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Fate and behavior of organic compounds in an artificial saturated subsoil under controlled redox conditions: The sequential soil column system

Michael Nay, Mario Snozzi* & Alexander J. B. Zehnder
Department of Microbiology, Swiss Federal Institute for Environmental Science and Technology, Überlandstr. 133, 8600 Dübendorf, Switzerland (author for correspondence, e-mail: snozzi@eawag.ch)*

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Abstract

A system was developed to investigate the fate and behavior of anthropogenic organic contaminants at concentrations present in polluted subsoils and aquifers. A sequential soil column system was constructed to simulate redox conditions from methanogenic, sulfate-reducing, denitrifying, to aerobic conditions which normally occur in a leachate pollution plume. This system allowed the simulation of subsurface pollution with a range of xenobiotics and the observation of the microbial response to this contamination. After an adaptation period of up to about 7 months, 2,4-dichlorophenol and 2-nitrophenol were eliminated and perchloroethene disappeared almost completely in the methanogenic column. Toluene was partially transformed under sulfate-reducing conditions, and nearly completely in the nitrate-reducing column. The same applied to naphthalene under denitrifying and aerobic conditions. Aerobically, a fraction of benzene was transformed, and 1,4-dichlorobenzene decreased to very low residual concentrations in one system. No significant transformation of 1,1-dichloroethene could be seen.

Introduction

During the last decade, leachate pollution plumes (LPP's) downstream of hazardous waste sites have been investigated extensively (e.g. Christensen et al. 1989; Lesage et al. 1990; Lyngkilde & Christensen 1992; Kerndorff et al. 1993; Christensen et al. 1994; Johnston et al. 1996). Strongly reducing conditions are usually established next to a site. With distance they are influenced by abiotic and biotic processes such as dilution and transformation. LPP's represent longitudinal redox cascades including methanogenic, sulfate-reducing, iron-reducing, manganese-reducing, nitrate-reducing, and aerobic conditions (e.g. Lovley 1991; Lyngkilde et al. 1991; Lyngkilde & Christensen 1992) (Figure 1). Such LPP's can cover very large areas downgradient from the site (e.g. Schött 1992).

Several compounds are readily biodegradable in the laboratory but only very slowly or not at all in the environment (Roberts et al. 1982). For example benzene (BEN), perchloroethene (PER), and trichloroethene (TRI) have been found to persist in LPP's even decades after initial pollution (Freedman & Gossett 1989; Kerndorff et al. 1993; Nay 1994). However, in the laboratory, these compounds have been reported to be biodegradable under different redox conditions (e.g. Holliger 1995; Lovley et al. 1997).

In the subsurface, biodegradation may be limited by the lack of nutrients such as nitrogen or phosphorous, or of electron acceptors such as sulfate, nitrate, or oxygen. Additionally, bioavailability may be restricted by sorption effects (Alexander 1994; Pignatello & Xing 1996). Since leachates often contain some 50 or more different organic as well as inorganic compounds, bacterial metabolism may be inhibited by toxic effects (e.g. Burback et al. 1994; Dolan & McCarty 1995). "Natural" concentrations of the contaminants investigated are usually below 1 μg l^{-1} (Kowalewski 1995).

Biological transformations and sorption effects have been studied in laboratory soil columns, simulating a specific set of environmental conditions (e.g. Kuhn et al. 1985; Christensen et al. 1989; van der

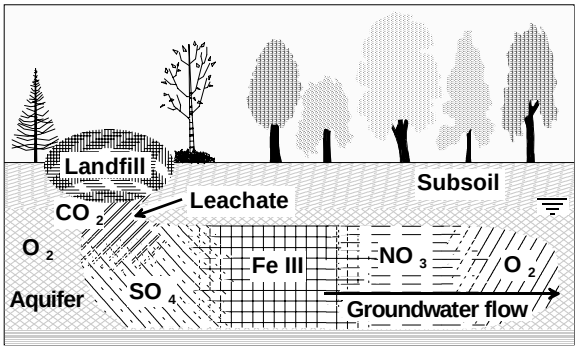

Figure 1. Scheme of a contamination plume as a result of a leaching landfill. Redox conditions present are characterized by methanogenic – (CO_2), sulfate-reducing – (SO_4), iron- (and manganese-) -reducing – (FeIII), nitrate-reducing – (NO_3), and aerobic conditions (O_2). The same texture for the different redox conditions is used for all figures.

Meer et al. 1992; Bosma et al. 1994; Langenhoff et al. 1996). Recently, a single column has been investigated, containing a redox gradient from aerobic to anaerobic (Hess et al. 1996). However, little has been done to simulate longitudinal redox sequences as present in LPP's. We have therefore developed a sequential soil column system (SSCS; Figure 2) which allows to separate and control different redox conditions from anaerobic to aerobic. Selected parameters such as nutrient concentrations or flow rates can be varied. Xenobiotics tested in this study are present in household LPP's (Kerndorff et al. 1993) as well as in aquifers polluted by hazardous waste sites (Nay 1994).

Materials and methods

Sequential soil column system

Each of the two SSCS's used consisted of four glass tubes (inner diameter (Ø) 2.5 cm, length 16 cm, net volume about 60 ml; Figure 2). These were wet-packed with washed, inoculated quartz sand (grain size 0.3 to 0.9 mm; Zimmerli AG, Zürich, Switzerland) and closed with Viton® stoppers (Maagtechnik, Dübendorf, Switzerland). Glass wool between matrix and stoppers prevented clogging of the connecting stainless-steel HPLC-tubings (1/16″ × 1/25″; ict AG, Basel, Switzerland). Stainless steel T-unions (1/16″; BGB-Analytik AG, Rothenfluh, Switzerland) were inserted for addition of xenobiotics and electron acceptors. Sample ports were installed directly before and after each column.

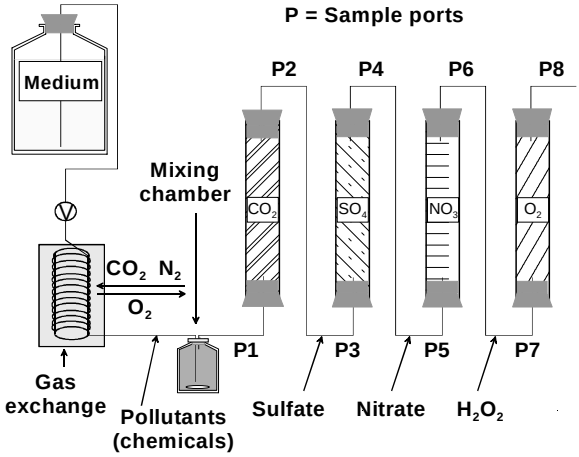

Figure 2. Scheme of the sequential soil column system. Medium flow was regulated by a peristaltic pump. Dissolved oxygen was replaced by passing the medium through silicon tubings placed in a gas-exchange chamber containing an atmosphere of nitrogen and carbon dioxide. A mixing-chamber ensured constant input concentrations of the xenobiotics. Soil columns had sample ports right before each inlet and right behind each outlet. The corresponding electron acceptors were introduced by a syringe pump to each column. The redox conditions established were methanogenic (CO_2), sulfate-reducing (SO_4), nitrate-reducing (NO_3), and aerobic conditions (O_2).

All substrates and electron acceptors were continuously added in constant concentrations. Medium flow (about 60 ml d^{-1}) was regulated by a peristaltic pump system (MCP-CA8) with ismaprene® tubings (Ø 0.38 mm; Ismatec SA, Glattbrugg, Switzerland). Xenobiotics and electron acceptors were added by a syringe pump (Harvard Apparatus Mod. 55 5920; Indulab AG, Gams, Switzerland). Glass syringes for addition of xenobiotics and electron acceptors were equipped with custom-made stainless steel pistons with Teflon seals.

Silicon tubings (0.5 mm × 0.5 mm × 5 m; Maagtechnik, Dübendorf, Switzerland) in a gas exchange chamber (99.5% N_2, 0.5% CO_2) allowed to remove oxygen from the passing medium (Zeyer et al. 1986). A mixing-chamber (glass, about 35 ml volume) in front of the first column ensured a constant input concentration of the feed.

For sampling, a 30 ml glass syringe (Fortuna hypodermic syringe; Huber AG, Reinach, Switzerland) was connected to the HPLC-tubing with a piece of maprene tubing (Maagtechnik, Dübendorf, Switzerland). The syringe was filled by the pressure of the medium flow during approximately 12 h, interrupting the continuous flow of the bulk solution and thus disturbing the conditions in the following columns.

Therefore, every sample series was started at the last sample port (P8) and progressed stepwise toward port 1. A recovery time of about 12 h between single samples and a minimum recovery time of 5 days undisturbed operation were allowed between sample series.

Organisms

Inocula were collected at the drainage system (shaft # S11) of a landfill (Riet, Winterthur, Switzerland). Material was obtained from leachate channels (sample 1a: Jan. 1995; 1b: Sept. 1995) and the leachate collecting pool under these channels (sample 2a: Jan. 1995; 2b: Sept. 1995). Additionally, anaerobic primary sludge was used from the municipal wastewater treatment plant Glatt (Jan. 1995: sample 3). During hydrogeological examination, drill core samples were taken from a recultivated landfill near Riet, dating back to the 1920's (Sept. 1995: sample 4).

These samples were mixed (1 : 1 : 1) in a nitrogen atmosphere (SSCS1: 1a, 2a, 3; SSCS2: 1b, 2b, 4). Quartz sand (300 ml) was inoculated with about 10 ml of the resulting material and added to the columns. The SSCS's were operated in the dark to prevent growth of photosynthetic organisms. Temperature was kept constant at 25 ± 1 °C.

Medium and xenobiotics

The following medium was prepared (final concentrations, in mg l^{-1}): NaCl (350), KCl (298), NH_4Cl (53), $MgCl_2 \cdot 6\ H_2O$ (102), $CaCl_2 \cdot 2\ H_2O$ (37), K_2HPO_4 (8.7), $Na_2Wo_4 \cdot 2\ H_2O$ (10^{-3}), $Na_2SeO_3 \cdot 5\ H_2O$ (10^{-3}), Na-acetate (33). The trace element solution contained (final concentrations, in μg l^{-1}): Na_2-EDTA (1000), $FeCl_3 \cdot 6\ H_2O$ (540), $CoCl_2 \cdot 6\ H_2O$ (38), $MnCl_2 \cdot 6\ H_2O$ (20), $ZnCl_2$ (13.6), Na_2MoO_4 (7.3), H_3BO_3 (1.2), $NiCl_2 \cdot 6\ H_2O$ (4.8), and $CuCl_2 \cdot 2\ H_2O$ (0.85). After autoclaving, a vitamin solution (1 : 1000; Egli & Weilenmann 1989) was added after filter sterilization (FP 030/3; Schleicher & Schüll, Dassel, Germany). The medium was buffered by the addition of about 1 g l^{-1} small calcite stones.

Xenobiotics tested were (in final concentrations, μg l^{-1}): perchloroethene (PER, 100), 1,1-dichloroethene (DCE; 100), 1,4-dichlorobenzene (DCB; 150), 2,4-dichlorophenol (DCP; 100), 2-nitrophenol (NP; 100; Riedel de Häen, Seelze, Germany), benzene (BEN; 700), toluene (TOL; 300), naphthalene (NAP; 80). The compounds were first dissolved in ethanol and filled up with water to one liter. Final ethanol content in the system was about 12.5 μl l^{-1} (9.9 mg l^{-1} = 215 μM).

Electron acceptors and acetate at the corresponding column inlets were (final concentrations, in mg l^{-1}): sulfate-reducing: $(NH_4)_2SO_4$ (225) and Na-acetate (3.3); nitrate-reducing: $NaNO_3$ (400) and Na-acetate (3.3); aerobic: H_2O_2 (17) and Na-acetate (1.65).

Analytical methods

Volatile compounds were quantified with a Hewlett Packard 5890 Series II gas chromatograph (GC) (Hewlett Packard AG, Urdorf, Switzerland). Analytical columns (J&W Scientific; BGB-Analytik AG, Rothenfluh, Switzerland) were a DB-5.625 (0.32 mm Ø × 30 m length, 0.5 μm film thickness) at the flame ionization detector (FID) and a DB-624 (0.32 mm × 60 m, 1.8 μm) at the electron capture detector (ECD).

Twenty ml samples were extracted with 0.6 ml of hexane (Burdick & Jackson Brand) and tetrachloroethane and *m*-xylene as the internal standards. Three μl of the organic phase were injected splitless but switched to split mode (98%) 0.5 min after injection. Carrier and auxiliary gases were He (flow rate 1.2 ml min^{-1}) and N_2 (30 ml min^{-1}), respectively, for both detectors. The flame of the FID was maintained by H_2 (30 ml min^{-1}) and air (400 ml min^{-1}). All gases (impurities less than 40 ppm; Carbagas, Zürich, Switzerland) were flushed through a gas filter system (Hewlett Packard AG, Urdorf, Switzerland).

The ECD (injector 250 °C, detector 300 °C) oven program started at 60 °C (kept 4 min) with ramps at 110 °C (3 min), 170 °C (17 min), and 240 °C (10 min). FID program (injector 200 °C, detector 250 °C) started at 40 °C (5 min), with ramps at 100 °C (5 min) and 240 °C, (10 min). Temperature increase was always 15 °C min^{-1}.

Analytical errors and recoveries were lower than 5% (except DCP: 7.5%) and between 86 and 114% (except DCP and NP: 46 and 61%). Detection limits were 15 μ g l^{-1} or lower.

High performance liquid chromatography (HPLC) was conducted with a high-precision pump MD 480, a diode array detector UVD 340 and a GINA 50-autosampler (Gynkotek, Regensdorf, Switzerland). A Waters Novapak C_{18} stainless steel analytical column (Ø 3.9 mm, length 300 mm; Waters, Rupperswil, Switzerland) and the corresponding precolumn were used.

Eluent a) for reversed phase chromatography was a 10 mM phosphate buffer (NaH_2PO_4, H_3PO_4, pH

3.0), eluent b) consisted of a mixture of 90% methanol (HPLC grade) and 10% eluent a). Each run (with 10 to 100 μl of sample) started with 82% eluent a) and 18% eluent b) (flow rate: 1.0 ml min^{-1}; wavelength 220 nm). Eluent b) was continuously increased to 38% until min 10, to 40% until min 15, to 80% until min 40, and finally to 90% until min 47. Analytical errors were lower than 5%, detection limits were 200 $\mu g\ l^{-1}$ or below.

Ion chromatography (IC) was performed with a gradient pump GP40, a conductivity detector CD20, and an autosampler AS3500. Analytical column was an IonPac® AS11 (10–32; Ø 4 mm) with an IonPac® precolumn AG11 (10–32; Ø 4 mm) and an IonPac® ATC-1 (10–32). The suppressor was an ASRS-I 4 mm anion self regenerating suppressor (all equipment: Dionex, Olten, Switzerland).

Eluent A) consisted of 60 mM borate ($Na_2B_4O_7$), eluent B) was a 1 mM borate solution (Ammann & Rüttimann 1995). Eluents were degassed by vacuum-ultrasonification (10 min) and flushed with helium (5 min). Runs (injection volumes 5 to 100 μl) started with 2% eluent A) and 98% eluent B) (flow rate 1.0 ml min^{-1}). Eluent A) was increased to 6% until min 4 and to 98% until min 16.

Each sample (1.5 ml) was supplemented with NaOH (100 μl, 1 M), shaken, and centrifuged (about 14000 g, 5 min). Supernatants were filtered (4 mm nylon filters, 0.5 μm; Semadeni, Ostermundingen, Switzerland) and filled into GC vials which were sealed with Teflon septa (Schmidlin AG, Neuheim, Switzerland). Between 10 and 100 μl of sample were injected for a run. Analytical errors were lower than 5%. Detection limits were 1 mg l^{-1} or below.

Oxygen was quantified by a microprocessor OXI-meter 196 (Wissenschaftlich-Technische Werkstätten, Weilheim, Germany). Sulfide was measured by a photometric method (Anonymous 1983).

If not specified, all chemicals were obtained from Fluka AG, Buchs, Switzerland. All solutions were prepared with deionized water (Seradest-Serapur system; Seral, Ransbach-Baumbach, Germany). To avoid overloaded figures, error bars are not shown.

Results

Transformations

In SSCS1, most of the xenobiotics were transformed after 5 to 6 months of operation. Figures 3 and 4 show results obtained about 8 and 11 months after inoculation. DCP and NP were detected only at the inlet of the system. About 99% of the PER added was removed under methanogenic conditions. No further transformation was seen in the other columns. The residual concentrations of TOL were about 25 and 4% of the feed after the sulfate- and the nitrate-reducing column, respectively. About 60% of the NAP added were found after the nitrate-reducing, less than 1% after the aerobic column. Aerobically, DCB and BEN decreased to less than 1% and about 15% of their initial concentrations. No significant turnover of DCE was observed.

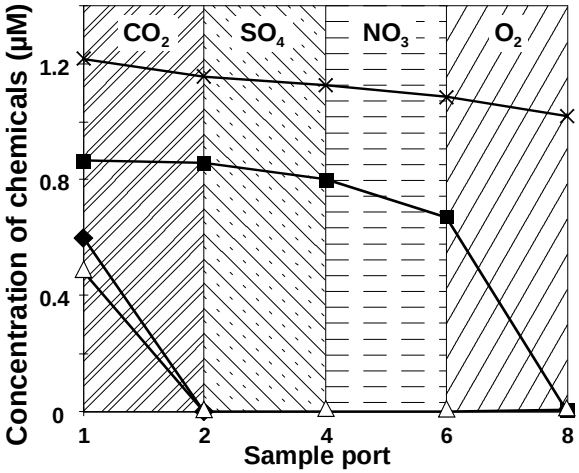

Figure 3. Chlorinated hydrocarbons in SSCS1 8 months after start. △, PER; ×, DCE; ■, DCB; ♦, DCP.

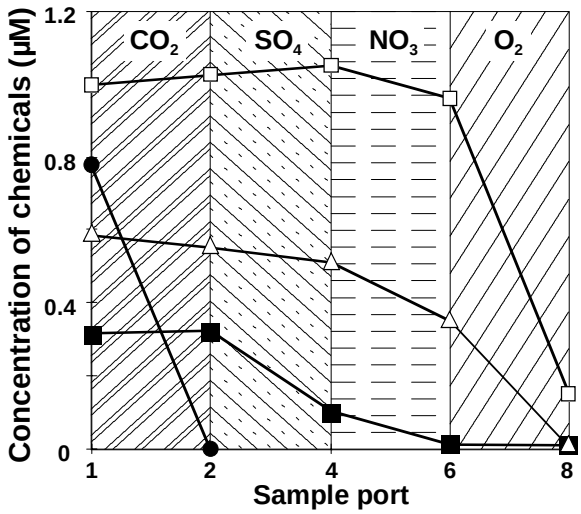

Figure 4. Non chlorinated hydrocarbons in SSCS1 11 months after start. □, BEN (concentration × 10^{-1}); ■, TOL (concentration × 10^{-1}); △, NAP; ●, NP.

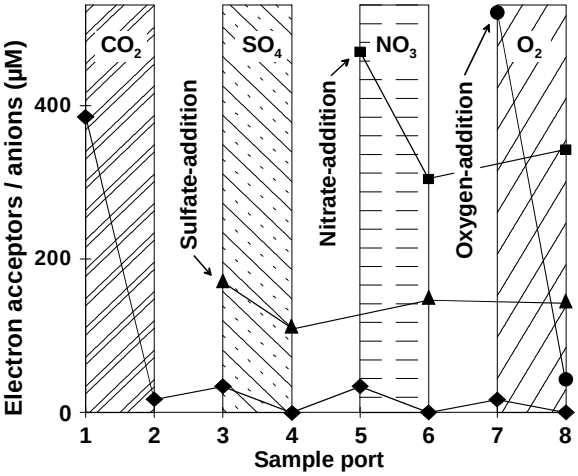

Figure 5. Anions in SSCS1 about 8 months after start. ♦, acetate; ▲, sulfate (concentration $\times$ 10^{-1}); ■, nitrate (concentration $\times$ 10^{-1}); ●, H_2O_2.

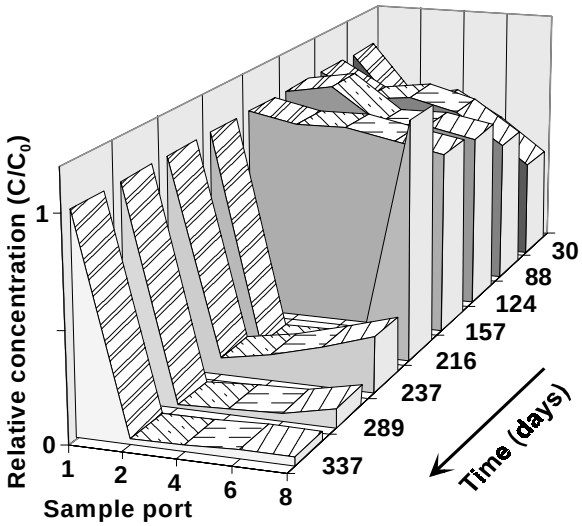

Figure 6. PER in SSCS2. X-axis: sample ports; Y-axis: ratio c/c_0; Z-axis: time (d). Different redox conditions are indicated by the texture of the columns.

At sample port (P5) (nitrate-reducing column inlet), the TOL concentration was about 30% smaller than after the sulfate-reducing column (P4). NAP and DCB were almost 20% lower at the inlet of the aerobic column (P7) than at P6. Additionally, formation of a gray material directly behind the nitrate introduction clogged tubings every 2 to 3 months. This material was inspected by phase-contrast microscopy and consisted of a large number of bacteria, indicating presence of biofilm. No particulate materials were seen. However, only few bacteria were present in the medium supplied to this part of the tubing. These findings suggest increased substrate consumption in the tubings, probably after addition of electron acceptors.

Acetate and electron acceptors

Acetate decreased to about 4% of the initial concentration of 0.4 mM in the methanogenic column. Acetate added to the inlets of the other columns was almost completely removed. Between 60 and 180 μM sulfate was reduced to sulfide in the sulfate-reducing column. Sulfide disappeared again nearly completely concomitant with some increase of sulfate in the nitrate-reducing column. Nitrate removal under denitrifying conditions was between 90 and 210 μM. Traces of nitrite after the nitrate-reducing column were reoxidized under aerobic conditions (Figure 5). Between 10 and 25% of the initial concentration of oxygen supplied to the aerobic column (ca. 1.1 mM) were still present at its outlet.

Long term behavior of SSCS

The methanogenic column of SSCS2 was saturated with PER after 3 to 4 months of operation, the whole matrix after approximately 5 months (Figure 6). PER transformation started under methanogenic conditions. Two months later, sulfate- and nitrate-reducing columns released only very low amounts of PER, but this pollutant was still present at the aerobic outlet in almost initial concentrations. After another 4 months all outlet concentrations remained below 5% of the PER fed (Figures 6 and 7). Traces of TRI, the first metabolite of reductive dechlorination, were detected but remained below the quantification limit of 10 nM.

After 3 months, methanogenic and sulfate-reducing columns were almost saturated by TOL. At the denitrifying column outlet, TOL did not exceed 40% of the concentration added, indicating concurrent saturation and transformation in this column. More than 1 year after incubation, a residual concentration of about 30% of the TOL fed was released after sulfate-reducing conditions but completely consumed in the nitrate-reducing column (Figure 8).

Matrix saturation with NAP was achieved after 5–6 months before this compound was converted nearly completely under aerobic conditions. Four months after inoculation, DCP was eliminated in the methanogenic column. NP was always found in the inlet, but never in the outlet of the methanogenic column. Removal of BEN under aerobic conditions

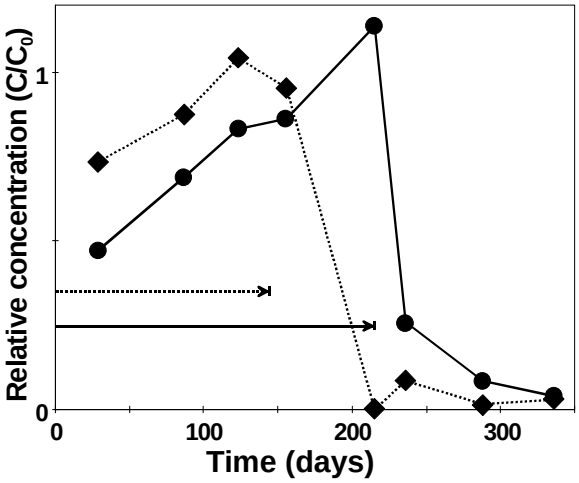

Figure 7. PER in SSCS2 at sample ports 2 (end of methanogenic column; ♦, and dotted line) and 8 (end of aerobic column; ●, and solid line) (see also Figure 6).

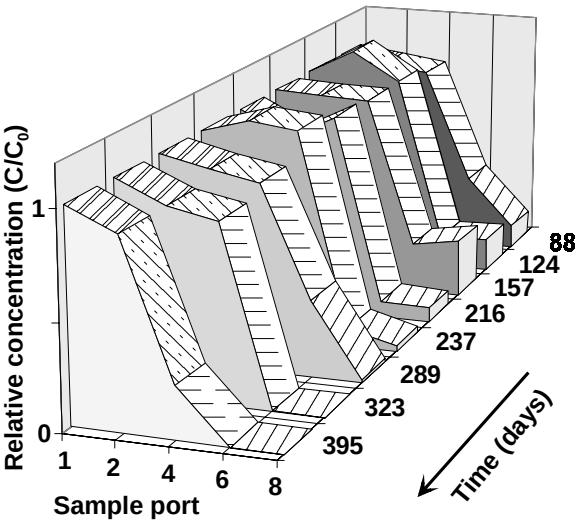

Figure 8. TOL in SSCS2. *X*-axis: sample ports; *Y*-axis: ratio c/c_0; *Z*-axis: time (d). Different redox conditions are indicated by the texture of the columns.

started only after more than 5 months of operation. Occasionally but inconsistently, some transformation of BEN was also observed under nitrate-reducing conditions. DCB and DCE were not significantly transformed in SSCS2.

Discussion

Locally, subsurface environments can be very heterogeneous (e.g. grain size, clay lenses, organic matter). This reduces the predictability of local groundwater flow conditions and behavior of xenobiotics. But considering its huge extension, an aquifer can still be regarded as a more or less homogeneous system. The SSCS allowed to control basic parameters such as the medium flow rate or concentrations of compounds. Therefore, it could be used as a suitable instrument for investigation of important biotic and abiotic processes on xenobiotics.

Redox conditions

Sequential redox conditions as present in LPP's were reproduced within the SSCS, including methanogenic, sulfate-, nitrate-reducing, and aerobic conditions. They were maintained by addition of the respective electron acceptors in excess from lower to higher redox potentials. This prevented a change of redox conditions towards higher potentials within a column. After 4 to 6 months a redox steady state developed in the SSCS.

Significant sulfate- and nitrate-reduction were first observed after about 2 to 3 months of operation. In both SSCS's, a stable redox steady state had developed after 4 to 6 months. Sulfide was partly reoxidized to sulfate in the nitrate-reducing column. Some sulfide may also have been precipitated within the soil matrix (Schachtschabel 1982).

Microbial activity

The behavior of the pollutants followed roughly three steps. First, the matrix of SSCS was saturated either partly (e.g. TOL) or fully (e.g. PER). The biomass was probably not yet able, not adapted, or too small to consume significant amounts of xenobiotics under the given conditions. Microbial growth, combined with adaptation processes, may have taken place as a second step after stabilization of redox conditions. Third, substrates were transformed. For example, PER almost completely saturated the matrix of the SSCS within 4 months. The biomass responsible for transformation was established about 6 months after inoculation. Another 4 months were required to wash out PER from the subsequent columns.

Transformations

All compounds tested except DCE in both SSCS's and DCB in SSCS2 were converted, most of them to low or not measurable amounts. Although biodegradation in columns has often been reported to take place in the first few centimeters (e.g. Schraa et al. 1986; van der

Meer et al. 1992; Hess et al. 1996), TOL in the sulfate-reducing, NAP in the nitrate-reducing, and BEN in the aerobic column were not fully eliminated. All compounds are known to be biodegradable under one or more of the conditions tested (e.g. Mihelcic & Luthy 1988; Mihelcic & Luthy 1991; Beller et al. 1996; Lovley et al. 1997).

Recently, Langenhoff et al. (1996) have reported that benzoate addition increased NAP transformation under nitrate-reducing conditions, indicating the need of a primary substrate. Since acetate was completely consumed in the sulfate- as well as in the nitrate-reducing column, its absence could have limited NAP degradation. On the other hand, poorly biodegradable metabolites can also restrict conversion of compounds. For example, Burback et al. (1994) have reported that the presence of 4-CP – as recalcitrant metabolite of DCP – limits degradation of TOL. Inhibitory effects by vinyl chloride (VC) – a metabolite of PER and TRI – are known (e.g. Dolan & McCarty 1995). Therefore, products which were not detectable by the methods used may have lowered turnover of certain compounds.

SSCS and environmental conditions

The heterogeneity of natural subsurface structures creates specific niches which change in space and time and may be distinctly different from their surroundings (e.g. locally high amounts of organic carbon compounds, iron- or manganese oxides). Methanogenic bacteria have been found to be restricted to the methanogenic zone. Sulfate-reducing bacteria (SRB) were present under methanogenic and sulfate-reducing conditions. However, iron- and manganese-reducers were observed all over the plume with no significant difference in their numbers and only little activity, even under corresponding redox-conditions. Nitrate reducers were almost found in similar numbers all over the plume except in the methanogenic zone. But denitrification took place only in nitrate-reducing zones with high concentrations of this electron acceptor (Ludvigsen et al. 1995).

Although pollution plumes are more or less stable over a couple of years (Lyngkilde & Christensen 1992; Albrechtsen et al. 1995), dynamic systems such as LPP's react to external influences such as stormwater events and addition of compounds. Therefore, no complete steady state may be reached at any site. Compared to such a plume, SSCS's were more used as systems in steady state. The reaction of a SSCS on changes of conditions such as different flow rates or omission and addition of the electron donor will be presented in a further publication.

But SSCS's may also become designed as tools to develop or test remediation technologies with small scale systems. Polluted groundwater may be decontaminated with a sequential "*ex-situ*" system, containing the conditions required for safe (bio-) transformation. Sequential systems may also be modified for investigation of ecological processes within a microbial population. Variations in the composition of bacterial populations may be examined during establishment of a steady state or as a response to changed conditions.

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