrations of the target compounds. Group II involved MP and SP processes in summer and CW process in winter, which showed moderate removals of NOR, OTC, TCN and ROX. Group III covered MP and SP processes in winter, which displayed rather limited removals for the most 12 antibiotics. In summary, for all three groups, better removals were achieved in AS process in both seasons and CW process in summer.

To better elucidate the seasonal differences in AS and CW processes, Fig. 3 compared the removal efficiencies of CTC, ROX, OFL and SMX in the AS and CW in summer and winter. The results

![Fig. 2. PCA scores of the AS and CW processes in different seasons: PC1 vs PC2; (circles indicate possible sample groups according to their origin).](image-url)
indicate that higher and more stable removals of the four antibiotics were achieved in summer in both AS and CW processes. With the influent concentrations of ROX varying remarkably (Table 2), its removals in the CW in summer ranged from 60% to 98%, whilst some negative removals were observed in winter, which was classified into group II in PCA analysis. The removal of micropollutants in the CW is a result of complex physico-chemical and microbial interactions including substrate sorption, plant uptake, and biological degradation (Hussain et al., 2011). Apart from the poor biological degradation activity in winter, both desorption of substrate-bound compounds and the potential cleavage of conjugates in winter could contribute to the negative removals (Clara et al., 2005; Lindberg et al., 2005). These results imply that biodegradation and plant uptake might be two main removal pathways in the CW, which exhibited higher removal rates in summer (Hijosa-Valsero et al., 2010). In general, the AS outperformed the CW in terms of antibiotics removal. Apart from the homogeneous mass transfer and diverse microbial community in the AS, the unstable performance of the CW was probably due to a high fluctuation in the influent quantity and quality as well as a low management level.

3.4. Correlation of nitrification and antibiotics removal in AS and CW

Ammonia monooxygenase (AMO) is reported as an important role in the degradation of micro-pollutants, indicating the removal of antibiotics in biological wastewater treatment process does not only depend on the chemical characteristics of the target compounds, but also relies on the nitrifying activity (Han et al., 2009; Suarez et al., 2010). Correlations of effluent ROX and NH$_3$–N concentrations in AS and CW processes in summer were investigated in Fig. 4. Good linear relationships were obtained between effluent NH$_3$–N and ROX concentrations for both AS and CW processes (with $R^2$ values 0.91 and 0.84, respectively). With the deterioration of nitrifying activity (i.e. increase of effluent NH$_3$–N concentrations), elevated ROX concentrations were observed in the AS and CW processes. Negligible biodegradation rates of micro-pollutant under anoxic conditions were also verified by Dorival-García et al. (2013). Nitrifying bacterium usually facilitates the enrichment of various non-specific mono-and dioxygenase enzymes associated with both heterotrophic and autotrophic microorganisms, which may promote the degradation of antibiotics in WWTPs (Golet et al., 2003; Han Tran et al., 2009). Based on these facts, the maintenance
of stable nitrifying activity in the AS and CW processes is an important prerequisite for the effective removal of antibiotics in conventional and ecological wastewater treatment processes.

3.5. Impact of WWTF effluent on a receiving river

The impact of the effluent discharged from a typical WWTF, employing the AS (anoxic/oxic) process with a treatment capacity of 2500 m³/d, on the water quality of a local receiving river was further assessed. Fig. 5 shows the variation of the concentrations of six most abundant antibiotics along the receiving river at the upstream (~1 km from the outlet), mixing, and downstream (1 km from the outlet) sections. In the summer season, ERY-H₂O, OFL and CLA were at the same concentration range (ca. 10 ng/L) in the upstream section, then increased rapidly to ca. 100 ng/L in the mixing section, and thereafter dropped considerably in the downstream section. Other three antibiotics (CTC, OCT and ROX) showed a similar trend of variation along the river. It is seen that the discharged effluent had an obvious impact on the river water quality in terms of antibiotics micro-pollution. In winter, higher antibiotic concentrations were observed in the mixing and downstream sections, probably due to the inhibited microbial activity at a low temperature and/or limited dilution effect by river water. In particular, the concentrations of ERY-H₂O, OFL, CLA and ROX reached 224, 207, 251 and 209 ng/L in the mixing section, respectively, which were about two times than those in summer. In the downstream section, the OFL and CLA concentrations in summer and winter exceeded 70.0 ng/L, much higher than those detected in the upstream section.

4. Conclusions

This study investigated the removal of 19 antibiotics in 20 WWTFs throughout a country located in eastern China. The target antibiotics were almost all detected in the influent and effluent samples with detection frequencies of >90%. CLA, OFL, ROX and ERY-H₂O were found to be the dominant antibiotics with maximum concentrations reaching 6524, 5411, 1293 and 957 ng/L, respectively; while the concentrations of TIA, SMR, STZ, SMN, SML and SFX were below 10 ng/L. Although the effluent concentrations of target antibiotics were obviously lower than the influent ones (except CIP), their removals were generally incomplete. The removal efficiency of antibiotics showed a strong dependence on the specific wastewater treatment process. PCA results showed that the AS and CW showed lower effluent concentrations than the MP and SP. In addition, both AS and CW exhibited higher removal efficiencies of target antibiotics in summer than in winter, indicating the important role of biological degradation. Correlations of effluent ROX and NH₃–N concentrations in AS and CW processes in summer were also observed. The incomplete removal and subsequent discharge of residual antibiotics to the aquatic environment highlight the need to optimize the design and operation of WWTFs in rural areas.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.jenvman.2016.04.037.
References


