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BIODEGRADABILITY PROPERTIES OF SULFONAMIDES IN ACTIVATED SLUDGE

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Abstract—Twelve different sulfonamides were selected for a biodegradation study using a respirometric screening test and an activated sludge simulation test. A simple bacterial growth inhibition test was applied to show that the sulfonamides did not affect the bacteria at the concentration levels used. None of the compounds were degraded in the screening test, leading to the conclusion that sulfonamides cannot be classified as readily biodegradable. In the simulation test, primary degradation of mixtures of four compounds at concentration levels of 250 to 500 μg/L were tested and analyzed using high-performance liquid chromatography. Biodegradation occurred after lag phases of 7 to 10 d at 20°C when nonadapted sludge was applied. Test compounds were degraded within a few days. At 6°C, degradation lag phases and degradation rates were three to four times longer. Degradation curves fit well to the logistic growth model, indicating growth of specific degraders in the test system. Sulfonamide adapted bacterial cultures were able to degrade either the same compounds as previously added or four other sulfonamides in a rapid and uniform way ($t_{½}$ from 0.2 to 3 d). This finding shows that if capable of degrading one sulfonamide substance, these bacteria may also degrade many other sulfonamides. In practice, this implies that because the biodegradation rate is found to be identical for several sulfonamides in the sludge, the compounds may be assessed as a group by studying only a few compounds in applications such as environmental fate assessments. The mechanism for inducement of sulfonamide adaptation to the bacteria was not revealed in this study.

Keywords—Drugs Sulfonamides Biodegradation Activated sludge

INTRODUCTION

Recent findings of drug residues in the environment have increased interest in the environmental fate, or biodegradation and distribution, of these compounds [1–3]. Since the number of existing active substances is very high, it seems an overwhelming task to investigate all these compounds to a degree that would make environmental fate assessment feasible. Therefore, it would be advantageous to assess structurally related substances instead of single compounds whenever possible. One example of a large, structurally related group of drugs is the sulfonamides, which are used for numerous medical purposes, such as diuretics, tuberculostatics, antileprotic drugs, and oral hypoglycemic drugs. Furthermore, several other applications result in the formation of sulfonamides. One example is sulfonylurea herbicides such as chlorsulfuron, which is metabolized to sulfonamide [4,5]. As reported recently, phenyl sulfonamides are used for numerous other purposes, such as corrosion inhibition or production of polymers [6]. Sulfanilamide (or 4-aminobenzenesulfonamide), the best-known compound from this group of chemicals, was discovered as an antibacterial agent in the 1930s. Since then, more than 5,000 different sulfonamides were developed and are still widely used [7]. Therefore, the sulfonamides were selected in the current study as an example of a large group of drugs and for the purpose of investigating whether biodegradation of different sulfanilamides varies significantly.

All sulfonamides are N-substituted derivatives of the substance sulfanilamide (Fig. 1). The compounds are odorless and white or slightly colored powders that are characterized by low solubility in water (S = 0.1–5 g/L) and organic solvents (log K_{ow} = −0.5). Depending on pH, the NH_2 substituent is protonated or the R_1SO_2NHR is deprotonated with partition coefficients of pK_{a1} = 2 to 3 and pK_{a2} = 4.9 to 10.4 for the two equilibriums. Therefore, sulfonamides will occur in either neutral form or, if pH ≫ pK_{a2}, as anions under natural conditions. The sulfonamides are structural analogs of *p*-aminobenzoic acid used by the bacteria in the synthesis of folic acid, which is important for the synthesis of DNA. The main mechanism of sulfonamides is to compete with *p*-aminobenzoic and by this inhibit the growth of the bacteria rather than kill them [7]. Sulfonamides are typically excreted via urine 1 to 2 d after administration. In urine, the compounds occur as the parent compound and as a derivative with an acetylated NH_2 group. The ratio between these two fractions varies between the different substances [8–13]. A biodegradation study of N-acetylated sulfadiazine in manure shows that sulfadiazine is reactivated (the acetyl moiety attached during metabolism to the sulfonamide is cleaved by bacteria so that the parent compound again is present) after excretion [14]. Although no evidence exists, it is likely that the sulfonamides after administration end up in the sewer systems and eventually reach the sewage treatment plants (STPs).

As numerous sulfonamides are used, the bacteria in the STPs might be exposed continuously to different sulfonamides at low concentrations. Over a long time, the resulting overall picture could be that the bacteria are exposed more or less permanently to various sulfonamides but only periodically to each single substance. Whether the bacteria are able to degrade a compound depends on its successful adaptation. The question is whether the bacteria are able to degrade a newly introduced sulfonamide when it has been exposed only to other, different sulfonamides. An understanding of the microbial adaptation is needed to answer this question. Wiggins et al. [15] showed that adaptation is controlled by several factors. The most significant aspect is that microbial adaptation is due to preferential growth of specific degraders that are present at such low con-

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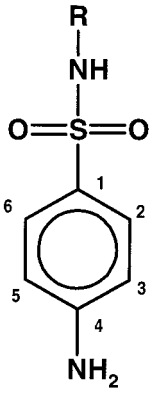


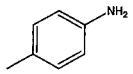
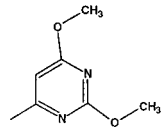
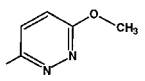
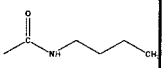
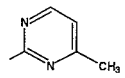
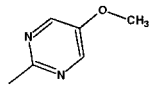
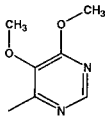
Name	R	Name	R
Sulfacetamide		Sulfadimidine	
Sulfabenzamide		Sulfadimethoxine	
Sulfamethoxy-pyridazin		Sulfanilamide	
Carbutamide		Sulfamerazine	
Sulfadiazine		Sulfameter	
Sulfapyridine		Sulfadoxine	

Fig. 1. Chemical structures of sulfonamides used in the current study.

centrations that biodegradation is initially insignificant when a new chemical is introduced. In other words, the lag phase reflects the time needed for these specific degraders to proliferate to an extent where degradation of the chemical is detectable. In the case with several sulfonamides occurring periodically, the question is whether these degraders can degrade only the specific substances on which they proliferated or whether they also are able to degrade other related substances. In competent bacteria, any sulfonamides could, for example, induce a sulfonamide hydrolase, and hydrolysis of a common structural part of the molecule would then be the crucial first degradative step. In this case, the bacteria will be described as sulfonamide adapted in the following.

The aim of the present study was to answer this question by showing that 12 different sulfonamides were more or less identical with respect to their biodegradability in activated sludge from an STP. A series of tests simulating biodegradation in activated sludge was performed with the purpose of describing the kinetics of the degradation pattern before and after adaptation of the sludge. After biodegradation had occurred in unadapted sludge, the sludge was considered as sulfonamide acclimated, and the same sludge was respiked either with the same drug as used for adaptation or with other sulfonamides than those used for adaptation. As high-performance liquid chromatography (HPLC) analysis was used to monitor biodegradation, only the primary degradation was followed, but it will be referred to as biodegradation in the current study.

MATERIALS AND METHODS

Chemicals

The chemical structures of the sulfonamides used in the current study are shown in Figure 1. All drugs were of analytical grade (purity >99%). The chemicals used were sulfadiazine (Unikem A/S, Copenhagen, Denmark), carbutamide (Aldrich, Milwaukee, WI, USA), sulfameter and sulfabenzamide (Riedel-de Haën, Seelze, Germany), and other sulfonamides (Merck, Darmstadt, Germany). Aniline was used as a reference compound in the tests and was obtained from Merck. Chemicals used for preparation of test media were purchased from Merck. All chemicals used for HPLC analysis were of HPLC grade and supplied by Merck. All solutions were prepared in Mili-Q® water (Millipore, Bedford, MA, USA).

Sampling and preconditioning of activated sludge

All tests were performed with activated sludge from the primary aeration tank at a pilot scale activated sludge sewage treatment plant receiving municipal wastewater (Institute of Environmental Science and Technology, Lyngby, Denmark). Preconditioning of the sludge began within 1 h of collection. Preconditioning (aeration) took 20 to 24 h at room temperature.

Bacterial toxicity test

The International Organization for Standardization (ISO) 15522 [16] was used without any modifications to perform all toxicity tests with sludge bacteria. The toxicity of sulfanilamide, sulfadiazine, and sulfacetamide were tested at five different concentration levels (range 1–400 mg/L) in duplicate test flasks. 3,5-Dichlorophenol was tested as a reference substance.

Exponential growth rate constants, R (per hour), of the bacteria at each concentration were determined as the slope of a log (OD_{530}) versus time calculated by linear regression analysis. Growth inhibition was calculated using Equation 1:

$$I(\%) = \frac{R_0 - R_C}{R_0} \cdot 100 \quad (1)$$

where I is the extent of inhibition (%) and R_0 and R_c are growth rates in controls and solutions with test substance added.

Biodegradation tests

Screening tests. Screening tests were performed according to the guidelines in ISO 9408 [17]. The tests were performed in Oxytop® respirometers for measurement of biochemical oxygen demand (WTW, Weilheim, Germany). The tests were performed on sulfanilamide, sulfadiazine, sulfameter, and sulfabenzamide. Sodium acetate was used as a readily biodegradable reference compound. All compounds were tested in duplicates at concentrations corresponding to theoretical oxygen demands of 40 mg/L.

Activated sludge system. A degradation experiment simulating activated sludge systems, using aerated reactors (height = 50 cm, diameter = 5 cm) were performed as previously described by Nyholm and co-workers [18,19]. Undiluted, preconditioned sludge was added to the reactors without further handling. Sludge concentrations ranged from 3.2 to 3.5 g SS/L. All 12 substances were tested at 20 ± 2°C. Sulfanilamide, sulfacetamide, and sulfadiazine were also tested at 6 ± 1°C. In the first test series, the biodegradation of three mixtures consisting of (1) sulfacetamide, sulfabenzamide, sulfamethoxypyridazine, and carbutamide; (2) sulfadiazine, sulfapyridine, sulfadimidine, and sulfadimethoxine; and (3) sulfanilamide, sulfamerazine, sulfameter, and sulfadoxine were tested in three sets of duplicate reactors. When the added compounds were degraded, the test compounds were respiked twice. A second series of tests was performed at 20 ± 2°C, where two mixtures consisting of (1) sulfadiazine, sulfadimidine, and sulfadimethoxine and (2) carbutamide, sulfadoxine, sulfameter, and sulfanilamide were tested in two separate sets of duplicate reactors. One set was sulfonamide adapted with mixture 1, and after degradation mixture 2 was added. In the second set, the opposite took place: The reactor was adapted with mixture 2 and respiked with mixture 1 after mixture 2 was degraded. In all tests, the compounds were added in concentrations ranging from 250 to 1,000 μg/L. Experiments with 250 to 500 μg/L of aniline were performed as controls in parallel to all degradation tests with sulfonamides. Biodegradation rate calculations on data from the first spike of the test compounds was based on the logistic degradation model (Eqn. 2) as presented by, for example, Simkins and Alexander [20]:

$$S = \frac{S_0 + X_0}{1 + (X_0/S_0)\exp(k \cdot t \cdot (S_0 + X_0))} \quad (2)$$

where S and S_0 are concentrations of substrate (μg/L) at the time t and at the start of the experiment, respectively. The initial amount of substrate degraders, X_0, has the units of μg/L, which reflects that X_0 corresponds to the amount of substrate needed to develop the specific degraders. The rate constant, k, has the units of L (μg/d).

To correct for differences of initially added drug concentrations between the duplicate measured concentrations, S, in the biodegradation, all reactors were divided by the initial concentration, S_0, and the dimensionless concentrations were calculated as $C = S/S_0$. Using Equation 2 and replacing S and S_0 by C and C_0, the computer software GraphPad Prism, Version 2.01 [21], was used on the duplicate data sets to fit nonlinear regression estimates of C_0, X_0, and k.

On the second and third spike, degradation was assumed to follow simple first-order degradation kinetics (Eqn. 3) because the biomass of specific degraders was already developed, and therefore the data no longer fit the logistic degradation model:

$$\frac{dS}{dt} = -k_1 \cdot t$$
$$\Updownarrow$$
$$S = S_0 \cdot e^{-k_1 \cdot t} \quad (3)$$

where S_0 and S are concentrations of substrate at the beginning or at time t and k_1 is the first-order rate constant. Using this model, half-lives, $t_{½}$ can be calculated as $t_{½} = \ln(2)/k_1$.

Chemical analysis

Prior to all analyses, samples were centrifuged for 10 min (3,500 *g*) and filtered through a 0.45-μm syringe filter (Minisart® 17598, Sartorius AG, Goettingen, Germany). Chemical analysis was made on a high-performance chromatography system (Waters 2690, Milford, MA, USA), equipped with a photodiode detector (Waters 996), a sample cooler, and a column heater. A Spherisorp ODS2 (Phenomenex, Torrance, CA, USA) C-18 chromatographic column was used, 125 × 4.6 mm, particle size 5 μm. The eluent consisted of acetonitril and 16.7 mM glacial acetic acid adjusted to pH = 5 with 4 M NaOH 17.5:82.5. The flow rate was 1 ml/min, and detection was performed at 280 nm. Injection volumes of 50 μl were used, and temperatures of the samples and the column were 4 and 40°C, respectively.

A simple experiment was set up to validate the analytical technique. Three standard solutions consisting of approx. 4 mg/L each of sulfacetamide, sulfabenzamide, sulfamethoxipyridine, and carbutamide were weighed out separately. Each of these was diluted to five concentrations ranging from 100 to 2,000 μg/L. The sorption of the sulfonamides to the sludge was evaluated by diluting 50 ml of each standard solution in either 50 ml of 3 g SS/L of activated sludge or 50 ml of water. After 1 h of thorough mixing, samples were analyzed using the procedure described previously. The relative repeatability and recovery were calculated at each concentration, and on the basis of the linear regression lines, limits of detection and quantification were calculated. All calculations were performed as described by Miller and Miller [22].

RESULTS

Chemical analysis

Analyses of water or activated sludge with added concentrations of sulfacetamide, sulfabenzamide, sulfapyridine, and carbutamide in concentrations from 0.05 to 1.10 mg/L are summarized in Table 1. As indicated, limits of detection for all biodegradation experiments are much lower than the initially added concentrations of sulfonamides. The average recoveries were close to 100%, leading to the important conclusion that the absorption of sulfonamides to activated sludge is negligible. Measured liquid concentrations could thus be considered total concentrations in the test solutions.

Toxicity

Calculated inhibitions of sulfonamides at different concentrations are shown in Figure 2. More than 20% bacterial inhibition was found for sulfadiazine (10 mg/L), whereas for sulfacetamide and sulfanilamide (up to 400 mg/L), very little

Table 1. Data used for validation of the applied high-performance liquid chromatography method for analysis of sulfonamides in activated sludge solutions

Compound	Retention time (min)	Repeatability (activated sludge) % recovery	Average recovery activated sludge	Detection limit (μg/L)	Quantification limit (μg/L)
Sulfacetamide	1.63	±1.4% (n = 15)	106%	19	67
Sulfabenzamide	2.57	±2.2% (n = 15)	99%	33	110
Sulfapyridine	3.03	±2.2% (n = 15)	98%	36	119
Carbutamide	8.37	±3.8% (n = 15)	98%	52	174

or no inhibition of the bacteria was measured, and calculation of effect concentrations (e.g., EC50 values) was not possible. The EC50 values of 3,5-dichlorophenol ranged from 5.8 to 12.2 mg/L, which is in accordance with the guideline [16]. The findings lead to the overall conclusion that the biodegradation experiments (up to 0.5 mg/L test solutions) were not affected by bacterial toxicity using the current toxicity test.

Biodegradation in screening test

In the screening test system, none of the sulfonamides tested were degraded within the test period of 28 d. According to the regulations, it can hereby be concluded that none of the compounds tested may be classified as readily biodegradable [23].

Biodegradation in aerated reactors

All 12 test compounds were degraded after an initial lag phase of 7 to 10 d at 20 ± 2°C. After the lag phase, elimination of the test compounds occurred during 5 to 10 d. Parameters from all the curve fits to the logistic model are listed in Table 2. As observed, a good fit of the initial substrate concentration was obtained, whereas the 95% confidence intervals for k and X_0 reveal that these parameters were determined with greater uncertainties. As the primary added compounds were degraded, readdition of the same test compounds was performed on days 13.9 and 18.0 after original test start, resulting in rapid degradation of the second exposure of test compounds. Half-lives, $t_{½}$, ranged from 0.2 to 4.1 d but with no lag phase (Table 3). On the second consecutive readdition of test compounds on days 17.9 and 25.9, similar degradation rates ($t_{½}$ = 0.2–0.7 d) were obtained. In Figure 3, some examples of degradation curves are given for sulfacetamide, sulfabenzamide, sulfamethoxypyridazine, and carbutamide. Readdition of test compounds to the aerated reactors was also performed with different mixtures of sulfonamides than those initially added. The biomass of specific degraders was thus developed with sulfadiazine, sulfadimidine, and sulfadimethoxine and then used 14 d later to degrade carbutamide, sulfadoxine, sulfameter, and sulfanilamide. Again the degradation occurred rapidly with half-lives, $t_{½}$, in the range of 0.4 to 1.7 d (Table 4). In a similar experiment where adaptation was developed on carbutamide, sulfadoxine, sulfameter, and sulfanilamide, rapid degradation of sulfadiazine, sulfadimidine, and sulfadimethoxine was also obtained ($t_{½}$ = 0.9 d). This leads to the conclusion that a specific sulfonamide structure may be degraded by specific degraders or enzymes (e.g., hydrolase) induced by bacterial adaptation to other sulfonamides.

Not surprisingly, the results from the degradation experiment at 6 ± 1°C (see Fig. 4) showed longer lag phases and slower degradation rates than at 20 ± 2°C. Data fit too poorly to the logistic growth model ($0.83 > r^2 > 0.95$) to enable calculations of confidence intervals for the three parameters.

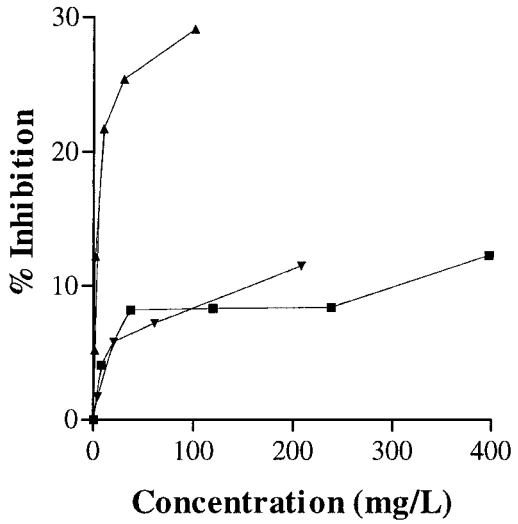


Fig. 2. Inhibition of activated sludge bacteria by sulfacetamide (■), sulfadiazine (▲), and sulfanilamide (▼).

DISCUSSION

The biodegradability of the 12 different sulfonamides in nonadapted reactor systems was found to be relatively homogeneous. Furthermore, uniform degradation curves, but with substantially lower half-lives after adaptation, were obtained when sulfonamides were readded. This was observed when adaptation was performed with both previously unexposed sulfonamides and previously exposed sulfonamides. These findings lead to the important conclusion that degradability of most sulfonamides can be considered as uniform in STPs; therefore, there is no need to make detailed investigations of single drugs for, as an example, an environmental fate assessment of sulfonamides. Taking this conclusion into consideration, the rest of the discussion pertains to degradation of sulfonamides in general rather than specific compounds.

In the current study, a standard method for screening of biodegradability and a test simulating STPs were used as tools for generation of data to assess the biodegradability of sulfonamides. The results from the screening tests showed that the drugs were not mineralized; it is not possible to know whether primary degradation had occurred. Because the toxicity tests showed that the tests were performed at noninhi-

Table 2. Parameters and 95% confidence limits for logistic fits of data from biodegradation reactors at 20 ± 2°C. When two sets of parameters are shown, the first line indicates data from experiments where the same compounds were respiked after degradation was completed, and the second line consists of data from experiments where new compounds were readded after completion of degradation

	Parameters in logistic fit			
Compound	S_0 (mg/L)	X_0 (mg/L)	k[L/(mg/d)]	r^2
Sulfacetamide	0.97 ± 0.04	(1.2 ± 5.2) × 10^{-5}	1.09 ± 0.43	0.93
Sulfabenzamide	0.92 ± 0.03	(1.5 ± 7.9) × 10^{-8}	1.68 ± 0.50	0.94
Sulfamethoxypyridazine	0.90 ± 0.05	(0.8 ± 1.1) × 10^{-2}	0.73 ± 0.21	0.97
	0.32 ± 0.02	(1.2 ± 1.8) × 10^{-2}	1.73 ± 0.79	0.96
Carbutamide	0.83 ± 0.03	(1.0 ± 6.4) × 10^{-8}	1.99 ± 0.69	0.94
	0.36 ± 0.07	(0.60 ± 4.1) × 10^{-4}	2.60 ± 2.07	0.95
Sulfadiazine	1.01 ± 0.03	(0.8 ± 1.6) × 10^{-5}	1.37 ± 0.24	0.99
	0.49 ± 0.03	(0.8 ± 7.2) × 10^{-6}	3.92 ± 2.89	0.97
Sulfapyridine	0.91 ± 0.06	(1.5 ± 2.8) × 10^{-3}	0.88 ± 0.27	0.96
Sulfadimidine	0.92 ± 0.06	(0.5 ± 2.6) × 10^{-5}	1.55 ± 0.64	0.96
	0.45 ± 0.02	(0.2 ± 1.7) × 10^{-5}	3.95 ± 2.89	0.97
Sulfadimethoxine	0.95 ± 0.06	(1.1 ± 2.2) × 10^{-3}	0.81 ± 0.25	0.96
Sulfanilamide	1.10[a]	1.2 × 10^{-11}[a]	2.20[a]	0.97
	0.50 ± 0.04	(1.4 ± 3.2) × 10^{-2}	0.92 ± 0.61	0.95
Sulfamerazine	0.97 ± 0.04	(0.3 ± 1.5) × 10^{-8}	1.93 ± 0.53	0.98
Sulfameter	0.94 ± 0.05	(0.6 ± 1.2) × 10^{-3}	0.92 ± 0.26	0.97
	0.42 ± 0.03	(0.4 ± 3.0) × 10^{-4}	3.2 ± 2.62	0.97
Sulfadoxine	1.00 ± 0.07	(0.3 ± 1.4) × 10^{-5}	1.31 ± 0.54	0.95
	0.31 ± 0.03	(0.8 ± 6.0) × 10^{-5}	3.66 ± 2.78	0.96

[a] No 95% confidence intervals were obtained.

biting concentrations, it is evident that sulfonamides can not be characterized as readily biodegradable. Results from the aerated reactors showed that the sulfonamides were degraded only after an adaptation period. Therefore, it was not surprising that no degradation occurred in those tests using unadapted biomass. It should also be mentioned that the biomass level is much lower in the screening test than in the aerated reactors, and this may also result in this difference.

Bacteria species in STPs might be adapted to sulfonamides, as they are believed to be continuously exposed from different sources. Therefore, as concluded by several authors [23–28], the ready biodegradability screening tests are unsuitable for prediction of biodegradation in the environment. This statement is, of course, important also for drugs and drug residues. Therefore, the many papers (e.g., Richardson and Bowron [29] or Al-Ahmad et al. [30]) assessing the environmental degradation of drugs using tests such as the Organization for Economic Cooperation and Development 301D [31] may not be used to conclude that drugs are persistent in the environment. Likewise, applying unadapted sludge might also, in the case of drugs, overestimate the potential biodegradation.

The selection of the current reactor system used in this study was based on three main arguments. First, it is a standardized and easily implemented method. Second, bacteria from the most relevant environment, STPs, are used. Finally, it does not require complicated analytical techniques. Three main factors are believed to complicate the interpretation of the results. (1) With the HPLC technique used, only the primary degradation of the test compounds is followed, possible metabolites formed during degradation might not be detectable, and complete mineralization cannot be proved. (2) Biodegradation rate data obtained in the current study are based on experiments performed at concentrations much higher than could be expected in the STP. Ternes and co-workers measured up to 1 μg/L of sulfamethoxasole in German sewage water [32]. (3) Most of the biodegradation tests in the current study were performed at a temperature (20 ± 2°C) higher than average temperatures in Danish STPs.

Analysis of the chromatograms did not indicate formation of any metabolites during degradation of the test compounds. However, with the HPLC methods used here, only metabolites with ultraviolet absorbance at λ = 280 nm and with similar

Table 3. Degradation half-lives in adapted biodegradation reactors at 20 ± 2°C where the compounds used for adaptation were respiked twice

	First respike			Second respike		
Compound	$t_{1/2}$ (d)	r^2	n	$t_{1/2}$ (d)	r^2	n
Sulfacetamide	0.6	0.89	8	0.5	0.75	22
Sulfabenzamide	0.4	0.94	8	0.3	0.76	14
Sulfamethoxypyridazine	0.2	0.99	6	0.2	0.98	16
Carbutamide	1.3	0.99	6	0.4	0.78	18
Sulfadiazine	1.6	0.94	20	0.7	0.83	12
Sulfapyridine	1.5	0.91	19	0.6	0.81	12
Sulfadimidine	1.5	0.96	12	0.6	0.81	12
Sulfadimethoxine	4.1	0.46	10	0.4	0.73	10
Sulfanilamide	3.8	0.70	13	0.4	0.67	12
Sulfamerazine	1.7	0.23	12	0.7	0.79	12
Sulfameter	0.4	0.59	11	0.6	0.87	12
Sulfadoxine	0.6	0.47	11	0.7	0.85	12

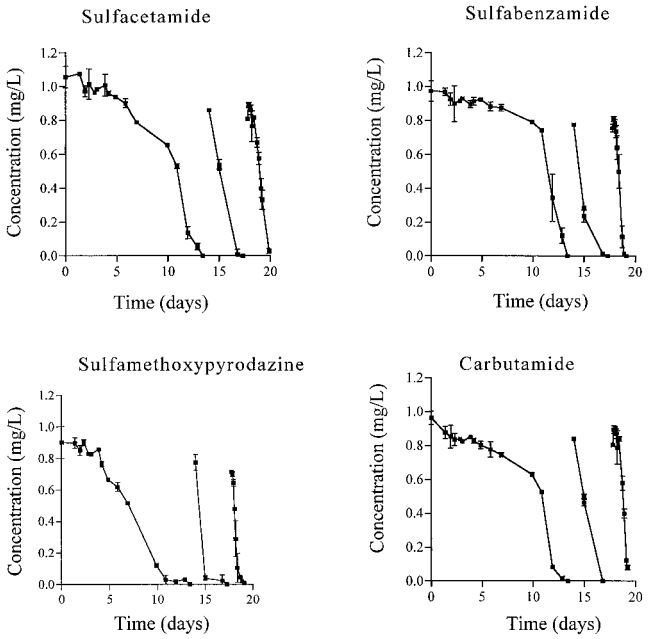


Fig. 3. Biodegradation of sulfacetamide, sulfabenzamide, sulfamethoxypyrodazine, and carbutamide in a reactor experiment at 20 ± 2°C. All compounds were readded twice during the experiment. Error bars indicate standard deviations calculated on duplicate reactors.

or lower lipophilicity than the sulfonamides would have been detected. Therefore, the possible formation of more polar metabolites during degradation of sulfonamides cannot be excluded. Researchers have shown cleavage of the amine–sulfur bond followed by formation of several intermediates during mineralization of sulfonamide in a pure culture of three bacterial species [33,34]. Recent findings by Knepper et al. [6] have shown formation of several metabolites during degradation of sarkosin-*N*-(phenylsulfonyl). Both findings are made in systems with much lower biomass than used in current reactor systems. Even though metabolites are easier mineralized at high than at low bacterial concentrations, we cannot exclude that nondegraded metabolites occurred in the current experiments.

At first glance, the results from the first spikes in reactor experiments seem rather homogeneous. Considering the small chemical differences in the substances and the relative uniformity of the biodegradation curves, it seems unlikely that the range over 10 decades of the substrate-specific parameter X_0 has a microbiological basis. Furthermore, it is seen that at high k values, low X_0 values are obtained, and a correlation

Table 4. Degradation half-lives in adapted biodegradation reactors at 20 ± 2°C where other compounds than used for adaptation were spiked

Sulfonamides used for adaptation	Sulfonamides spiked after adaptation	$t_{1/2}$ (d)	r^2	N
Sulfadiazine Sulfadimidine Sulfadimethoxine	Carbutamide	1.4	0.71	26
	Sulfadoxine	1.4	0.80	28
	Sulfameter	0.4	0.95	28
	Sulfanilamide	1.7	0.78	28
Carbutamide Sulfadoxine Sulfameter Sulfanilamide	Sulfadiazine	0.9	0.85	13
	Sulfadimidine	0.9	0.87	13
	Sulfadimethoxine	0.9	0.82	11

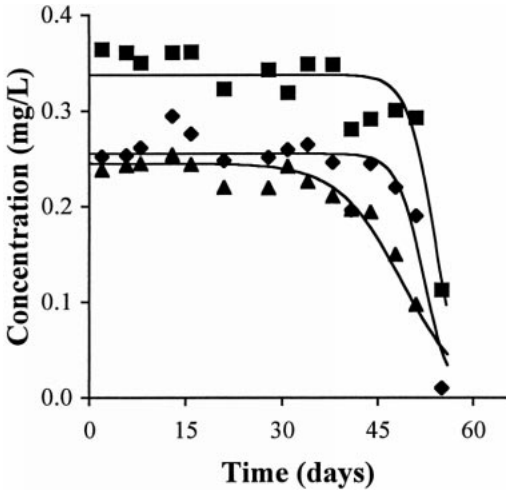


Fig. 4. Biodegradation of sulfanilamide (♦), sulfacetamide (■), and sulfadiazine (▲) at 6 ± 1°C.

between k values and $\ln(X_0)$ reveals a high degree of linearity ($k = 0.29–0.09 \cdot \ln(X_0)$; $R^2 = 0.92$), indicating that k and X_0 are dependent. It is believed that this dependency does not have any microbiological basis but rather is related to statistical uncertainties of the curve fit. Here it should be emphasized that with the results presented, a more significant way to interpret the parameter X_0 is to consider it not as a measure of biomass but rather as a rate constant with little or no biological relevance. However, despite this weakness, the agreement between the measured concentrations and the model fits proves that the observed degradation of the sulfonamides is linked to bacterial growth. This conclusion is strengthened by the finding that degradation rates tend to increase at each readdition of test compounds to the reactors. The results obtained in this kinetic-based analysis are confirmed by the found presence of soil bacteria, which are capable of growing on sulfonamide as the sole carbon source [34].

Apparently, a population of specific sulfonamide degraders is proliferating to artificially high concentrations during the lag period in the current reactor system. The experimental setup in this investigation differs from the conditions in STPs where chemical concentrations are too low to provide such high growth of these degraders. By comparing the results obtained in this study with other chemicals made with the current reactor test system, it is seen that sulfonamides are equally or more persistent than compounds normally accounted for as recalcitrant. For example, pentachlorophenol, known as persistent, was degraded with a half-life, $t_{½}$, of 2.9 d in the same test system at a concentration of 1 mg/L following first-order kinetics but without a preceding lag period [18]. Considering that typical retention times in STPs range from 8 to 12 h [35], there is reason to believe that sulfonamides may pass STPs, as they do not tend to sorb to the sludge. This concern is further confirmed by the fact that in many countries the temperature is much lower than used in current experiments. The few reactor experiments at 6 ± 1°C showed that lag periods and removal rates increased three to five times compared to tests performed at 20 ± 2°C.

CONCLUSIONS

Results from toxicity tests showed that the biodegradation tests were not performed at concentrations where the sulfonamides inhibited the bacteria. The sulfonamides were not de-

graded in the screening tests, indicating that these substances could not be classified as readily biodegradable. Primary degradation of the different sulfonamides studied in activated sludge reactors occurred after a lag period of 6 to 12 d in activated sludge at 20 ± 2°C and of 34 to 47 d at 6 ± 1°C. Degradation was completed within 2 to 4 d (20°C) and 12 to 30 d (6°C), respectively. Data fit the logistic growth model ($r^2 > 0.93$), and therefore the lag period was believed to reflect the time needed for specific degrading microorganisms to grow to an extent where degradation was detectable. At 20°C, readded sulfonamides were rapidly removed from the adapted sludge following first-order degradation kinetics (half-lives, range 0.2–4.1 d). The specific degraders developed during exposure to four different sulfonamides could easily degrade four other sulfonamides (half-lives, range 0.4–1.4 d), indicating that the bacteria during the lag period has acquired general properties needed for degradation of several sulfonamides. Analytical detection limits restricted current tests to be performed at concentrations much higher than the expected sub-ppb levels in STPs. Therefore, artificially high amounts of specific degraders may be present in the test systems, leading to unrealistically high degradation rates. Current results from the activated sludge reactors show that biodegradation compared to other known recalcitrant compounds (e.g., pentachlorophenol) is so slow that sulfonamides may pass the sewage treatment systems because of nonsorbing properties.

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