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In situ and laboratory studies on the fate of specific organic compounds in an anaerobic landfill leachate plume, 1. Experimental conditions and fate of phenolic compounds

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Abstract

The transformation of specific organic compounds was investigated by in situ and laboratory experiments in an anaerobic landfill leachate pollution plume at four different distances from the landfill. This paper presents the experimental conditions in the in situ microcosm and laboratory batch microcosm experiments performed and the results on the fate of 7 phenolic compounds. Part 2 of this series of papers, also published in this issue, presents the results on the fate of 8 aromatic compounds and 4 chlorinated aliphatic compounds.

The redox conditions in the plume were characterized as methanogenic, Fe(III)-reducing and NO_3^--reducing by the redox sensitive species present in groundwater and sediment and by bioassays. With a few exceptions the aquifer redox conditions were maintained throughout the experiments as monitored by redox sensitive species present in groundwater during the experiments, by redox sensitive species present in the sediment after the experiments and by bioassays performed after the experiments.

Transformation of nitrophenol was very fast close to the landfill in strongly reducing conditions, while transformation was slower in the more oxidized part of the plume. Lag phases for the nitrophenols were short (maximum 10 days). Phenol was only transformed in the more distant part of the plume in experiments where NO_3^-, Fe(III) and Mn(IV) reduction was dominant. Lag phases for phenol were either absent or lasted up to 2 months. Dichlorophenols were only transformed in experiments representing strongly reducing, presumably methanogenic, redox

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conditions close to the landfill after lag phases of up to 3 months. Transformation of *o*-cresol was not observed in any of the experiments throughout the plume.

Generally, there was good accordance between the results obtained by in situ and laboratory experiments, both concerning redox conditions and the fate of the phenolic compounds. However, for phenol and *2,4*-dichlorophenol, transformation was observed in some in situ experiments but not in the corresponding laboratory experiments. In some experiments, this could be explained by differences in the redox conditions developing during the experiments. Nitrophenols were apparently transformed abiotically in the most reduced part of the plume, at 2 m from the landfill.

1. Introduction

Landfill leachate contains a variety of specific organic pollutants including phenolic, aromatic, and chlorinated aliphatic hydrocarbons (Christensen et al., 1994). These compounds constitute only a minor fraction of the leachate, but due to their potential health risk (Brown and Donnelly, 1988) much concern is associated with these groups of compounds.

In the leachate pollution plume the specific organic pollutants may migrate through different environments as the redox level changes from methanogenic conditions close to the landfill to more oxidized conditions further downgradient of the landfill. As reviewed by Christensen et al. (1994), a redox zone sequence of methanogenic, sulphate-reducing, iron(III)-reducing, manganese(IV)-reducing, NO_3^--reducing and aerobic conditions may be formed in leachate plumes. The changing redox conditions may indicate that the degradability of the specific organic compounds may change with distance from the landfill (Ghiorse and Wilson, 1988).

Only few reports exist on the degradation of specific organic compounds in landfill leachate plumes, and much work still needs to be done (see Christensen et al., 1994). This is primarily because many compounds have not been studied yet but also because most reported studies have been performed in the laboratory and it is still unclear how well laboratory experiments resemble field conditions.

Gillham et al. (1990) described an in situ microcosm (ISM) for in situ biotransformation studies. This technique has also been used, for example, by Acton and Barker (1992) and Holm et al. (1992). Compared to field injection experiments (e.g., Barbaro et al., 1992), the ISM has the advantage that the actual redox conditions during the experiment can be monitored quite precisely and that dilution does not influence the fate of the compounds. The ISM technique was chosen for this project because it was considered specifically suitable for leachate plumes, where the redox conditions may change over short distances, and where many of the compounds of interest may already be present in the plume.

The purpose of this study was to investigate under field conditions the fate of a mixture of specific organic leachate pollutants in an aquifer affected by landfill leachate. A total of 10 ISM's were installed in the pollution plume at Vejen Landfill (Denmark) at 4 different distances from the landfill. In this plume methanogenic, Fe(III)/Mn(IV)-reducing and denitrifying redox zones previously have been identified by Lyngkilde and Christensen (1992a). In order to compare the results obtained in situ to results from

more traditional experiments, also a number of laboratory batch microcosm (LBM) experiments were set up with sediment and groundwater from the same locations. Seven phenolic, 8 aromatic and 4 chlorinated aliphatic compounds were selected as model compounds for the study. This paper describes the experiments and presents the results with respect to redox conditions, biological conditions and the fate of the phenolic compounds. A following paper [Nielsen et al. (1995 in this issue) which is further referred to as Part 2] focuses on the fate of aromatic and chlorinated aliphatic compounds.

2. Materials and methods

2.1. Field site

The study was performed in a shallow, unconfined sandy aquifer 1 km north of Vejen City in Jutland, Denmark. The aquifer consists mainly of glacial meltwater sand with clay and silt inhomogeneities. The main fractions of the sediment are coarse sand ($\sim 80\%$) and fine sand ($\sim 20\%$). Clay and silt constitute $< 1\%$ (Nielsen and Christensen, 1994a). The thickness of the aquifer is ~ 10 m and the water table is located 3–5 m below the ground surface. The base of the upper aquifer consists of a layer of clay and silt. The pore velocity is 150–200 m yr^{-1} and the temperature is $\sim 10°C$. The hydrogeology is described in detail by Bjerg et al. (1992). The aquifer is influenced by landfill leachate from Vejen Landfill, resulting in elevated concentrations of non-volatile organic carbon (NVOC) in the groundwater. The degradation of the organic matter has

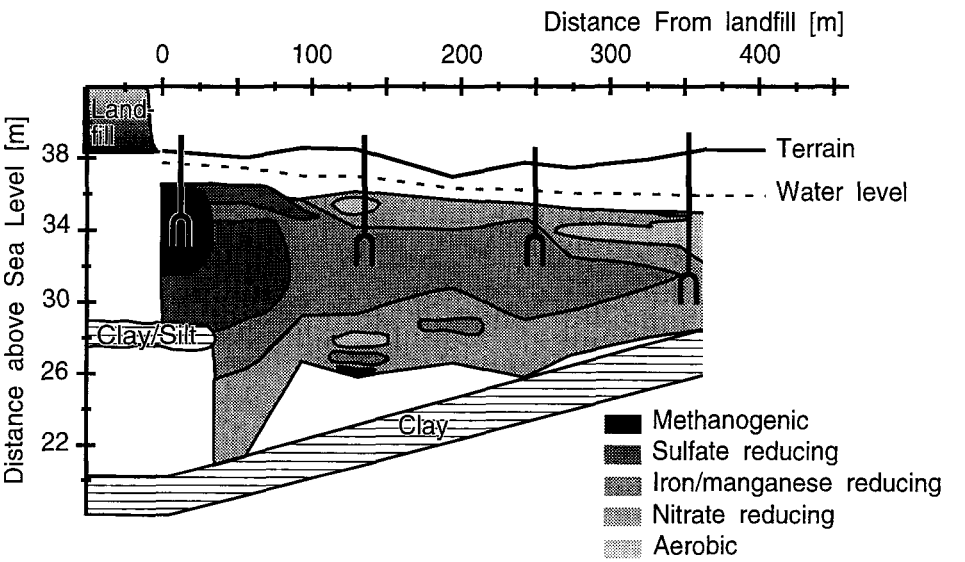

Fig. 1. Redox zones in the leachate plume (Lyngkilde and Christensen, 1992a) and location of ISM's installed in the aquifer. Groundwater and sediment for LBM experiments were collected adjacent to the ISM's.

resulted in the formation of a sequence of redox zones in the aquifer (Fig. 1), which has been described previously by Lyngkilde and Christensen (1992a) and by Heron and Christensen (1994). Specific organic compounds (e.g., benzene isomers, toluene, xylenes, naphthalenes and phenol isomers) have been detected in the groundwater downgradient from the landfill by Lyngkilde and Christensen (1992b) at distances up to 100 m. This study took place at four distances from the landfill (2, 135, 250 and 350 m) representing different redox zones, as indicated in Fig. 1.

2.2. *Specific organic compounds*

The specific organic compounds studied consisted of a mixture of typical landfill pollutants: phenolic hydrocarbons (phenol, *o*-cresol, *2,4*-dichlorophenol, *2,6*-dichlorophenol, *4,6-o*-dichlorocresol, *o*-nitrophenol, *p*-nitrophenol, pentachlorophenol), aromatic hydrocarbons (benzene, toluene, *o*-xylene, nitrobenzene, naphthalene, biphenyl, *o*-dichlorobenzene, *p*-dichlorobenzene, phenanthrene, dibenzothiophene, fluorenone) and chlorinated aliphatic hydrocarbons (*1,1,1*-trichloroethane, tetrachloromethane, trichloroethene, tetrachloroethene, *1,1,2,2*-tetrachloroethane). The chemicals were all analytical grade. The initial concentration of each compound was $\sim 150\ \mu g\ L^{-1}$ except for phenanthrene, dibenzotiophene and fluorenone, which due to a low solubility in water were used at an initial concentration of $\sim 30\ \mu g\ L^{-1}$. The latter three compounds are not reported further because strong sorption to the aquifer material hampered the measurements and the data interpretations.

2.3. *In situ microcosms (*ISM*'s)*

The ISM was originally presented by Gillham et al. (1990) and essentially consists of a cylinder which is open at the bottom and screened at the top (see Fig. 2). The ISM was

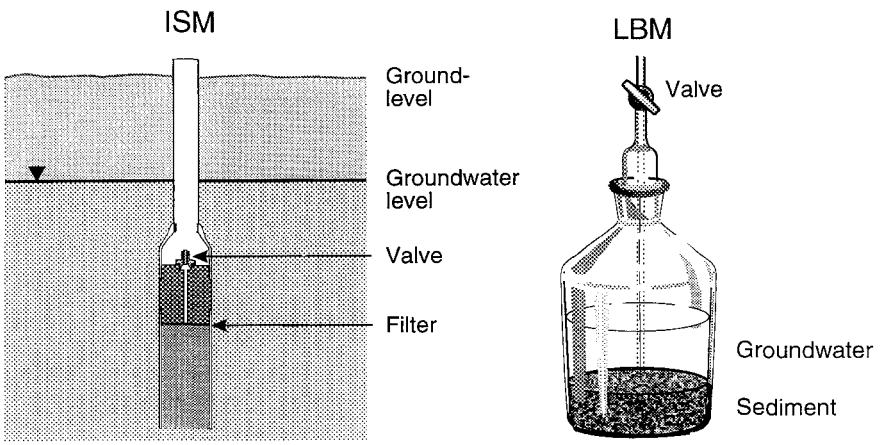

Fig. 2. Sketch of an in situ microcosm (ISM) and a laboratory batch microcosm (LBM). The ISM is not in scale.

installed 5 m below ground level through a cased borehole in the aquifer beside the filter tip of a drive point piezometer. The ISM isolates ~ 2 L of aquifer material including both sediment and groundwater. Five litres of groundwater were collected from the drive point piezometer into a Tedlar® bag and spiked with specific organic compounds and a tracer (^3H_2O). For biologically deactivated control experiments performed at 2 and 350 m from the landfill 250 mg L^{-1} formaldehyde were added to the water. For biologically deactivated control experiments performed at 135 and 250 m from the landfill 2 g L^{-1} NaN_3 were added. The ISM was loaded with the spiked water through a stainless-steel pipe (4-mm I.D.) corresponding to ~ 7 pore volumes. After loading of the ISM, the iron tube connected to the ISM was flushed with argon and capped to prevent O_2 diffusion into the microcosms through the sampling valve. The argon atmosphere in the iron tube was maintained during the experiment. At the distance of 350 m from the landfill a small amount of dissolved O_2 in the groundwater (max. 0.6 mg L^{-1}) was removed by addition of an equivalent amount of SO_3^{2-}. Samples for specific organic compounds, NO_3^-, NO_2^-, SO_4^{2-}, NH_4^+, Fe(II), Mn(II), CH_4, pH, NVOC, ATP (adenosine triphosphate) and AODC (acridine orange direct counts) were collected by a syringe. Sampling was approximately once per week during a period of 3–6 months. At the end of the experiment, the ISM was pulled out of the ground and the sediment inside the ISM was characterized [for TOC (total organic carbon), OXC (oxidation capacity), Fe(II) and Fe(III)], and used in bioassays as described below.

2.4. Laboratory batch microcosms (LBM's)

The LBM's consisted of 2.5-L glass bottles equipped with a glass valve used in sampling (see Fig. 2). The microcosms were prepared in an anaerobic glovebox (Coy®). An LBM contained a suspension of 1 kg of wet sediment and 1.3 L of water collected at the same depth as the ISM's were installed and within a horizontal distance from the ISM of < 0.5 m. The sediment was collected in aluminum tubes with a Waterloo piston sampler (Starr and Ingleton, 1992). Groundwater was collected by the drive point piezometer used to obtain groundwater for loading of the ISM and transferred directly into sterilized glass bottles flushed with N_2 and capped with glass stoppers. The LBM's were spiked with the mixture of specific organic compounds. A small amount of O_2 in the groundwater (max. 0.6 mg L^{-1}) from the 350-m distance from the landfill was reduced by addition of an equivalent amount of SO_3^{2-}. For control experiments formaldehyde (250 mg L^{-1}) or NaN_3 (2 g L^{-1}) was added to the water in the same manner as discussed for the ISM experiments. The LBM's were incubated in the dark at groundwater temperature (10°C) during a period of 5–6 months. During the incubation, the LBM's were kept submerged in anaerobic water (< 0.1 mg L^{-1} O_2), where the oxygen was removed by addition of a large excess of SO_3^{2-}. The bottles were shaken gently 3 times per week. Prior to sampling, sediment was allowed to settle and by opening the valve, a water sample was pushed out of the microcosms into a sample bottle by the over-pressure of N_2 in the LBM. The over-pressure was maintained by forcing 99.998% N_2 gas into the LBM's through an O_2 filter (R*3-11*®, BASF). Samples were characterized by the same chemical and biological parameters as the ISM's. After the experiment, sediment from selected microcosms was sampled and characterized [OXC, Fe(II) and Fe(III)].

2.5. Bioassays

Bioassays, incubated at 10°C, were performed to identify if methanogenesis, Fe(III) reduction and denitrification took place in the aquifer.

Methane bioassays: 10 mL of groundwater were transferred directly into evacuated 13-mL Venoject® test tubes with butyl rubber stoppers and the production of CH_4 was measured in the headspace. For control assays, H_2SO_4 was used as a sterilizing agent.

Fe(III)-reduction assays: 20 g wet weight (ww) of sediment were incubated with 30 mL of groundwater in 58-mL serum bottles and the development of dissolved Fe(II) was followed by the ferrozine method. For control assays chloroform was added. For further details see Albrechtsen et al. (1995).

Denitrification assays: 15 g ww of sediment were incubated with 20 mL of groundwater in 58-mL serum bottles and the denitrification was monitored as formation of N_2O after addition of 6 mL acetylene to each bottle. For control assays formaldehyde was added.

2.6. Sediment chemistry

The oxidation capacity related to Fe-oxides and -hydroxides in the sediment was determined by an 8 m*M* Ti(III)–50 m*M* EDTA (ethylene diamine tetraacetic acid) extraction followed by redox titration with dichromate and quantification of the extracted amount of Fe. The amount of ion-exchangeable Fe(II) was determined by an anaerobic 24-h extraction at 20°C (1 *M* $CaCl_2$ at pH 7.0) followed by Fe(II) quantification. Fe(II) and Fe(III) soluble in 0.5 *M* HCl were determined using a 24-h extraction at 20°C. Fe(III) was calculated as total Fe minus Fe(II) measured in the same extract. Fe(II) and Fe(III) soluble in 5 *M* HCl were determined using a 21-day extraction at 20°C. This extraction completely dissolves crystalline iron oxides, siderite and magnetite. Fe(II) and Fe(III) bound to clays and silicates will be partly dissolved, see Heron et al. (1994a, 1994b).

2.7. Analytical procedures

Samples for Cl^-, NO_3^-, NO_2^-, SO_4^{2-}, Fe^{2+}, Mn^{2+}, NH_4^+, CH_4, pH, alkalinity, electrical conductivity and NVOC were analyzed either immediately or as preserved samples by techniques standard to the laboratory (Lyngkilde and Christensen, 1992a). Sulphide was analyzed immediately by a sulphide-selective electrode (Radiometer® F*1212*S) and N_2O was analyzed by gas chromatography (Bjerg et al., 1995). Dissolved O_2 was measured immediately by the Winkler method modified to 12-mL volumes. Concentrations of phenolic compounds were measured with a Carlo Erba® gas chromatograph equipped with FID (flame ionization detector) and ECD (electron capture detector) on organic extracts from 10-mL water samples as described by Nielsen and Christensen (1994b). AODC and ATP in water and sediment were measured on preserved samples by epifluorescence microscopic counting after acridine orange staining (AODC) and by the luciferin–luciferase principle (ATP) —for further details see Albrechtsen et al. (1995). Activity of ^3H_2O was quantified by liquid scintillation

counting on a Packard® Tri-Carb *2000* CA Liquid Scintillation Analyzer on 1 mL of water mixed with 5 mL of Optiphase® "HighSafe" *3* Scintillation liquid. Sediment-bound TOC was determined as CO_2 evolved during dry combustion in a LECO® oven after removal of inorganic carbon by treatment with 6% H_2SO_3.

3. Results and discussion

3.1. Experimental conditions

3.1.1. Groundwater and sediment chemistry

Based on the groundwater chemistry of the plume, Lyngkilde and Christensen (1992a) proposed the redox zones shown in Fig. 1. However, three years have elapsed since that study and the redox conditions need to be specifically addressed at each location. The groundwater and sediment chemistry at the four studied locations in the plume is summarized in Table 1.

At 2 m from the landfill, reduced inorganic species [CH_4, NH_4^+, Fe(II)] dominated among the dissolved species, and the sediment content of solid electron acceptors [Fe(III)-and Mn(IV)-oxides extracted by Ti(III)–EDTA] was below detection limits. Since also SO_4^{2-} was absent in the groundwater, the only available electron acceptor at this location was CO_2, strongly indicating that methanogenesis and fermentation were the only possible redox processes.

At 135 m, SO_4^{2-} and traces of Fe(III)-oxides indicated less reducing conditions. CH_4 and NH_4^+ were far less significant. Absence of dissolved sulphide indicated that SO_4^{2-} reduction was not significant but previous detection of small amounts of pyrite, acid volatile sulphide and elemental sulphur suggest that SO_4^{2-} reduction once occurred at this distance (Crouzet et al., 1995). Dissolved Fe(II) was high but the origin of the observed Fe(II) was not specifically known (Heron and Christensen, 1994). Thus, the actual redox conditions cannot be diagnosed from the groundwater and sediment chemistry.

At 250 m, the absence of O_2 and NO_3^- indicated reducing conditions. Both SO_4^{2-} and Fe(III)-and Mn(IV)-oxides were present. No signs of SO_4^{2-} reduction were observed since dissolved and solid sulphides were below detection limits in this part of the aquifer. Thus, Fe(III) and Mn(IV) reductions were the most plausible processes, supported by the detection of dissolved Mn and both dissolved and solid Fe(II). See also Heron and Christensen (1994).

At 350 m, reduced inorganic species were practically absent, and NO_3^- and small amounts of dissolved oxygen appear in solution, indicating NO_3^--reducing conditions. Fe(III) dominated in the aquifer solids.

3.1.2. Aquifer microbiology

The numbers of microorganisms measured as AODC in the sediments ranged from $7.1 \cdot 10^6$ to $55 \cdot 10^6$ cells per g dry weight [$(g\ dw)^{-1}$], see Table 1 and were within the range of values previously reported for the uncontaminated part of the aquifer (Albrecht-

Table 1
Characteristics of groundwater and sediment in the Vejen Landfill leachate plume at the four distances studied

Distance from landfill (m)	2	135	250	350
Groundwater chemistry:				
Cl^- (mg L^{-1})	463	213	76.3	41
NO_3^- (mg L^{-1})	< 0.2	< 0.2	< 0.2	22
NO_2^- (mg L^{-1})	< 0.2	< 0.2	< 0.2	< 0.2
SO_4^{2-} (mg L^{-1})	< 0.1	44	72	34
Fe(II) (mg L^{-1})	17	27	5	0.3
Mn(II) (mg L^{-1})	5.7	0.67	0.46	< 0.1
NH_4^+ (mg L^{-1})	299	18.1	1.03	0.32
O_2 (mg L^{-1})	< 0.5	< 0.5	< 0.5	0.6
CH_4 (mg L^{-1})	23.7	3.1	0.15	– [a]
Sulphide (mg L^{-1})	< 0.1	< 0.1	< 0.1	– [a]
pH, water	6.5	5.7	6.2	5.6
TAL (meq L^{-1})	34.3	1.38	3.24	– [a]
Conductivity (mS cm^{-1})	4.6	0.91	0.63	0.3
NVOC (mg L^{-1})	107	23	6.1	5.8
Sediment chemistry:				
OXC (μeq gdw^{-1})	< 4	4	18	31
Fe extracted by Ti(III)–EDTA (μmol gdw^{-1})	< 2	2	17	32
Mn extracted by Ti(III)–EDTA (μmol gdw^{-1})	0.02	0.02	0.14	0.49
Total Fe [b] (μmol gdw^{-1})	39	28	70	62
% Fe(II) of total Fe	90	35	28	7
% Fe(III) of total Fe	10	65	72	93
TOC (% C)	0.088	0.019	0.027	0.044
Groundwater microbiology:				
ATP, average (pg mL^{-1})	53	18	17	15
ATP, range (pg mL^{-1})	36–69	– [c]	– [c]	15–15
AODC, average (10^6 mL^{-1})	6.24	0.80	1.28	1.07
AODC, range (10^6 mL^{-1})	2.97–9.50	– [c]	– [c]	0.93–1.21
Sediment microbiology:				
ATP, average (pg gdw^{-1})	1,104	927	90	411
ATP, range (pg gdw^{-1})	145–2,063	206–1,447	28–122	123–577
AODC, average (10^6 gdw^{-1})	21.1	30.5	26.7	32.8
AODC, range (10^6 gdw^{-1})	18.5–23.7	10.3–52.2	7.1–54.9	16.5–48.1

AODC = acridine orange direct counts; ATP = adenosine triphosphate; EDTA = ethylene diamine tetraacetic acid; NVOC = non-volatile organic carbon; OXC = oxidation capacity; TAL = total alkalinity; TOC = total organic carbon.
[a] Not analyzed.
[b] Determined as the sum of 5 *M* HCl-extracted Fe and Fe from pyrite (Heron et al., 1994a).
[c] Only one sample available.

sen and Winding, 1992). The ATP content of the sediment ranged from 28 to 2000 pg (g dw)$^{-1}$ in accordance with other observations from similar aquifers, including uncontaminated aquifers, although different extraction methods have been used (Webster et al.,

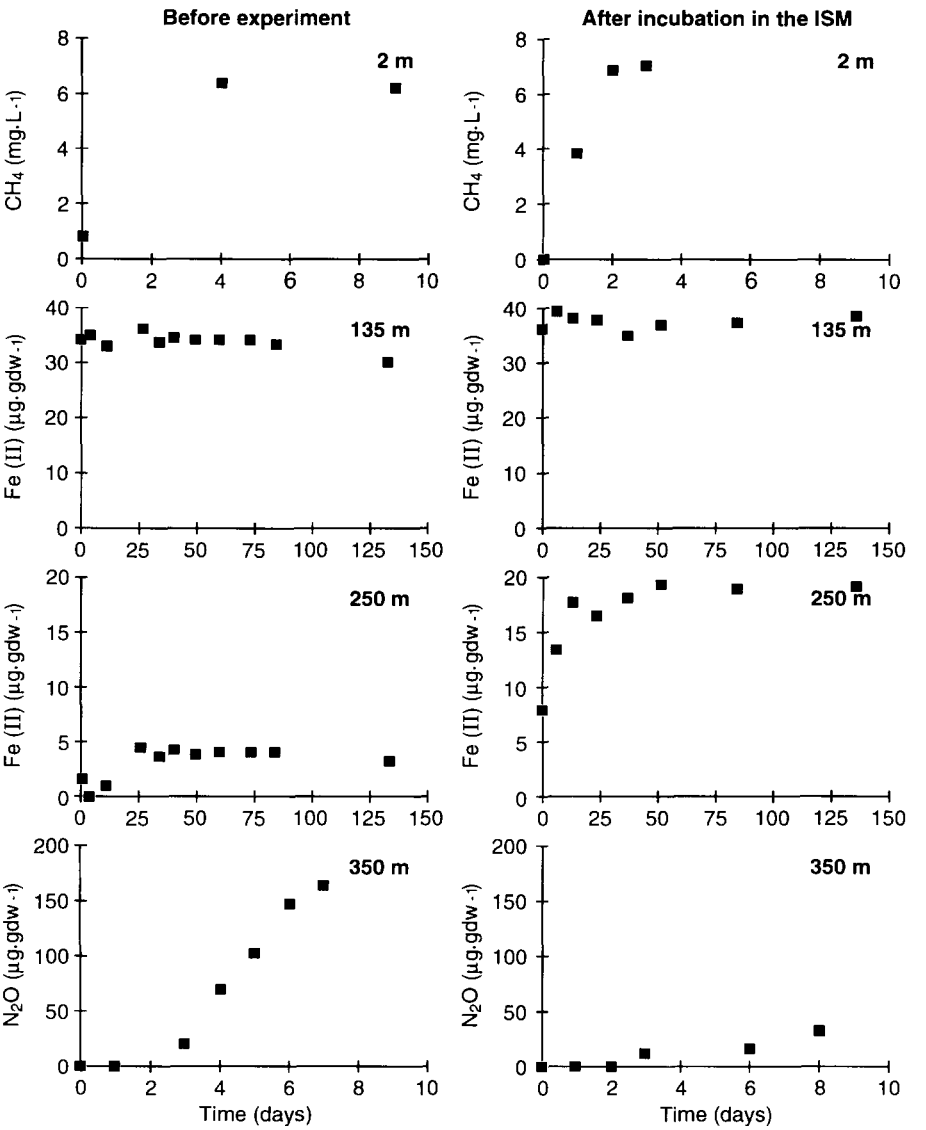

Fig. 3. Concentrations of CH_4, Fe(II) and N_2O as function of time in bioassays performed before and after in situ microcosm experiments (ISM's B, D, F and J) at the four distances from the landfill: 2 m, methane assay; 135 and 250 m, Fe(III)-reduction assay; and 350 m, denitrification assay.

1985; Balkwill et al., 1988; Beloin et al., 1988). Thus, the density of the microbial biomass was not significantly affected by the leachate.

Prior to, as well as after the ISM experiments, bioassays were set up to evaluate if the microorganisms in the investigated sediments were able to perform the relevant redox processes using the electron acceptors present (Fig. 3). Before experiments, the bioassays showed CH_4 production in the samples from a distance of 2 m from the landfill, no

significant Fe(II) production in the samples from 135 m from the landfill, Fe(II) production at 250 m and NO_3^- reduction at 350 m. In all cases the observed activities were microbiologically mediated, since the killed controls were inactive (data not shown).

The assays demonstrated microbial potential for the redox processes expected from the chemical characterizations and were in general in accordance with the redox zones previously proposed by Lyngkilde and Christensen (1992a) (Fig. 1), except for the samples at 135 m from the landfill, where no significant Fe(III) reduction was observed. This is probably due to the low content of Fe(III) on the sediment (Table 1).

3.1.3. Microbiological conditions during experiments

Although the free-living biomass, as often reported (e.g., Harvey et al., 1984; Godsy et al., 1992a; Harvey and Barber II, 1992), only constituted a minor fraction of the total biomass in the aquifer (Table 1), the biomass (AODC and ATP) was followed in the water phase, since it was not possible to collect sediment samples during the ISM and LBM experiments. In most cases the bacterial number (AODC) in the water samples remained constant during the ISM and LBM experiments (data not shown), supporting that the microbial population was not significantly influenced by the experimental conditions. The metabolically active biomass (measured as ATP content) remained constant in the water samples during the incubation of both ISM and LBM experiments, indicating that the experimental setup and addition of the specific organic compounds did not affect the total activity of the microbial population. A minor decrease in the ATP content in the water samples at the beginning of LBM experiments may have been caused by the disturbance during the setup of the experiment.

Sediment-associated ATP contents were compared for sediment collected before the ISM experiments and sediment collected from inside the ISM after the experiment. The ATP content decreased during the ISM experiments at distances of 135 and 350 m from the landfill and only increased (8–17 times) in the ISM's at 250 m from the landfill. In the ISM's at 2 m from the landfill the ATP content was not investigated. Since no major growth (AODC, ATP) was observed in the water samples and the sediment-associated ATP content decreased in 7 out of 9 investigated ISM's, it was concluded that generally no significant biomass was built up in the ISM's during the experiment.

Since AODC and ATP detect the whole microbial population, minor populations may have changed in numbers during the experiments and therefore results of bioassays performed before the experiments were compared with results of bioassays performed after the experiments on sediment collected inside the ISM's. The bioassays (Fig. 3)

Fig. 4. Activities of the tracer, concentrations of redox-sensitive parameters and phenolic hydrocarbons as function of time in four in situ microcosm experiments (ISM's B, D, F and J) and four laboratory batch microcosm experiments (LBM's B*1*, D*2*, E*2* and J*1*) at the four investigated distances from the landfill. For NO_3^-, Fe(II), Mn(II), SO_4^{2-} and CH_4, the *vertical arrows* are *Y*-axes. All activities of the tracer and concentrations of the phenolic compounds are normalized to the initial concentration of ~ 150 Bq mL^{-1} and ~ 150 μg L^{-1}, respectively. For LBM experiments the concentrations of CH_4 are corrected for evaporation into the headspace. C_0 = initial concentration; $C_n < x$ = concentration was less than the detection limit, x, throughout the experiment; – = compound was not analyzed for.


2 m
135 m
250 m
350 m
ISM B
LBM B1
ISM D
LBM D2
ISM F
LBM E2
ISM J
LBM J1
Tracer
NO_3^-
Fe^{++}
Mn^{++}
SO_4^{--}
CH_4
Phenol
o-Cresol
2,4-Dichloro-phenol
2,6-Dichloro-phenol
4,6-o-Di-chlorocresol
o-Nitrophenol
p-Nitrophenol
C·C$_0^{-1}$
Time (days)

showed that the potential for CH_4 production was maintained in ISM's installed 2 m from the landfill. The Fe(II) production remained absent in the sediment from ISM's installed 135 m from the landfill, which is in accordance with the very low content of Fe(III) on the sediment. In this part of the aquifer (Table 1), furthermore, the ISM's may have been depleted in available carbon, since addition of acetate or acetate plus Fe(III) [but not Fe(III) alone] stimulated the Fe(II) production in the sediment collected from the ISM's after the ISM experiments (data not shown). In the ISM's at 250 m from the landfill Fe(II) production was observed before and after the termination of the ISM experiments. In fact, the Fe(II) production was significantly higher in the sediment from the ISM, which was in accordance with a higher content of sediment-associated ATP (data not shown). The addition of the specific organic compounds and maybe a release of organic matter from the sediment during installation of the ISM may have caused the increase in Fe(III) reduction. This hypothesis is supported by the observations made by Albrechtsen et al. (1995) who found that addition of acetate to the sediment collected prior to the installation of the ISM's stimulated the Fe(III) reduction. During the ISM experiments 350 m from the landfill the denitrification rate decreased in some cases. However, the ratios in denitrification rates among ISM's remained the same.

3.1.4. Redox conditions and pH during experiments

At 2 m from the landfill: Sulphide was below the detection limit of 0.1 mg L^{-1} throughout the experiments and, in accordance with the low content of sediment-bound Fe(III) and Mn(IV) (see Table 1), production of dissolved Fe(II) or dissolved Mn was observed during neither the ISM nor LBM experiments (Fig. 4). The bioassays showed a clear potential for CH_4 production in the ISM's (concentrations of CH_4 in the ISM could not be determined due to the suction technique used in sampling of these ISM's). For LBM experiments the measured concentrations of CH_4 were corrected for volatilization into the increasing headspace ($K_H = 0.54$ atm m^3 mol^{-1}; Pankow, 1986), see Part 2. The concentration of CH_4 decreased slightly in the beginning of the experiment, but remained constant after ~ 1 month (Fig. 4) as opposed to the control experiments, which showed further decrease in CH_4 concentration (data not shown). It was likely that weak methanogenesis appeared during the LBM incubations.

At 135 m: Methanogenesis was not considered to be of importance in the ISM or LBM experiments because CH_4 remained constant and low throughout all experiments. Sulphide was below the detection limit of 0.1 mg L^{-1} throughout all the experiments. SO_4^{2-} concentration was constant in the LBM experiments while it apparently decreased at the end of the ISM experiments. Concentrations of SO_4^{2-} were only measured 3 times during the ISM experiments because signs of SO_4^{2-} reduction have never been observed in this part of the aquifer (H.-J. Albrechtsen unpublished data, 1994) and only strongly limited amounts of water were available for sampling. Thus, SO_4^{2-} reduction was not of importance in the LBM experiments, while in the ISM experiments it cannot be ruled out. After an initial drop, the concentration of dissolved Fe(II) increased slowly during both ISM and LBM experiments as an indication of Fe(III) reduction in the experiments. However, the initial drop of dissolved Fe(II) indicates oxidation during setup or sediment handling, leading to precipitation of amorphous and thus readily reducible Fe(III). In the LBM with the initial oxidation, dissolved and ion-exchangeable Fe(II)

increased relative to control experiments, indicating that Fe(III) reduction took place. It is in agreement with the observations by Albrechtsen et al. (1995) that Fe(III) availability is a limiting factor for the microbial Fe(III) reduction in this part of the aquifer. In the ISM's, clear indication of Fe(III) reduction was observed, since dissolved, ion-exchangeable and HCl-extractable Fe(II) increased relative to the control (sediment data not shown). In the ISM experiments the concentration of Mn(II) increased relative to controls, which decreased throughout the experiments (data not shown), indicating that also Mn(IV) reduction took place in the ISM's. This is in contrast to the low amount of Ti(III)–EDTA-extractable manganese in the sediment, suggesting that Mn(IV) is depleted. It is unclear, whether the observed increase in dissolved Mn is due to Mn(IV) reduction or to a release of solid Mn(II) from the sediment caused by slight changes in the chemical conditions during sediment handling. In the LBM experiments, dissolved Fe(II) increased while Mn(II) remained constant, indicating that only Fe(III) reduction occurred during this incubation. No significant change in the Fe(II)–Fe(III) composition of the sediment was observed.

At 250 m: Methanogenesis was not considered to be of importance in the ISM or LBM experiments because CH_4 was < 0.3 mg L^{-1} throughout all experiments. SO_4^{2-} concentration was constant during the LBM experiments while it seemed to decrease at the end of the ISM experiments. Sulphide was below the detection limit of 0.1 mg L^{-1} throughout all experiments. Thus, SO_4^{2-} reduction was not of importance in the LBM experiments, while it cannot be ruled out in the ISM experiments. For the ISM's, concentrations of dissolved Fe(II) increased slightly (Fig. 4), but significantly compared to the controls which decreased throughout the experiments (data not shown). Ion-exchangeable and HCl-extractable Fe(II) showed that Fe(III) reduction occurred (data not shown). This was supported by bioassays, and substantial amounts of Fe(III)-oxides extracted from the sediment by Ti(III)–EDTA, indicating that Fe(III) was not a limiting factor. No sign of Mn(IV) reduction was seen, even though small amounts of Mn(IV) was present in the sediment (Table 1). Thus, Fe(III) reduction was the main electron-accepting reaction during the ISM incubation. No significant redox processes were observed in the LBM incubations, since no reduced species occurred in solution and no consumption of electron acceptors was observed.

At 350 m: The concentration of O_2 was measured frequently during both ISM and LBM experiments. Concentration of O_2 never exceeded the detection limit of 0.1 mg L^{-1} during the LBM experiment, while a small amount was measured (max. 0.8 mg L^{-1}) at the very end of the ISM experiments (data not shown). During both ISM and LBM experiments, the concentration of NO_3^- decreased slowly, indicating that NO_3^- reduction was going on during the experiments. Dissolved Fe(II) was low throughout the experiment, indicating that Fe(III) reduction was not significant in the ISM. Dissolved Mn increased significantly in some cases during both ISM and LBM experiments, showing that Mn(IV) reduction was going on. This is in agreement with the presence of Mn(IV) in the sediment and the anoxic conditions (Table 1), and previous studies into simultaneous Mn(IV) reduction and NO_3^- reduction (Lovley, 1991). The relative importance of NO_3^- reduction vs. Mn(IV) reduction in this particular case cannot be addressed, since the manganese distribution among Mn(II) and Mn(IV) in the sediment is unknown. In short, NO_3^- reduction was dominant at the distance of 350 m, and

Mn(IV) reduction and NO_3^- reduction was observed simultaneously in a few of the incubations.

pH was measured frequently throughout the ISM and LBM experiments. In all experiments pH remained constant and close to the level measured in the aquifer prior to the experiments. See Table 1. The ISM and LBM data are not shown.

3.1.5. Hydraulic conditions during ISM *experiments*

The activity of the tracer (^3H_2O) was constant during the experimental periods of all of the ISM experiments presented in Fig. 4, showing that the ISM's were hydraulically well functioning. All ISM results discussed in this paper originate from hydraulically well-functioning ISM's. Results from 6 ISM's (in addition to the 10 ISM's included in this paper) were discarded because the activity of the tracer decreased during the experiment. Five of those experiments were control experiments which had been spiked with a biocide (NaN_3 or formaldehyde). The high ratio of malfunctioning control ISM's was probably caused by the addition of the biocide which may have changed the density of the water inside the ISM, resulting in exchange of water with the aquifer during the incubation.

3.2. Fate of phenolic hydrocarbons

3.2.1. Examples of fate curves

Examples of the fate of the seven studied phenolic hydrocarbons in experiments representing the four distances from the landfill are shown in Fig. 4. In both ISM and LBM experiments the concentration of the nitrophenols dropped very fast at the distance of 2 m from the landfill while in the more distant parts of the plume the decrease was slower. In many experiments concentrations of the other phenols dropped slightly at the beginning of the experiment, but remained constant or almost constant during the rest of the experiment. In two ISM's, however, the concentrations decreased further after a period. In the ISM installed at 2 m from the landfill the concentration of *2,6*-dichlorophenol was almost constant until day 70 whereafter the concentration decreased slowly. In the ISM experiment at 250 m from the landfill, the concentration of phenol dropped 80% after a period of ~ 50 days.

3.2.2. Compound transformation

During the ISM and LBM experiments, the phenolic compounds could be influenced by sorption to the sediment, biotransformation and chemical transformation. Evaporation to the headspace during the LBM experiments was not of importance since the studied phenols have very low Henry constants ($K_H < 3 \cdot 10^{-5}$ atm m^3 mol^{-1}; Montgomery and Welkom, 1990) and the maximum compound loss was calculated to be 0.1% during the LBM incubations.

The influence of sorption on the fate of the phenolic compounds was small in the main part of the experiments, as the compound concentration after a minor initial drop remained constant throughout the experiments. This is in good consistence with the low K_{ow}-values for the phenolic compounds (Montgomery and Welkom, 1990) and the very

low content of organic carbon on the sediment (Table 1). However, in some sediments, especially those at the 250-m distance from the landfill, the initial drop was followed by a very slow decrease in the compound concentration (e.g., *2,4*-dichlorophenol in Fig. 4). The same levelling off was observed in the control experiments (data not shown) and it was not possible directly to distinguish between sorption and non-biological transformation. A simple correlation between the compound loss of the phenols and their respective K_{ow}-values (including consideration of their dissociation) could, however, not be established as was the case for the aromatic compounds (see Part 2), but because the shape of the fate curves for the phenols was fairly similar to that of the fate curves of the aromatic compounds, and although this cannot be clearly documented, sorption is considered to be the reason for the slow levelling off.

When the concentration of a compound decreased more quickly (e.g., *p*-nitrophenol; Fig. 4) and the fate curve differed significantly from fate curves for the other compounds studied, the compound was considered to be transformed as discussed in further details in Part 2 for the aromatic compounds. The lag phase was defined as the time period from the start of the experiment until $\sim 10\%$ of the compound had been transformed. The identification of lag phases was complicated by the sorption in the beginning of the experiments and therefore short lag phases (< 10 days) could generally not be determined. Table 2 summarizes the transformation of the seven phenolic compounds studied in all ISM and LBM experiments performed.

The two nitrophenols studied were transformed at all the four studied distances from the landfill without lag phases or with lag phases shorter than 10 days. The transformation was very fast ($> 95\%$ transformed in a few days) in the most reduced part of the plume close to the landfill but the rate, especially for *p*-nitrophenol in ISM experiments, tended to decrease with distance from the landfill. In reduced environments, the nitro group of aromatic compounds is assumed to be reduced to the corresponding aniline group (e.g., Macalady et al., 1986). This reaction may have been responsible for the transformation of *o*-and *p*-nitrophenol in the aquifer as will be discussed in more details in Section 3.2.3.

Phenol was not transformed close to the landfill (2 and 135 m) but was clearly transformed in some ISM's in the more distant part of the plume (250 and 350 m) after lag phases up to ~ 2 months. No significant transformation of *o*-cresol was observed at any of the studied distances from the landfill. Phenol and *o*-cresol previously have been shown to be transformed in laboratory batch experiments under methanogenic conditions (e.g., Godsy et al., 1992b) and denitrifying conditions (Flyvbjerg et al., 1993), and phenol and *p*-cresol to be degraded under Fe(III)-reducing conditions (Lovley and Lonergan, 1990). Since phenol and *o*-cresol have been transformed in other anaerobic experiments, it is somewhat surprising, that *o*-cresol was not transformed in any part of this aquifer and that phenol was only transformed in few ISM's in the more oxidized part of the leachate plume.

Significant transformation of the chlorinated phenols was observed in some experiments close to the landfill, mainly at the distance of 2 m. Lag phases were up to 3 months. The transformation of chlorophenols may have been caused by a reductive dechlorination as observed in methanogenic laboratory batch experiments by Gibson and Suflita (1986).

Table 2
Transformation of phenolic hydrocarbons in an aquifer influenced by landfill leachate at four distances from the landfill as determined by in situ experiments (ISM's) and laboratory batch experiments (LBM's)

Distance from landfill (m)	Location [a]	Governing redox conditions	Phenol	*o*-Cresol	*2,4*-Dichloro-phenol	*2,6*-Dichloro-phenol	*4,6-o*-Dichloro-cresol	*o*-Nitro-phenol	*p*-Nitro-phenol
ISM *experiments*:									
2	A	methanogenesis	– [b]	–	*1*	*2* (30)	–	*3*	*3*
	B	methanogenesis	–	–	–	*2* (50)	–	*3*	*3*
	C	methanogenesis	–	–	*1*	*2* (30)	–	*2*	*3*
135	D	Fe(III) reduction	–	–	–	–	*1*? [c]	*3*	*2*
250	E	Fe(III) reduction	*2* (70) [d]	–	–	–	*1*?	*3*	*2*
	F	Fe(III) reduction	*2* (50)	–	–	–	–	*3*	*2*
	G	Fe(III) reduction	*2*	–	–	–	–	*3*	*2*
350	H	NO_3^- reduction	*2* (30)	–	–	–	–	*2*	*1*
	I	NO_3^- reduction	–	–	–	–	–	*2*	*1*
	J	NO_3^-, Mn(IV) reduction	–	–	–	–	–	*2*	–

LBM *experiments*:									
2	B*1*	supposedly methanogenesis	–	–	–	–	–	*3*	*3*
	B*2*	supposedly methanogenesis	–	–	–	–	–	*3*	*3*
	B*3*	supposedly methanogenesis	–	–	*2* (90)	–	*1*	*3*	*3*
135	D*1*	Fe(III) reduction	–	–	–	–	–	*1*	*1*
	D*2*	Fe(III) reduction	–	–	–	–	–	*1*	*1*
250	E*1*	no processes observable	–	–	–	–	–	*2*	*1*
	E*2*	no processes observable	–	–	–	–	–	*2*	*1*
	E*3*	no processes observable	–	–	–	–	–	*2*	*1*
350	H*1*	NO_3^- reduction	–	–	–	–	–	*1*	*1*
	J*1*	NO_3^-, Mn(IV) reduction	–	–?	–	–	–	*2*	*1*

1 = slow transformation (< 80% transformed in 2 months); *2* = fast transformation (> 80% transformed in 2 months); *3* = very fast transformation (> 90% transformed in 10 days). [a] ISM's were installed at different locations in the aquifer. Aquifer material for LBM experiments was collected adjacent to the ISM's at the same locations. [b] No transformation observed. [c] ? indicates that the results did not allow for conclusive statements because of analytical uncertainties. [d] Lag phases (days) of > 10 days are given in parentheses.

3.2.3. Variation in compound transformation

Since two or three ISM and LBM experiments were set up at each of the investigated distances from the landfill, the results give a preliminary indication of the variation in the transformation of the phenolic compounds in the aquifer. Within each distance from the landfill transformation of *o*- and *p*-nitrophenol generally was quite similar, while that of *2,4*-dichlorophenol, phenol and *4,6-o*-dichlorocresol varied (see Table 2).

At 2 m from the landfill, variation in transformation of *2,4*-dichlorophenol in LBM experiments was observed (see Fig. 5) although aquifer material was collected within only 0.5 m of distance. The concentration of *2,4*-dichlorophenol remained constant throughout the experimental period in two LBM experiments (B*1* and B*2*) while it decreased by > 95% in the LBM with B*3* aquifer material after a period of 3 months. Redox conditions were very similar in the three LBM experiments, since CH_4 developed

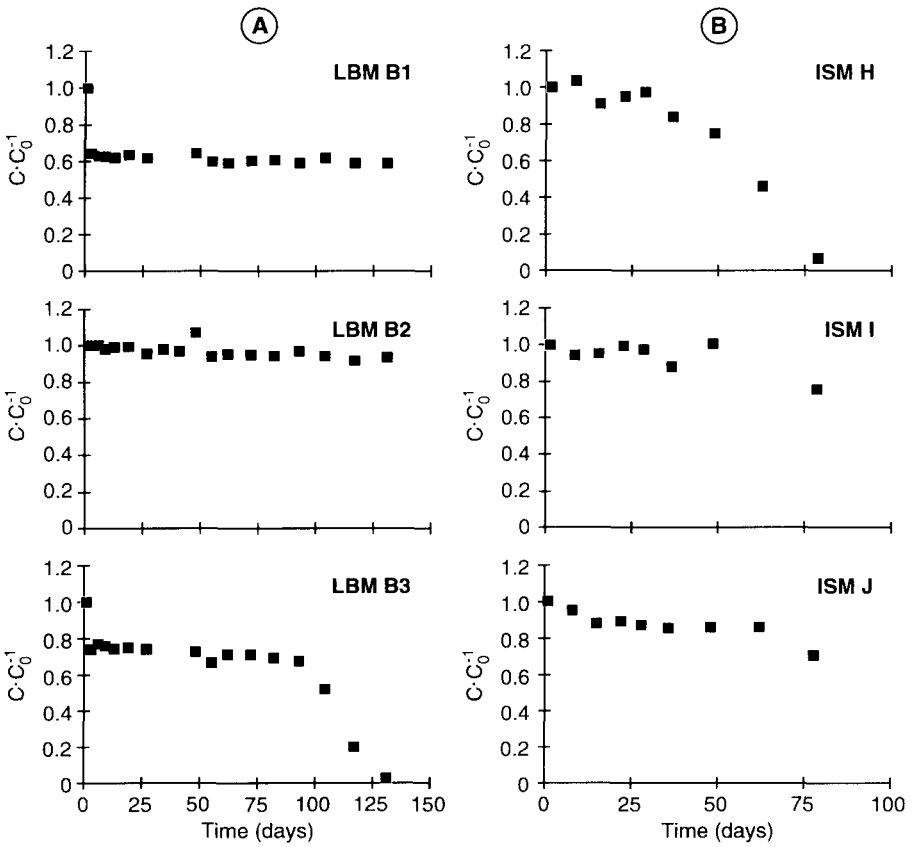

Fig. 5. Concentrations of two phenolic compounds as function of time. A. *2,4*-Dichlorophenol in three LBM's loaded with aquifer material collected 0.5 m from each other at a distance of 2 m from the landfill. B. Phenol in three ISM's installed < 10 m from each other at a distance of 350 m from the landfill. All concentrations are normalized to the initial concentration.

in the same way, but the ATP content reached a level of ~ 50 pg mL^{-1} in B*3*, twice as high as in B*1* and B*2*.

At the distance of 350 m from the landfill, three ISM's were installed within 10 m (locations H, I and J). At location H, the concentration of phenol decreased 95% after a lag phase of 1 month, while the concentration remained almost constant throughout the experiment at locations I and J (see Fig. 5). The microbial activity at location H differed significantly from the two other locations, since NO_3^- reduction during the experiment was faster (data not shown) and denitrification as measured in the bioassays was faster. The denitrification rates in the bioassays from location H were 2–5 times faster than in sediment from locations I and J before the installation of ISM's, and 7–38 times faster with sediment collected from inside the ISM's after the experiments. The ATP in the water samples collected from the ISM during the experiment and the ATP content of the sediment from location H was not significantly higher than in sediment from location I, but higher than in sediment from location J.

Although the number of experiments at each distance from the landfill were limited and relatively few compounds were transformed during the experiments, this study indicates that different experiments incubated under the same conditions may have a different outcome. These differences may either be caused by local variations in the aquifer or by experimental variations, which have not been investigated in this study, or by a combination of both. Local variations in the transformation of the same phenolic compounds in an aerobic part of the same aquifer have been observed by Nielsen and Christensen (1994b).

3.2.4. Biological vs. non-biological transformation of nitrophenols

In order to determine whether the observed transformations were biological or non-biological, the results from biologically active LBM experiments were compared with results from biologically deactivated LBM experiments. Since almost all control ISM's had hydraulic problems, as discussed earlier, no comparison will be made for the ISM experiments.

Fig. 6 shows the concentration of *o*-nitrophenol as a function of time in four biologically active experiments (LBM's B*1*, D*1*, E*1* and H*1*) and in their respective control experiments at the four distances from the landfill. In contrast to the biologically active LBM's, no ongoing redox processes [methanogenesis, Fe(III), Mn(IV), or NO_3^- reduction] were detected during the control experiments. Furthermore, the biological activity was low in the control LBM's, as measured by the ATP content (1–70 pg mL^{-1}, close to the detection limit). The control experiments were not considered to be sterile, but substantially inhibited by the biocides added. The very fast transformation of *o*-nitrophenol in both control-and biologically active experiments at 2 m from the landfill strongly indicates that non-biological processes were of importance. At the distances of 135 and 350 m from the landfill no transformation of *o*-nitrophenol was observed in the control experiment while a significant transformation was observed in the biologically active LBM's, indicating that the transformation was associated with biological activity in this part of the plume. At 250 m from the landfill, *o*-nitrophenol was clearly transformed in the biologically active experiments. In the control experiment, the concentration of *o*-nitrophenol decreased slowly, but clearly more than the

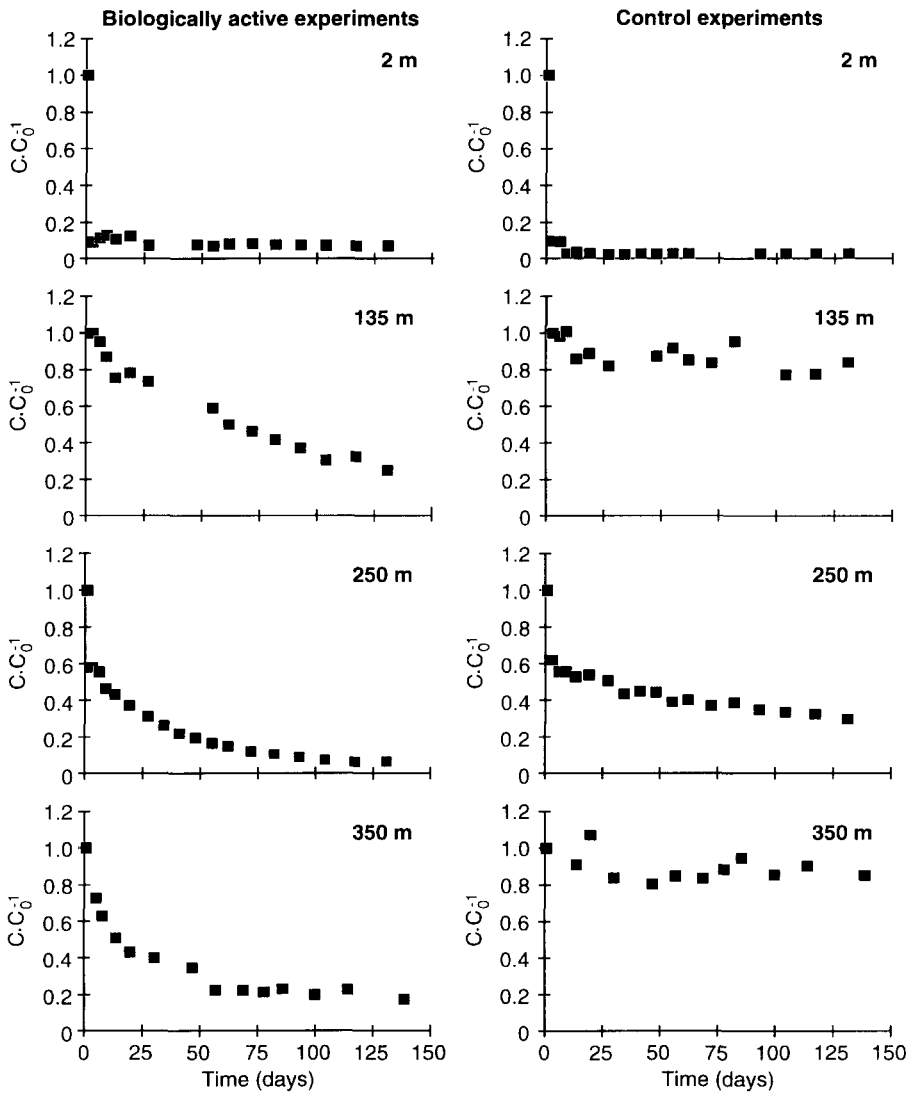

Fig. 6. Concentrations of *o*-nitrophenol as function of time in biologically active laboratory batch experiments (LBM's B*1*, D*1*, E*1* and H*1*) and their respective laboratory batch control experiments at the four investigated distances from the landfill. All concentrations are normalized to the initial concentration.

other compounds studied (data for the other compounds not shown), indicating that also abiotic transformation was going on. However, since the control experiments were not totally sterile, inhibited biotransformation cannot be ruled out as an explanation. Similar statements can be made for *p*-nitrophenol, since the fates of *o*-and *p*-nitrophenol were very similar. As discussed by Dunnivant et al. (1992) abiotic reduction of nitroaromatic compounds may be a significant transformation process under redox conditions typical

for methanogenic and SO_4^{2-} – reducing conditions. This is in good consistence with the observations in this study of non-biological transformation in the strongly reduced part of the aquifer. Furthermore, Heijman et al. (1993) have observed abiotic reduction of nitroaromatic compounds in the presence of Fe(II) formed during dissimilatory Fe(III) reduction. This supports the observation in this study of abiotic transformation of nitrophenols at 250 m from the landfill where high concentrations of reduced Fe was present, but is inconsistent with the lack of transformation in the control experiment at 135 m from the landfill where significant amounts of Fe(II) also were present.

3.2.5. Comparison of ISM*'s and* LBM*'s*

The fates of *o*-and *p*-nitrophenol were rather similar in the ISM and LBM experiments although transformation tended to be somewhat faster in the ISM experiments (see Table 2). Phenol and *2,6*-dichlorophenol were transformed in certain ISM experiments but not in any of the corresponding LBM experiments. The observed differences in results obtained in the ISM and LBM experiments may have been caused by the differences in the two setups of experiments, but the variations in transformation of the compounds as discussed above may also be an explanation. However, at the distance of 250 m from the landfill, the differences in phenol transformation may be caused by the fact that Fe(III) reduction was going on in the ISM experiment but not in the LBM experiment.

Although the differences in transformation in ISM and LBM experiments in some cases could be explained by differences in the redox conditions evolving during the experiments, the results stress that the different types of experiments may produce different results and that transformation of certain compounds in this anaerobic plume would have been overlooked if only LBM experiments had been performed. On the other hand, the LBM experiments were useful for more detailed interpretations, as the fate curves were very well established due to the simplicity of the experimental setup.

4. Conclusions

In situ and laboratory experiments were established on the fate of organic hydrocarbons in an anaerobic leachate plume at four distances from the Vejen Landfill. The different distances from the landfill represent the changing redox conditions typically found in leachate plumes.

The experimental conditions were carefully described by the composition of groundwater and sediment with respect to redox sensitive species and microbial activities. Close to the landfill (2 m downgradient) methanogenic conditions existed, while the conditions at 135 and 250 m downgradient the landfill were predominantly Fe(III)-reducing. However, the microbial activities as well as the availability of electron acceptor were much less at 135 m than at 250 m. Farthest away from the landfill (350 m) the redox conditions were NO_3^--reducing. In one of the experimental locations at 350-m distance, also Mn(IV) reduction was observed. In most cases, the redox conditions in the in situ and in the laboratory experiments developed in the same way, but a few exceptions were observed in cases where aquifer redox conditions were not maintained

in the laboratory experiments. This emphasizes the importance of careful monitoring of the redox conditions in long-term anaerobic fate experiments.

The fate of the phenolic compounds reported in this experiment depended strongly on the specific compound and on the location in the plume. Sorption of the compounds to the aquifer material was only modest.

o-Nitrophenol and *p*-nitrophenol were transformed both in in situ and in laboratory experiments at all the investigated distances from the landfill without lag phases or with lag phases shorter than 10 days. The transformation was very fast ($< 95\%$ in a few days) close to the landfill under methanogenic conditions, but tended to be slower at higher redox levels in the more distant parts of the plume. Non-biological transformation apparently was dominant under strongly reducing conditions at 2 m from the landfill.

o-Cresol was not transformed in any of the performed experiments and seems to be recalcitrant in this anaerobic leachate plume.

Phenol transformation was observed in some in situ experiments in the more distant parts of the plume, where Fe(III)-reducing and NO_3^--reducing conditions were dominant. None of the laboratory experiments showed transformation of phenol. The transformation of phenol often appeared after lag phases of 1–2 months.

Transformation of *2,4*-and *2,6*-dichlorophenols was only observed in the methanogenic part of the plume. Transformation often appeared after lag phases of 1–3 months. Transformation of *2,6*-dichlorophenol was only observed in the in situ experiments.

Very little and inconsistent transformation was observed for *4,6-o*-dichlorocresol, indicating that the compound is nearly recalcitrant in the studied leachate plume.

The comparison of results obtained in in situ experiments and laboratory experiments was complicated by variation in transformation of some of the compounds, but the transformation in in situ experiments tended to be somewhat faster and to include more compounds than in the corresponding laboratory experiments. In some experiments this could be explained by differences in the redox conditions developing during the experiments.

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