

Fate and biodegradability of sulfonated aromatic amines

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Abstract

Ten sulfonated aromatic amines were tested for their aerobic and anaerobic biodegradability and toxicity potential in a variety of environmental inocula. Of all the compounds tested, only two aminobenzene-sulfonic acid (ABS) isomers, 2- and 4-ABS, were degraded. The observed degradation occurred only under aerobic conditions with inocula sources that were historically polluted with sulfonated aromatic amines. Bioreactor experiments, with non-sterile synthetic wastewater, confirmed the results from the aerobic batch degradation experiments. Both ABS isomers were degraded in long-term continuous experiment by a bioaugmented enrichment culture. The maximum degradation rate in the aerobic bioreactor was 1.6–1.8 g l⁻¹ d⁻¹ for 2-ABS and a somewhat lower value for 4-ABS at hydraulic retention times (HRT) of 2.8–3.3 h. Evidence for extensive mineralization of 2- and 4-ABS was based on oxygen uptake and carbon dioxide production during the batch experiments and the high levels of chemical oxygen demand (COD) removal in the bioreactor. Furthermore, mineralization of the sulfonate group was demonstrated by high recovery of sulfate. The sulfonated aromatic amines did not show any toxic effects on the aerobic and anaerobic bacterial populations tested. The poor biodegradability of sulfonated aromatic amines indicated under the laboratory conditions of this study suggests that these compounds may not be adequately removed during biological wastewater treatment.

Introduction

Sulfonated aromatic amines are used as precursors for sulfonated azo dyes, optical brighteners, pesticides, ion-exchange resins, pharmaceuticals, and concrete plasticisers. The presence of a sulfonated group makes these compounds highly water-soluble. Therefore, during production processes sulfonated aromatic amines are easily discarded via the water system. Sulfonated aromatic amines are found in river and surface waters (Zerbinati et al. 1997). Furthermore, polar aromatic sulfonates, containing the sulfonated

aromatic amines, were found in industrial wastewater and sewage, in river Rhine and in water relevant for drinking water production (Lange et al. 1995a; Lange et al. 1998; Knepper et al. 1999).

One of the important pollution sources of sulfonated aromatic amines are sulfonated azo dyes. Sulfonated aromatic amines are formed during the reduction of sulfonated azo dyes under anaerobic conditions (Figure 1). It is estimated that during the dyeing process, sulfonated reactive dyes are fixed for 50–90% and the remaining 10–50 will end up in the wastewater (O'Neill et al. 1999). The discharge of these sulfonated azo dyes has not only

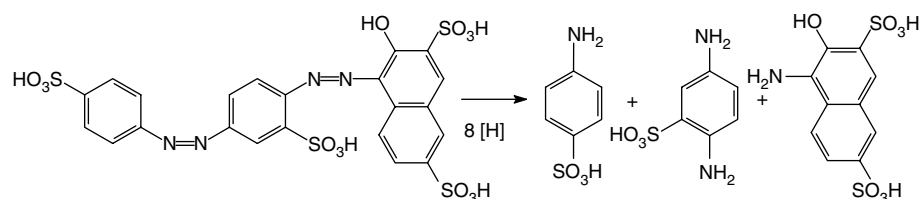


Figure 1. Anaerobic reduction of sulfonated azo dye Ponceau S (Acid Red 112, C.I. 27915) and the formation of sulfonated aromatic amines.

a negative aesthetic effect but also some azo dyes and their degradation products, sulfonated and unsulfonated aromatic amines, are toxic or even carcinogenic (Chung & Cerniglia 1992; Oh et al. 1997). However, due to their poor lipophilicity, sulfonated aromatic amines are considered less dangerous than their unsulfonated analogues (Jung et al. 1992).

Concerning the anaerobic biodegradability of sulfonated aromatic amines, only limited data is available in literature indicating that none of these compounds tested were degraded (Kuhn & Suflita 1989; Razo-Flores et al. 1996). Under aerobic conditions, some sulfonated aromatic amines are degraded and used as carbon and energy source by specific strains. However, these specific strains only have a narrow substrate range and therefore only a limited number of different compounds are degraded (Hooper 1991; Kertesz et al. 1994; Cook et al. 1998; Tan & Field 2000). Also sulfonated aromatic amines can be used as a sulfur source if no other sulfur compound is present, which are unrealistic for natural and wastewater treatment environments (Zurrer et al. 1987; Ruff et al. 1999). Additionally, another non-biological process for the removal of sulfonated aromatic compounds is autoxidation, resulting in the formation to colored polymers (Zerbinati et al. 1997; Kudlich et al. 1999). In practice, sulfonated aromatic amines are not readily degraded and drinking water-producing facilities have problems in removing the sulfonated aromatic amines via their treatment systems (Lange et al. 1995b).

The fate of the sulfonated aromatic amines formed from sulfonated azo dyes, in aerobic, as well in anaerobic biological treatment systems is still unclear (Stolz 2001). Limited attention has previously been paid towards aromatic sulfonates and intensified investigation of the occurrence and fate of substituted sulfonated aromatics in aquatic

environments is required (Reemtsma 1996). Therefore, the aim of this study was to investigate the fate of sulfonated aromatic amines in biological wastewater treatment systems. Biodegradation and toxicity of sulfonated aromatic amines were tested under anaerobic and aerobic conditions. The biodegradability was then confirmed in a long-term laboratory-scale non-sterile bioreactor experiment. The aim of the bioreactor experiment was to simulate aerobic wastewater treatment conditions and evaluate the biodegradation of the three ABS isomers.

Materials and methods

Media

The basal medium used in the batch experiments contained (mg l^{-1}): NaHCO_3 (5000); NH_4Cl (280); $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ (10); K_2HPO_4 (250); $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ (100); yeast extract (100); H_3BO_3 (0.05); $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$ (2); ZnCl_2 (0.05); $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ (0.05); $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$ (0.03); $\text{NH}_4\text{SeO}_3 \cdot 5\text{H}_2\text{O}$ (0.05); $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$ (2); $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$ (0.05); $\text{NaSeO}_3 \cdot 5\text{H}_2\text{O}$ (0.1); EDTA (1); resazurin (0.2); and 36% HCl (0.001 ml l^{-1}). The concentrations of trace metals and NaHCO_3 were 10- and 15-fold lower for the bioreactor experiments.

For the aerobic biodegradation experiments in which carbon dioxide was measured as mineralization product, a medium modified from (Feigel & Knackmuss 1988) was used. This medium contained (mg l^{-1}) K_2HPO_4 (3000); $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ (1000); $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ (100); NH_4NO_3 (500); K_2SO_4 (100); 10 ml $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$ (concentration 6.27 g l^{-1} , pH 2); H_3BO_3 (1.0); $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ (0.5), ZnCl_2 (0.2); $4\text{H}_2\text{O}$ (0.14); $\text{NaMoO}_4 \cdot 2\text{H}_2\text{O}$ (0.06), $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$ (0.02); $\text{NaSeO}_3 \cdot 5\text{H}_2\text{O}$ (0.06), $\text{NiCl}_2 \cdot \text{H}_2\text{O}$ (0.02). For the batch experiments, the sulfonated aromatic amines

were added from sterilized and neutralized (pH = 7.0) stock solutions.

Inoculum sources

Activated sludge was obtained from a wastewater treatment plant in Aarle-Rixtel (The Netherlands). This plant treats domestic and industrial wastewater, including effluents from several textile industries. Aerobic Rhine sediment was collected along side the banks of the river near Lexkesveer in Wageningen (The Netherlands). The sediment was suspended in 500 ml water and sieved (pore size 1.5 mm). Anaerobic granular sludges, from a full-scale upflow anaerobic sludge blanket (UASB) reactor treating wet oxidized industrial effluent of Shell Nederland Chemie (Moerdijk, The Netherlands) and from a full-scale UASB reactor treating effluent of an alcohol distillery at Nedalco (Bergen op Zoom, The Netherlands), were used for the experiments. All biomass sources were stored at 4 °C before usage.

Chemicals

Sulfonated aromatic amines 2-ABS, 3-ABS, 4-ABS, 2,4-diaminobenzenesulfonic acid (2,4-DABS), 3-amino-4-hydroxybenzenesulfonic acid (3-A-4-HBS), 1-aminonaphthalene-4-sulfonic acid (1-AN-4-S), 1-aminonaphthalene-5-sulfonic acid (1-AN-5-S), 2-aminonaphthalene-1,5-disulfonic acid (2-AN-1,5-DS), 3-aminonaphthalene-2,7-disulfonic acid (3-AN-2,7-DS), and 8-aminonaphthol-3,6-disulfonic acid (8-ANO-3,6-DS) were purchased from Aldrich (Milwaukee, USA), Sigma (St. Louis, USA), Fluka (Neu-Ulm, Switzerland) and Riedel-de Haën (Seelze, Germany) at the highest purity commercially available.

Analyses

The aromatic amines, used in the biodegradation assays, were analyzed spectrophotometrically with a Spectronic 60 spectrophotometer (Milton Roy Analytical Products Division, Oostende, Belgium) at their absorbance maxima; 2-ABS, 3-ABS and 4-ABS at 236, 236 and 248 nm and for 2,4-DABS, 1-AN-4-S, 2-AN-1,5-DS and 3-AN-2,7-DS the values were 220, 317, 247, and 247 nm, respectively. Liquid samples were centrifuged (7833 × *g*, 5.0 min) and diluted in a 0.10 M sodium phosphate

buffer solution (pH 7.0) and measured in a 1.0 cm 100-QS (Hellma Benelux, The Hague, The Netherlands) quartz cuvette.

The three ABS isomers, used in the bioreactor experiment, were analyzed with high performance liquid chromatography (HPLC). Influent and effluent samples were centrifuged (7833 × *g*, 5.0 min) and 10 μl samples were injected with a Marathon autosampler (Separations, Hendrik Ido Ambacht, The Netherlands). A 0.5% acetic acid solution (pH 5.9) was used as liquid phase and was pumped (Separations High Precision Pump Model 104, Separations, Hendrik Ido Ambacht, The Netherlands) at a flow rate of 300 μl min⁻¹ through the system. Samples and liquid phase were first degassed (Separations GT-103, Hendrik Ido Ambacht, The Netherlands) and separation of the compounds occurred in two reverse phase C18 temperature-controlled (20 °C) columns (200 × 3 mm, Chromosphere C18, Chrompack, Bergen op Zoom, The Netherlands). The ABS isomers were detected spectrophotometrically with a Spectroflow 783 UV detector (Kratos Analytical, Hendrik Ido Ambacht, The Netherlands) at 248 nm.

Sulfate was measured by HPLC using a Vydac-anion exchange column (302IC405, Mojave, USA) 250 × 4.6 mm inner diameter, which was temperature-controlled at 20 °C. Sulfate was detected with a Waters 431 conductivity detector (Waters Millipore, The Netherlands). As liquid phase potassium biphthalate solution (0.018 M) was used and a flow of 1.2 ml min⁻¹ was applied.

Headspace contents of oxygen, carbon dioxide and nitrogen were measured by gas chromatographic techniques. A sample (100 μl) was injected, with a pressure-locked gas syringe (Dynatech Precision Sampling Corporation, Louisiana, USA), in a Packard Becker 433 chromatograph (Delft, The Netherlands) gas chromatograph. Two parallel-connected columns (split 1:1), one with a 1.2 m × 2 mm steel column packed with molecular sieve 5A (mesh 60–80) and the other with Teflon packed Chromsorb (1.5 m × 2 mm) columns were used to separate the gases. The temperatures of the column, injection port and the flame ionization detector were 40 °, 110 °, 125 °C, respectively. Helium was used as the carrier gas (20 ml min⁻¹). All gas samples analyses were conducted after calibration with standards of known amounts. Chemical oxygen demand (COD) and volatile suspended

solids (VSS) were determined according to the Standard Methods (APHA et al. 1995).

Bioreactor

The bioreactor was originally used as the aerobic post treatment to degrade aromatic amines formed during the azo dye reduction of the sulfonated azo dye Mordant Yellow 10 (MY10). This azo dye was treated in a sequential anaerobic/aerobic setup. The aerobic reactor was successfully bioaugmented with a 4-ABS-degrading enrichment culture derived from Rhine sediment (Tan et al. 2000). For further investigation the aerobic reactor was disconnected from the anaerobic reactor 146 days after bioaugmentation and further fed with the three ABS isomers. The aerobic reactor had a volume of 1.0 l and was aerated at a flowrate of 75 l.h⁻¹ such that an oxygen concentration of 3.0–3.5 mg.l⁻¹ was maintained. A small secondary settler was connected to the reactor to retain the biomass. Occasionally unknown but insignificant amounts of biomass were accidentally wasted with the effluent. The sludge retention time therefore could not be calculated but was extremely long. The operational parameters and ABS isomer concentrations in the influent of the aerobic reactor are shown in Table 1.

Assays

Aerobic biodegradation experiments were described previously (Tan et al. 1999b). As inoculum for the aerobic degradation experiment, Rhine sediment (5–10% w/v) or activated sludge from Aarle-Rixtel (5–10% v/v) were used. The following sulfonated aromatic amines, 2-ABS, 3-ABS, 4-ABS, 2,4-DABS, 1-AN-4-S, 3-AN-2,7-DS, and 2-AN-1,5-DS, were tested. The target concentrations applied in the biodegradation experiments

were 100 mg l⁻¹ of sulfonated aromatic compound and were daily analyzed via UV measurements. Mineralization was followed in time by measuring oxygen uptake, carbon dioxide production and sulfate production. For these experiments the modified Feigel medium (Feigel & Knackmuss 1988) was used and experiments were conducted in duplicate.

Anaerobic biodegradation experiments were described previously (Tan et al. 1999b). Granular sludge was added to a 117 ml batch bottle together with basal medium (25 ml) containing the sulfonated aromatic amine (100–200 mg l⁻¹). The bottles were flushed with N₂/CO₂ (70/30%) and were stored on a shaker (50 strokes per minute) in a temperature controlled room (30 °C). The sulfonated aromatic amines, 2-ABS, 3-ABS, 4-ABS, 2,4-DABS, and 3-A-4-HBS, were tested with Shell granular sludge (1.0–2.0 g VSS l⁻¹) as inoculum source. The sulfonated aromatic amines, 2-ABS, 3-ABS, 4-ABS, 2,4-DABS, 1-AN-4-S, 1-AN-5-S, and 3-A-4-HBS were tested with Nedalco granular sludge (1.0–2.0 g VSS l⁻¹). Methane was measured as parameter for biodegradation and compared with methane production in control batches without addition of sulfonated aromatic amine. The anaerobic biodegradation experiments were conducted in triplicate.

The anaerobic toxicity assay was described previously (Tan et al. 1999a) and conducted in triplicate. For this experiment Nedalco granular sludge (2.0 g VSS l⁻¹) was used. The sulfonated aromatic amines tested were 4-ABS, 1-AN-4-S, 1-AN-5-S, and 8-ANOH-3,6-DS. Acute aerobic toxicity of sulfonated aromatic amines to Aarle-Rixtel activated sludge (aerated overnight to remove residual substrates) was measured (20 min, in triplicate) via dissolved oxygen concentration (DO, WTW, Ochten, The Netherlands) with a

Table 1. Operational parameters of the aerobic bioreactor fed with ABS isomers (values are averages during the period)

Period (days)	2-ABS (mg l ⁻¹)	3-ABS (mg l ⁻¹)	4-ABS (mg l ⁻¹)	ABS (mg l ⁻¹)	HRT (h)
I 0–104	–	–	150	150	8.8
II 105–155	96	–	110	206	10.7
III 156–189	76	51	79	206	14.9
IV 190–294	150	–	111	261	7.7
V 295–380	204	–	160	364	3.3

2-aminobenzenesulfonic acid (2-ABS), 3-aminobenzenesulfonic acid (3-ABS), 4-aminobenzenesulfonic acid (4-ABS).

Microprocessor Oximeter 196 (Weilheim, Germany). Closed bottles of 294 ml were filled with 275 ml of sludge were mixed with a magnetic stirrer and in a temperature controlled of 20 ± 2 °C room. Endogenous respiration was initially measured before substrates were added. Afterwards, 1.5 ml acetate solution (10.0 g l^{-1} , pH 7.0) and 12.0 ml of a sulfonated aromatic amine stock solution (25.0 g l^{-1} , pH 7.0) were added. Subsequently, the oxygen uptake rate was compared with the oxygen uptake rate without addition of the sulfonated aromatic amine. The sulfonated aromatic amines tested were 2-ABS, 3-ABS, 4-ABS, 2,4-DABS, 1-AN-4-S, and 3-AN-2,7-DS.

The kinetic parameters of the sludge in the aerobic reactor were determined with a respirometer. In a 1.2 l bottle oxygen concentrations were measured with an oxygen probe (WTW, Ochten, The Netherlands). The oxygen concentration was computer controlled, if the concentration became below 3.0 mg l^{-1} compressed air was blown (500 ml min^{-1}) into the bottle until a concentration of 6.0 mg l^{-1} was reached. The pH was controlled between 6.0 and 8.0. The respiration bottle was filled with 950 ml of effluent of the aerobic bioreactor and 2.0 ml NH_4Cl solution (48.0 g l^{-1}) as a nitrogen source. Furthermore, 1.0 ml of a micro nutrient solution was added and 30 ml of sludge of the reactor ($4.12 \text{ g VSS l}^{-1}$). As sole

organic carbon source 2-ABS was supplied as an aliquot of 100 ml with a concentration of 5.0 g l^{-1} . The respiration rate was calculated from the time course of the DO, which was logged, onto a computer. Periodically, samples were taken to determine the concentration of the sulfonated aromatic amines and sulfate. The oxygen uptake rate (OUR), 2-ABS and sulfate analyses were used to estimate the parameters of a (growth) Monod model, i.e. the biomass-yield (Y), the substrate affinity constant (K_s) and the maximum specific growth rate (μ_{max}). In this model additional oxygen consumption by chemical oxidation of sulfonate group to sulfate was taken into account. This experiment was conducted four-times and average values of the parameters were calculated.

Results

Biodegradation and toxicity experiments

Under anaerobic conditions none of the tested compounds (2-ABS, 3-ABS, 4-ABS, 2,4-DABS, 1-AN-4-S, 1-AN-5-S, and 3-A-4-HBS) were degraded by the two inoculums tested after periods ranging from 100 to 180 days. Additionally, 3-A-4-HBS was studied in a batch anaerobic re-circulation reactor and no degradation was observed after 117 days based on HPLC measurements.

Table 2. Aerobic biodegradability of selected sulfonated aromatic amines (– no degradation observed)

Inoculum	Rhine Sediment			Aarle-Rixtel sludge		
	Recovery S-SO_4^{2-} (% S)	Uptake O_2 (% COD) ^a	Recovery CO_2 (% COD) ^a	Recovery S-SO_4^{2-} (% S)	Uptake O_2 (% COD) ^a	Recovery CO_2 (% COD) ^a
2-ABS	120 ± 13	62 ^b	48 ^b	108 ± 3	68 ± 19	61 ± 8
3-ABS	98 ^c	52 ^c	52 ^c	–	–	–
4-ABS	104 ± 13	45 ± 25	34 ± 14	125 ± 6	65 ± 20	52 ± 25
2,4-DABS	–	–	–	–	–	–
1-AN-4-S	–	–	–	–	–	–
3-AN-2,7-DS	–	–	–	–	–	–
2-AN-1,5-DS	–	–	–	–	–	–

^aCorrected for the oxygen uptake and carbon dioxide production due to endogenous substrate respiration.

^bNo standard deviation because gas measurements were not performed in duplicate.

^cDegradation occurred only in one replicate of a duplicate batch experiment; 2-aminobenzensulfonic acid (2-ABS), 3-aminobenzensulfonic acid (3-ABS), 4-aminobenzensulfonic acid (4-ABS), 2,4-diaminobenzenesulfonic acid (2,4-DABS), 1-aminonaphthalene-4-sulfonic acid (1-AN-4-S), 3-aminonaphthalene-2,7-disulfonic acid, and 2-aminonaphthalene-1,5-disulfonic acid (2-AN-1,5-DS).

Under aerobic conditions, of all the sulfonated aromatic amines tested (2-ABS, 3-ABS, 4-ABS, 2,4-DABS, 1-AN-4-S, 3-AN-2,7-DS, and 2-AN-1,5-DS), only 2- and 4-ABS were readily degraded (Table 2). Decrease of UV absorbance at the absorbance maximum, high recovery of sulfur from the sulfonate group as sulfate, good carbon dioxide recovery, and significant uptake of oxygen clearly demonstrated the mineralization of 2- and 4-ABS. Aerobic degradation of 2-ABS isomers was observed with only two of the inocula tested (Rhine sediment and Aarle-Rixtel sludge). Aerobic biodegradation of 4-ABS was also tested inocula from the humus layer of soil, polluted harbor sediment, and activated sludge from a plant treating domestic wastewater but no degradation was observed. The anaerobic and aerobic toxicity experiments did not show any toxicity of the sulfonated aromatic amines tested (anaerobic 4-ABS, 1-AN-4-S, 1-AN-5-S, and 8-ANOH-3,6-DS; aerobic 2-ABS, 3-ABS, 4-ABS, 2,4-DABS, 1-AN-4-S, and 3-AN-2,7-DS). Even at concentrations up to 1.0 g l^{-1} , no inhibition was observed. These concentrations are in excess of those expected in wastewater discharges. Therefore, the sulfonated aromatic amines are not expected to have an inhibitory effect on sequential biological treatment system used to treat sulfonated azo dye containing wastewaters.

Bioreactor

The aerobic bioreactor in this study was originally used as the aerobic post treatment in a sequential anaerobic/aerobic bioreactor that treated the sulfonated azo dye MY10. This reactor was exposed to 4-ABS, one of the degradation products of MY10. However, 4-ABS was only degraded after bioaugmentation with a 4-ABS degrading enrichment culture derived from Rhine sediment (Tan et al. 2000). Afterwards, the anaerobic stage was disconnected and the aerobic stage was used for the continuous degradation of sulfonated aromatic amines, especially the ABS isomers. The main results of the aerobic bioreactor are shown in Figure 2. This figure is divided into four graphs, which will be discussed separately.

In Figure 2a, the load of the ABS isomers and the HRT are shown. During period I, 4-ABS was fed to the reactor. In period II, 2-ABS was added together with 4-ABS. During period III, 3-ABS

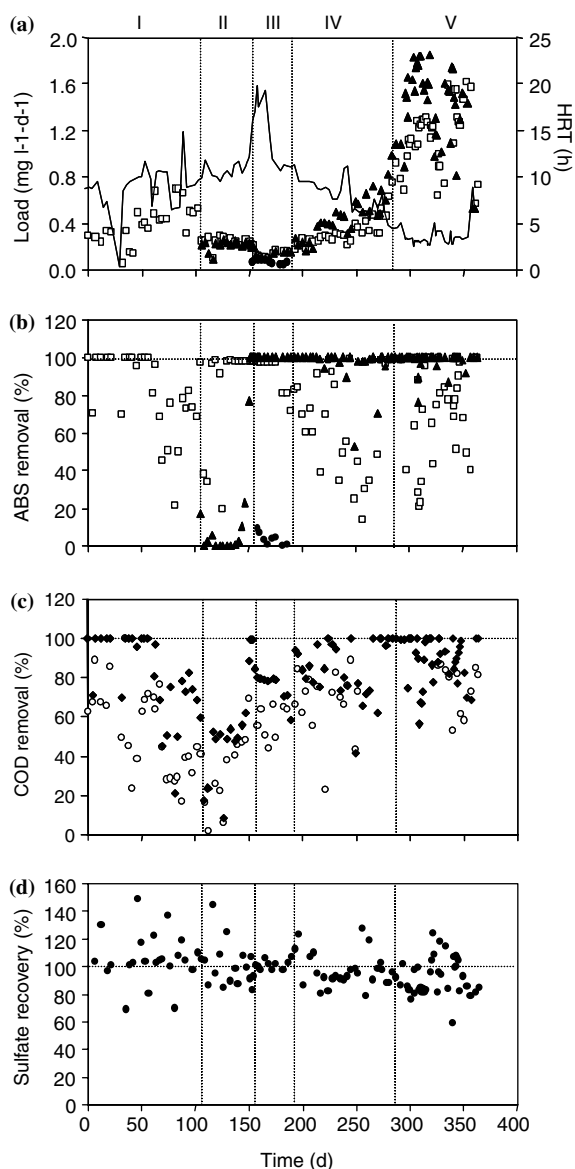


Figure 2. a-d. (a) Loading rate of 2-ABS (▲), 3-ABS (●), and 4-ABS (□) and hydraulic retention time (HRT (h), —); (b) Percentage removal 2-ABS (▲), 3-ABS (●), and 4-ABS (□); (c) COD removal percentage measured (◆) and calculated (○); (d) Percentage of sulfate recovery (●) and average (---) during the period.

was fed to the reactor together with the two other ABS isomers (Table 1). Finally, in the last two periods, 2- and 4-ABS were fed at increasing loading rates to determine the maximum loading rate.

Figure 2b indicates the removal efficiencies of the reactor. Since the reactor was originally bio-

augmented with a 4-ABS degrading enrichment culture, degradation of this compound was expected and observed in period I. Due to problems with sludge settling at the end of period I, lower removal efficiencies were observed. Therefore, using higher flow rates and adjusting the tubing, the settler configuration was changed, and this resulted in a higher removal efficiency of 4-ABS in the next periods. In period II, 2-ABS was also added to the influent. Thirty days after starting this addition, degradation of 2-ABS could clearly be detected. These results were confirmed during batch experiments with sludge from the bioreactor and with the original enrichment culture used to bioaugment the reactor (results not shown). Surprisingly, the original bioaugmented 4-ABS-degrading enrichment culture was still able to degrade 4-ABS and could also degrade 2-ABS after 2 years of storage in the refrigerator (4 °C).

Introduction of 3-ABS in period III did not result in degradation of 3-ABS. Also this result was confirmed in batch experiments with bioreactor sludge and the original enrichment culture. Bioreactor sludge and the original bioaugmented 4-ABS degrading enrichment culture were not able to degrade 3-ABS (results not shown). In period IV and V only 2- and 4-ABS were added in the reactor. During the period IV, the HRT was decreased and during period V the influent concentrations of these compounds were gradually increased from 80 to 200 mg l⁻¹. High removal efficiencies of 2- and 4-ABS were still observed. The maximum degradation rates achieved were around 1.6–1.8 g ABS l⁻¹ d⁻¹ (Figure 2a) for both compounds. Sometimes lower removal efficiencies of 4-ABS were incidentally observed during period IV and V. The lower efficiencies were again due to problems occurring in the settler, which were most probably aggravated by the decrease of the HRT. The 4-ABS degradation was more affected by settler problems than 2-ABS degradation.

Figure 2c shows the COD removal of the reactor. The measured and calculated COD removal during the five periods is depicted in this figure and shows the same trend. The calculated COD removal was determined by the difference in the theoretical influent and effluent COD concentrations of the ABS isomers. The measured COD was sometimes lower, indicating occasional accumulation and discharge of degradation intermediates. The batch degradation

experiments of 2- and 4-ABS (Table 2) indicated a complete mineralization of the isomers. The desulfonation of 2- and 4-ABS in the reactor was also observed and is shown in Figure 2d. The average recovery of sulfur from the sulfonate group as sulfate-S was high (99 ± 19%) during all five periods.

The bioreactor, fed with sulfonated aromatic amines containing wastewater, showed high removal efficiencies even at a total ABS concentration of 364 mg l⁻¹ and at an HRT of 2.8–3.3 h. The maximum volumetric degradation rate achieved during the experiments was 1.6–1.8 g l⁻¹ d⁻¹ for 2-ABS and 1.3–1.5 g l⁻¹ d⁻¹ for 4-ABS.

Kinetic parameters

The OUR, 2-ABS and sulfate analyses during the kinetic assay are shown in Figure 3, together with the model fit according to a growth Monod model. Figure 3 clearly showed the mineralization of 2-ABS by formation of the mineralization product, sulfate, and the increase in OUR. The model was used to estimate the following values for the kinetic parameters: $Y = 0.17$ mg VSS mg⁻¹ COD, $K_s = 28$ mg COD l⁻¹ and $\mu_{max} = 0.077$ h⁻¹. Although the model fit the measurements reasonably well, the initial linear increase of the OUR cannot be explained by growth alone as this would have resulted in an exponential increase of the OUR. An explanation for this phenomenon could be due to either a shortage of trace metals or nutrients for the microorganisms or mass transfer limitation of oxygen to the bacteria. Therefore, the estimated value for μ_{max} may be an underestimate.

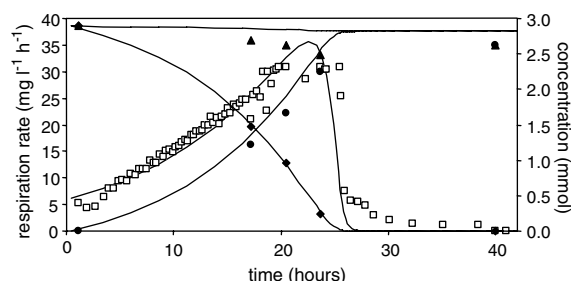


Figure 3. Respiration rate (\square) of 2-ABS degrading culture together with the removal of 2-ABS (\blacklozenge) and sulfate production (\blacklozenge) and mass balance (\blacktriangle) based on 2-ABS removal and sulfate production (lines represents the model fit).

Discussion

The aim of this study was to evaluate the fate of sulfonated aromatic amines. Biodegradation and toxicity of sulfonated aromatic amines were tested with natural bacterial populations used as inocula to predict their fate in wastewater treatment systems and in aquatic environment. The results under the laboratory conditions used in this study showed that the tested sulfonated aromatic compounds were not toxic and generally were not readily biodegraded with the tested inocula from a variety of environmental sources. The poor biodegradability observed in this study is in agreement with the observation that many sulfonated aromatic amines accumulate in the environment as evidenced by their occurrence in surface waters where they are a substantial polluting factor (Malle 1978; Zerbinati et al. 1994; Lange et al. 1995b; Zerbinati et al. 1997). Other dye metabolites such as unsulfonated aromatic amines are relatively stable in aquatic conditions and are poorly degraded under anaerobic and aerobic wastewater treatment conditions (Ekici et al. 2001). Therefore, both sulfonated and unsulfonated aromatic amines are an important group environmental pollutants formed during reduction of (sulfonated) azo dyes that can potentially pass through biological treatment systems (Pinheiro et al. 2004).

None of the sulfonated aromatic amines tested were degraded anaerobically. This is in agreement with the limited experimental data available in the literature (Kuhn & Sufliata 1989; Razo-Flores et al. 1996). However, there are a few reports of other sulfonated aromatic compounds being degraded under anaerobic conditions. For example 4-sulfobenzoate was eliminated under sulfate reducing conditions (Kuhn & Sufliata 1989). Furthermore, there is evidence that linear alkylbenzenesulfonic acids and other sulfonated aromatic compounds are utilized and degraded as a sulfur source under sulfur limiting anaerobic conditions (Denger et al. 1996; Cook 1998; Jensen 1999; Prats et al. 1999).

While most sulfonated aromatic amines persisted in the degradation assays, aerobic degradation of a few simple structures was observed. Aerobic degradation of 2- and 4-ABS isomers was demonstrated in batch experiments as well in the bioreactor experiment. The aerobic degradation of the related 3-ABS isomer could not be consistently

observed during the batch experiments and it was not degraded in the bioreactor experiment. Aerobic degradation of these three isomers by isolated cultures has been described (Thurnheer et al. 1986; Feigel & Knackmuss 1988; Perei et al. 2001). The degradation of 4-ABS was possible by a co-culture of two stains, *Hydrogenophaga palleronii* and *Agrobacterium radiobacter*, that collaborated to mineralize this compound (Feigel & Knackmuss 1988, 1993). Molecular analysis of a 4-ABS degrading enrichment culture developed at our laboratory also showed a co-culture of two strains (unpublished). The fact that two strains are involved in the degradation of 4-ABS could indicate that it is not easy to mineralize this compound. However, a single culture of *Pseudomonas paucimobilis* degraded also 4-ABS (Perei et al. 2001).

In this study, the aerobic degradation of the sulfonated aromatic amines was only observed with inocula sources that were historically polluted with sulfonated aromatic amines. Therefore, the conclusion can be made that inoculation source, which have been exposed to these compounds, such as the Rhine (Malle 1978; Lange et al. 1995a) and activated sludge from Aarle-Rixtel (treating textile wastewater), are a good source for specialized xenobiotic degradation microbial activity. Similar results were obtained for a bioreactor treating 3-nitrobenzenesulfonic acid and 3-ABS, in which degradation occurred only with activated sludge originating from plants treating industrial wastewater (Kolbener et al. 1994).

Aerobic biodegradation of aminonaphthalene-sulfonic acids (ANS) was not found in this study. ANS that were aerobically degraded have the amino and sulfonic acid substituents located next to each other on the same aromatic ring or both substituents on different aromatic rings (Tan & Field 2000). Two compounds tested in this study did meet the first criteria but had also an extra sulfonated group as substituent and this may be the reason for their recalcitrance (Reemtsma & Jakobs 2001).

Both anaerobic and aerobic toxicity experiments were conducted at fairly high concentrations of sulfonated aromatic amines (1.0 g l^{-1}). The results obtained from the toxicity experiment show that sulfonated aromatic amines do not have any toxic effect on the anaerobic and aerobic microbial populations tested. Due to the high

concentrations of sulfonated aromatic amines tested it may be expected that under real wastewater treatment conditions the anaerobic and aerobic bacterial populations, which are required for the removal of sulfonated azo dyes, are not inhibited by the formed sulfonated aromatic amines. Due to the presence of a ionizable sulfonate group, the sulfonated aromatic amines are highly soluble. Therefore, bacteria do not easily take up these compounds, which maybe accounting for their non-toxicity. For the same reason sulfonated aromatic amines are less genotoxic and carcinogenic than their unsulfonated analogues (Jung et al. 1992).

The continuous aerobic bioreactor used in this study was able to successfully degrade 2- and 4-ABS. However, this system was not able to degrade 3-ABS. The microorganisms capable of degrading these two ABS isomers remained in the reactor for a long period (380 days). To our knowledge this is the first bioreactor with natural bioaugmented enrichment culture that is able to degrade these compounds under non-sterile wastewater treatment conditions (Tan & Field 2000). Only Thurnheer et al. (1988), who operated a chemostat at high HRT inoculated with five isolated cultures could degrade seven benzene-sulfonic acids, including the three ABS isomers (Thurnheer et al. 1988). However, a chemostat is not comparable with the activated sludge system tested here, which is more comparable to wastewater treatment systems.

The kinetic assay experiments clearly showed an increase in OUR coupled with the removal of 2-ABS and the production of the mineralization product sulfate. The results confirm extensive mineralization of 2-ABS by the sludge from the aerobic bioreactor. The OUR data were used to estimate kinetic parameters for growth. However since OUR increased in a linear rather than exponential pattern, a shortage of either a nutrient or mass transfer limitation of oxygen was assumed to have occurred. Thus the simulated μ_{max} may be an underestimate. A better approach to obtain these parameters might be to use experiments in which the sludge retention time of a continuous bioreactor is varied and effluent substrate concentrations are measured (Tchobanoglus & Burton 1991).

Ruff and co-workers showed that fourteen sulfonated aromatic compounds, five of which

were sulfonated aromatic amines, were degraded by a mixed culture under sulfur limited conditions (Ruff et al. 1999). Therefore, they presumed that the capacity to degrade these compounds extensively is widespread in the environment. However, the results from our experiments showed contradictory results. The reason for this may be a lack of sulfur limitations in the tested sediments or wastewater treatment environments.

Conclusions

This research clearly showed that 2- and 4-ABS were degraded in aerobic conditions by inoculum sources that were historically polluted with sulfonated aromatic amines. Biodegradation of the two isomers was furthermore confirmed in lab-scale activated sludge bioreactor and a respirometric experiment. However, biodegradation of other sulfonated aromatic amines of more complicated structures was not observed under aerobic and anaerobic conditions. These compounds showed no acute toxic effect for activated sludge and were not toxic for anaerobic granular sludge. Therefore, it is expected that many of these compounds have no negative influence and remain untreated in anaerobic and aerobic biological wastewater treatment systems. Since many different types of sulfonated azo dyes are currently utilized, a wide variety of sulfonated aromatic amines will be formed under anaerobic conditions that will not easily be biodegraded and will constitute an important part of the untreated COD fraction in sulfonated azo dye containing wastewater after biological treatment.

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