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Sorption and biodegradation of sulfonamide antibiotics by activated sludge: Experimental assessment using batch data obtained under aerobic conditions

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ABSTRACT

This study investigated the adsorption, desorption, and biodegradation characteristics of sulfonamide antibiotics in the presence of activated sludge with and without being subjected to NaN_3 biocide. Batch experiments were conducted and the relative contributions of adsorption and biodegradation to the observed removal of sulfonamide antibiotics were determined. Three sulfonamide antibiotics including sulfamethoxazole (SMX), sulfadimethoxine (SDM), and sulfamonomethoxine (SMM), which had been detected in the influent and the activated sludge of wastewater treatment plants (WWTP) in Taiwan, were selected for this study. Experimental results showed that the antibiotic compounds were removed via sorption and biodegradation by the activated sludge, though biodegradation was inhibited in the first 12 h possibly due to competitive inhibition of xenobiotic oxidation by readily biodegradable substances. The affinity of sulfonamides to sterilized sludge was in the order of SDM > SMM > SMX. The sulfonamides existed predominantly as anions at the study pH of 6.8, which resulted in a low level of adsorption to the activated sludge. The adsorption/desorption isotherms were of a linear form, as well described by the Freundlich isotherm with the n value approximating unity. The linear distribution coefficients (K_d) were determined from batch equilibrium experiments with values of 28.6 ± 1.9, 55.7 ± 2.2, and 110.0 ± 4.6 mL/g for SMX, SMM, and SDM, respectively. SMX, SMM, and SDM desorb reversibly from the activated sludge leaving behind on the solids 0.9%, 1.6%, and 5.2% of the original sorption dose of 100 μg/L. The sorbed antibiotics can be introduced into the environment if no further treatments were employed to remove them from the biomass.

1. Introduction

Antibiotics have been recently investigated as a source of emerging environmental contaminants. Researchers are increasingly concerned with prevalent exposure of pathogenic microorganisms to antibiotics that may lead to development of multi-resistant strains and antibiotics-resistant genes in the bacteria and to diminished effectiveness of conventional antibiotics (Boxall et al., 2003; Cabello, 2006; Hernando et al., 2006; Kummerer, 2004; Schwartz et al., 2003). As an important sector in prescription pharmaceuticals, antibiotic substances are employed mainly for protection from infection and diseases

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and as growth promoters in veterinary clinics. Among the major classes of antibiotics, sulfonamide antibiotics were the first antimicrobial drugs utilized worldwide. Sulfonamides are amphoteric that they have both functional groups that can donate and accept a proton. Transfers of protons occur through protonation and deprotonation of the groups depending on the solution pH. In general, sulfonamides possess amine group ($-NH_3^+$) of $pK_{a1} = 2.5$ and sulfonamide group ($-SO_2NH-$) of $pK_{a2} = 5-11$. Hence, a sulfonamide compound can be cationic, neutral, or anionic depending on the solution pH relative to the pK_a values of the compound (Thiele-Bruhn, 2003; Sarmah et al., 2006). Sulfonamide antibiotics excreted by human or animals were found to enter wastewater treatment plants (WWTPs) through the sewage system (Ingerslev and Halling-Sørensen, 2000; Rabolle and Spliid, 2000). Antibiotics were detected in the influents to wastewater treatment plants, and they were not completely removed by the activated sludge processes (Linberg et al., 2006; Xu et al., 2007; Lin et al., 2009; Kasprzyk-Hordern et al., 2009; Loganathan et al., 2009; Zorita et al., 2009; Gros et al., 2010; Ort et al., 2010; Plosz et al., 2010a, 2010b; Zuccato et al., 2010). These compounds were found at concentrations ranging from ng/L to a few μg/L. In WWTPs, the activated sludge may act as a reservoir that interacts with the compounds through sorption and biodegradation (Clara et al., 2004; Xia et al., 2005). Antibiotics including sulfonamides, fluoroquinolone, macrolides, and trimethoprim have been found in the activated sludge and the digested sludge in most WWTPs (Golet et al., 2002; Göbel et al., 2005a, 2005b; Kinney et al., 2006; Yang and Lin, 2009; Nieto et al., 2010). They have been detected at levels of micrograms per kilogram in sewage sludge. To date, little has been quantified for the interactions, biodegradation and sorption, of sulfonamide antibiotics with activated sludge. An aim of this work is to investigate the fate of sulfonamides as they are subjected to the activated sludge process. Experiments were designed and carried out to assess the relative contributions of adsorption, desorption, and biodegradation in the observed removal of sulfonamide antibiotics. Three sulfonamide antibiotics were selected for this investigation that had been previously found in both the domestic wastewater and in the activated sludge (Lin et al., 2009; Yang and Lin, 2009). In this research, sorption kinetics, sorption isotherms, and effects of chemical speciation on the fate of sulfonamide antibiotics are presented. In addition, measured solid–water distribution coefficients (K_d) are compared with values predicted based on octanol–water distribution coefficient (K_{ow}) to provide insight on the interaction of antibiotics with the biomass.

2. Materials and methods

2.1. Chemicals and reagents

Sulfamethoxazole (SMX), sulfadimethoxine (SDM), and sulfamonomethoxine (SMM) were purchased from Sigma–Aldrich (St. Louis, MO, USA). HPLC-grade methanol, formic acid (FA), and sodium azide (NaN_3) were from Merck (Darmstadt, Germany). Milli-Q water (18.2 MΩ) was produced from a Millipore purification system (Billerica, Calif., USA). Individual stock solutions of sulfonamide antibiotics were prepared by dissolving 1 mg of each compound in 1 mL of methanol in amber bottles and stored in the dark at −20 °C until use. Working solutions of 1 mg/L and 0.1 mg/L were prepared by dilution of stock solutions prior to each experimental run.

2.2. Activated sludge

The activated sludge sample was collected from an aerobic sequence batch reactor (SBR) of a wastewater treatment plant in the food manufacturing complex of Uni-President Enterprises Corporation in Taiwan. The wastewaters were generated from processes manufacturing instant noodles, tea beverages, and dairy products. Treatment processes employed in the plant included screen, equalization, dissolved air flotation, acidification, upward-flow anaerobic sludge bed process, aerobic process (via SBR), and final clarification. The flow rate, chemical oxygen demand (COD), pH, and suspended solid (SS) of the wastewater influent were 3500 m^3/d, 3200 mg/L, 5–11, and 660 mg/L, respectively. The pH in the sampled SBR was 6.8, within the range of 6.6–7.1 typically observed for several other plants located at Northern, Central, and Southern Taiwan. After sampling, the activated sludge was cultivated under aerobic conditions in 20-L batch reactors using a synthetic wastewater feed (COD = 300 mg/L) comprising: $C_{12}H_{22}O_{11}$ (sucrose), 268 mg/L; $(NH_4)_2SO_4$, 134 mg/L; $MnSO_4.H_2O$, 2.68 mg/L; $MgSO_4.7H_2O$, 21.4 mg/L; $FeCl_3.6H_2O$, 0.134 mg/L; $CaCl_2$, 3.8 mg/L; KH_2PO_4, 141 mg/L; and K_2HPO_4, 287 mg/L (Yang et al., 2003). Cultivation was carried out at 25 ± 0.5 °C, pH of 6.8–7.0, and dissolved oxygen (DO) at 3 mg/L in the reactor. Aeration was by means of a digital mass flow meter (XFM; AALBORG, USA) and a porous diffuser with agitation at 90 rpm. Preexisting sulfonamides in the sludge sample were analyzed for and not found (i.e. <detection limit of 0.5 μg/kg). The activated sludge thus prepared was used in biodegradation, adsorption, and desorption experiments.

2.3. Biodegradation experiments

In batch biodegradation experiments, a 1.5-L glass beaker containing 1 L of activated sludge suspension was spiked with 100 μg each of the sulfonamide standards. The operating conditions were 25 ± 0.5 °C, pH of 6.8, and with DO of 3 mg/L in the reactor. The aeration and mixing (JLT6; VELT, Italy) were the same as during cultivation of the activated sludge. The synthetic wastewater was introduced daily. Aqueous samples (500 μL) were taken at designated time intervals and analyzed by liquid chromatography tandem mass spectrometry (LC/MS/MS).

2.4. Sorption experiments

Batch experiments were conducted to reveal the sorption of sulfonamide antibiotics with the activated sludge. Adsorption and desorption experiments with a sterilized sludge were conducted separately for SMX, SMM, and SDM. Activated sludge samples were diluted to 2.56 g MLSS/L with DI water and dispensed into 1-L screw top Erlenmeyer flasks. Sodium azide was added (1.0 g/L) to inhibit microbial activity of the sludge and minimize the loss of sulfonamide in the solution due to biodegradation. The mixture with the added biocide was agitated by a magnetic stirrer (SP135935, Barnstead-Thermolyne, USA) at 90 rpm for sorption kinetics and

isotherm measurements. All adsorption and desorption studies were conducted in triplicate at each concentration level at 25 °C in a programmable temperature chamber (GTH225, Giant force, Taiwan). Sulfonamide solutions of varied concentration were prepared for experiments, e.g., 100 μg/L for adsorption experiments, and 10 μg/L, 50 μg/L, 100 μg/L, 200 μg/L, 300 μg/L, and 500 μg/L for isotherm determinations. The needed equilibration time for the sterilized activated sludge and sulfonamides was determined by observed kinetics in the adsorption experiment, in which aqueous samples (500 μL) were taken over 48 h of the experiment and analyzed by LC/MS/MS. The equilibration of 12 h was used in subsequent isotherm experiments. Two blank experiments, one with the reactor system containing sulfonamides without sludge and another containing sludge without sulfonamides, were carried out to ensure that no sorption of sulfonamides occurred on the flask surface and that no contamination of sulfonamides was introduced by the sampled sludge.

2.5. Desorption experiments

After adsorption equilibrium, the sludge suspension was centrifuged at 3200 rpm for 15 min. The supernatant was removed and the residual sludge was re-suspended by addition of 1 L of DI water to initiate the desorption experiment. Test conditions of the desorption experiments were identical to those in the adsorption experiments.

2.6. Analytical methods

Aqueous samples from the sorption and biodegradation experiments were immediately filtered through a polyrinylidene difluoride (PVDF) syringe filter of 0.22 μm in pore size (Millipore, Billerica, Calif., USA) and stored at −20 °C until analysis. The analytical methods adopted for chromatographic separation of analytes and mass spectrometric measurements of the aqueous samples were as previously reported (Lin and Tsai, 2009). An Agilent 1200 module (Agilent, Palo Alto, CA, USA) equipped with a 150 × 4.6 mm ZOBRAX Eclipse XDB-C18 column (5 μm, Agilent, Palo Alto, CA, USA) was employed to separate the analytes. A binary gradient with a flow rate of 1.0 mL/min was used. Mobile phase A contained 0.1% formic acid (v/v) in water. Mobile phase B contained 0.1% formic acid (v/v) in methanol. The sample injection volume was 25 μL and the autosampler was operated at room temperature. The mass spectrometric measurements were taken on a triple quadrupole mass spectrometer (Sciex API 4000; Applied Biosystems, Foster City, CA, USA) equipped with electrospray ionization (ESI). Detection was performed in positive mode. The curtain gas, nebulizer gas, turbo gas at 10, 50, 60 L/h, respectively, and ion spray voltage at 5.0 kV, heated capillary temperature at 400 °C, and collisionally activated dissociation of 7 were used.

2.7. Sorption and desorption isotherm

Three isotherms including the Freundlich, linear, and Langmuir isotherms were applied to describe the sulfonamide adsorption/desorption equilibrium. During sorption experiments, sulfonamide concentrations in the aqueous phase were monitored and were used to determine their partition onto the sludge, q (μg of compound sorbed/g of sludge), according to:

$$q = \frac{(C_0 - C_e)V}{XV} = \frac{C_0 - C_e}{X}$$

Where C_0 is the initial sulfonamide concentration (μg/L), C_e the residual sulfonamide concentration (μg/L) in the aqueous

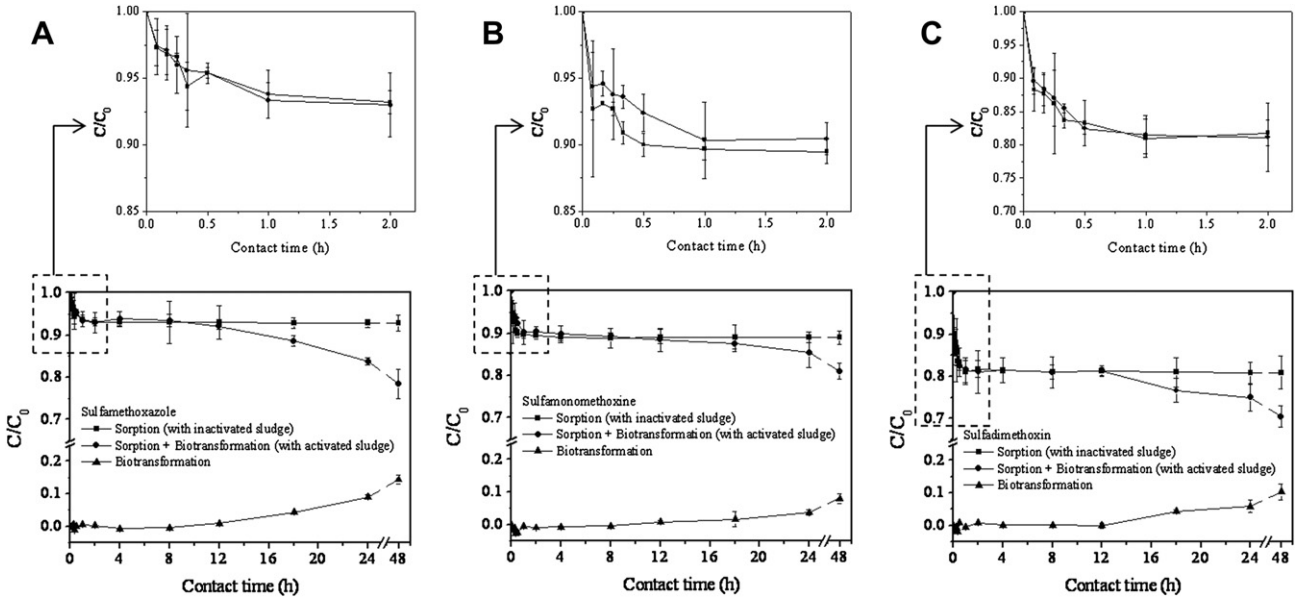
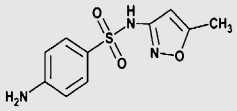
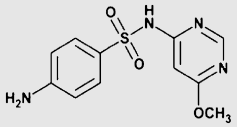
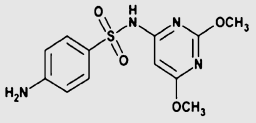


Fig. 1 – Removal of antibiotic compounds (SMX, (A); SMM, (B); SDM, (C)) from aqueous solution by activated sludge via sorption and biodegradation (circle, ●) and by NaN_3-treated activated sludge via sorption only (square, ■), with low-lying curves (triangle, ▲) indicating the effect of biocide, i.e., the presence of biodegradation without the biocide. Conditions: initial sulfonamide concentration at 100 μg/L, sludge concentration at 2.56 g MLSS/L, 25 °C, pH 6.8.

Table 1 – Molecular structure, CAS registry number, and physico-chemical properties of sulfonamide antibiotics.

Name	Abbreviation	Molecular structure	CAS number	Formula	M.W. (g/mol)	Solubility[a] (mg/L)	Log K_{ow}[a]	pK_a[a]
Sulfamethoxazole	SMX		723-46-6	$C_{10}H_{11}N_3O_3S$	253.3	610 (37 °C)	0.89	1.9/5.7
Sulfamonomethoxine	SMM		1220-83-3	$C_{11}H_{12}N_4O_3S$	280.3	4030 (25 °C)	0.70	2.0/6.0
Sulfadimethoxine	SDM		122-11-2	$C_{12}H_{14}N_4O_4S$	310.3	343	1.63	2.1/6.3

a Solubility, Log K_{ow}, and pK_a are from references: (Kan and Petz, 2000; Qiang and Adams, 2004; Sakurai and Ishimitsu, 1980; http://www.syrres.com/what-we-do/databaseforms.aspx?id=386; http://logkow.cisti.nrc.ca/logkow/search.html).

phase at a specific moment, V the suspension volume (L), and X is the concentration (mg/L) of the sludge.

The Freundlich model describes the relationship of sorption density of compounds on solid surface and the equilibrium concentration in liquid phase empirically (Aboul-Kassim and Simoneit, 2001):

$$q_{eq} = K_f C_{eq}^{1/n}$$

Where q_{eq} (μg/g) is the amount of compounds adsorbed onto the sludge at equilibrium, K_f ($\mu g^{1-n} mL^n/g$) the Freundlich adsorption coefficient, C_{eq} (μg/L) the equilibrium concentration in the liquid phase, and $1/n$ the measure of nonlinearity. The Freundlich isotherm can be linearized in the logarithmic form:

$$\log q_{eq} = \log K_f + \frac{1}{n}\log C_{eq}$$

The experimental data were fitted to the Freundlich isotherm and the coefficients (K_f and $1/n$) were determined.

A linear isotherm results when the constant $1/n$ of the Freundlich model approximates unity. The linear isotherm describes well the partition of a compound at low mass loading or when there is no specific bonding between the adsorbate and the adsorbent (Aboul-Kassim and Simoneit, 2001):

$$q_{eq} = K_d C_{eq}$$

Where K_d (mL/g) is the distribution coefficient, and q_{eq} and C_{eq} are as previously defined. Thus, K_d also defines the ratio of concentration in the aqueous phase to that in the solid phase.

The parameter K_{OM} (mL/gVSS) describes the sorption potential of a compound toward organic matter (OM); it reflects the compound's distribution toward the sludge solid phase and is related to K_d by:

$$K_{OM} = \frac{MLSS}{VSS} \times K_d$$

Where MLSS is the mixed liquor suspended solids concentration (mg/L), VSS the volatile suspended solids concentration (mg/L), and K_d the linear distribution coefficient (mL/g) as previously defined.

The Langmuir model describes the monolayer sorption of a compound onto a surface with a finite number of identical sites without surface diffusion, as:

$$q_{eq} = \frac{Q\,b\,C_{eq}}{1 + bC_{eq}}$$

Where Q (μg/g) indicates the binding strength, b (L/mg) the maximum amount of the compound adsorbed per amount of sludge, and q_{eq} and C_{eq} are as previously defined. The Langmuir model can be rewritten and plotted linearly as C_{eq}/q_{eq} vs. C_{eq}:

$$\frac{C_{eq}}{q_{eq}} = \frac{1}{Qb} + \frac{C_{eq}}{Q}$$

2.8. *Speciation of sulfonamide in solution*

The amphoteric sulfonamides with functional groups that readily undergo acid–base equilibrium processes (Sakurai and Ishimitsu, 1980):

$$H_3N^+C_6H_4SO_2NHR \leftrightarrow H_2NC_6H_4SO_2NHR \leftrightarrow H_2NC_6H_5SO_2N^-R$$

At pH 6.8 (>pK_2) used in this study, anionic sulfonamides were the predominant form. Sulfonamide speciation is a function of solution pH relative to its pK_a values. The extent of various amphoteric forms (neutral and charged species) in the solution can be determined by the following equations:

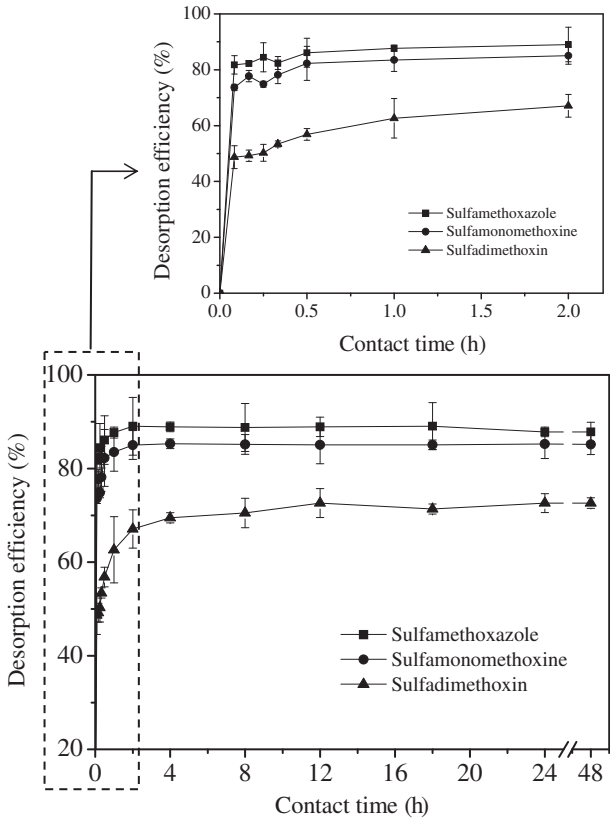


Fig. 2 – Desorption of test sulfonamides from NaN_3-dosed activated sludge over time (sulfonamide initially at 100 μg/L, sludge at 2.56 g/L, 25 °C, pH 6.8).

$$\text{The fraction of neutral}(\phi_n) = \frac{1}{1 + 10^{\text{pH}-\text{pK}_{\text{a, acid}}} + 10^{\text{pK}_{\text{a, base}}-\text{pH}}}$$

$$\text{The fraction of anion } (\phi_-) = \phi_n \, 10^{\text{pH}-\text{pK}_{\text{a, acid}}}$$

$$\text{The fraction of cation } (\phi_+) = \phi_n \, 10^{\text{pK}_{\text{a, base}}-\text{pH}}$$

3. Results and discussion

3.1. *Interaction of sulfonamides with activated sludge*

Batch incubation experiments were conducted to determine the interaction of sulfonamide antibiotics with activated sludge at pH 6.8, 25 °C, activated sludge concentration of 2.56 g MLSS/L, and initial sulfonamide concentration of 100 μg/L. The concentration changes of SMX, SMM, and SDM with time are presented in Fig. 1A, B, and C, respectively. The circles show changes upon contact with activated sludge without the biocide; the squares show changes upon contact with the sterilized sludge (with the biocide); whereas the triangles illustrate the net effect of biodegradation (i.e., square minus circle). For each sulfonamide, the concentration changes in the first 2 h of contact with the sludge (active and sterilized) are highlighted as magnified plots in Fig. 1, which reveal within this initial period a relatively rapid decrease to a stable concentration. For each sulfonamide, the rapid decreases (see enlarged plots of Fig. 1) in both active and sterilized sludges are very similar, which suggest the initial decreases are due to the adsorptive removal of sulfonamides from the aqueous phase by the solid phase; these decreases dampen to steady values over the next 10 h. In the ensuing 36 h (i.e. period 12–48 h), the concentrations of sulfonamides (see main plots of Fig. 1) in contact with the active sludge decrease gradually while the concentrations of sulfonamides in contact with the sterilized sludge remain unchanged. Thus, Fig. 1 appears to have shown an apparent point of transition at 12 h when the sulfonamide concentrations in the active sludge suspension begin to deviate from those of the sterilized suspension and decrease continually. These continual decreases of the antibiotics are attributed to biodegradation by the live activated sludge. Therefore, the removal of sulfonamides from aqueous solution was explained in two stages: the first stage between 0 and 12 h when adsorptive removal occurs predominantly reaching 6.5%, 11%, and 19% removal of SMX, SMM, and SDM, respectively, at the end of 12 h and the second stage between 12 and 48 h when biodegradative removal continues reaching 24.0%, 18.8%, and 29.5% removal of SMX, SMM, and SDM, respectively, at the end of 48 h. Thus, the sorption of sulfonamide antibiotics onto the activated sludge is a vital initial step for the removal of antibiotics. This step was followed by continual removal via biodegradation. The role of biodegradation became significant after 12 h, when the sulfonamides have fully established sorption equilibrium with the activated sludge. The lag phase before the onset of biodegradation of the antibiotics (2–10 h) could be due to readily biodegradable substrates in the reactor that could cause competitive inhibition on xenobiotic oxidation (Plosz et al., 2010b).

3.2. *Interaction of sulfonamides with sterilized activated sludge*

3.2.1. *Adsorption of sulfonamides*

As shown in Fig. 1, the adsorptive removals of (SMX, 7.2%; SMM, 11%; and SDM, 19%) over the initial hours were modest compared to the removals of tetracycline (75–95%) (Kim et al., 2005) and other pharmaceutical compounds (Clara et al., 2004; Urase and Kikuta, 2005). Sulfonamides with low n-octanol–water distribution coefficients (log K_{ow}) dissolve relatively well in water. According to their acid dissociation constants (all pK_1 and $pK_2 < 6.8$ as shown in Table 1), the predominant sulfonamide species would be the anionic form at the study pH of 6.8. Other studied compounds could exist in a neutral form at pH 6.8 that were more amenable to adsorptive removal (Sakurai and Ishimitsu, 1980; Schwarzenbach et al., 2003). Neutral sulfonamide species with log K_{ow} values of 0.70–1.63 can more readily partition onto the activated sludge (Wang et al., 1993). Contrarily, organic compounds in their anionic forms adsorb less due to electrostatic repulsion by the negatively charged surface of the activated sludge (Mikkelsen and Keiding, 2002). The observed order of adsorptive removal, i.e., SDM (19%) > SMM (11%) > SMX (6.5%) consistently follows the order of their predominance in neutral form (i.e., the neutral fractions to total for SDM (24.0%, pK_2 of 6.3) > SMM (13.7%, pK_2 of

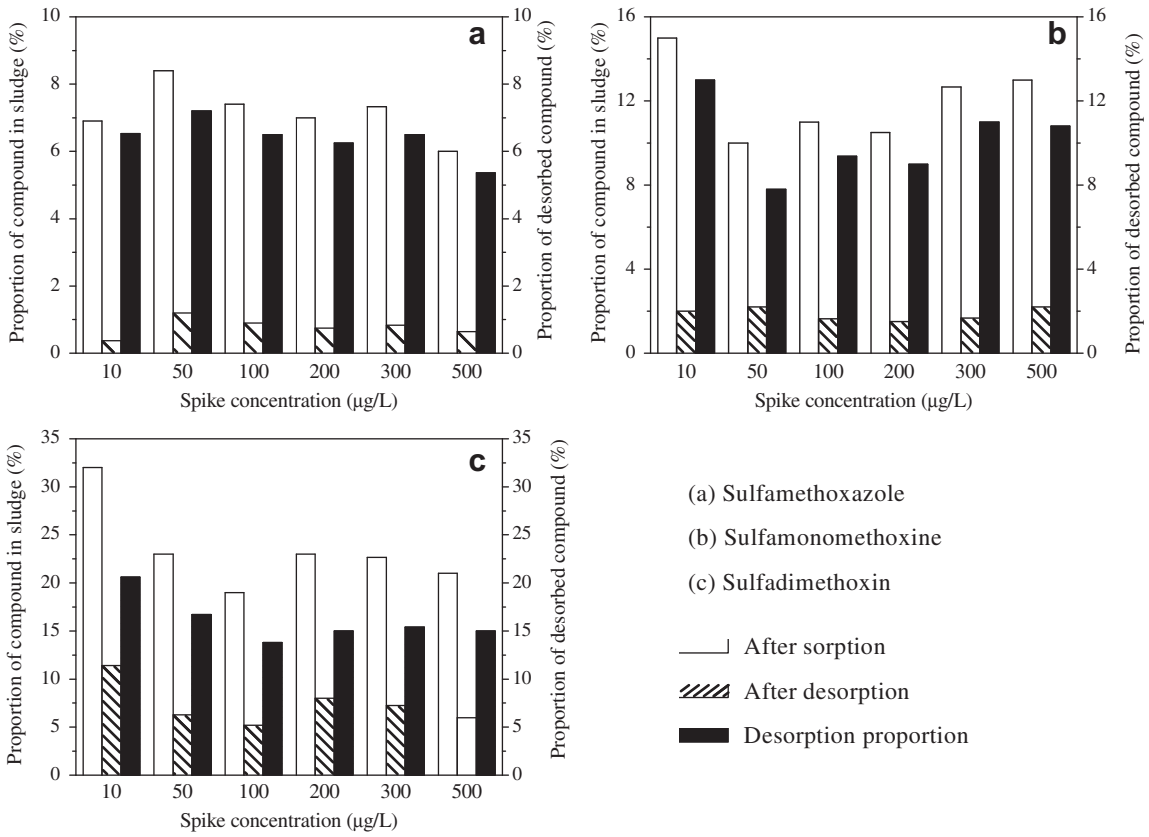


Fig. 3 – Amounts (%) of spike sulfonamides that partitioned to NaN_3-dosed activated sludge after adsorption (blank column) and after subsequent desorption (hatched column) equilibria, along reversibly desorbed amount shown (filled column). Conditions: sulfonamide initially at 10–500 μg/L, sludge at 2.56 g/L, 25 °C, pH 6.8.

6.0) > SMX (9.1%, pK_2 of 5.7)), which is explained by the increasing predominance of the anionic form and the increasing charge repulsion in the same order. It should be noted that a high pH employed above the pK_2 of a diprotic acid (which the studied sulfonamides are) would result in increasing predominance of the anionic form. Thus, the adsorptive removal of sulfonamides is generally lower than other investigated pharmaceutical compounds (Clara et al., 2004; Kim et al., 2005; Urase and Kikuta, 2005).

3.2.2. *Desorption of sulfonamides*

Initial SMX, SMM, and SDM loadings on the sterilized activated sludge were 2.9 μg/g, 4.3 μg/g, and 7.8 μg/g, respectively. Desorption of the compounds over time is shown in Fig. 2. The aqueous concentrations of sulfonamides increased rapidly during the first 0.5 h; they approach steady values in the next 2 h and then held constant for the next 2 day.

Fig. 3 presents the percentages of sulfonamide compounds partitioned onto the sterilized sludge after equilibrating (for 12 h) the sludge (2.56 g/L) with varying quantities of sulfonamides (10–500 μg/L) and again the percentages after re-equilibrating (24 h) the loaded sludge once more with deionized water (desorption). Taking the spike dose of 100 μg/L as an example, the percentages of SMX, SMM, and SDM that adsorbed onto the sludge at equilibrium were 7.4%, 11%, and 19%, respectively. After desorption equilibrium with deionized water, most of the SMX, SMM, and SDM were found to have desorbed to the aqueous phase leaving behind 0.9%, 1.6%, and 5.2% (relative to the original dose of 100 μg/L), respectively, on the solid phase. Thus, the residual sulfonamides still in the sludge were 0.4 μg/g, 0.6 μg/g, and 2.0 μg/g for SMX, SMM, and SDM, respectively. These results suggest that, in the absence of biodegradation, the partition of sulfonamides to the activated sludge is reversible, as reported by other researchers for surfactant and azoprotein compounds (Guellil et al., 2001; Conrad et al., 2006).

3.3. *Adsorption/desorption isotherms*

3.3.1. *Adsorption/desorption equilibrium*

To determine the time required to reach adsorption/desorption equilibrium for each sulfonamide, batch experiments were carried out to bring SMX, SMM, and SDM into contact with sterilized activated sludge. Fig. 1 shows the sorption of sulfonamides with sterilized activated sludge (square symbol) as a function of contact time. Sorption equilibrium was reached within 8 h, once reaching it the aqueous sulfonamide concentrations remained little changed for as long as the study period of 48 h. Thus, equilibration time of 12 h was adequate and used in this study for sorption of SMX, SMM, and SDM to the sludge. The study compounds showed similar desorption kinetics, achieving equilibrium in the first few hours (Fig. 2). To maintain consistency, desorption experiments were performed with a contact time of 24 h. Based on the observed time required for

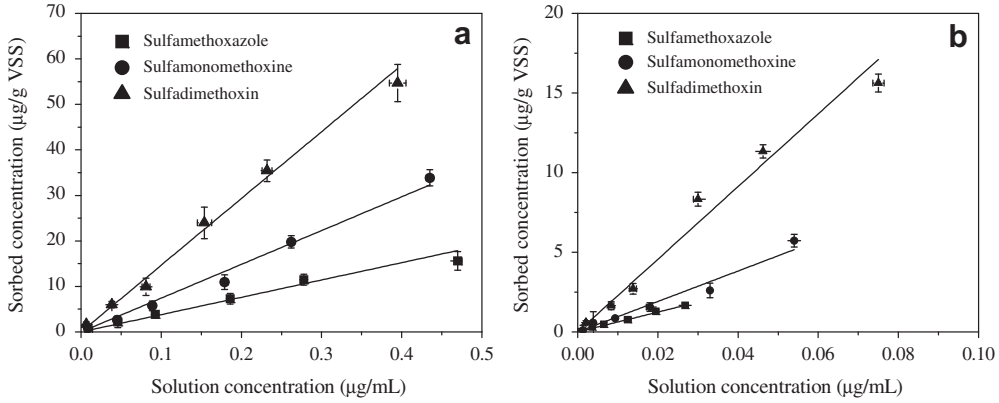


Fig. 4 – (a) Linear adsorption isotherm and (b) linear desorption isotherm of sulfonamides with NaN_3-dosed activated sludge as adsorbent. Conditions: sulfonamide initially at 10–500 μg/L, sludge at 2.56 g/L, 25 °C, pH 6.

sorption equilibrium (8–12 h) and a lag time of 12 h prior to biodegradation, the hydraulic retention time (4–6 h) provided by conventional activated sludge processes in typical WWTPs may not have allowed sufficient time for the antibiotics to be substantially biodegraded.

3.3.2. *Sorption isotherms*

Freundlich and Langmuir adsorption isotherms were used to describe sorption equilibrium for the sulfonamides. While the Langmuir isotherm fitted poorly, the Freundlich isotherm described the data well with the n value close to 1, indicating a linear relationship between the sorption density (μg/g VSS) and the equilibrium concentration (μg/mL). The linear adsorption isotherms for SMX, SMM, and SDM with activated sludge are shown in Fig. 4(a). The fitted coefficients K_f and $1/n$ of the Freundlich model as well as the fitted distribution coefficients (K_d) of the linear model are presented in Table 2. Over the studied concentration range, the Freundlich isotherms for the free sulfonamides were linear with the $1/n$ value approximating unity. A linear isotherm is common where the adsorbate concentration is low relative to the adsorptive capacity of the solid, i.e., adsorption conditions far below saturation. However, extending the concentrations used in this study to higher values in order to observe adsorption saturation characterized by the Langmuir isotherm is not warranted, because the concentrations would be considerably higher than those typically encountered in the natural or engineered aquatic systems.

Linear distribution coefficients for the sulfonamide antibiotics were determined by applying the Freundlich isotherm with $1/n = 1$ to the experimental results, and the fitted K_d values (in lieu of K_f) for SMX, SMM, and SDM are presented in Table 2. The parameter K_d, indicating the affinity of sulfonamide for the activated sludge, was found to be 28.6 ± 1.9, 55.7 ± 2.2, and 110 ± 4.6 mL/g for SMX, SMM, and SDM, respectively. In addition to K_d, the distribution coefficient normalized to the content of organic matter (K_{OM}) is also presented in Table 2. Because the activated sludge comprised 75% of organic matter, the K_{OM} values were expected to be greater than the K_d values. Ternes et al. (2004) determined that the K_d values of selected pharmaceuticals in their study to be between <1 and 500 mL/g. Field experiments were conducted by Göbel et al. (2005b) to investigate the sorption behavior of sulfonamides, such as sulfapyridine and SMX, in the presence of activated sludge. The K_d values were determined to be between 114 and 295 mL/g for sulfonamides, which were higher than the K_d values obtained in the present work. This might be due to other processes in the field study, such as biotic or abiotic degradation, resulting in reduced concentrations of sulfonamides in the field experiments (Sarmah et al., 2006).

Table 2 – Parameters of adsorption and desorption isotherms (mean ± SD) for sulfonamide antibiotics with NaN_3-dosed sludge as adsorbent.

Compounds	Freundlich parameters			Linear parameters		
Sorption	K_f (μg^{1-n} mL^n/g)	$1/n$	R^2	K_d (mL/g)	K_{om} (mL/g VSS)	R^2
Sulfamethoxazole	35.2 ± 1.2	0.93 ± 0.09	0.98	28.6 ± 1.9	38.1 ± 2.6	0.99
Sulfamonomethoxine	79.2 ± 1.1	1.04 ± 0.01	0.98	55.7 ± 2.2	74.2 ± 2.9	0.99
Sulfadimethoxine	133.6 ± 1.1	0.94 ± 0.06	0.99	110.0 ± 4.6	146.6 ± 6.2	0.99
Desorption	K_f (μg^{1-n} mL^n/g)	$1/n$	R^2	K_d (mL/g)	K_{om} (mL/g VSS)	R^2
Sulfamethoxazole	77.4 ± 1.3	1.05 ± 0.06	0.98	46.7 ± 1.0	62.3 ± 1.4	0.99
Sulfamonomethoxine	106.5 ± 1.2	1.01 ± 0.03	0.97	71.7 ± 3.6	95.6 ± 4.7	0.99
Sulfadimethoxine	190.9 ± 1.0	0.94 ± 0.01	0.95	171.1 ± 3.8	228.1 ± 5.1	0.98

3.3.3. *Desorption isotherms*

Desorption isotherms were determined similarly according to the procedure used for adsorption isotherms. The desorption isotherms are presented in Fig. 4(b) with parameters summarized in Table 2. The desorption data agree well with the Freundlich isotherm with 1/*n* approximating unity, indicating linear isotherms. The average 1/*n* values for desorption are 1.05 ± 0.06, 1.01 ± 0.03, and 0.94 ± 0.01 for SMX, SMM, and SDM, respectively. The K_d values of all sulfonamides in adsorption are lower than those in desorption, and are listed in Table 2.

3.4. *Comparison of observed* K_d *with* $K_{d,\ predicted}$ *derived from* K_{ow}

Xia et al. (2005) suggested that for pharmaceutical compounds the K_d values could be predicted based on their K_{ow} values according to the equation: $\log K_{d,\ predicted} = 0.58 \log K_{OW} + 1.14$, which was developed by Dobbs et al. (1989) for chlorinated organic compounds. The $K_{d,\ predicted}$ values for SMX, SMM, and SDM were 45.3, 35.2, and 122, respectively, in comparison to experimentally determined K_d of 28.6, 55.7, and 110, respectively. Apparently, the $K_{d,\ predicted}$ values and K_d values are reasonably close. That adsorption constants, K_d, can be predicted with K_{OW} values is helpful for assessing the role of adsorption of sulfonamides in conventional biological wastewater treatment processes.

4. Conclusions

This study has presented a quantitative assessment of the sorption of sulfonamide antibiotics to activated sludge. Adsorption of sulfonamide antibiotics to activated sludge occurs initially that accounts for the early removal of the antibiotic compounds from the water column. The compounds adsorb onto the activated sludge relatively quickly in the first 2 h. After the first 12 h that allows adsorption equilibrium as well as presumed acclimation of the microbes to the antibiotics, biodegradation of the antibiotic compounds occurs in the ensuing study period of 36 h (i.e., 12–48 h) resulting in at the end residual concentrations of SMX, SMM, and SDM at 76%, 81%, and 70%, respectively. The initial 12-h period prior to the onset of biodegradation could be due to readily biodegradable substrates in the reactor that caused competitive inhibition on xenobiotic oxidation.

At the experimental pH 6.8, the sorption affinity of sulfonamide antibiotics to activated sludge follows the order of SDM > SMM > SMX, which is consistent with the order of abundance in neutral sulfonamide species that adsorb more readily to the sludge. The adsorption/desorption isotherms were well described by the Freundlich model in the linear regime. The K_d values determined from batch equilibrium experiments are in good agreement with model values predicted based on K_{ow} of the sulfonamide compounds. The K_d values were 28.6 ± 1.9, 55.7 ± 2.2, and 110.0 ± 4.6 mL/g for SMX, SMM, and SDM, respectively.

Desorption results indicate that SMX, SMM, and SDM desorb extensively to the aqueous phase leaving behind 0.9%, 1.6%, and 5.2% (of the original dose of 100 μg/L), respectively, on the solid phase. The results affirm that sorption of sulfonamides to the activated sludge is reversible and rapid relative to their biodegradation. Indeed, the contact time required for the activated sludge to degrade sulfonamide antibiotics is longer than the hydraulic retention time of 4–6 h provided by conventional activated sludge processes in domestic wastewater treatment plants. The reversibility in the sorption of the studied sulfonamides implies that they can be released from the activated sludge upon release to the natural aquatic environment. Residual sulfonamides pose a potential risk for the environment if no suitable processes were to eliminate them from the sludge.

REFERENCES

Aboul-Kassim, T.A.T., Simoneit, B.R.T., 2001. Pollutant-solid Phase Interactions: Mechanism, Chemistry and Modeling. Springer-Verlag, Berlin, Heidelberg, pp. 129–132.

Boxall, A.B.A., Kolpin, D.W., Halling-Sorensen, B., Tolls, J., 2003. Peer reviewed: are veterinary medicines causing environmental risks? Environ. Sci. Technol. 37 (15), 286A–294A.

Cabello, F.C., 2006. Heavy use of prophylactic antibiotics in aquaculture: a growing problem for human and animal health and for the environment. Environ. Microbiol. 8 (7), 1137–1144.

Clara, M., Strenn, B., Saracevic, E., Kreuzinger, N., 2004. Adsorption of bisphenol-A, 17β-estradiole and 17α-ethinylestradiole to sewage sludge. Chemosphere 56 (9), 843–851.

Conrad, A., Cadoret, A., Corteel, P., Leroy, P., Block, J.-C., 2006. Adsorption/desorption of linear alkylbenzenesulfonate (LAS) and azoproteins by/from activated sludge flocs. Chemosphere 62 (1), 53–60.

Dobbs, R.A., Wang, L., Govind, R., 1989. Sorption of toxic organic compounds on wastewater solids: correlation with fundamental properties. Environ. Sci. Technol. 23 (9), 1092–1097.

Göbel, A., Thomsen, A., McArdell, C.S., Alder, A.C., Giger, W., Theiss, N., Löffler, D., Ternes, T.A., 2005a. Extraction and determination of sulfonamides, macrolides, and trimethoprim in sewage sludge. J. Chromatogr. A 1085 (2), 179–189.

Göbel, A., Thomsen, A., McArdell, C.S., Joss, A., Giger, W., 2005b. Occurrence and sorption behavior of sulfonamides, macrolides, and trimethoprim in activated sludge treatment. Environ. Sci. Technol. 39 (11), 3981–3989.

Golet, E.M., Strehler, A., Alder, A.C., Giger, W., 2002. Determination of fluoroquinolone antibacterial agents in sewage sludge and sludge-treated soil using accelerated solvent extraction followed by solid-phase extraction. Anal. Chem. 74 (21), 5455–5462.

Gros, M., Petrovic, M., Ginebreda, A., Barcelo, D., 2010. Removal of pharmaceuticals during wastewater treatment and environmental risk assessment using hazard indexes. Environ. Int. 36 (1), 15–26.

Guellil, A., Thomas, F., Block, F.-C., Bersillon, J.-L., Ginestet, P., 2001. Transfer of organic matter between wastewater and activated sludge flocs. Water Res. 35 (1), 143–150.

Hernando, M.D., Mezcua, M., Fernandez-Alba, A.R., Barcel, D., 2006. Environmental risk assessment of pharmaceutical residues in wastewater effluents, surface waters and sediments. Talanta 69 (2), 334–342.

Ingerslev, F., Halling-Sørensen, B., 2000. Biodegradability properties of sulfonamides in activated sludge. Environ. Toxicol. Chem. 19 (10), 2467–2473.
Kan, C.A., Petz, M., 2000. Residues of veterinary drugs in eggs and their distribution between yolk and white. J. Agric. Food Chem. 48 (12), 6397–6403.
Kasprzyk-Hordern, B., Dinsdale, R.M., Guwy, A.J., 2009. The removal of pharmaceuticals, personal care products, endocrine disruptors and illicit drugs during wastewater treatment and its impact on the quality of receiving waters. Water Res. 43 (2), 363–380.
Kim, S., Eichhorn, P., Jensen, J.N., Weber, A.S., Aga, D.S., 2005. Removal of antibiotics in wastewater: effect of hydraulic and solid retention times on the fate of tetracycline in the activated sludge process. Environ. Sci. Technol. 39 (15), 5816–5823.
Kinney, C.A., Furlong, E.T., Zaugg, S.D., Burkhardt, M.R., Werner, S.L., Cahill, J.D., Jorgensen, G.R., 2006. Survey of organic wastewater contaminants in biosolids destined for land application. Environ. Sci. Technol. 40 (23), 7207–7215.
Kummerer, K., 2004. Resistance in the environment. J. Antimicrob. Chemother. 54 (2), 311–320.
Lin, A.Y.C., Tsai, Y.T., 2009. Occurrence of pharmaceuticals in Taiwan's surface waters: impact of waste streams from hospitals and pharmaceutical production facilities. Sci. Total. Environ. 407 (12), 3793–3802.
Lin, A.Y.C., Yu, T.H., Lateef, S.K., 2009. Removal of pharmaceuticals in secondary wastewater treatment processes in Taiwan. J. Hazard. Mater. 167 (1–3), 1163–1169.
Linberg, R.H., Olofsson, U., Rendahl, P., Johansson, M.I., Tysklind, M., Andersson, B.A.V., 2006. Behavior of fluoroquinolones and trimethoprim during mechanical, chemical, and active sludge treatment of sewage water and digestion of sludge. Environ. Sci. Technol. 40 (3), 1042–1048.
Loganathan, B., Philips, M., Mowery, H., Jones-Lepp, T.L., 2009. Contamination profiles and mass loadings of macrolide antibiotics and illicit drugs from a small urban wastewater treatment plant. Chemosphere 40 (1), 70–77.
Mikkelsen, L.H., Keiding, K., 2002. Physico-chemical characteristics of full scale sewage sludges with implications to dewatering. Water Res. 36 (10), 2451–2462.
Nieto, A., Borrull, F., Pocurull, E., Marce, R.M., 2010. Occurrence of pharmaceuticals and hormones in sewage sludge. Environ. Toxicol. Chem. 29 (7), 1484–1489.
Ort, C., Lawrence, M.G., Rieckermann, J., Joss, A., 2010. Sampling for pharmaceuticals and personal care products (PPCPs) and illicit drugs in wastewater systems: are your conclusions valid? A critical review. Environ. Sci. Technol. 44 (16), 6024–6035.
Plosz, B.G., Leknes, H., Liltved, H., Thomas, K.V., 2010a. Diurnal variations in the occurrence and the fate of hormones and antibiotics in activated sludge wastewater treatment in Oslo, Norway. Sci. Total. Environ. 408 (8), 1915–1924.
Plosz, B.G., Leknes, H., Thomas, K.V., 2010b. Impacts of competitive inhibition, parent compound formation and partitioning behavior on the removal of antibiotics in municipal wastewater treatment. Environ. Sci. Technol. 44 (2), 734–742.
Qiang, Z., Adams, C., 2004. Potentiometric determination of acid dissociation constants (pKa) for human and veterinary antibiotics. Water Res. 38 (12), 2874–2890.
Rabolle, M., Spliid, N.H., 2000. Sorption and mobility of metronidazole, olaquindox, oxytetracycline and tylosin in soil. Chemosphere 40 (7), 715–722.
Sakurai, H., Ishimitsu, T., 1980. Microionization constants of sulphonamides. Talanta 27 (3), 293–298.
Sarmah, A.K., Meyer, M.T., Boxall, A.B.A., 2006. A global perspective on the use, sales exposure pathways, occurrence, fate and effects of veterinary antibiotics (VAs) in the environment. Chemosphere 65 (5), 725–759.
Schwartz, T., Kohnen, W., Jansen, B., Obst, U., 2003. Detection of antibiotic-resistant bacteria and their resistance genes in wastewater, surface water, and drinking water biofilms. FEMS Microbiol. Ecol. 43 (3), 323–335.
Schwarzenbach, R.P., Gschwend, P.M., Imboden, D.M., 2003. Environmental Organic Chemistry. John Wiley & Sons Publishers, Hoboken, NJ, pp. 254–256.
Ternes, T.A., Herrmann, N., Bonerz, M., Knacker, T., Siegrist, H., Joss, A., 2004. A rapid method to measure the solid-water distribution coefficient (Kd) for pharmaceuticals and musk fragrances in sewage sludge. Water Res. 38 (19), 4075–4084.
Thiele-Bruhn, S., 2003. Pharmaceutical antibiotic compounds in soils – a review. J. Plant Nutr. Soil Sci. 166 (2), 145–167.
Urase, T., Kikuta, T., 2005. Sorption and biodegradation of 17β-estradiol by acclimated activated sludge under anaerobic conditions. Water Res. 39 (7), 1289–1300.
Wang, L., Govind, R., Dobbs, R.A., 1993. Sorption of toxic organic compounds on wastewater solids: mechanism and modeling. Environ. Sci. Technol. 27 (1), 152–158.
Xia, K., Bhandari, A., Das, K., Pillar, G., 2005. Occurrence and fate of pharmaceuticals and personal care products (PPCPs) in biosolids. J. Environ. Qual. 34 (1), 91–104.
Xu, W., Zhang, G., Li, X., Zou, S., Li, P., Hu, Z., Li, J., 2007. Occurrence and elimination of antibiotics at four sewage treatment plants in the pearl river delta (PRD), South China. Water Res. 41 (19), 4526–4534.
Yang, S.F., Lin, C.F., 2009. Distribution of antibiotics in municipal sewage sludge. In: The 3rd IWA-ASPIRE Conference, Oct. 18–22, Taipei, Taiwan.
Yang, P.Y., Su, R., Kim, S.J., 2003. EMMC process for combined removal of organics, nitrogen and an odor producing substance. J. Environ. Manage. 69 (4), 381–389.
Zorita, S., Martensson, L., Marthiasson, L., 2009. Occurrence and removal of pharmaceuticals in a municipal sewage treatment system in the south of Sweden. Sci. Total. Environ. 407 (8), 2760–2770.
Zuccato, E., Castiglioni, S., Bagnati, R., Melis, M., Fanelli, R., 2010. Source, occurrence and fate of antibiotics in the Italian aquatic environment. J. Hazard. Mater. 179 (1–3), 1042–1048.