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Fate of sulfonamide antibiotics in contact with activated sludge – Sorption and biodegradation

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ABSTRACT

The sorption and biodegradation of three sulfonamide antibiotics, namely sulfamethoxazole (SMX), sulfadimethoxine (SDM), and sulfamonomethoxine (SMM), in an activated sludge system were investigated. Experiments were carried out by contacting 100 μg/L of each sulfonamide compound individually with 2.56 g/L of MLSS at 25 ± 0.5 °C, pH 7.0, and dissolved oxygen of 3.0 ± 0.1 mg/L in a batch reactor over different periods of 2 d and 14 d. All sulfonamides were removed completely over 11–13 d. Sorptive equilibrium was established well within the first few hours, followed by a lag period of 1–3 days before biodegradation was to deplete the antibiotic compounds linearly in the ensuing 10 days. Apparent zeroth-order rate constants were obtained by regression analysis of measured aqueous concentration vs. time profiles to a kinetic model accounting for sorption and biodegradation; they were 8.1, 7.9, and 7.7 μg/L/d for SDM, SMX, and SMM, respectively, at activated sludge concentration of 2.56 g/L. The measured kinetics implied that with typical hydraulic retention time (e.g. 6 h) provided by WWTP the removal of sulfonamide compounds from the wastewater during the activated sludge process would approximate 2 μg/L.

1. Introduction

Antibiotics are known as antibacterials and they are pharmaceuticals used to treat infections caused by bacteria. Among major classes of antibiotics, sulfonamides are used worldwide for human and animals. Significant amounts, 45–75%, of ingested sulfonamides are excreted within 24 h and they enter wastewater treatment plants (WWTPs) through the sewage system (Calamari et al., 2003; Radke et al., 2009). Prior studies found sulfonamide antibiotics in both the influent and effluent of WWTPs at ng/L to μg/L concentrations, which suggested incomplete removal of the compounds by the activated sludge processes (Batt et al., 2007; Brown et al., 2006; Carballa et al., 2007; Gobel et al., 2005a; Gros et al., 2010; Karthikeyan and Meyer, 2006; Kasprzyk-Hordern et al., 2009; Lin and Tsai, 2009; Lin et al., 2009; Miege et al., 2008; Nakada et al., 2007; Plosz et al., 2010a; Senta et al., 2008; Xu et al., 2007; Yang et al., 2005; Zuccato et al., 2010). Of increasing concern are routine exposures of bacteria to antibiotics at large, which may contribute to the emergence of multi-resistant strains and antibiotics-resistant genes in the bacteria that leads to diminishing effectiveness of antibiotics (Hernando et al., 2006; Kummerer, 2004; Le-Minh et al., 2010; Rysz and Alvarez, 2004). Antibiotics are emerging environmental contaminants with growing concern regarding their fate and behavior in activated sludge processes. In WWTPs,

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the activated sludge may act as a reservoir interacting with the compounds through sorption and biodegradation (Clara et al., 2004; Xia et al., 2005). Particularly, sulfonamides were detected at μg/kg levels in activated and digested sludges of most WWTPs (Gobel et al., 2005a, b; Kinney et al., 2006; Nieto et al., 2010; Xu et al., 2007; Yang and Lin, 2009). Though sorption and biodegradation of sulfonamides occur in aerobic activated sludge processes, the relative removals by these mechanisms have not been clearly identified. Using ultrasound, Loffler et al. (2005) extracted pharmaceuticals in sediment and assessed their biodegradability in the sediment. The biodegradation of antibiotics by activated sludge has been determined based on the removal of antibiotics in the aqueous and solid phases in comparison to sterilized sludge (Ingerslev and Halling-Sørensen, 2000; Kim et al., 2005; Li and Zhang, 2010; Wu et al., 2009). In this study, we extracted antibiotics in the activated sludge solids using ultrasound and delineated the sorption and biodegradation mechanisms offered by the biomass. Three frequently occurring sulfonamide antibiotics were selected for this study (Lin et al., 2009; Yang and Lin, 2009). Our objectives were to determine the distribution of sulfonamides in the aqueous and solid phases, the concentration changes of the compounds in both phases over time, and the sorption and biodegradation mechanisms contributed by the activated sludge for removal of the compounds.

2. Experimental section

2.1. Chemicals and reagents

Sulfamethoxazole (SMX), sulfadimethoxine (SDM), and sulfamonomethoxine (SMM) were from Sigma–Aldrich (St. Louis, MO, USA). HPLC-grade methanol, formic acid (FA), acetone, sodium azide (NaN_3), and LiChrolut® EN SPE cartridges were from Merck (Darmstadt, Germany). Phenyl-$^{13}C_6$-Sulfamethazine (1 g/L in methanol) from Cambridge Isotope Laboratories (Andover, MA, USA) was used as a surrogate standard. Milli-Q water (18.2 MΩ) was produced from a Millipore purification system (Billerica, CA, 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 dark at −20 °C until use. Working solutions of 1 mg/L and 0.1 mg/L were prepared by dilution of stock solutions with methanol/water (25/75, v/v) prior to each experimental run. A working surrogate standard solution (0.1 mg/L) was prepared by diluting the standard solution (1 g/L in methanol).

2.2. Preparation of the activated sludge

The activated sludge sample was collected from an aerobic sequencing batch reactor (SBR) of a wastewater treatment plant in a food manufacturing company in Taiwan. The wastewater was generated from processes manufacturing instant noodles, tea beverages, and dairy products. Treatment at the plant included screening, equalization, dissolved air flotation, acidification, upward-flow anaerobic sludge bed processing, aerobic processing (via SBR), and final clarification. The flow rate, chemical oxygen demand (COD), pH, and suspended solids (SS) of the wastewater at the plant were typically 3500 m^3/d, 3200 mg/L, 5–11, and 660 mg/L, respectively. Brought to the laboratory, the activated sludge was cultivated in a 20-L aerobic batch reactor with a synthetic wastewater of composition: $C_{12}H_{22}O_{11}$ (sucrose), 268 mg/L; $(NH_4)_2SO_4$, 134 mg/L; $MnSO_4 \cdot H_2O$, 2.68 mg/L; $MgSO_4 \cdot 7H_2O$, 21.4 mg/L; $FeCl_3 \cdot 6H_2O$, 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 conditions were 25 ± 0.5 °C, pH 6.8–7.0, and dissolved oxygen (DO) of 3.0 ± 0.1 mg/L. Aeration was by a porous diffuser regulated with a digital mass flow meter (XFM; AALBORG, USA); agitation was by a stirrer operated at 90 rpm. The sludge sample was analyzed for pre-existing sulfonamides and none of the study sulfonamides were detected (i.e. < detection limit of 0.5 μg/kg).

2.3. Sorption and biodegradation experiments

Fig. 1 illustrates the reactor and air supply system. Air was delivered by an air compressor that was controlled by a programmable logic controller (PLC) with a DO meter (DO-80C; HOTEC, Taiwan). The PLC was equipped with two digital inputs from float switches, i.e. low level and high level, which regulated the DO in the reactor between 2.9 and 3.1 mg/L. Synthetic wastewater was introduced daily and controlled by a digital dosing pump (P100; FIRSTEK, Taiwan). For experiments, six 1.5-L glass beakers each containing 1 L of activated sludge suspension were simultaneously spiked with 100 μg of a sulfonamide standard. Sorption and biodegradation experiments were performed for 2 d in one trial and 14 d in another at 25 ± 0.5 °C, pH 7.0, DO of 3.0 ± 0.1 mg/L, and MLSS of 2.56 g/L. The six available batches were fewer than 126 required to incubate concurrently to obtain one measurement per day over 14 d in triplicates (i.e. 14 d × 3 replicates × 3 compounds). Therefore, they were divided into two sets of triplicate batches; they were removed alternately (one set at each sampling day) to analyze the liquid and solid contents for remaining antibiotics and the set was immediately replaced with triplicate batches for a longer incubation period (an increase by two days in each subsequent replacement). The two sets were alternately removed, analyzed, and replaced until a full record of data was collected for the incubation period of 14 d. The procedure was repeated for other sulfonamides in turn. The 2-day trial was done similarly in duplicate only with incubation times of 0.25, 0.5, 1, 2, 24, and 48 h.

2.4. Analysis of sulfonamide antibiotics in the liquid and solid phases

For aqueous content analysis, a slurry sample of about 0.5 mL was taken from the removed batch and filtered through a 0.22-μm polyvinylidene difluoride (PVDF) filter/syringe (Millipore, Billerica, CA, USA) and stored at −20 °C until analysis by liquid chromatography tandem mass spectrometry (LC/MS/MS). For solids content analysis, the entire suspension was centrifuged to collect the solids. The solids portion was freeze-dried, ground in an electric mortar grinder (RM 200; Retsch, U.K.), and sieved (<0.5 mm) to obtain a homogeneous specimen. It

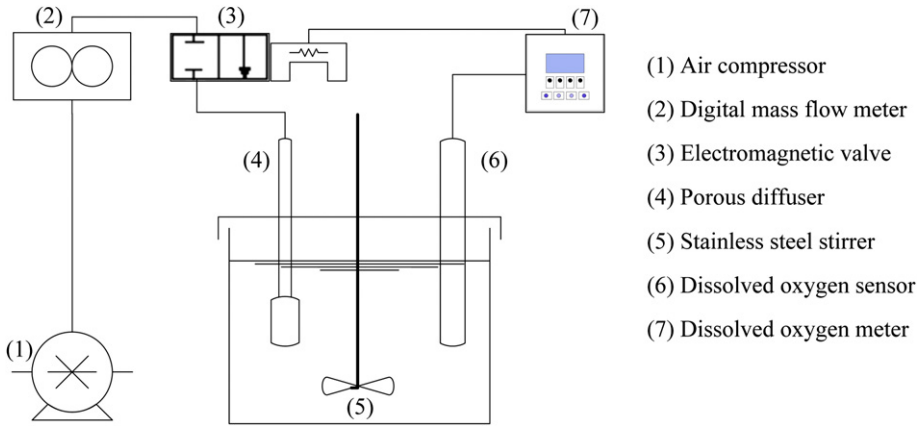

Fig. 1 – Experimental reactor system showing batch reactors with programmable logic controller regulating air supply.

was extracted by solvents four times each for 5 min – the first and second times with 30 mL and 15 mL of methanol, respectively, and the third and fourth times with 15 mL of acetone each. The surrogate standard was spiked into the slurry just before the first extraction. The extraction with solvents was assisted with ultrasonication (VCX-750; Sonics & Materials Inc., USA) as described by others (Yang and Lin, 2009). The ultrasonicated slurry was centrifuged at 3000 rpm for 10 min; the supernatant was removed and then evaporated using a nitrogen stream under the fume hood. The concentrated extract was diluted with Milli-Q water for solid phase extraction (SPE) before LC/MS/MS analysis. LiChrolut® EN cartridges with 200 mg and 3 mL of hydrophilic-hydrophobic balance were employed for SPE. Following sample passage, the cartridge was dried using a nitrogen stream for 1 h. The cartridge was then eluted with 4 mL of methanol and the solvent was evaporated using a nitrogen stream. The residue was reconstituted to 1 mL, dissolved in 25% methanol solution (aqueous), and filtered through a 0.22-μm PVDF filter/syringe before LCMS/MS analysis.

The analytical methods used for chromatographic separation of analytes and mass spectrometric measurements of the aqueous samples were described previously (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. The mass spectrometric measurements were made with a triple-quadrupole mass spectrometer (Sciex API 4000; Applied Biosystems, Foster City, CA, USA) equipped with electrospray ionization (ESI).

Phenyl-$^{13}C_6$-sulfamethazine was used as a surrogate standard to reduce matrix effects and possible losses in the analytical procedures. Sulfonamide recoveries were determined using analyte-to-surrogate area ratios and the results presented as mean ± standard deviation. Table 1 shows the recovery and limit of detection (LOD) of sulfonamide antibiotics in the aqueous phase and in the activated sludge solid phase. The recoveries from both the liquid and solid phases were higher than 95%. The LOD was defined as the minimum concentration in the linear range with a signal-to-noise ratio of 3. The LOD was determined to be 1.0 ng/L for the test sulfonamides in the liquid samples, and 0.1 ng/g (dry weight) for SMM and 0.5 ng/g (dry weight) for both SMX and SDM in the solid activated sludge samples. The quantification method for sulfonamides showed recovery of 98% and LOD of 0.1 ng/g dw.

2.5. *Control experiments*

Hydrolysis and volatilization of the study sulfonamides (with small Henry's Law constants of 1.3×10^{-14} to 6.4×10^{-13} atm-m^3/mole; Thiele-Bruhn, 2003) during experiments were checked and ruled out. Their removal through volatilization was examined in two blank experiments: first with the reactor system containing sulfonamides without sludge and then with the system containing sludge without

Table 1 – Recovery and limit of detection for sulfonamide antibiotics in aqueous phase and solid activated sludge phase.

Compounds	Aqueous phase ($n = 3$)		Activated sludge ($n = 3$)		
	Recovery (%)	LOD (ng/L)	Spiked 10 (ng/g) Recovery[b] (%)	Spiked 100 (ng/g) Recovery[b] (%)	LOD[a] (ng/g d.w.)
Sulfamethoxazole	103.0 ± 7.2	1.0	98.6 ± 6.2	99.8 ± 5.9	0.5
Sulfamonomethoxine	108.7 ± 2.8	1.0	100.1 ± 4.5	97.3 ± 8.0	0.1
Sulfadimethoxine	107.0 ± 2.4	1.0	99.5 ± 3.6	95.4 ± 7.1	0.5

a LOD: Limit of detection; LOD as minimum concentration within the linear range having a signal-to-noise ratio of 3.
b Recovery determined by area ratio of analyte to surrogate standard; presented as mean ± standard deviation.

the sulfonamides. Experiments were also conducted to ensure that no sorption of sulfonamides occurred on the flask surface and no sulfonamides were introduced by the sampled sludge. Thus, elimination of sulfonamides in the liquid phase would be due to sorption and/or biodegradation (Radke et al., 2009; Roger, 1996).

3. Results and discussion

3.1. Removal of sulfonamides by activated sludge in two days

Fig. 2 shows the concentration changes of sulfonamides in the aqueous and solid phases over 48 h of contact in the slurry. Within the first hour, aqueous concentrations of SMX, SMM, and SDM rapidly decreased to mass fractions (relative to the spike amount of 100 μg/L) of 93.4 ± 2.7%, 88.1 ± 0.8%, and 80.5 ± 2.8%, respectively, while their solid-bound counterparts increased to 6.2 ± 0.6%, 11.8 ± 4.2%, and 19 ± 2.8%. After the first 2 h, 92.1 ± 1.9%, 87.9 ± 5.6%, and 80.7 ± 3.4% of SMX, SMM, and SDM, respectively, remained in solution while the rest of the individual compounds partitioned onto the solid phase, measured at 6.8 ± 2.4%, 11.9 ± 5.1%, and 18.7 ± 5.4% of the total added amounts. The combined amounts from separately measured contents in the liquid and solid phases after 2 h accounted for 98.9%, 99.8%, and 99.4% of the spiked amount of SMX, SMM, and SDM, respectively, indicating good mass balance and absence of losses in this period. The initial decrease from the aqueous phase was attributed to sorption of sulfonamide to the solid phase, essentially establishing sorption equilibrium in just 1 h. After the first day, the total sulfonamide concentrations began to show slight decreases and reached at the end of 2 d 92.1 ± 2.7%, 93 ± 5.5%, and 89 ± 4.6% of the initial concentrations of SMX, SMM, and SDM, respectively. After 2 d, the total masses of sulfonamide compounds in the system were no longer conserved, apparently due to the onset of biodegradation. It should be noted that our previous work of contacting sulfonamides with azide-dosed sludge showed complete conservation of added compounds and no sign of biodegradation after 2 d (Yang et al., 2011).

3.2. Removal of sulfonamides by activated sludge over a longer period of two weeks

The fate of sulfonamides in an activated sludge suspension was further examined over a longer period of two weeks. Fig. 3 shows the aqueous and the solid phase concentrations of SMX, SMM, and SDM under the specified contact conditions. While the aqueous portion of the sulfonamides continually decreased over the incubation period, the solid-bound portion increased during the first 2–3 d and then decreased more gradually than the aqueous concentration did. The composite concentration of the two phases (top curve) thus showed an initial lag period in the first 2–3 d, after which it continued to decrease till complete disappearance (>99% removal) at the end of 14 d. For example, SMX in the aqueous phase continually decreased from 81.2 ± 6.4% to 0.22 ± 0.03% from day 1–14. During this period, the sorbed fraction increased from 0% to 19.1 ± 2.5% within 2 d and then decreased to 0.41 ± 0.04% in the ensuing 12 d (i.e. day 2–14). The accumulation of sulfonamides in the solid phase coincided with a lag phase before biodegradation was to occur. The cumulative removal of SMX via biodegradation increased from 1.3 ± 0.2% (day 1) to 99.4 ± 3.8% (day 14). Both other SMM and SDM displayed similar trends under the same incubation conditions. After 14 d of contact, the residual amounts of SMM in the aqueous and solid phases were 0.14 ± 0.04% and 0.23 ± 0.02%, respectively, suggesting 99.6 ± 2.4% removal via biodegradation. Similarly for SDM, the residual amounts in the aqueous and solid phases were 0.28 ± 0.01% and 0.31 ± 0.07%, suggesting 99.4 ± 3.7% removal via biodegradation.

When compared to our previous work with azide-dosed experiments (Yang et al., 2011), Fig. 3 reveals a period of 1–3 d required for the activated sludge to reach maximum sulfonamide loading, indicating a continual addition of sulfonamide during this period. The maximum mass fractions of SMX, SMM, and SDM in the activated sludge solids were 19.1 ± 2.5% (day 2), 15.5 ± 5.7% (day 3), and 22.3 ± 6.2% (day 1), respectively, in comparison to 7.2 ± 1.3%, 11 ± 1.2% and 19 ± 2.9%, respectively, previously reported for sodium azide-treated solids (Yang et al., 2011). Apparently, the sorption affinity of sulfonamides to live activated sludge was stronger than to the sterilized. This was attributed to

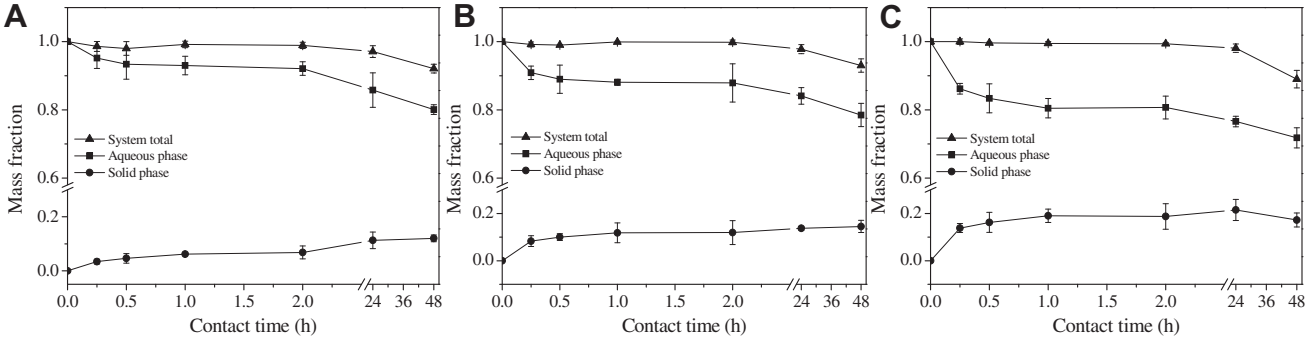

Fig. 2 – Measured mass changes of sulfonamide antibiotics (SMX, A; SMM, B; SDM, C) in the aqueous (square, ■) and solid activated sludge phase (circle, ●) over 2 days of incubation, with sum of both phases shown (triangle, ▲). Mass fraction shows the remaining sulfonamide concentration in the aqueous/solid phase relative to the added amount. Conditions: initial sulfonamide concentration at 100 μg/L, activated sludge concentration at 2.56 g MLSS/L, 25 °C, and pH 7.0.

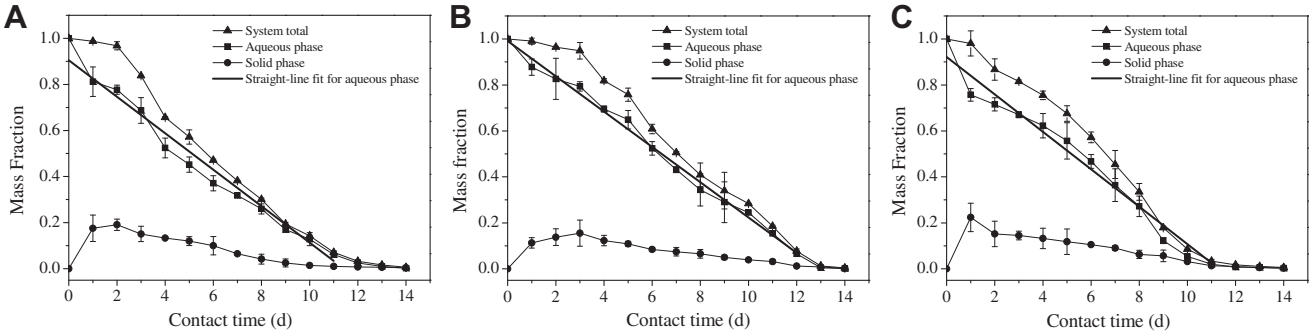

Fig. 3 – Measured mass changes of sulfonamide antibiotics (SMX, A; SMM, B; SDM, C) in the aqueous (square, ■) and solid activated sludge phase (circle, ●) over 2 weeks of incubation, with sum of both phases shown (triangle, ▲). Mass fraction shows the remaining sulfonamide concentration in the aqueous/solid phase relative to the added amount. Conditions: initial sulfonamide concentration of 100 μg/L, activated sludge concentration of 2.56 g MLSS/L, 25 °C, and pH 7.0.

extracellular polymeric substances (EPS) being continually produced and expected to be more abundant in the active, live activated sludge (Ng et al., 2011) that enhanced sorption, as the microbes were supplemented daily with nutrients during the long incubation. EPS are extracellular organics that are tightly bound to the biological flocs, and they can provide increased accommodation of the sulfonamide organics with the flocs (Laspidou and Rittman, 2002; Meng et al., 2007). Thus, the initial lag period in Fig. 3 was likely due to continual sorptive addition of the antibiotic compound to the biomass when biodegradative removal was not operative. This lag period was due to microbial acclimation or suppression of xenobiotic degradation (i.e. slower degradation of the antibiotic compound due to strong competition from available and readily biodegradable nutrients) (Yang et al., 2011).

3.3. *Kinetic model*

The concentration profiles in the aqueous and solid phases, i.e. the continual removal from the aqueous phase and the initial accumulation in the solid phase followed by more gradual removal, are consistent with several mechanisms at work:

- Sorptive removal of sulfonamide from the aqueous phase from start
- Accumulation of sorbed sulfonamide on the solid phase at the initial days during which sorptive increase is greater than biodegradative removal, resulting in the rise of solid-bound sulfonamide
- Onset of biodegradation that removed sulfonamide in the solid phase after acclimation or initial suppression
- Continual biodegradation on the solid phase and ongoing sorptive equilibrium that resulted in continual removal of sulfonamides from the suspension

The mechanism can be modeled mathematically. Specifically, the mass balance for antibiotic compound A due to sorption to and desorption from the biomass is given by:

$$\frac{dC_A}{dt} = -k_1 C_A C_S + k_{-1} C_{AS} \quad (1)$$

The mass balance of biomass-bound antibiotic AS due to sorption, desorption, and biodegradation on the solid is:

$$\frac{dC_{AS}}{dt} = k_1 C_A C_S - k_{-1} C_{AS} - k_2 C_{AS} \quad (2)$$

subjected to concentration conditions:

$$C_{S0} = C_S + C_{AS} \quad (3)$$

$$C_{AS} = 0 \text{ at } t = 0 \quad (4)$$

where: C_A = aqueous concentration of antibiotic compound (μmol/L); C_S = available site concentration in the biomass (μmol/L); $C_{AS} = S_L S_d$ = biomass-bound antibiotic compound concentration (μmol/L); S_L = biomass loading (g/L); S_d = available active site density (μmol/g) of the biomass; $C_{S0} = S_L S_{d0}$ = total active site concentration (μmol/L); S_{d0} = total active site density (μmol/g); k_1 = second-order sorption rate constant (L/μmol/min); k_{-1} = first-order desorption rate constant (1/min); k_2 = first-order rate constant of biodegradation (1/min).

The kinetic model depicts the reactive solid-bound sulfonamide undergoing biodegradation while being continually replenished by the aqueous sulfonamide via sorptive equilibrium between the aqueous and solid phases. Since sorptive equilibrium is rapid (within hours as in Fig. 2) relative to the entire period (2 weeks), we propose the pseudo-steady-state hypothesis for the solid-bound reactive substrate, i.e. $dC_{AS}/dt = 0$, and obtain the pseudo-steady-state concentration for C_{AS} as:

$$C_{AS} = \frac{k_1 C_A C_{S0}}{(k_{-1} + k_2) + k_1 C_A} \quad (5)$$

The last equation can be substituted into the first mass balance equation to arrive at:

$$\frac{dC_A}{dt} = \frac{-k_1 k_2 C_A C_{S0}}{(k_{-1} + k_2) + k_1 C_A} \quad (6)$$

Two limiting cases may manifest:

i) When $k_1C_A >> k_{-1} + k_2$, resulting in a pseudo-zeroth-order rate expression:

$$\frac{dC_A}{dt} = -k_2C_{S0} = -k_{p0} \quad (7)$$

ii) When $k_1C_A << k_{-1} + k_2$, resulting in a pseudo-first-order rate expression:

$$\frac{dC_A}{dt} = \frac{-k_1k_2C_AC_{S0}}{k_{-1} + k_2} = -k_{p1}C_A \quad (8)$$

where k_{p0} (mol/L/d) and k_{p1} (1/d) are pseudo-zeroth-order and pseudo-first-order rate constants, respectively. It should be noted that the pseudo-steady-state condition assumed for the biomass-bound antibiotic compounds (AS) does not impose that the intermediate remains constant over the entire reaction period but it does suggest that its rate of change is much slower than that of the parent compound in the aqueous phase. This is essentially complied for day 3 onward, as evident in the middle and low curves of Fig. 3. Further, the model assumes full speed of biodegradation once the sulfonamide is on the biomass. This assumption has not been built into the model and thus the model would not account for the initial lag period due to acclimation or competitive suppression. Thus, one must temper the evaluation of kinetic concentration profiles with considerations that during the first few days sorptive equilibrium is being established and that acclimation or competitive inhibition of biodegradation may be occurring.

The aqueous concentration of each sulfonamide disappearing over 14 d by contact with activated sludge was fitted to both the pseudo-zeroth and pseudo-first order expressions derived under the specified limiting conditions. The degradation of sulfonamide compounds conformed to the pseudo-zeroth order kinetics (Regression coefficient r^2: 0.973, SMX; 0.993, SMM; 0.974, SDM) better than to pseudo-first order kinetics (r^2: 0.882, SMX; 0.864, SMM; 0.791, SDM). Fig. 3 shows the pseudo-zeroth order fit (straight line) with rate constants of 8.1 μg/L/d, 7.9 μg/L/d, and 7.7 μg/L/d for SDM, SMX, and SMM, respectively. It should be cautioned that the observed apparent zeroth-order kinetics is valid only for the test concentration condition, i.e. 100 μg/L of sulfonamide, which is subject to change into first-order if the test sulfonamide concentration were significant lower (e.g. low enough for $k_1C_A << k_{-1} + k_2$).

Fig. 4 plots the cumulative removal of sulfonamides via biodegradation alone over the course of incubation. The sigmoidal curve is characteristic of a response over time that develops, accelerates, and then approaches a steady value. The shape of the removal curve agreed well with the biodegradation process in that the initial depressed removal was attributed to the lag phase prior to the onset of fully active biodegradation. The lag phase was due to microbial acclimation to the xenobiotic compounds or due to competitive inhibition on the biodegradation of the xenobiotic compounds by readily biodegradable substrate initially present in the water (Plosz et al., 2010b). Beyond the initial lag period (day 2 or 3), biodegradation occurred at constant rates (day 2 or 3 through day 12) as evidenced by the linear part of the removal curve until complete depletion of the antibiotic compounds (>95% at day 12). Based on the removal vs. time profiles, the biodegradability was very close among the test compounds with this slightly decreasing order: SDM > SMX > SMM. This is reflected in the order of the fitted pseudo-zeroth order rate constants of 8.1 μg/L/d, 7.9 μg/L/d, and 7.7 μg/L/d for SDM, SMX, and SMM, respectively.

The apparent zeroth-order kinetics implies that the removal of sulfonamides from wastewater depends on the rate constants of the individual compounds (7.7–8.1 μg/L/d in this study) where the rate constants ($k_{p0} = k_2C_{S0}$) would depend on available active site concentration that in turn depends on the activated sludge concentration being deployed, i.e. the apparent rate constant being proportional to the employed MLSS. The observed zeroth-order kinetics was further ascertained by two additional incubation experiments – one in which 50 μg/L of sulfonamide (each separately) were incubated for 7 d and another in which 10 μg/L of sulfonamide (each separately) were incubated for 3 d, all at 2.56 g/L MLSS. At the end of the periods, remaining aqueous sulfonamide concentrations were analyzed and none were found. This lends further support to zeroth-order kinetics of the process. Were it first-order with initial rate constant of 8/d, one would expect 57% (i.e. $e^{-0.08(7)}$) or 29 μg/L remaining from 50 μg/L after 7 d and 79% ($e^{-0.08(3)}$) or 7.9 μg/L remaining from 10 μg/L after 3 d. Note that an observed rate of 8 μg/L/d at an initial concentration of 100 μg/L would translate to a first-order rate

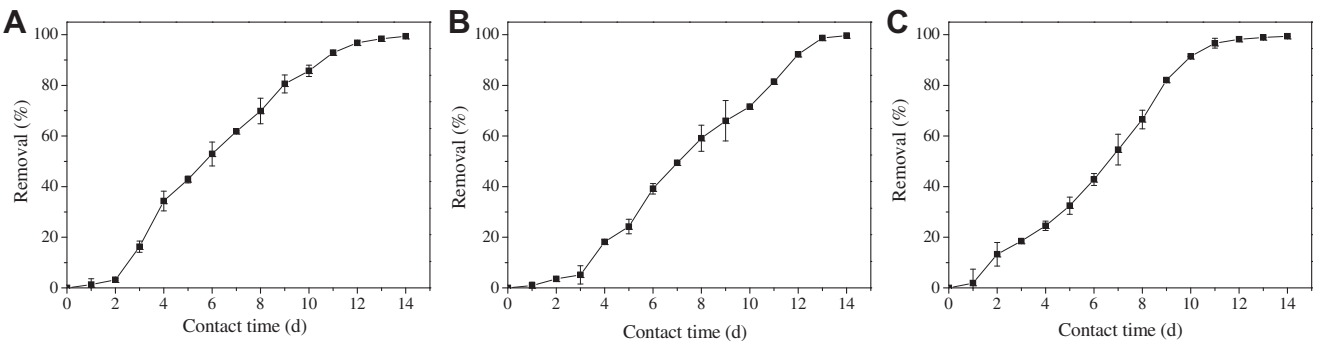

Fig. 4 – Cumulative biodegradative removal of sulfonamides (SMX, A; SMM, B; SDM, C) from the activated sludge system during incubation (net effect of biodegradation as determined by: spike amount minus aqueous phase amount minus solid phase amount). Conditions: initial sulfonamide of 100 μg/L, activated sludge concentration (MLSS) of 2.56 g/L, 25 °C, pH 7.0.

constant of 0.08/d. The complete disappearance in both cases would more plausibly argue for a constant zeroth-order rate constant of 8 μg/L/d, which would have depleted the compounds completely within the periods of both cases as observed.

For the studied MLSS of 2.56 g/L that is common in WWTP with a hydraulic retention time of 6 h, the zeroth-order process would remove only a portion (e.g. 2 μg/L) of the sulfonamide from the wastewater, which would not be complete for a wastewater with higher influent concentration.

4. Conclusions

By dosing sulfonamides SMX, SMM, and SDM into the aerated activated sludge suspension and delineating the changes of the antibiotic amounts in the aqueous and biomass over the incubation trials of 2 and 14 d, we found sorptive equilibrium between the compounds and the solids being established within the first 2 h followed by an onset of biodegradation that continued until complete removal of the compounds in the system at the end of 14 d. During incubation, the aqueous concentrations of the antibiotics showed much retarded rates of removal in the initial days (1–3 d) and afterward accelerated to apparently linear rates of removal at 7.9 (SMX), 7.7 (SMM), and 8.1 (SDM) μg/L/d until complete removal at the end of 12–14 d; meanwhile, the amounts on the solids accumulated to their maxima in the first days (again 1–3 d) and afterward decreased at much more gradual rates than their aqueous counterparts until complete removal at the end of 12–14 d. These concentration behaviors resulted in cumulative removals of the antibiotics from the activated sludge in the sigmoidal form over time. The observed apparent zeroth-order rate constant predicts removal of sulfonamide compounds at 8 μg/L/d by the acclimated activated sludge of 2.5 g/L, though expectably higher proportional to higher MLSS encountered. This implies removal of 2 μg/L within the hydraulic retention time of 6 h operated at many WWTP, and the removal would not be complete if the influent sulfonamide concentrations were higher.

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