

Metabolism Studies of Phenylsulfonamides Relevant for Water Works

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Polar organic contaminants in surface water for drinking water purposes are relevant if they are poorly degradable, and analyses of such compounds are challenging because they are extremely water soluble. The problem is enhanced with more polar and unknown metabolites such as Sarkosin-*N*-(phenylsulfonyl) (SPS) which was detected in the Rhine river constantly in concentrations between 0.5 and 2 $\mu\text{g/L}$. The ubiquitous presence of SPS in the water was initially hypothesized to be due to the microbial degradation in sewage plants of 6-[methyl(phenylsulfonyl)amino]hexanoic acid (HPS) which is extensively used in Germany as a corrosion inhibiting agent. Soil suspension as well as closed-bottle degradation experiments with sewage water confirmed this hypothesis. Immobilized cell systems simulated the behavior in underground passage. HPS was totally degraded within hours, while SPS and other metabolites such as 4-[methyl(phenylsulfonyl)amino]butanoic acid (BPS) and methyl(phenylsulfonyl)amide (MPS) were identified. These studies revealed that phenylsulfonamides should be included in the group of drinking water relevant compounds because of their positive identification in drinking water itself. Even if the toxic effects of the investigated phenylsulfonamides have not yet been substantiated possibly also due to their inadequate biodegradability, their presence in the environment is a concern in general and therefore should be minimized.

Introduction

To be assured of a safe drinking water supply from water works, infiltration of surface water is of crucial importance to gain knowledge about the relevance of polar organic contaminants present and their behavior during drinking water production. Due to their good solubility in water, the more persistent polar compounds are the focus of interest. Whereas contamination by the "classical" contaminants such as organochlorines has been improved by more or less simple treatment (e.g. flocculation or granular activated carbon (GAC) filtration) and avoidance, the polar substances cannot be dispensed of that easily (1). It is known that the analysis of such substances itself is quite challenging, and the problem is further enhanced if polar metabolites are to be analyzed. This is due to their unknown structures and the lack of reference compounds.

A typical example of a strategy for the detection of such unknowns was the identification of a compound which

appeared regularly in the GC/MS-chromatograms as an intensive peak during screening of the German river Main for persistent polar organic compounds (2). Utilizing four different esterification methods for carboxylic acids to form methyl-, ethyl-, isopropyl-, and *n*-butyl esters, almost the same EI-mass spectra were obtained differing only in the appearance of one fragment ion. Since the fragmentation pattern was typical for *N*-methylated sulfonamides in addition to the information obtained from the additional fragment, it was highly possible that the unknown compound was sarkosin-*N*-(phenylsulfonyl) (*N*-methyl-*N*-phenylsulfonylglycine, SPS, CAS number 46376-16-3, Figure 1). Because SPS was not commercially available, it was synthesized, and the unknown compound was clearly identified as SPS by LC/ES-MS and GC/MS after derivatization (2). The identification of SPS in this study was performed parallel with investigations done by Stan and co-workers (3) who detected SPS in the groundwater of sewage fields in Berlin, Germany.

SPS at levels of >1000 mg/L (LC_{50}) was found not to be toxic to fish, and at 179 mg/L (EC_{50}) is only slightly toxic to algae (2); however, it has some inhibiting effects on various kinds of enzymes such as the aldose reductase (4). A literature survey on the behavior and utilization of SPS revealed that it has already been patented in Japan for its plant growth retarding behavior (5). Its application as such has not been exploited in Germany (6), and thus its ubiquitous presence in the surface waters could not be explained (2, 7). Accordingly, possible precursor compounds of SPS were examined (Table 1). There are various possibilities of adding additional groups to the phenylsulfonamide structure leading to compounds with totally different features, resulting in a widespread field of possible applications (8). Additional functional groups at the phenylring such as an aminofunction in the 4-position lead to compounds with pharmaceutical usage ranging from chemotherapeutics to oral antidiabetics (9). But these precursor compounds can be ruled out because a metabolic pathway in which functional groups at the aromatic ring are removed have not yet been reported.

A more plausible explanation is the β -oxidation of a long chain *N*-methyl-*N*-alkylcarboxyphenylsulfonamide. The only compound with these features and with a widespread application in Germany is 6-[methyl(phenylsulfonyl)amino]hexanoic acid (HPS, CAS number 46948-72-5), which was patented in Germany in combination with diethanol amine as corrosion inhibiting agent (10, 11). Thus, the formation of SPS from HPS could be explained through degradation via a 2-fold β -oxidation (Figure 1). If this would be the source of SPS in the environment, the intermediate metabolite 4-[methyl(phenylsulfonyl)amino]butyric acid (BPS, CAS number 46779-81-1) other than HPS should have been detected depending on the half-life in the surface water samples. The formation of BPS from HPS has already been described in 1919 (12), and only BPS was detected in the excreted urine and SPS was not measured at all.

During the monitoring program set up for SPS, the samples were also investigated for HPS and the presumed metabolite BPS (7). Thereby SPS and BPS were measured frequently in the river Rhine at various points covering its entire length in concentrations up to 2.0 $\mu\text{g/L}$. Based on the concentrations measured at the sampling point Wiesbaden (Table 2), an approximate transport of 150 t/a for both compounds in river Rhine has been estimated. They were likewise present in all of the randomly investigated German rivers with the highest values of up to 47 $\mu\text{g/L}$ BPS and 12 $\mu\text{g/L}$ SPS detected at the river Rodau where some metalwork industries are located. In the selected urban sewage effluents totaling 36

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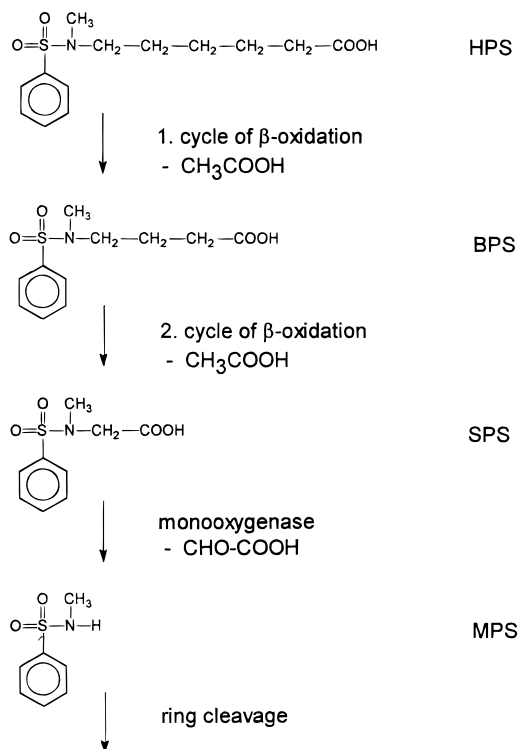


FIGURE 1. Postulated degradation pathway of HPS over BPS to SPS and from SPS to MPS.

areas, other than SPS (found in 66% of the samples at concentrations ranging from 0.16 $\mu\text{g/L}$ to 70 $\mu\text{g/L}$), BPS was detected in almost every sewage outlet in the same concentration range as SPS. These concentrations of SPS were comparatively lower than the concentrations found in the outlet of an industrial sewage plant where maximum values of up to 2,600 $\mu\text{g/L}$ could be determined (13).

Since the presence of the presumed parent compound HPS was never proven, the occurrence of SPS and BPS in the environment had to be investigated as well as its relevance for waterworks producing drinking water from surface water. This present study aims to simulate the metabolization of HPS through various microbial degradation experiments.

Experimental Section

Chemicals. All solvents and chemicals were analytical reagent grade. The reference compounds SPS, BPS, and HPS were synthesized as described in previous papers (2, 7). The identity and purity of the synthesized sulfonamides were proven by elemental analysis and after derivatization with diazomethane by GC-MS at the retention times and qualifying and quantifying ions mentioned under Analysis.

Sampling and Sample Preparation. Most of the investigated sewage and surface water samples, unless otherwise indicated, were taken randomly. The samples were treated and analyzed immediately after sampling or stored at 4 $^{\circ}\text{C}$ for not longer than 48 h. All samples were filtered through a glass fiber filter (0.45 μm , prewashed with methanol and MILLI Q water). The filtrates (1 L) were adjusted to pH 2 by adding sulfuric acid and filtered under vacuum through glass cartridges filled with a mixture of 0.1 g of LiChrolut(R)EN (Merck) and 0.25 g of RP-C₁₈-material (Merck). This mixture has been shown to have higher recoveries for polar acidic compounds compared with individual solid-phase extraction materials (2). The cartridges were conditioned with methanol and water prior to filtration. After drying the adsorbent material under a gentle stream of nitrogen for 45 min, the enriched carboxylic acids were eluted from the cartridge with

methanol. The extracts were evaporated under nitrogen to dryness and redissolved in 700 μL of *n*-hexane. Derivatization was performed with 0.1–0.2 mL of diazomethane in ether. The derivatization reaction was stopped after 1 h by the addition of 1–2 droplets of 15% (v/v) acetic acid in acetone. Finally, 10 μL heptadecanoic nitrilo acid (500 ng/ μL in *n*-hexane) was added as internal standard, and the volume was filled up to 1 mL with *n*-hexane.

Neutral MPS was extracted from a 100 mL sample and 30 g of sodium chloride with 5 mL of methyl-*tert*-butyl ether in a liquid/liquid-extractor at 1100 U min^{-1} for 20 min. After cooling at 5 $^{\circ}\text{C}$ for 30 min the organic phase was removed and the extraction repeated. The extracts were concentrated under nitrogen gas to 100 μL , and then heptadecanoic nitrilo acid (10 ng/ μL in *n*-hexane) was added to a final concentration of 1 ng/ μL . For the analysis of drinking water, the extracts were further concentrated under nitrogen from 1 mL to 100 μL . All extracts were stored at -12°C prior to analyses.

Mass Spectrometry. GC/MS. A mass spectrometer (Hewlett-Packard 5971) run in the selected ion monitoring mode was used to analyze the samples. Samples were likewise injected into a gas chromatograph (Hewlett-Packard 5890 Series II) equipped with a 30 m XTI-5 column (film thickness 0.25 μm , 0.25 mm ID) (Restek) with helium as the carrier gas. Injections (2 μL) were made in the splitless mode while maintaining the injection port temperature at 250 $^{\circ}\text{C}$. The initial temperature of the column was 50 $^{\circ}\text{C}$ which was held for 2 min, followed by a 10 $^{\circ}\text{C/min}$ ramp to 120 $^{\circ}\text{C}$, then a 6 $^{\circ}\text{C/min}$ ramp to 235 $^{\circ}\text{C}$ held for 2 min, and finally a 30 $^{\circ}\text{C/min}$ ramp to 290 $^{\circ}\text{C}$ held for 10 min.

The sulfonamides were analyzed at the following retention times (Rt) and qualifying and quantifying ions:

HPS: Rt: 39.4 min; $m/z = 77$ (52%), 141 (83%), 158 (52%), 184 (100%)

BPS: Rt: 33.5 min; $m/z = 77$ (100%), 130 (95%), 141 (90%), 184 (54%)

SPS: Rt: 26.7 min; $m/z = 77$ (70%), 102 (25%), 141 (71%), 184 (100%)

MPS: Rt: 12.1 min; $m/z = 77$ (100%), 141 (27%), 171 (39%)

LC/MS. A liquid chromatograph (Perkin-Elmer)/electrospray mass spectrometer (Perkin-Elmer-Sciex API 150) was used. Samples (100 μL) were injected manually to the LC/MS equipped with a Hypersil ODS (Merck) HPLC-column (250 \times 3.6 mm) at a flow rate of 0.4 mL/min at a column temperature of 40 $^{\circ}\text{C}$. The running solvent was a mixture of methanol/water (50:50, v/v). The mass spectrometer was run in the negative ionization mode at an ionization voltage of -3000 V and an orifice voltage of -30 V . The scan range was from $m/z = 120$ –300.

Analysis. For the development of the method, five-point calibration was used over the entire method, while for quantification, determination of the concentrations was performed only via a three-point-calibration in the expected range. The calibration curve was routinely checked by the addition of standards of known concentrations. Calibration was done in the range of 0.05 to 5 $\mu\text{g/L}$ (0.1 to 8 $\mu\text{g/L}$ for the degradation experiments). Quantification was performed through the mean value of the four quantifier ions (three in case of MPS), whereas the standard deviation was less than 3%. The recoveries for all the investigated sulfonamides were determined through five extractions in the range of 0.1 (0.25 $\mu\text{g/L}$ for HPS) and 1.5 $\mu\text{g/L}$. Thereby the following recovery values were obtained: HPS, 99.8 \pm 2.6%; BPS, 97.2 \pm 5.5%; SPS, 88.6 \pm 2.9%; MPS, 40.5 \pm 3.0%. The limit of detection (LOD) of the phenylsulfonamides was calculated as 0.03 $\mu\text{g/L}$ in drinking and surface waters and 0.1 $\mu\text{g/L}$ in sewage water and in the soil suspension experiments.

Soil Suspension Experiments. Fractions from a pristine soil were taken from the vertical profile between 10 and 15

TABLE 1: General Structure of Substituted Phenylsulfonamides, Their Biochemical Effects and the Thereby Resulting Application

structure	compound	X	Y	Z	effect	application
	HPS	H	CH ₃	(CH ₂) ₅ COOH	not known	in combination with diethanol amine as corrosion inhibition agent
	BPS	H	CH ₃	(CH ₂) ₃ COOH	not known	not known
	SPS	H	CH ₃	CH ₂ COOH	inhibition of plant growth	not known
	MPS	H	CH ₃	H	not known	not known
	a)	NH ₂	H	variable	antibacterial irreversible insertion in folic acid	chemotherapeutics
	b)	variable	H	H	urine inducer	diuretics
	c)	variable	H	CONHC ₄ H ₉	decreasing blood sugar	oral antidiabetics
	d)	H	H	C ₄ H ₉	neurotoxic	polymer blends

^a *p*-Aminobenzenesulfonamides. ^b Ring substituted benzenesulfonamides. ^c Phenylsulfonamideureas. ^d *n*-Butylbenzenesulfonamide.

TABLE 2: Monitoring of the Various Phenylsulfonamides during Different Subsequent Steps of Drinking Water Production Out of Surface Water

sampling point	c ^a (µg/L)							
	HPS		BPS		SPS		MPS	
	min. –max.	mean	min. –max.	mean	min. –max.	mean	mean	
river Rhine	nd	nd	0.04–0.33	0.12	0.18–0.87	0.47	0.06	
after sedimentation/aeration	nd	nd	nd –0.26	0.10	0.23–0.92	0.55	nm	
after flocculation/rapid sand filtration	nd	nd	nd –0.19	<0.03	0.04–0.99	0.43	nm	
after activated carbon filtration	nd	nd	nd	nd	0.03–0.65	0.20	0.03	
after underground passage	nd	nd	nd	nd	0.08–0.19	0.13	0.05	
after slow sand filtration/disinfection	nd	nd	nd	nd	nd – 0.07	<0.03	nd	

^a All values except MPS are based on 24 samples taken randomly every 14 days in 1997; MPS is a mean value of *n* = 3, LOD = 0.03 µg/L. nd = not detected. nm = not measured in this period.

cm. The soil composition was as follows: sand (34.4%), silt (32.5%), and clay (31.0%) with a humus content of 3.6%. After sieving and homogenization, soil with grain size of less than 2 mm was used for the further experiments. The water content was obtained by drying at 105 °C, and in the soil suspensions, water content was adjusted to give a soil-to-water ratio of 1:0.7. Three hundred grams of soil was mixed with the corresponding water spiked with HPS at an initial concentration of 6.04 mg/L and shaken in a rotary-shaker. The first sample was taken at day one after 1 min and then every 30 min. From day two until day 30, samples were taken at least once per day. For each sampling, 5 g of soil suspension was centrifuged at 2000 rpm for 30 min. From the supernatant, 600 µL were dried under a gentle stream of nitrogen at 65 °C while the pellet was dissolved in 700 µL acetone and then derivatized with 150 µL of the diazomethane solution and analyzed for HPS, BPS, and SPS using GC/MS as described earlier in the text. All tests were done in duplicates.

For the soil spiked with SPS, the initial concentration was adjusted to 6.58 mg/L SPS, and the eluents were divided into two portions. One portion was analyzed for SPS as described earlier, while the other portion was analyzed for MPS without derivatization.

Degradation Studies Using Sewage Water. The filtered sewage water (pH 7.9, DOC 9.0 mg/L and conductivity 190 mS/m) was spiked with HPS (final concentration of 6.35 mg/L) and stirred with a magnetic stirrer at 150 rpm to obtain a homogeneous mixture. The same procedure as for the soil suspension experiments was followed. To check for the background concentration, untreated water was also analyzed and served as control.

Testfilter Experiments. Based on the concept of Sontheimer (13, 14) a testfilter was developed where a spiked river Rhine water sample (pH 8.5 ± 0.05, DOC 2.2 ± 0.2 mg/L and conductivity 65 ± 5 mS/m) was aerated in a storage tank and then pumped into a filter column filled with SIRAN-Carrier (Schott, 023/02/300). These SIRAN-Carriers for the immobilization of microorganisms were made of native, porous

sintered glass (diameter: 2–3 mm, surface: 80 m²/L or 0.2 m²/g, porous diameter: 60–300 µm, weight: 450 g/L) which is chemically inert and easy to clean. The water was pumped continuously at a rate of 15 mL/min in a closed loop consisting of a 10 L storage tank, a pump (CONCEPT b, ProMinent), a glass column (diameter: 5 cm, height: 23 cm, volume: 310 mL), and a membrane pump to aerate over a glass frit. The individual components were connected via Teflon tubings (diameter: 6 mm). The whole testfilter was covered with aluminum foil to avoid exposure to daylight to prevent growth of algae. As a preparatory phase, river Rhine water was taken and pumped through the system for a period of 50 days. Every week thereafter, freshwater from river Rhine was added. For the degradation experiments 10 L Rhine river water was spiked with HPS at concentrations of 10 and 100 µg/L. The same procedure (sampling rate and analysis) as described for the soil suspension experiments was followed. To check for the background concentration, untreated river Rhine water was also analyzed and served as control. All experiments were conducted at 20 °C. Besides the analysis for sulfonamides, the water in the testing device was analyzed for "sum" parameters at different periods to monitor the microbial degradation.

Results and Discussion

Analysis of Phenylsulfonamides. Because all the investigated phenylsulfonamides were not commercially available, they were synthesized in our laboratory (2, 7) except for MPS which was given as reference compound by Dr. F. Karrenbrock (GEW, Cologne, Germany). A mass spectrum of HPS-methyl ester typical for all phenylsulfonamides is given in Figure 2. The tentative identification of each fragment is likewise given in the same figure. Besides the typical fragments resulting from the phenylsulfonamide structure (*m/z* = 77, 141, and 184), one characteristic fragment at *m/z* = 158 can be assigned to (CH₃)N⁺(CH₂)₅COOCH₃. In the mass spectra of the other investigated sulfonamides the mass of this particular fragment ion was shifting depending on the chain length. The

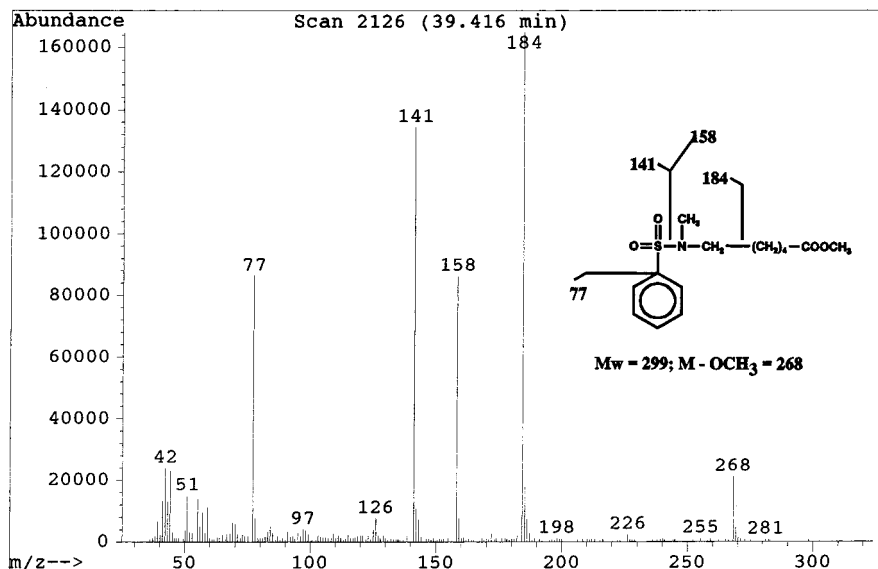


FIGURE 2. EI-GC/MS-spectrum of HPS-methyl ester and the tentative identification of fragment ions.

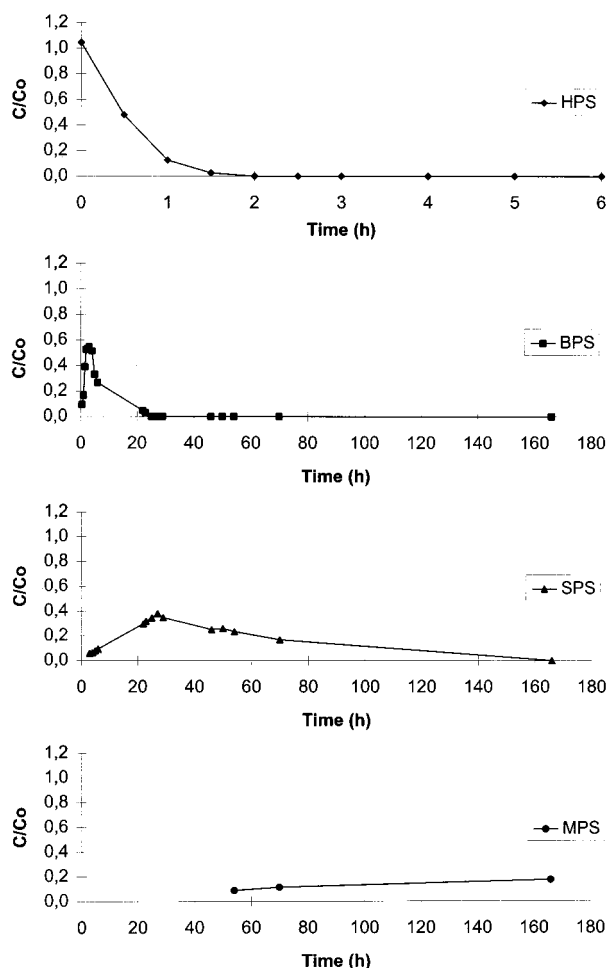


FIGURE 3. Degradation of HPS spiked into soil/water suspensions.

molecular ion was never obtained under these experimental conditions and thus has to be analyzed by LC/ES-MS without derivatization.

Degradation Experiments. The degradation of HPS in the soil-water system spiked with the compound was extremely rapid (Figure 3). After shaking for half an hour, the concentration of HPS was reduced by more than 50%, and after 2 h it was already below the detection limit. Simulta-

neously with the degradation of HPS, the formation of BPS was observed. Thus, the concentration of BPS increased to an almost steady level of 50% of the theoretically possible initial concentration of carbon (C_0) before it decreased slowly after 1 day with the concomitant formation of SPS. This compound was quite stable under these conditions over a period of more than 30 h. The decrease in SPS levels was observed, and at the same time, a yet unknown metabolite of HPS was also detected. This structure was presumed to be methyl (phenylsulfonylamide) (MPS) by its mass spectrum and was confirmed through the use of a reference compound. It was hypothesized that the formation of MPS from SPS by microbial degradation was via a monooxygenase (Figure 1). To elucidate the complete metabolism pathway for SPS, an additional experiment where SPS instead of HPS was spiked to the water/soil suspension was performed, however, only the formation of MPS was observed which was immediately degraded (data not shown). Other metabolites possibly arising from ring cleavage of MPS such as the very polar sulfonated dicarboxylic acids were not detected at all by LC/ES-MS. It may be possible that these compounds were mineralized immediately; however, this was not confirmed since labeling experiments were not performed.

To validate that the observed metabolism steps were mainly due to microbial degradation, parts of the soil and the soil suspension were autoclaved as described in Ternes et al. (15). HPS was not degraded at all in the autoclaved soil, while in the unsterilized soil the predicted metabolites were detected (data not shown). In all soil suspension experiments the initial molar concentrations of HPS (C_0) could not be obtained even taking into account all the molar concentrations of HPS and the other detected metabolites. This may be due to adsorption effects in the soil suspensions, the formation of unknown metabolites or by the formation of activated metabolites of the β -oxidation, which could not be confirmed even after analysis using hyphenated techniques such as LC/ES-MS. Similarly, for the water samples, formaldehyde (10% v/v) was added, and HPS was found not to be degraded at all (data not shown). This reinforces the hypothesis that degradation of HPS was mainly due to microbial action.

The degradation of HPS to SPS in sewage water was much slower as shown in the experiments with soil (Figure 4). This might be possibly due to the lower microbial density or other compounds acting either as alternative substrates or as inhibitors. The results obtained from the microbial degrada-

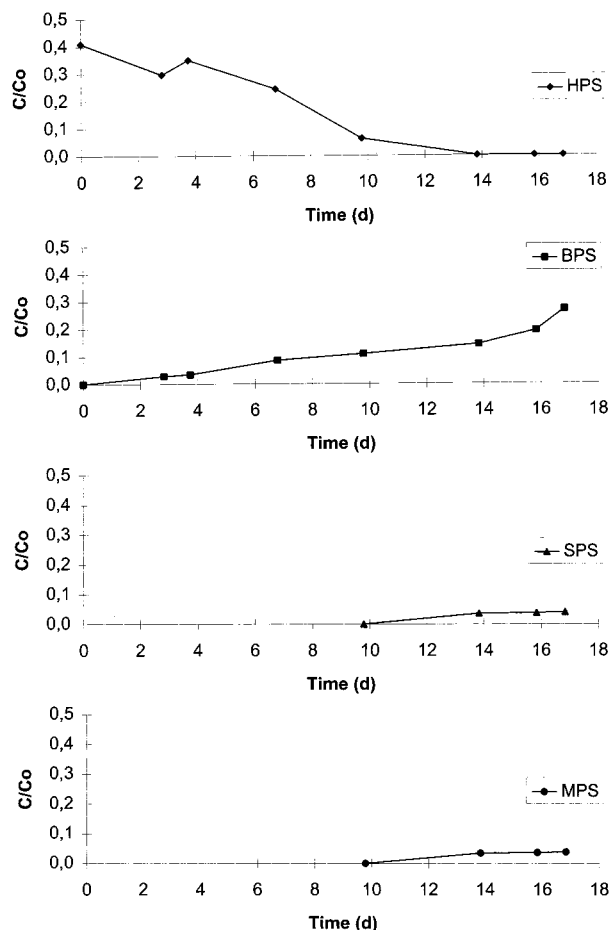


FIGURE 4. Degradation of HPS spiked into municipal sewage water.

tion studies were ascertained by a Ready Biodeg OECD 301A test. Using this test, SPS was shown to be less than 10% biodegradable (2).

To prove the results obtained from these model studies and correlate the relevance of sulfonamides for waterworks, additional testfilter experiments were done for the simulation of the subsoil passage. The testfilter concept has been successfully applied in Germany for the last 20 years to determine the nonbiodegradable fraction of the total organic load in biologically treated industrial wastewater (13) as well as to study the degradability of single compounds such as aromatic sulfonates (16). This testfilter system was spiked with HPS at concentrations of 10 and 100 $\mu\text{g/L}$ in river Rhine water as preparatory phase. In the individual samples taken at different time intervals totaling 30 days, HPS, BPS, SPS, and MPS were detected. With the initial concentration of 10 $\mu\text{g/L}$ HPS (Figure 5a) the results obtained from the soil suspension experiments were confirmed, while the result was different when the initial concentration was 100 $\mu\text{g/L}$ (Figure 5b). At both concentrations HPS was metabolized completely after 2 h resulting in the formation of BPS which was further degraded to SPS. In the experiment with 10 $\mu\text{g/L}$, SPS reached its maximum value after 3 days with a C/C_0 ratio of 0.75 and then steadily decreased to a C/C_0 ratio of 0.1 after 27 days. Again the formation of MPS was observed, but the concentration never exceeded a C/C_0 ratio of 0.1. The sum of the detectable sulfonamides after 27 days yielded in a C/C_0 ratio of 0.15. This experiment was repeated three times, obtaining reproducible results.

The degradation of SPS, however, did not proceed in the same rate when the initial concentration of HPS was 100 $\mu\text{g/L}$. Again the formation of BPS was observed but over a longer period of time resulting to a delayed formation of

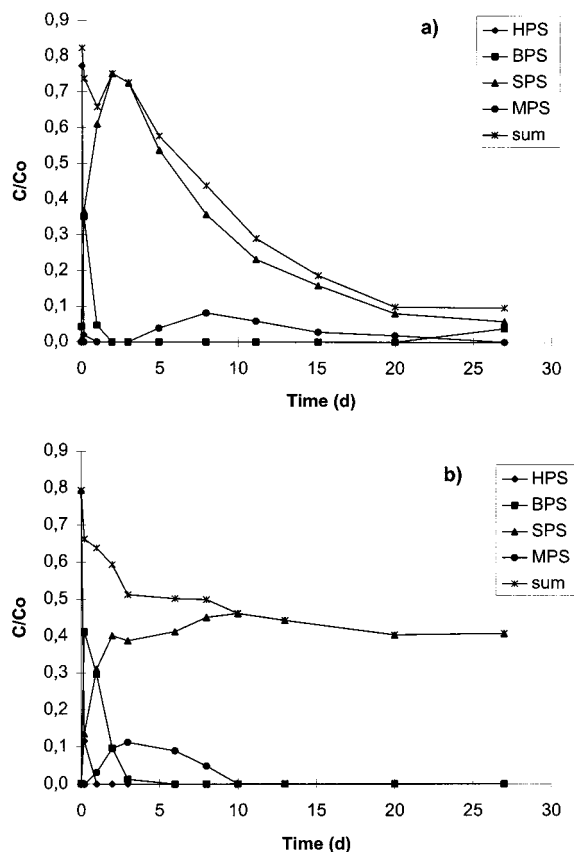


FIGURE 5. Degradation of HPS in the testfilter based on *siran* glass beads: (a) initial HPS concentration, 10 $\mu\text{g/L}$, and (b) initial HPS concentration, 100 $\mu\text{g/L}$.

SPS. SPS never exceeded a C/C_0 ratio of 0.45 after which steady state was reached. Formation of MPS was detected only after the first 10 days. Other metabolites could not be detected even if the concentration of the samples were multiplied by a factor of 10 utilizing either GC/MS after derivatization or LC/ES-MS. A possible explanation for this observation is the inhibition of the enzyme responsible for the further degradation of SPS as a result of an initial higher concentration of the compound itself. This may be similar to feedback inhibition, a regulation mechanism in microbial systems. The nondegradation of SPS has likewise been observed in several wastewater treatment plants, and thus this observation on the testfilter is not confined to this study. The possibility of biofilm deterioration as a result of exposure to higher levels of SPS could be not disregarded either since the viability of the cells within the biofilms was not monitored. The effect of concentration on degradation particularly on the enzyme systems should be further studied in detail.

Behavior of Phenylsulfonamides during Subsequent Steps of Drinking Water Purification. Water treatment steps such as sedimentation and aeration seemed to have negligible influence on the reduction of SPS and BPS in water; however, subsequent flocculation and rapid sand filtration (4 m h^{-1}) reduced BPS to below the detection limit of $0.03 \mu\text{g/L}$ (Table 2). A significant reduction of SPS was also observed. Subsequent filtration through granulated activated carbon (GAC) led to the total elimination of BPS and 50% reduction of SPS. An underground passage of about 180 m of the such treated raw water further reduced the SPS concentration. MPS, which was not measured as frequently as SPS, seemed to be not affected by these purification steps. Finally, slow sand filtration ($0.2\text{--}0.3 \text{ m h}^{-1}$) of this water with ensuing disinfection with chlorine dioxide led to the reduction of SPS

below the detection limit of 0.03 µg/L and the total elimination of MPS.

From 19 different drinking water samples, all taken from water works which were using bank filtrated or artificial infiltrated raw water for production, SPS was detected only in five of the drinking water samples. The concentrations were always below 0.1 µg/L except for one, where no GAC was applied, and values of up to 3 µg/L were measured for this particular sample.

The comprehensive degradation assays performed in combination with the results obtained from the measurements in sewage, surface, and drinking waters, showed that SPS did not come from usage of the compound per se. This verified the assumption it is highly likely that it was a metabolite arising from the degradation of HPS. After a more thorough analysis, it was shown that possible mother compounds for SPS other than HPS, as shown in Table 1, proved to be negative due to a different metabolism pattern and/or to a lesser production rate. HPS as source for SPS was confirmed through the detection of the other theoretically postulated intermediates such as BPS and MPS which were detected in almost all of the investigated sewage plant effluents and surface waters. HPS as the only source of SPS was furthermore confirmed by new measurements of SPS in the river Main in Germany. Accordingly, a significant reduction in SPS levels was monitored after closure of an HPS producing factory that was discharging its sewage (after treatment) into the river Main (17).

The phenylsulfonamides seemed to have been discharged into surface waters over several years. The individually observed ratios between BPS, SPS and MPS were dependent upon the initial amounts of HPS as well as the microbial activity and therefore should be reported as group parameter. These classes of compounds are typical examples of down-the-drain-chemicals and it may not be adequate at all to analyze only for the parent compounds while excluding the metabolites.

The degradation studies simulated the behavior of the phenylsulfonamides in sewage plants as well as during subsoil passage. Initial attempts to study the microbiology of the system have been done to study the specific microorganisms involved in the process.

SPS has been found to be poorly biodegradable and drinking water relevant and thus is recommended to be included in the group of environmentally relevant compounds. HPS, BPS, and MPS seemed not to be relevant for drinking water but there is still lack of toxicological data to assess their relevance. The enormous investigative effort for

the identification of such polar compounds is worthwhile since there are still many unknown but relevant compounds present in the environment as it was shown exemplary for the phenylsulfonamides in this work.

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