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Behavior of chemical contaminants under controlled Redox conditions in an artificial sequential soil column system and in batch cultures

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Abstract

A leachate pollution plume was simulated in a sequential soil column system (SSCS), defined by a longitudinal redox cascade from methanogenic, sulfate-, nitrate-reducing to aerobic conditions. A mixture of contaminants, including compounds such as perchloroethene (PER), 1,1-dichloroethene (DCE), 1,4-dichlorobenzene (DCB), 2-nitrophenol (NP), and benzene (BEN) was supplied to the methanogenic column; their conversion was followed during passage through the SSCS. After establishment of a steady state, parameters were changed to simulate changes in the environment and to follow the reactions of the system. Acetate, initially introduced as an anaerobically easy degradable carbon source, was first omitted and later added again to the system. Furthermore, the medium flow rate and by that the load of the test compounds was increased 4 fold. Changes transiently increased the residual concentrations of most of the xenobiotics. Within seven months after acetate omission, turnover of contaminants had almost returned to the original steady state. Flow and load increase resulted in increased transformation of compounds such as PER, NP, and BEN. After readdition of acetate, the turnover of most of the xenobiotics increased within the next five weeks. Microbial populations for the anaerobic batch cultures, obtained from the specific columns, were used to investigate metabolites formed and conversion kinetics of the xenobiotics. Almost the same conversion pattern was found in batches and columns, except no transformation of DCE and DCB was detected in the SSCS, though both compounds were converted in batch cultures.

Introduction

Many hazardous chemicals are completely biodegradable in the laboratory but persist in the environment (Roberts et al. 1982; Kerndorff et al. 1993). Knowledge about the fate of such compounds in leachate pollution plumes (LPP's) is important, but extensive investigations in the field are costly in terms of both time and money (SRU 1989; LFU BW 1993). Experimental results obtained from the environment are often difficult to interpret since factors such as heterogeneities of the matrix (Schachtschabel et al. 1982; LFU BW 1993) and changing hydraulic conditions such as stormwater infiltration (Kempf et al. 1986) can affect flow field and actual chemical composition of groundwater.

Despite these interferences, LPP's downstream from landfills or waste sites represent almost steady state, spatially defined redox gradients including methanogenic, sulfate-, iron-, manganese-, nitrate-reducing, and aerobic conditions (Lovley 1991; Lyngkilde et al. 1991; Bourg 1992; Lyngkilde & Christensen 1992; Christensen et al. 1994).

Landfill leachates can contain high concentrations of acetate (> 1 g l^{-1}) (Christensen et al. 1989; Blakey et al. 1992; Millot & Courant 1992). As an easily degradable electron donor it enhances the development of anaerobic conditions and can be beneficial for microbial reductive dechlorination (Holliger 1992; Picardal et al. 1995; Doong et al. 1997; Nay et al. 1999).

We have recently examined a redox cascade in an artificial sequential soil column system (SSCS), oper-

ated with a range of model compounds. Redox conditions were established and controlled by adding the specific electron acceptors in excess to the subsequent columns. Acetate as an easy degradable carbon source supported the development of anaerobic conditions. All parameters such as substrate concentrations or flow rate of the bulk solution were held constant. A steady state established and enabled the observation of (bio-) transformations under controlled redox conditions. Most of the contaminants were transformed almost completely in this system. Intermediates and metabolites, if produced, could not be detected by the analytical methods used, since the xenobiotics were introduced in concentrations close to those found in LPP's (between 80 and 150 μl^{-1}, except toluene (250 μl^{-1}) and benzene (550 μl^{-1})) (Nay et al. 1999).

In the environment the parameters influencing behavior of LPP's are not constant. This can influence the degradation of the xenobiotics and be one of the reasons why they persist. We therefore extended our work and investigated the reaction of the system to changing conditions. In this paper we report the influence of changes of environmental conditions such as loading and flow rate, omission, and addition of acetate on transformations during passage through the SSCS. Batch experiments were also performed under the respective redox conditions to study the production of metabolites and to analyze transformation kinetics.

Materials and methods

Sequential soil column system

The SSCS consisted of a sequence of four glass columns (net volume about 60 ml each), containing quartz sand as the matrix and microbial populations obtained from the drainage system of the landfill Riet (Winterthur, Switzerland). A steady state of a sequential redox cascade, consisting of methanogenic, sulfate- nitrate-reducing, and aerobic conditions, was established within 15 months of operation. For more details on the construction and maintenance of the SSCS see experimental details for SSCS2 (Nay et al. 1999).

Batch experiments

Experiments were designed for methanogenic, sulfate- and nitrate-reducing conditions. Glass serum flasks (net volume approximately 580 ml) were filled with about 2.5 g of calcite stones (for carbonate-bicarbonate buffer system), 10 g of washed quartz sand (grain size 0.3 to 0.9 mm; both Zimmerli AG, Zurich, Switzerland), and medium (500 ml) under strictly anaerobic conditions. After inoculation, the cultures were closed gas proof with Viton® stoppers (Maagtechnik, Duebendorf, Switzerland). Sterile controls were autoclaved (121 °C, 1 bar, 1 h) twice within two days. For each condition, two cultures and one control were operated for 4 months on a laboratory shaker (100 rpm) at 25 ± 1 °C in the dark to prevent growth of photosynthetic organisms. Sampling was performed with 10 ml glass syringes (Fortuna hypodermic syringe; Huber AG, Reinach, Switzerland).

Inocula

The inocula for the anaerobic batch cultures were obtained from 10-month old precultures, maintained under the respective redox conditions. The precultures (two for each condition) had been inoculated with material from the drainage system of the Riedt landfill and with active column material from the SSCS's, operated for 5 and 13 months under the respective redox conditions (Nay et al. 1999). All steps were performed in a nitrogen atmosphere.

Medium and xenobiotics

The medium previously described by Nay et al. (1999) was used for the SSCS. Compositions of the batch culture media are given in Tables 1 and 2. Trace elements and vitamins (Nay et al. 1999) were added before and after sterilization (121 °C, 1 bar, 1 h), respectively. The carbon and energy sources benzoate (1 mM) and acetate (about 0.7 mM) were added again to the cultures at days 51 and 92. At the same time, also electron acceptors ($FeSO_4$ respectively $NaNO_3$) and xenobiotics were supplied as well at the initial concentrations. To prepare stock solutions, the xenobiotics were first dissolved in ethanol (Table 1; Nay et al. 1999).

The batch cultures were monitored for the presence of possible conversion products of the xenobiotics. Intermediates tested for were trichloroethene (TRI), *cis*- and *trans*-1,2-dichloroethene, chlorobenzene, 2-chlorophenol, 4-chlorophenol (CP), 2-aminophenol (AP), phenol, 1-naphthol, salicylate, salicyl aldehyde, benzaldehyde, and benzyl alcohol.

Table 1. Final concentrations in μM of the xenobiotics added to the batch experiments and SSCS

Xenobiotics	PER	DCE	DCB	DCP	NP	BEN	TOL	NAP
[1] Day 1	0.7	1.5	2.7	2.5	2.9	7.7	3.8	2.7
Day 51	0.2	0.2	1.0	1.9	3.5	5.1	2.7	2.3
Day 92	1.2	2.2	2.0	1.5	2.2	3.8	3.3	2.3
[2]	0.6	1.0	1.0	0.6	0.7	7.0	2.5	1.0

[1] Xenobiotics added to batch cultures at the days indicated. Final ethanol concentration was 39.2 mg l^{-1} (850 μM).
[2] Xenobiotics in SSCS in μM in the medium, constant during all experiments. Final ethanol concentration was 9.9 mg l^{-1} (215 μM).

Table 2. Final concentrations in mg l^{-1} of the batch culture media. Acetate (0.7 mM) and benzoate (1 mM) were added as carbon sources. Final contents of the electron acceptors were 9.5 mM SO_4^{2-} and 9.4 mM NO_3^-

Conditions	Methanogenic	Sulfate-reducing	Nitrate-reducing
Mineral salts	NaBr (410)	Na_2SO_4 (570)	$NaNO_3$ (340)
	KBr (480)	K_2SO_4 (700)	KNO_3 (40)
	NH_4Br (100)	$(NH_4)_2SO_4$ (130)	NH_4NO_3 (80)
	$MgBr_2$ (150)	$MgSO_4 \times 7H_2O$ (120)	$MgSO_4 \times 7H_2O$ (120)
	$CaBr_2$. aq. (50)[a]	$CaBr_2$. aq. (50)[a,b]	$Ca(NO_3)_2 \times 4H_2O$ (50)

[a] ($CaBr_2$. aq.: $H_2O < 15\%$).
[b] Added after autoclaving by filter sterilization (FP 030/3; Schleicher & Schüll, Dassel, Germany) to prevent precipitation of $CaSO_4$ during sterilization.

Analytical methods

Samples were prepared as described before (Nay et al. 1999). Xenobiotics and possible metabolites were monitored by gas chromatography (GC) and high pressure liquid chromatography (HPLC); electron acceptors and acetate and benzoate were determined by ion chromatography (IC). Analytical errors were below 5% (except for 2,4-dichlorophenol (DCP): with a 7% error). Recoveries (GC) of all xenobiotics were between 86 and 113% (except DCP: 59%, and nitrophenol (NP): 71%). Quantification limits of GC, HPLC, and IC analysis were less than 250 nM, 5 μM, and 20 μM, respectively. To avoid busy figures, error bars are omitted. Although analytical errors for AP standards (together with all other xenobiotics and possible metabolites in deionized water) were only 4%, polymerization of aminoaromatic compounds (Paris & Wolfe 1987) in the experimental set-up or during preparation or storage of the samples may have partially prevented detection of AP.

Occasionally, an enzymatic quantification method (Böhringer, Mannheim, Germany) was used for verification of the acetate measurements by IC (differences less than 10%). Oxygen contents were determined with a microprocessor OXI-meter 196 (Wissenschaftlich-Technische Werkstätten, Weilheim, Germany).

All chemicals were purchased from Fluka AG (Buchs, Switzerland) unless otherwise specified. All solutions were prepared with deionized water (Seradest-Serapur system; Seral, Ransbach-Baumbach, Germany) and, when necessary, degassed with nitrogen (Carbagas, Zurich, Switzerland).

Results

Column studies

The sequential soil column system (SSCS) used here corresponds to the SSCS2 described in detail before (Nay et al. 1999). It was run for 15 months before influent and flow conditions were changed. The system converted perchloroethene (PER), 2,4-dichlorophenol (DCP), 2-nitrophenol (NP), benzene (BEN), toluene (TOL), and naphthalene (NAP), but not 1,1-dichloroethene (DCE) and 1,4-dichlorobenzene (DCB). From day 460 to day 819 acetate supply was stopped. The flow rate of the

medium was increased 4-fold at day 668 while the concentrations of all compounds were kept constant resulting in a 4-fold load.

Acetate dynamics

Acetate as an anaerobically easy degradable carbon source (input 400 μM; load 24 μmol d^{-1}) was initially added to each column to set the appropriate redox conditions, to serve as an electron donor for dehalogenation reactions, and to establish steady state conditions within the SSCS. Acetate was almost completely consumed in all columns during these initial steady state conditions (Nay et al. 1999); only very low acetate concentrations were detected after the individual columns. The omission of acetate in the feed resulted in concentrations of acetate of between 175 and 220 μM (10.5 to 13.2 μmol d^{-1}) after the methanogenic column. Production of acetate in the methanogenic column under these conditions was confirmed by ezymatic acetate determinations. The resulting input of acetate to the sulfate-reducing column was consequently consumed to residuals of less than 2 μmol d^{-1} (not shown). After the four-fold increase of flow and load at day 668, the residual acetate concentration at the methanogenic outlet decreased increased slightly to from 155 to 195 μM, equivalent to an increase of the output of from 37 to 47 μmol acetate d^{-1}.

Readdition of acetate to the feed (48 μmol d^{-1}) temporarily increased the acetate concentration after the methanogenic column to approximately 60 μmol d^{-1} which decreased to about 30 μmol d^{-1} five weeks after the change. At the same time 24 μmol acetate d^{-1} were added to each of the other columns where it was increasingly consumed. Nevertheless, five weeks after readdition complete degradation of acetate was only found in the aerobic column.

Dynamics of pollutants degradation

NP (input: 0.7 μM; load: 42 before/168 nmol d^{-1} after flow and load increase) was always eliminated in the methanogenic column. Less than 5% decrease of DCE and DCB was observed under all conditions tested in the SSCS experiment.

Omission of acetate resulted in a transient partial breakthrough of PER (input 0.6 μM; 35 nmol d^{-1}) (Figure 1a). After increasing flow and load (to 140 nmol d^{-1}) the residual concentration increased to about 30% of the feed (Figure 1b). Despite a higher removal rate (36 nmol d^{-1} before, almost 100 nmol d^{-1} after the change) PER was not fully eliminated under methanogenic conditions and not converted in subsequent columns. After acetate omission, traces of trichloroethene (TRI) below 12 nM (0.7 nmol d^{-1}) were always detected after the methanogenic column. The residuals of TRI increased up to 17 nM (4 nmol d^{-1}) after changing flow and load (not shown). Dichloro- and chloroethenes were analyzed for but not found.

DCP (input 0.6 μM; 35 nmol d^{-1}) was initially fully eliminated in the methanogenic column. Five weeks after acetate omission methanogenic conversion of DCP transiently decreased to less than 13 nmol d^{-1}. Five months after the change DCP was again almost completely transformed under these conditions (Figure 1c). Two months after changing flow and load to 140 nmol d^{-1}, only 17 nmol DCP d^{-1} were converted, but three months later the consumption rate had risen to about 50 nmol d^{-1} (Figure 1d). One week after readdition of acetate, methanogenic transformation of DCP was below 15 nmol d^{-1} but increased to about 30 nmol d^{-1} within the next four weeks (not shown). DCP was (partially) consumed in all other columns when supplied.

TOL (input 2.5 μM; 150 nmol d^{-1}) was first partially transformed under sulfate- and completely under nitrate-reducing conditions. Initially, acetate omission resulted in a partial breakthrough, but later less than 0.6 and 0.25 μM TOL were detected after the sulfate-reducing and the aerobic column, respectively (Figure 1e). Flow and load change (to 600 nmol TOL d^{-1}) again transiently increased the residual concentrations after the columns supplied with sulfate and nitrate. Later, TOL was completely removed under nitrate-reducing conditions (Figure 1f). When acetate addition was resumed, 425 nmol d^{-1} of TOL were measured after the sulfate-, and about 300 nmol d^{-1} after the nitrate-reducing column (not shown).

BEN was only transformed aerobically. Between 30 and 50% (125 to 210 nmol d^{-1}) of the initial concentration of 7 μM (about 420 nmol d^{-1}) were consumed after acetate omission. However, BEN conversion drastically increased to values from 60 to 80% (1.0 to 1.4 nmol d^{-1}) of the input of about 1.7 μmol d^{-1} after flow and load increase. Despite this higher removal rate, BEN was not completely consumed. The turnover decreased to less than 520 nmol d^{-1} immediately after readdition of acetate (data not shown).

Initially, NAP (input 1 μM, 60 nmol d^{-1}) could not be detected at the aerobic outlet. Acetate omission transiently resulted in an aerobic output of about

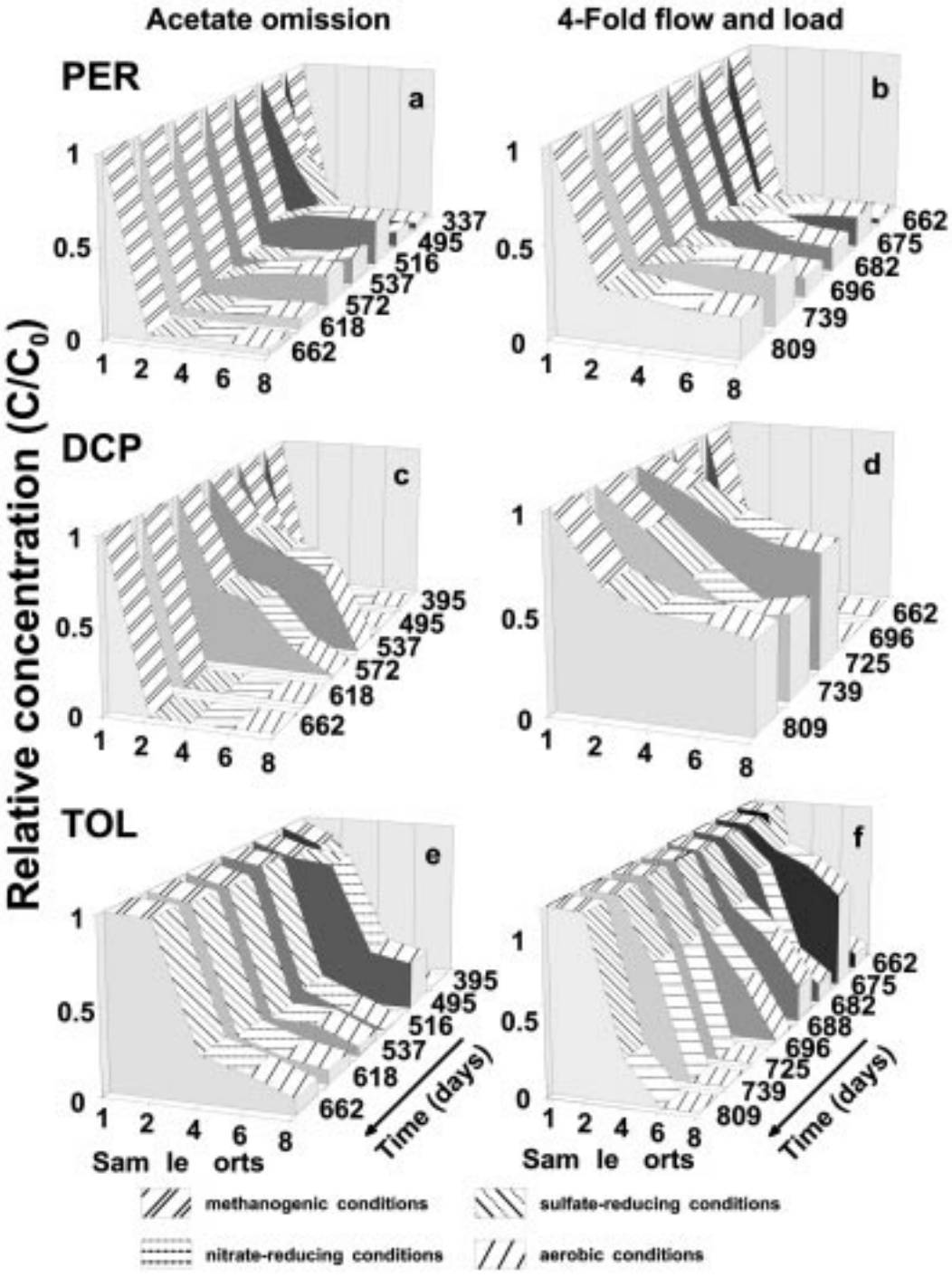

Figure 1. PER, DCP, and TOL in the SSCS after acetate omission (a, c, e) and after increase of flow rate and loading (b, d, f). Different redox conditions are indicated by the texture on top of the bars.

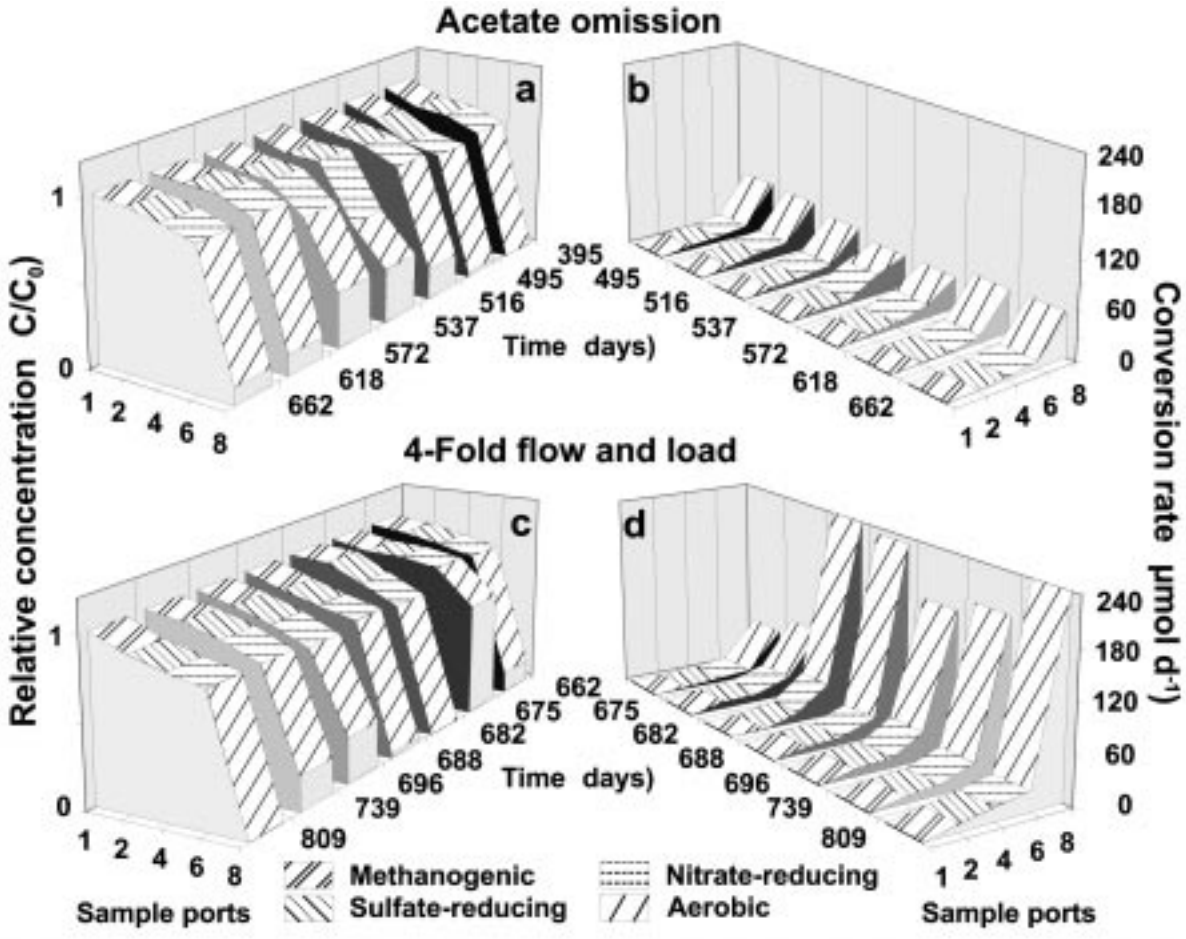

Figure 2. Transformation of NAP in the SSCS after acetate omission (a and b) and four fold increase of flow and load (c and d). Different redox conditions are indicated by the texture on top of the bars.

20 nmol NAP d^{-1} which later decreased to about 6 nmol d^{-1}. Change of flow and load first increased the residual concentration to about 70% (170 nmol d^{-1}) of the feed of 240 nmol d^{-1} before again complete transformation occurred (Figure 2). Resumed addition of acetate increased the output of NAP to more than 120 nmol d^{-1} within 5 weeks (data not shown). Occasionally but inconsistently, some NAP decrease was found under denitrifying conditions.

Chloride

DCE and DCB were hardly transformed in the SSCS. Complete dehalogenation of PER and DCP would result in a maximum chloride increase of about 4 μM. But the chloride content measured at the aerobic outlet did not significantly differ from the methanogenic inlet. Besides the problem of the high background level (about 14 mM chloride in the feed) this might also indicate that dechlorination of PER and DCP was not complete, and metabolites such as vinyl chloride (VC) and 4-chlorophenol (CP) may be present in the SSCS.

Batch culture studies

The highest removal rates of benzoate (42 μmol d^{-1} l^{-1}) were seen in the methanogenic batch cultures. The maximum benzoate and acetate turnover rates were more than 350 μmol carbon d^{-1} l^{-1} under methanogenic and about 250 μmol C d^{-1} l^{-1} under nitrate-reducing conditions. The transformation rates of all xenobiotics are given in Table 3. PER was only dechlorinated under methanogenic conditions (Figure 3), and TRI was always observed in traces (9 to 12 nM, not shown). Although searched for, further chloroethenes were not found. Consumption of DCE was

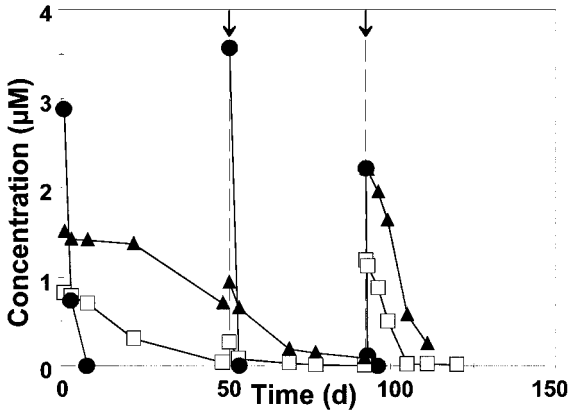

Figure 3. Transformation of PER (□), DCE (▲), and NP (*CIRCLE*) in a methanogenic batch culture. Arrows and dotted lines indicate addition of xenobiotics.

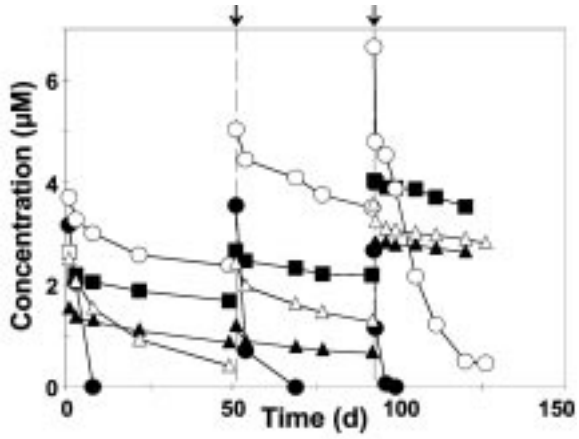

Figure 4. Transformation of DCE (▲), DCB (■), NP (*CIRCLE*), TOL (○), and NAP (△) in a nitrate-reducing batch culture. Arrows and dotted lines indicate addition of xenobiotics.

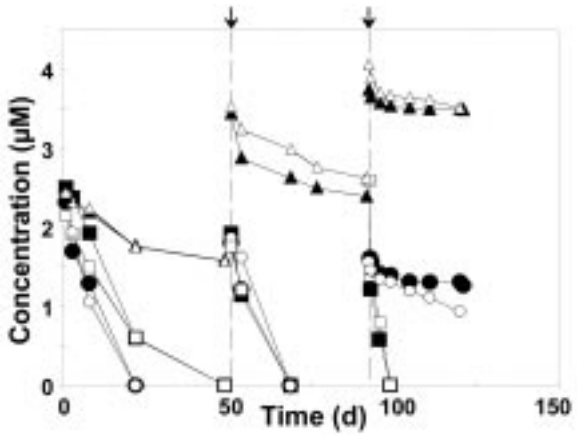

Figure 5. Transformation of DCP in the methanogenic (■, □), sulfate-reducing – (▲, △), and nitrate-reducing (●, ○) batch cultures. Arrows and dotted lines indicate addition of DCP.

seen in methanogenic (Figure 3) and sulfate-reducing cultures (not shown). DCP was consumed under all redox conditions (Figure 5), but the rates decreased in the denitrifying cultures. Conversion of DCP resulted in about stoichiometrical accumulation of CP in all batch cultures without further transformation (not shown). DCB consumption was observed in one sulfate- and in both nitrate-reducing batch cultures.

NP turnover rates were the highest observed during the batch experiments (Figure 4). NP did not significantly decrease in sterile controls. The reduction product 2-aminophenol (AP) accumulated in all cultures but started to decrease after seven to eight weeks of operation in one methanogenic and one denitrifying batch (not shown). TOL was transformed in one sulfate- and in both nitrate-reducing cultures (Figure 4). The concentration of NAP decreased at first in both sulfate-reducing batch cultures, but its conversion stopped almost completely after the 3rd addition of xenobiotics. Despite an initial decrease of the concentrations of NAP in the nitrate-reducing cultures, the rates decreased with time (Figure 4). Degradation of BEN was not seen in the batch cultures.

In the sterile controls, the concentrations of the chlorinated compounds decreased equivalent to 5 to 6 μM chloride without significant chloride increase, probably due to sorption or volatilization. For mass balance calculations, these losses were added to the chloride actually measured in the inoculated cultures. The resulting chloride recoveries were 53 and 58% under denitrifying, 72 and 75% under sulfate-reducing, and 83 and 98% under methanogenic conditions (not shown) and confirmed dechlorination reactions. The low recoveries of chloride under denitrifying conditions might be due to the overpressure formed under these conditions and subsequent loss of the present compounds during sampling and incomplete dechlorination.

Discussion

The SSCS allowed the simulation of a spatially defined redox cascade from anaerobic to aerobic conditions during 28 months. At the end of the first experimental phase of 15 months PER, DCP, NP, TOL, and NAP were transformed almost completely within the system and a steady state was established (Nay et al. 1999). The reaction of the system to changes, such

Table 3. Transformation rates (averages; in nmol d^{-1} l^{-1}) in the batch cultures. Losses in the corresponding sterile controls are subtracted. > indicates complete consumption of the compound before a second sample was taken

Substance flask	PER	DCE	DCB	DCP	NP	TOL 7 NAP	Addition of xenobiotics	
Methano-	11	10	0	81	353*	1	1	day 1
genic	56	23	3	256	>590*	2	0	day 51
1	94	92	1	211	1049*	3	2	day 92
Methano-	31	14	1	65	288*	1	1	day 1
genic	81	24	1	173	>542*	0	1	day 51
2	94	101	5	444	1519*	1	2	day 92
Sulfate	1	11	15	10	339	1	1	day 1
reducing	3	16	10	20	393*	7	7	day 51
1	1	3	0	3	>1184*	1	0	day 92
Sulfate	0	4	8	12	358	9	9	day 1
reducing	1	7	9	16	423*	5	15	day 51
2	0	20	14	13	>1085*	16	0	day 92
Nitrate	0	8	12	161	369	21	32	day 1
reducing	0	5	3	73	936*	30	16	day 51
1	1	2	11	16	320*	265	11	day 92
Nitrate	2	29	12	83	354	10	24	day 1
reducing	2	4	17	74	456*	11	14	day 51
2	2	0	31	0	52	43	13	day 92

* NP decreased too fast to measure defined rates. These rates were calculated per hour from the NP decrease between addition and sampling.

as omission of acetate, increase of flow and load, and readdition of acetate as an easily degradable carbon and energy source, has been tested.

Influence of electron donors in the SSCS

Before acetate was omitted from the feed, the acetate added to each column of the SSCS was almost completely consumed within the columns (Nay et al. 1999). After acetate omission, the amount of acetate measured after the methanogenic column increased, indicating acetate production under methanogenic conditions. The origin of the carbon in the acetate is not clear, but several possibilities exist. The acetate measured at the outlet of the methanogenic column corresponded roughly to the ethanol used to dissolve the xenobiotics (giving a load of approximately 13 μmol d^{-1} in the feed). This indicated the presence of acetogenic bacteria oxidizing ethanol to acetate (Dolfing 1988). But also cell lysis – (not measured) or transformation products of xenobiotics may have originated in acetate production. Since ethanol was not measured in our experiments, it is not possible to deduce whether it was already oxidized to acetate before the addition of acetate to the feed was stopped. We have no explanation as to why the newly formed acetate, although known as an anaerobically easily degradable carbon source (Holliger 1992; Picardal et al. 1995; Doong et al. 1997; Nay et al. 1999), was not consumed within the methanogenic column. This strange acetate dynamic may be an indication that under certain methanogenic conditions, acetate resists degradation.

While omission of acetate led to a transient decrease of transformation rates, it hardly affected the steady state turnover rates of the xenobiotics. However, after readdition of acetate to the column inlets, decreasing residual concentrations of most of the test compounds at the column outlets, indicated increased transformation of the pollutants. This observation confirmed that acetate can enhance anaerobic conversion rates of a range of contaminants. Despite the fact that most of the xenobiotics present can be biodegraded under one or more of the redox conditions present (Bosma et al. 1988), BEN and DCB were always detected at the aerobic outlet both in the presence and in the absence of acetate.

Flow and load increase

Increasing flow and load resulted in higher turnover rates of PER, NP, TOL, NAP, and BEN. This behavior indicated that a population under steady state conditions, supplied with low amounts of substrates, is able to immediately increase the turnover of these substrates without need to grow first. Recently, such a metabolic over-capacity has been observed under steady state conditions for the sugar uptake systems of *E. coli* in continuous cultures (Kovářová et al. 1996).

Not all compounds were converted with higher rates in the SSCS after changing flow and load. Causes may have been inhibitory effects by toxic metabolites such as CP or vinyl chloride (VC; Fogel et al. 1986; Rippen 1987). CP which is poorly biodegradable (Gibson & Suflita 1986; Kohring et al. 1989; Utkin et al. 1995) and can inhibit microbial metabolism (Battersby & Wilson 1989; Burback et al. 1994) accumulated in all batch cultures. Presence of CP in the SSCS must therefore be assumed and is also supported by the low changes in the chloride concentrations. Presence of CP may generally have limited degradation of other compounds. A strong breakthrough of DCP (and PER) after increased flow and load lowered the concentration of inhibitory CP (and possibly VC) in the bulk solution. Simultaneously, aerobic turnover rates of BEN and TOL increased. Inhibitory effects of CP (and probably VC) on the aerobic degradation of BEN and TOL may be the reason for this observation.

Transformations in batch cultures vs. SSCS

After each spiking, PER, DCP, and TOL were converted with increased rates in the batch cultures. These compounds were also transformed under the corresponding conditions in the SSCS. DCE was not consumed in the SSCS but in batch cultures under methanogenic and sulfate-reducing conditions. DCB, known to be degradable in the absence of oxygen (Bosma et al. 1988), was transformed in sulfate- and nitrate-reducing batch cultures but not in the SSCS. These findings also indicated that the degradation ability of initially identical microbial populations may change in different systems exposed to different mixes of chemicals.

Sequential systems in the laboratory vs. environmental plumes

The SSCS was constructed to simulate an environmental pollution plume and to investigate the influences of changing conditions on degradation of xenobiotics. It also enabled us to monitor the main processes from initial pollution of a sequential system until it reaches a steady state.

Our results showed that the highest efficiencies of microbial transformation of xenobiotics were obtained during steady state conditions. Among other factors, microbial activity seems to depend on the stability of the conditions present. Disturbances by external events such as oscillating groundwater tables or introduction of further compounds may additionally limit microbial conversion in environmental systems. Measures to prevent such changes in LPP's could help to keep the conditions for microbial transformation of pollutants as optimal as possible.

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