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Toxicity of xenobiotics during sulfate, iron, and nitrate reduction in primary sewage sludge suspensions

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ABSTRACT

The effect and persistence of six organic xenobiotics was tested under sulfate-, iron-, and nitrate-reducing conditions in primary sewage sludge suspensions. The xenobiotics tested were acenaphthene, phenanthrene, di(2-ethylhexyl)phthalate (DEHP), 4-nonylphenol (4-NP), linear alkylbenzene sulfonate (LAS), and 1,2,4-trichlorobenzene (1,2,4-TCB) added to initial analytical concentrations of 54–117 mg L^{-1}. The suspensions were incubated at 30 °C for 15 weeks and rates of sulfate, iron, and nitrate reduction were estimated from the time course of hydrogen sulfide accumulation, Fe(II) accumulation, and nitrate depletion, respectively. Chemical analysis showed that the xenobiotics were persistent under the different electron acceptor regimes for the duration of the experiment. This was partly attributed to low bioavailability and microbial toxicity of the xenobiotics. Rates of anaerobic respiration in control suspensions (without added xenobiotics) showed a weekly reduction potential of 0.84 mM SO_4^{2-}, 0.92 mM Fe(III), and 9.25 mM NO_3^-. All three processes were completely inhibited by 1,2,4-TCB (54 mg L^{-1}) whereas there was no significant ($P < 0.05$) toxicity of phenanthrene (109 mg L^{-1}) and DEHP (105 mg L^{-1}). Sulfate reduction was inhibited completely by LAS (105 mg L^{-1}), 76% by acenaphthene (54 mg L^{-1}) and 57% by 4-NP (117 mg L^{-1}), and likewise iron reduction was inhibited 62% by LAS and 55% by 4-NP (the latter though at $P < 0.10$). Nitrate reduction was not significantly inhibited by acenaphthene and 4-NP and furthermore was resistant to LAS toxicity (105 mg L^{-1}). Nitrate reduction also had the highest potential for mineralization of organic matter and thus was the most robust of the tested anaerobic processes in the sewage sludge suspensions.

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1. Introduction

In anoxic soil and sediments (i.e., without O_2), microbial degradation of organic carbon proceeds through sequential reduction processes, where anaerobic microorganisms use prevailing electron acceptors such as NO_3^-, Fe^{3+}, SO_4^{2-} and CO_2 (e.g., Peters and Conrad, 1996). The degradation potential of anaerobic processes is extensive, often with consortia of species contributing to the complete mineralization of complex organic compounds. In wastewater treatment systems, comparable microbial redox processes occur and notably methanogenesis is exploited during anaerobic digestion of sewage sludge (Appels et al., 2008). However, also sulfate, iron, and nitrate reductions occur during wastewater treatment and have been studied in terms of microbial activity, community composition and ecosystem function (Etchebehere et al., 2001; Ingvorsen et al., 2003; Thomsen et al., 2007).

During wastewater treatment, primary sewage sludge may adsorb and concentrate a range of organic contaminants (xenobiotics) that are undesired in the recycling of sewage sludge for environmental use such as application to agricultural fields (Jensen and Jepsen, 2005). This potential environmental impact of sewage sludge contaminants may be reduced due to microbial biodegradation of the xenobiotics. However, a range of xenobiotics are rather persistent and, in addition, the xenobiotics may have a toxic effect on the microbial consortia in the sewage systems (Haynes et al., 2009). Biodegradation potential and potential toxicity under methanogenic conditions have been tested for a range of xenobiotics known to occur in sewage sludge (e.g., Battersby and Wilson, 1989; Madsen et al., 1995). However, the interactions of xenobiotics and other anaerobic processes have not been extensively studied.

The present study compared the effect of six common organic xenobiotics on anaerobic respiration under sulfate-, iron-, and nitrate-reducing conditions in primary sewage sludge suspensions. The xenobiotics were chosen to represent polycyclic aromatic hydrocarbons (PAHs), phthalates, surfactants and chlorinated benzenes, which frequently occur in sewage sludge and for which ready anaerobic biodegradation was not expected (Haynes et al., 2009).

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2. Materials and methods

2.1. Sewage sludge inoculum and xenobiotics

Primary sewage sludge was obtained from a wastewater treatment plant at Lundtofte (Denmark), which mainly receives municipal wastes (Ucisik and Henze, 2008). The sludge was sampled in airtight 3 L glass jars that were completely filled and kept at 2 °C until used (after 4 wk). The sludge was washed by centrifugation (ISO, 1995a) and resuspended in a basal salts medium with (in g L^{-1} of distilled water): NaCl, 0.3; $MgCl_2 \cdot 6H_2O$, 0.2; NH_4Cl, 0.1; $CaCl_2 \cdot 2H_2O$, 0.1; KH_2PO_4, 0.4; $K_2HPO_4 \cdot 3H_2O$, 0.4. Oxygen was stripped from the medium by bubbling with N_2-gas and pH was adjusted to 7.0. The final concentration of dry solids was adjusted to 43 g L^{-1} as determined by drying (105 °C, 24 h). Prior to the use as inoculum, the resuspended sludge sample was allowed to equilibrate under oxygen-free conditions for 5 d at 30 °C.

Individual stock solutions of xenobiotics (~10 mg C mL^{-1}) were prepared with the following amounts in 20 mL of dichloromethane (DCM): acenaphthene, 214 mg; phenanthrene, 215 mg; 4-nonylphenol (4-NP), 243 mg; di(2-ethylhexyl)phthalate (DEHP), 283 mg; 1,2,4-trichlorobenzene (1,2,4-TCB), 529 mg; sodium dodecylbenzene sulfonate, 312 mg. The latter was included to represent linear alkylbenzene sulfonates (LAS). Selected physico-chemical properties of the xenobiotics are shown in Table 1.

2.2. Inoculation and incubation of anaerobic media

Media with SO_4^{2-}, Fe(III) or NO_3^- were prepared by supplementing the basal salts medium with Na_2SO_4 (final concentration, 20 mM SO_4^{2-}), amorphous $Fe(OH)_3$ (final concentration, ~120 mM Fe(III)) or $NaNO_3$ (final concentration, 30 mM NO_3^-). Amorphous $Fe(OH)_3$ was prepared from $FeCl_3$ as described by Ghiorse (1994). The media were autoclaved (121 °C, 20 min), cooled under an atmosphere of N_2-gas, and supplemented with non-chelated trace elements (1 mL L^{-1}) and vitamin mixture (V7 and V3, 1 mL L^{-1} each) as previously described (Widdel and Bak, 1992). Resazurin was added in trace amounts as a redox indicator, and the media were chemically reduced by Na_2S to ~0.4 mM total sulfide ($\sum H_2S = H_2S + HS^- + S^{2-}$). Each of the three media (4.2 L) was inoculated with 0.1 L of the resuspended sludge sample, corresponding to a final dry solids content of 1 g L^{-1}.

Incubation experiments with xenobiotics were done in 120 mL serum bottles with Teflon-coated butyl rubber stoppers (Apodan, Denmark) using the anoxic Hungate technique (Widdel and Bak, 1992). For addition of the xenobiotics to nominally 100 mg C L^{-1} (ISO, 1995a), a procedure for handling of poorly water-soluble organic compounds was used (ISO, 1995b). Hence, 1 mL of the stock solutions in DCM was transferred to serum bottles containing 0.1 g of powdered silica gel (60–200 mesh, i.e., 0.08–0.25 mm) and hereafter the DCM was evaporated (ISO, 1995b). Then, 100 mL aliquots of the inoculated media were anoxically transferred to the serum bottles. For each medium, 39 serum bottles were prepared. This comprised 30 bottles with xenobiotics (five replicates for each xenobiotic), three control bottles with silica gel only, three control bottles with silica gel and DCM, and three control bottles with silica gel and a solution of lactate and acetate (lactate/acetate; final concentration, 100 mg C L^{-1} each). Two of the five replicates with xenobiotics were directly frozen for chemical analysis of the initial xenobiotic concentration. The remaining replicates and the control bottles (i.e., a total of 27 bottles for each medium) were incubated in darkness at 30 °C on a thermostated rotary shaker (100 rpm).

The incubated suspensions were assayed for reduction of the added electron acceptors after 0, 1, 2, 4, 10 and 15 wk (samples representing time 0 were taken after incubation for <1 d). After the last sampling occasion, pH was measured in 10-mL aliquots of all suspensions. The bottles (with the remaining suspension) were then frozen for chemical analysis of the final concentration of xenobiotics.

2.3. Assays of anaerobic respiration and chemical analyses

Sulfate reduction in the incubations was estimated as the accumulation of $\sum H_2S$. At each sampling time ~2 mL of the incubations with SO_4^{2-} were withdrawn in a N_2-flushed syringe, filtered (0.45 μm) and transferred to 1 mL of 2% $ZnCl_2$ to precipitate the $\sum H_2S$ as ZnS (the precise amount of sample was determined gravimetrically). The mixture was frozen for subsequent ZnS analysis. To this end, two diluted samples (35 mL) were reacted with 2.8 mL of diamine reagent (Cline, 1969) and $\sum H_2S$ was determined spectrophotometrically at 670 nm (Milton Roy, Spectronic 301).

Iron reduction was assayed as the accumulation of HCl-extractable Fe(II), basically as described by Lovley and Phillips (1986). With an N_2-flushed syringe, 0.5 mL of the media with Fe(III) was transferred to 4.5 mL of 0.5 N HCl. After extraction for 15 min, the samples were filtered (0.45 μm) and frozen for Fe^{2+} analysis by the colorimetric ferrozine method (Stookey, 1970) using duplicate dilutions of the filtrates.

Nitrate reduction was assayed as the depletion of NO_3^-. Approximately 2 mL of the incubations with NO_3^- were withdrawn in a N_2-flushed syringe and filtered (0.45 μm). The filtrate was frozen for subsequent NO_3^- and NO_2^- analysis by single column anion chromatography as previously described by Elsgaard and Jørgensen (2002), but using a 20-μL injection loop. Retention times were 3.8 min for NO_2^- and 5.6 min for NO_3^-. Ammonium in the filtrates was measured colorimetrically by use of a microtiter plate method with four replicate wells for each analysis (Sims et al., 1995).

Initial and final concentrations of xenobiotics were evaluated by chemical analysis of samples from the start and the end of the incubation period. Samples were analysed by an accredited laboratory

Table 1
Physico-chemical properties of the tested xenobiotics.

Xenobiotic	Formula	CAS No.	MW[a] (g mol^{-1})	Log K_{ow}[b]	Water solubility (mg L^{-1})	Vapor pressure (Pa at 20–25 °C)	Refs.[c]
Acenaphthene	$C_{12}H_{10}$	83-32-9	154.2	3.9	3.9	0.3	[1,2]
Phenanthrene	$C_{14}H_{10}$	85-01-8	178.2	4.5	1.2	0.02	[1,3]
4-Nonylphenol (4-NP)	$C_{15}H_{24}O$	104-40-5	220.3	4.5	6	~0.3	[4]
Sodium dodecylbenzene sulfonate (LAS)	$C_{18}H_{29}SO_3Na$	25155-30-0	348.5	3.3	250×10^3	$(3–17) \times 10^{-13}$	[5]
Di(2-ethylhexyl)phthalate (DEHP)	$C_{24}H_{38}O_4$	117-81-7	390.6	7.5	3×10^{-3}	3.4×10^{-5}	[6]
1,2,4-Trichlorobenzene (1,2,4-TCB)	$C_6H_3Cl_3$	120-82-1	181.5	3.9–4.2	36	36	[7]

[a] MW, molecular weight.
[b] K_{ow}, octanol–water partition coefficient.
[c] Refs.: [1] – Anyakora (2007), [2] – Wei (1996), [3] – Haritash and Kaushik (2009), [4] – Hansen et al. (2002), [5] – HERA (2009), [6] – Pakalin et al. (2008), and [7] – van Wijk et al. (2006).

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