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Occurrence of sulfonamide antibiotics in sewage treatment plants

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The occurrence of sulfonamide antibiotics (SAs) was investigated in the six sewage treatment plants (STPs) of Beijing, China. Of the 13 objective antibiotics, sulfamethoxazole, sulfapyridine, sulfamerazine, sulfadiazine and sulfamethizol were detected in the influents with the average concentrations of 1.2 ± 0.45, 0.29 ± 0.25, 0.048 ± 0.012, 0.35 ± 0.52 and 0.33 ± 0.21 μg·L^{-1}, respectively, and those in the effluents were 1.4 ± 0.74, 0.22 ± 0.19, 0.021 ± 0.008, 0.22 ± 0.21and 0.01± 0 μg·L^{-1}, respectively. Sulfamethoxazole was the predominant compound detected, and was found in all wastewater samples with the other two compounds sulfapyridine and sulfamerazine. It should be noted that sulfadiazine was first reported in wastewaters, and the concentration levels of all detected compounds except for sulfamethizol (detected once in the effluent samples) in the influents were observed to be similar to those in the effluents. From the data in this study, it can be found that sulfamethoxazole, sulfapyridine, sulfamerazine and sulfadiazine could be partly removed in anoxic and aerobic treatment unit and *vice versa* in anaerobic process, which led to their low or even negative removal rates in the effluents. The increase on the concentrations of sulfamethoxazole and sulfapyridine in the effluents was found probably due to the biotransformation of their acetylated forms in anaerobic treatment unit. In addition, it was observed that the biodegradation of sulfamethoxazole and sulfapyridine could partly occur during the anoxic and aerobic process, while sulfamerazine was partly eliminated in the anaerobic and anoxic units.

biotransformation, concentration detection, sewage treatment plant, sulfonamide antibiotics

The occurrence and the potential impacts of pharmaceuticals in the environment have attracted increasing attention in recent years[1,2]. Antibiotics are the most widely used medicine, and rank among the most consumed ones. Because their residues in the environment promote the development and spread of bacterial resistance, the occurrence of SAs has caused particular concern of the environmental researchers[3,4]. At present, the contamination of antibiotics has been listed as the most important environmental problems in the first twenty years of the twenty-first century, and many basic researches are progressing quickly[5−14]. China is among the countries that produce and consume large quantities of antibiotics, however, few reports have been found about the environmental occurrence and fates of antibiotics in China.

Unchanged or metabolized human-use antibiotics are excreted via urine to the sewage, and are discharged into the environmental waters after the treatment process of STPs. To understand the environmental effects of residual antibiotics, it is necessary to investigate the occurrences and fates of antibiotics in STPs. Of several classes of antibiotics such as quinolones, macrolides and tetracyclines, SAs are the first antimicrobial drugs and are most widely used to treat bacterial infection and some fungal infections. Many studies have focused on the concentration level of some SAs in the STP effluents of the United States, Canada and some EU countries.

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And generally, the concentrations in effluents from STPs are up to low microgram/liter levels[5−14]. Also, limited papers reported the fate of some SAs in STPs. More than 55% sulfamethoxazole (SMX) [10,11] or its metabolite, N(4)-acetyl-SMX[12,13] can be eliminated in STPs, and their removals were explained by biodegradation. However, Lindberg et al.[14] reported that SMX cannot be effectively removed in STP. Thus, it is still unclear whether the SAs can be effectively removed by the treatment process of STP. In addition, present studies mainly focused on the occurrence of SMX and SPD in environment.

In this study, we determined the contamination levels of the 13 SAs, namely sulfisomidine (SIM), sulfamethoxazole (SMX), sulfamerazine (SMR), sulfathiazole (STZ), sulfadiazine (SDZ), sulfanilamide (SA), sulfamethizol (SMT), sulfadimidine (SDMD), sulfadimethoxine (SDM), sulfapyridine (SPD), sulfachloropyridiazine (SCP), sulfisoxazole (SIA) and sulfameter (SME) in the STPs of Beijing, China. And their removals were evaluated. Finally, the potential removal mechanism of SAs in STPs was discussed by analyzing the wastewater samples of entering and leaving the individual biological treatment units.

1 Experimental

Thirteen SAs (SIM, SMX, SMR, STZ, SDZ, SA, SMT, SDMD, SDM, SPD, SCP, SIA and SME) were all obtained from Sigma-Aldrich (St Louis, Mo), and their structures are shown in Figure 1. Surrogate standard, $^{13}C_6$-sulfamethazine ($^{13}C_6$-SMA, 90%), was obtained from Cambridge Isotope Laboratories (50 Frontage Road, MA, USA). Methanol, dichloromethane, ethyl

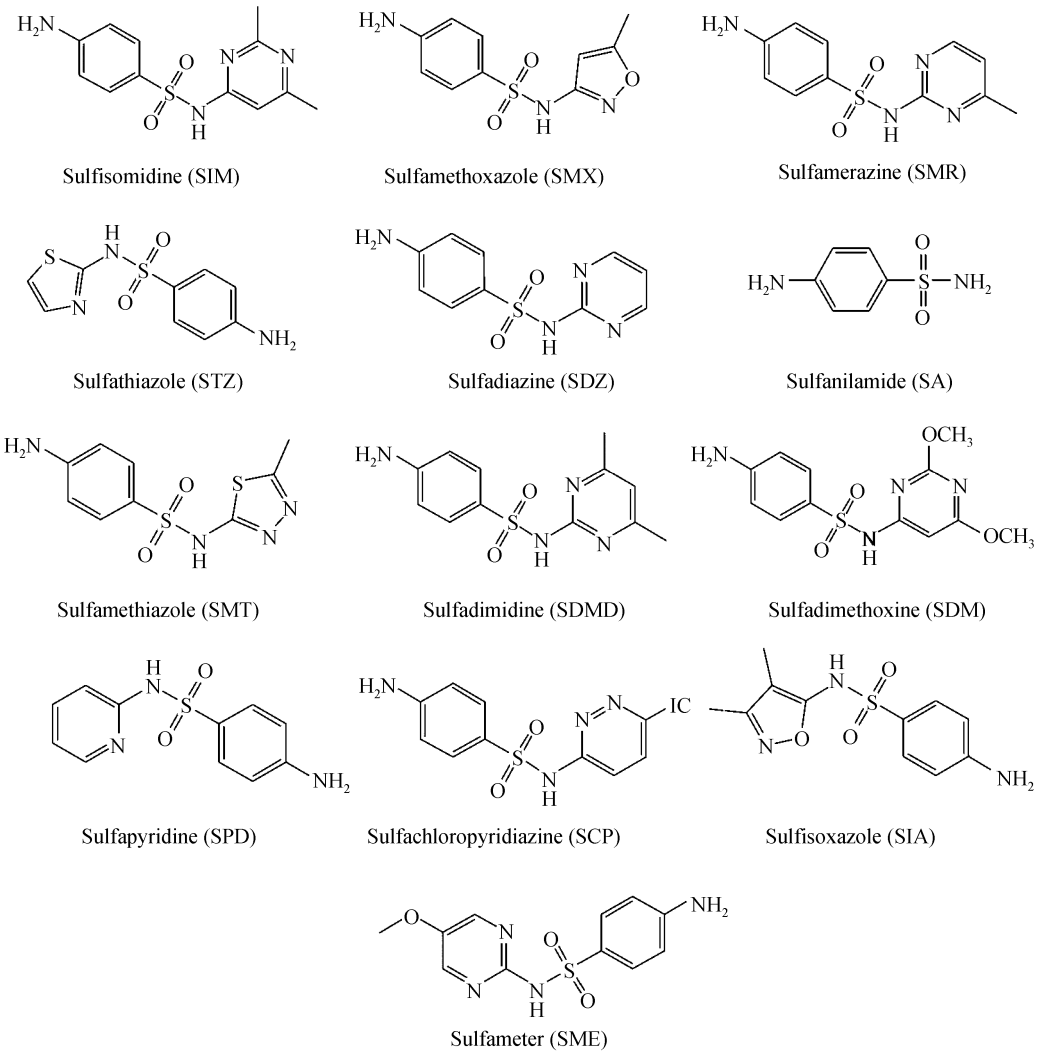

Figure 1 Structures of the objective SAs.

acetate, and hexane, being all of HPLC grade, were purchased from Fisher Chemical Co. (China). Solid-phase extraction cartridges (Oasis HLB, 500 mg/6 mL and Sep-Pak silica, 500 mg/3 mL) were purchased from Waters (Milford, MA, USA). Glass fiber pads (GF/C, 1.2 μm) were obtained from Whatman, Co. (Maidstone, UK). Stock solutions (1000 mg·L^{-1} in methanol) for all standard substances were prepared, and stored at −20℃.

1.1 Sample collection

Samples were taken in one week interval in September 2005, as well as in the last week of November, 2005 at the six main STPs of Beijing, China. The influents and effluents were all sampled in each sampling. The primary effluent and the effluents of the individual biological treatment units were sampled on March 15, 2006. These six STPs are all operated with primary and secondary treatment processes. All of the plants mainly receive domestic water. After collection, samples were immediately shipped back to the laboratory to be extracted.

1.2 Sample preparation

To avoid SPE cartridge plugging, suspended materials were removed by filtration with glass fiber pad (1.2 μm, Whatman GF/C). After filtration, the water samples (200 mL for various types of water except for final effluents (500 mL)) added with Na_2EDTA (0.5 g/L) and 50 ng·L^{-1} of surrogate standard were extracted through an Oasis HLB cartridge, previously conditioned with 6 mL of dichloromethane, 6 mL of methanol and 12 mL of distilled water at a flow rate of 5−10 mL/min. The cartridge was washed with 10 mL of distilled water to remove extra Na_2EDTA, and then was dried under a flow of nitrogen. Dichloromethane/methanol (2∶1, *v/v*; 6 mL) was used to elute the analytes from SPE cartridge. The extracts were dried under a gentle nitrogen stream. The dry residues were redissolved in ethyl acetate, and then 1.8 mL of hexane was added. The mixed solutions were applied to silica normal cartridges, which had been preconditioned with 4 mL of hexane. After the silica cartridges were rinsed with 3 mL of hexane, the analytes were eluted with 3 mL of hexane/ethyl acetate (90∶10, *v/v*) and 3 mL of ethyl acetate. The solution was evaporated to dryness under a gentle stream of nitrogen, and reconstituted with 0.5 mL of methanol for LC-MS analysis.

1.3 LC-MS analysis

Identification and quantification of analytes were carried out using an Alliance 2690 (Waters, USA) liquid chromatography equipped with a platform ZMD single quadrupole mass spectrometer (Micromass, Manchester, UK). The analytical column was a Capcell Pak C18 (250 mm×2.0 mm ID, 5 μm, Shiseido, Japan). Methanol (B) and water containing 20 mmol·L^{-1} CH_3COONH_4 (A) were used as mobile phase. 3% of methanol was held for 4 min, and then linearly increased by 10% in 1 min. It was then increased to 50% in 25 min, later to 65% in 2 min, held for 3 min, and finally it was brought back to 3% and held for 20 min until the next injection. The flow rate was kept at 0.2 mL·min^{-1}, and the injection volume was 2 μL.

The mass spectrometry was operated in electrospray positive ion mode (ESI+). The capillary voltage was set at 3.0 kV. The flow rates of desolvation gas and cone gas were set to 350 and 0 L·h^{-1}, respectively. The source temperature and desolvation gas temperature were held at 120 and 400℃, respectively. The selected ion monitoring (SIM) mode was used for quantitation, and for each compound, the protonated molecular, MH^+, and fragment ions are listed in Table 1.

Table 1 Protonated molecular, fragment ions and cone voltages for SAs

Compound	MH^+ (*m/z*)	Fragment ions (*m/z*)			Cone voltage (V)
$^{13}C_6$-SMA	285	186			30
SA	173	156	132		15
SCP	285	156	207		27
SDM	311	156	108	92	40
SDMD	279	124	156	186	34
SDZ	251	156	108	92	31
SIA	268	156	113	108	32
SIM	279	124	186	156	36
SME	281	156	126	108	34
SMR	265	156	172	110	36
SMT	271	156	108	92	30
SMX	254	156	108	92	45
SPD	250	156	184		28
STZ	256	156	108	92	32

2 Results and discussion

2.1 Quantitation and method performance

To automatically correct the losses of analytes during extraction or sample preparation, and to compensate for variations in instrument response from injection to in-

jection and matrix effects, one surrogate standard was used in this study. Surrogate standards should be the isotope compounds of objective SAs, however, because of their similar structures and the limited commercial availability of stable isotope compounds, only $^{13}C_6$-SMA was used as surrogate standard in this study. Recovery experiments were done by spiking standard solutions to an influent and effluent sample from Gaobeidian STP. Analyte addition was made with the criterion of at least three times the original concentration determined prior to the fortification experiment. The recovery of 13 SAs ranged between 62% and 102% with an RSD lower than 12% (Table 2). Because STP influent was the matrix-richest water sample, the matrix effects of individual biological treatment unit effluents should not be more severe than that of influents. When analyzing the samples of individual biological treatment units, we chose one to do duplicate analysis and fortification experiment. Duplicate analysis showed that the difference of detected concentrations of target SAs was all less than 10%, and the recovery rates were 60%—100%. In addition, no significant difference (<10%) was found by comparing the signal of surrogate standard in individual biological treatment unit effluent. The method detection limit (MDL) was estimated based on the peak-to-peak noise of the baseline near the analyte peak obtained by analyzing field samples and a minimal value of signal-to-noise of 3, and the MDLs of the objective SAs were 3—12 ng·L^{-1} and 1—5 ng·L^{-1} for the influent and effluent samples, respectively (Table 2).

Table 2 Recoveries (%, ±RSD, n=3) amd MDL (ng·L^{-1}) of the STP influent and effluent

Compound	Influent		Effluent	
	recovery ± RSD	MDL	recovery ± RSD	MDL
SA	63 ± 3.6	12	62 ± 7.2	5
SCP	84 ± 5.2	10	90 ± 5.1	4
SDM	83 ± 4.7	2	89 ± 3.4	1
SDMD	86 ± 6.1	6	86 ± 6.5	2
SDZ	86 ± 3.2	10	82 ± 5.2	2
SIA	62 ± 7.5	3	63 ± 6.6	1
SIM	98 ± 2.9	3	98 ± 3.0	1
SME	87 ± 4.3	8	88 ± 4.8	3
SMR	95 ± 6.9	5	91 ± 1.9	1
SMT	89 ± 4.8	5	96 ± 3.7	1
SMX	89 ± 9.1	10	82 ± 5.2	4
SPD	102 ± 9.6	5	100 ± 5.3	1
STZ	95 ± 7.6	8	98 ± 5.0	2

2.2 Occurrence of SAs in STPs

Thirteen objective SAs were analyzed in the influents and effluents collected from six STPs in the five samplings. Figure 2 shows the typical SIR-LC-MS chromatograms obtained from an influent and the corresponding effluent sample, and the analytical results are listed in Table 3. Five compounds, SMX, SPD, SMR, SDZ and SMT, were detected in at least one of the wastewater samples. In particular, SMX, SPD and SMR were found in all wastewater samples. SMX is one of the most frequently prescribed SAs in human medicine, and it is frequently found in the STPs in many countries[5−14]. In this study, average concentrations of SMX in the influents and effluents were 1.2±0.45 μg·L^{-1} and 1.4±0.74 μg·L^{-1}, respectively, which can be comparable to those in previous papers. In these papers, the concentrations in influents were usually at lower microgram/liter levels in the United States[6], Spain[10] and Switzerland[12], with the maximum concentration of 9.0 μg·L^{-1} reported in Germany[8], and concentrations in effluents were between 0.05 and 4.7 μg·L^{-1} [5−14]. SPD has been seldom investigated in environmental samples because it is rarely used as antimicrobial agent itself. However, it still has the possibility to occur in the sewage and environmental waters due to the wide application of sulasalazine, of which 10%—35% can be metabolized to SPD, and 20%—40% to N(4)-acetyl-SPD[15]. It has been reported that SPD was found in STP influents and effluents in Switzerland to be up to 0.15 and 0.35 μg·L^{-1}, respectively[12], and in STP effluents in Canada up to 0.30 μg·L^{-1} [9]. In this study, the detected concentration levels in the influents and effluents were 1.5 and 1.0 μg·L^{-1}, respectively, suggesting the relatively high usage of sulasalazine in China. It is interesting that SMR was first detected in STPs with the average concentration values of 0.048 ± 0.012 and 0.021 ± 0.008 μg·L^{-1} in the influents and effluents, respectively. Following SMX, SPD and SMR, SDZ was also frequently detected in the influents and effluents at the average concentrations of 0.35±0.52 and 0.22±0.21 μg·L^{-1}, respectively, which was higher than that (0.019 μg·L^{-1}) reported from the STP effluents of Canada[9]. In 22 of 30 influents, SMT was detected in influents with maximum concentration up to 0.71 μg·L^{-1}; however, it was only detected in one effluent with relatively low concentration (0.01 μg·L^{-1}), which was similar to that reported in previous study[8].

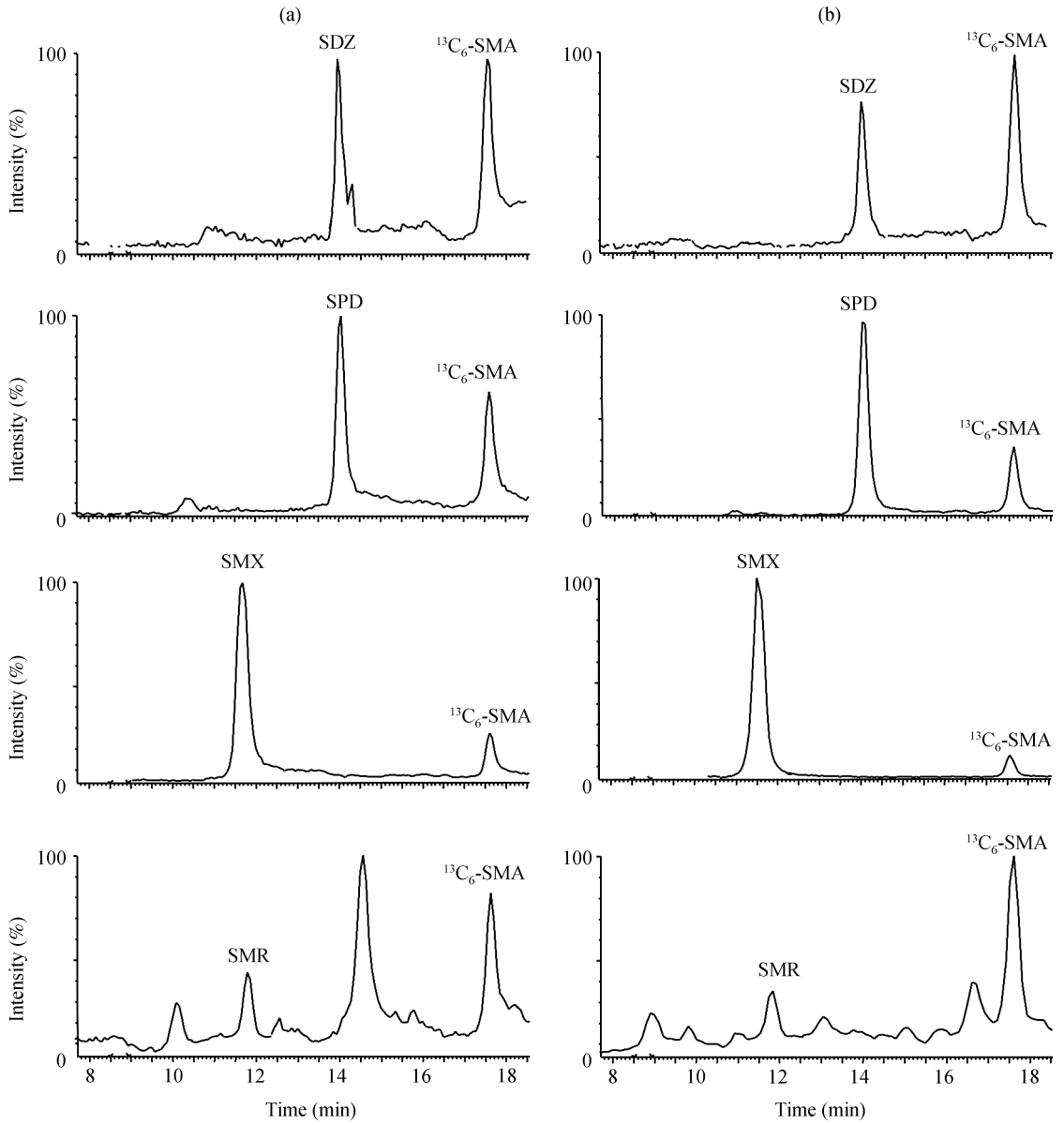

Figure 2 Typical SIR-LC-MS chromatograph of detected SAs in STP influent (a) and effluent (b).

2.3 Removal of SAs in STPs

By comparing the concentrations of SAs in the influents and effluents of each STP, we can find that SMT was almost completely removed, which is similar to the result reported by Yang et al.[6]. However, the removal rates for SMX, SPD, SDZ and SMR were usually relatively low, especially in the case of the former three compounds which were often negatively removed with the ranges of 61% to −463%, 76% to −215% and 100% to −200%, respectively. The removal of SMR ranged from 33% to 75%. Gobel et al.[12,13] reported that sulasalazine and/or its human metabolite N(4)-acetyl-SPD could be transformed to SPD during the biological treatment and N(4)-acetyl-SMX was also found to transform to SMX, indicating that the increased concentrations of SPD and SMX in the effluents of this study were probably due to the transformation from their metabolites. It could be observed that the removals of SMX, SPD and SDZ in STPs experienced high variability, which could be explained by the possible transformation and the simultaneous elimination of themselves during the biological treatment process[12,13]. Therefore, research needs still to be done about the behavior of SAs in STP, especially in the biological treatment process.

In this study, the fates of SAs were investigated dur-

Table 3 Concentration levels ($\mu g \cdot L^{-1}$) of detected SAs in 6 STPs during the five sampling campaigns

STP	Times	SMX		SPD		SMT		SDZ		SMR	
		infuent	effluent	infuent	effluent	infuent	effluent	infuent	effluent	infuent	effluent
Qinghe	1	1.5	2.6	0.39	0.52	0.15	n.d.	0.44	0.54	0.059	0.015
	2	1.4	1.4	0.32	0.22	n.d.	n.d.	0.24	0.12	0.054	0.017
	3	0.83	0.89	0.20	0.15	0.30	n.d.	0.23	0.15	0.037	0.012
	4	0.93	1.0	0.21	0.16	n.d.	n.d.	0.19	0.17	0.043	0.02
	5	1.4	1.7	0.40	0.28	0.26	0.01	0.31	0.25	0.056	0.032
Fangzhuang	1	1.3	1.3	0.23	0.15	0.36	n.d.	0.23	0.06	0.062	0.031
	2	1.2	0.81	0.23	0.13	0.59	n.d.	0.38	0.08	0.043	0.019
	3	0.95	0.62	0.15	0.06	0.71	n.d.	0.16	n.d.	0.034	0.016
	4	1.2	1.3	0.25	0.06	0.57	n.d.	0.18	n.d.	0.041	0.023
	5	1.4	0.60	0.49	0.12	0.64	n.d.	0.34	0.08	0.058	0.028
Jiuxianqiao	1	1.1	0.93	0.17	0.12	n.d.	n.d.	0.08	0.06	0.043	0.013
	2	1.0	1.1	0.19	0.15	0.66	n.d.	0.50	n.d.	0.038	0.014
	3	0.72	0.50	0.11	0.06	0.19	n.d.	n.d.	0.03	0.03	0.015
	4	0.71	0.80	0.15	0.08	n.d.	n.d.	0.17	0.11	0.04	0.018
	5	1.4	2.0	0.25	0.14	0.04	n.d.	0.29	0.12	0.063	0.042
Beixiaohe	1	1.6	1.2	0.36	0.19	0.36	n.d.	0.34	0.09	0.063	0.04
	2	1.2	1.2	1.5	1.0	0.56	n.d.	0.23	n.d.	0.038	0.012
	3	0.38	1.5	0.08	0.16	0.22	n.d.	n.d.	n.d.	0.042	0.022
	4	0.94	0.97	0.27	0.18	0.31	n.d.	0.20	0.19	0.029	0.015
	5	1.1	1.6	0.23	0.24	n.d.	n.d.	0.19	0.24	0.054	0.031
Gaobeidian	1	1.2	2.0	0.27	0.13	0.15	n.d.	0.25	n.d.	0.05	0.021
	2	0.88	1.1	0.22	0.20	0.13	n.d.	0.20	0.18	0.032	0.014
	3	0.71	4.0	0.20	0.63	0.24	n.d.	0.19	0.57	0.044	0.019
	4	0.43	0.64	0.14	0.09	n.d.	n.d.	2.9	0.96	0.04	0.015
	5	1.0	1.2	0.24	0.20	n.d.	n.d.	0.16	0.18	0.061	0.03
Lugouqiao	1	1.6	2.3	0.24	0.29	n.d.	n.d.	0.22	0.20	0.062	0.028
	2	1.5	1.8	0.29	0.26	0.14	n.d.	0.33	0.24	0.038	0.013
	3	1.8	1.0	0.16	0.12	0.12	n.d.	n.d.	n.d.	0.054	0.018
	4	2.1	2.3	0.28	0.22	0.14	n.d.	0.27	0.23	0.063	0.02
	5	2.4	0.94	0.36	0.17	n.d.	n.d.	0.25	0.18	0.073	0.03

n.d., Not detected.

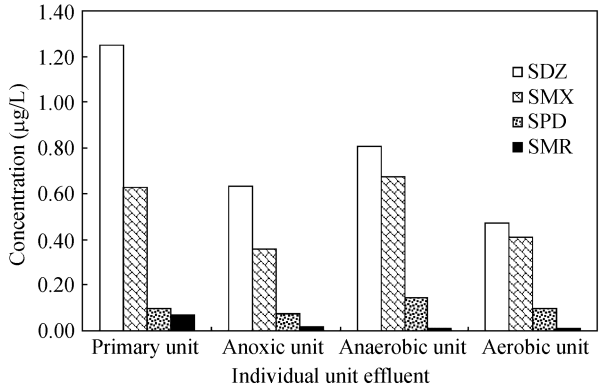

Figure 3 Concentration levels of SAs in the individual unit of the biological treatment process.

ing the biological treatment process by comparing the entering and leaving concentrations of each detected analyte in each individual unit, exemplified by the Gaobeidian STP. From Figure 3, it can be seen that SDZ, SMX, SPD and SMR were all eliminated to some extent during the anoxic process (Removal rates are 49%, 43%, 24% and 73%, respectively); however, in the following anaerobic unit, the concentrations of the former three compounds increased with the negative removal rates of −27%, −89% and −91%, respectively, indicating that the biotransformation from the acetylated forms of SMX and SPD may just occur here. For SDZ, the increase of concentration would be also due to some kind of biotransformation. The different behavior was observed for SMR, which was still effectively removed by 47% in the same biological treatment unit. Then in the sequent aerobic unit, SDZ, SMX and SPD were eliminated by 41%,

40% and 33%, respectively, but no concentration variation of SMR was found in this aerobic unit. Thus, SPD, SMX and SDZ were partly biodegraded during the anoxic and aerobic treatment process, while SMR also can be partly biodegraded in the anoxic and anaerobic units.

3 Conclusions

The concentration levels of SAs in STP in China (Beijing) were in the $\mu g \cdot L^{-1}$ levels, similar to those reported in other countries. The SMR was first detected in STP, generally with concentration level lower than those of other SAs; at the same time, we first reported the occurrence of SDZ in STP, which can be negatively removed in STP. Transformations of SMX and SPD were observed in the anaerobic process, and the biodegradation could occur during the anoxic and aerobic process. The results obtained in this study provided information for wastewater treatment to fulfill the increasing requirements on the quality of the final effluents.

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