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Anaerobic microbial redox processes in a landfill leachate contaminated aquifer (Grindsted, Denmark)

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Abstract

The distribution of anaerobic microbial redox processes was investigated along a 305 m long transect of a shallow landfill-leachate polluted aquifer. By unamended bioassays containing sediment and groundwater, 37 samples were investigated with respect to methane production, sulfate, iron, and manganese reduction, and denitrification. Methane production was restricted to the most reduced part of the plume with rates of 0.003–0.055 nmol CH_4/g dry weight/day. Sulfate reduction was observed at rates of maximum 1.8 nmol SO_4^{2-}/g dry weight/day along with methane production in the plume, but sulfate reduction was also observed further downgradient of the landfill. Iron reduction at rates of 5–19 nmol Fe(II)/g dry weight/day was observed in only a few samples, but this may be related to a high detection limit for the iron reducing bioassay. Manganese reduction at rates of maximum 2.4 nmol Mn(II)/g dry weight/day and denitrification at rates of 0.2–37 nmol N_2O–N/g dry weight/day were observed in the less reduced part of the plume. All the redox processes were microbial processes. In many cases, several redox processes took place simultaneously, but in all samples one process dominated accounting for more than 70% of the equivalent carbon conversion. The bioassays showed that the redox zones in the plume identified from the groundwater composition (e.g. as methanogenic and sulfate reducing) locally hosted also other redox processes (e.g. iron reduction). This may have implications for the potential of the redox zone to degrade trace amounts of organic chemicals and suggests that unamended bioassays may be an important supplement to other approaches in

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1. Introduction

Leachate from municipal landfills is rich in dissolved organic matter and inorganic species that may migrate into groundwater. Depending on the composition of the leachate and the redox-buffering capacity of the aquifer, a sequence of anaerobic redox zones may develop in the pollution plume (Christensen et al., 1994), and this sequence strongly affects the behaviour of the pollutants leaching from the landfill (e.g. Lyngkilde and Christensen, 1992b; Rügge et al., 1995). Microorganisms are believed to be responsible for many redox processes (Table 1) involved in a pollution plume, in particular the degradation of the organic matter, e.g. methane production, sulfate, iron, manganese, and nitrate reduction. Theoretically, the differences in energy release from the organic carbon oxidation by the different electron acceptors will be the controlling factor for the different redox environments developing downgradient from the carbon source. However, variations in geology and geochemistry may also affect the microbial redox processes and the redox conditions in the plume due to changes in flow conditions and availability of substrates, nutrients or electron acceptors.

Different approaches have been used to investigate redox processes and redox zones in aquifers. Often, distribution of dissolved redox-relevant species have been used (e.g. Baedeker and Back, 1979; Bjerg et al., 1995; Lyngkilde and Christensen, 1992a; Nicholson et al., 1983), but groundwater composition does not provide direct insight into the actual redox processes at a specific location in the aquifer, since dissolved species such as e.g. methane may have been produced upstream and been transported to the sampling location and thus may not reflect an occurring process at the specific location in the aquifer. Recently, measurement of dissolved hydrogen was used successfully to indicate the dominant redox reaction (Chapelle et al., 1995, 1996; Lovley et al., 1994). However, the hydrogen level cannot be used to determine the rates of the individual processes or the relative importance of simultaneous reactions. In the present study

Table 1
Electron accepting redox processes and assumed dissolved and solid intermediate and final products from the processes

Electron accepting processes	Intermediate and final products	
	Dissolved species	Solid species
$CO_2 + 8H^+ + 8e^- \rightarrow CH_4 + 2H_2O$	CH_4	
$SO_4^{2-} + 8H^+ + 8e^- \rightarrow S^{2-} + 4H_2O$	H_2S, HS^-, S^{2-}, S^0	FeS, FeS_2, S^0
$Fe^{3+} + e^- \rightarrow Fe^{2+}$	Fe^{2+}, Fe^{2+}-complexes	$FeCO_3$, FeS, FeS_2 Fe^{2+}-ion exchanged
$Mn^{4+} + 2e^- \rightarrow Mn^{2+}$	Mn^{2+}, Mn^{2+}-complexes	$MnCO_3$, Mn^{2+}-ion exchanged
$NO_3^- + 6H^+ + 5e^- \rightarrow 1/2N_2 + 3H_2O$	NO^{2-}, N_2O, N_2	

unamended laboratory bioassays with sediment and groundwater are used to estimate rates of redox processes in a pollution plume by measuring end products from the redox reactions. Since sulfate, iron, and manganese reduction may produce both dissolved and solid products (Table 1), sediment bound species have to be included in the rate estimations.

This study focuses on the leachate plume at the Grindsted landfill, Denmark. Based on the distribution of dissolved redox-sensitive parameters in groundwater samples, Bjerg et al. (1995) proposed a methanogenic/sulfate reducing zone close to the landfill, followed by iron, manganese, and nitrate reducing and aerobic zones further away from the landfill. The purpose of this study was to investigate the distribution of microbial redox processes in the leachate plume in order to investigate if various microbial redox processes are taking place at specific sampling locations, to estimate rates of the various redox processes, to compare the significance of the various redox processes, and to relate the results from the bioassays to the previously suggested redox zones based on groundwater composition.

2. Materials and methods

2.1. Site description

The aquifer is shallow with a groundwater level 1 to 3 m below ground surface. The aquifer is aerobic outside the leachate plume created by the old, abandoned municipal landfill in Grindsted, Denmark. The geology of the aquifer is rather complex as described by Heron et al. (1998). The transect of the aquifer under examination is 10–12 m deep and consists of two geological settings: a lower Tertiary, Miocene sand deposit and an upper Quaternary, Glacial meltwater sand deposit. The Quaternary sediment consists mainly of fine to medium and coarse-grained light-grey brown sand with gravel. The Tertiary layer consists of fine-grained slightly silty sand. Between the two layers, clay and silt are observed as discontinuous thin layers. Furthermore, a layer of brown coal and peat was observed between 120 and 180 m from the landfill and could be interpreted as deposited in a small pond or lake. These intermediate layers represent heterogeneities within the sandy aquifer and may affect the flow conditions as well as the redox processes.

The transect studied is contaminated over the entire depth at the border of the landfill. The leachate plume is recognizable as far as 200–250 m from the landfill and contains elevated concentrations of chloride, dissolved organic carbon, methane, ammonium, iron(II) and manganese(II) (Bjerg et al., 1995). The dissolved organic carbon in the leachate plume decreased with distance from the landfill from maximum concentrations of 12 000 μmol C/l at the border of the landfill to the background concentrations in the range of 100–250 μmol C/l at 170 m from the landfill.

2.2. Sampling

A total of 37 sets of sediment and groundwater samples were collected at different distances from 0 to 305 m downgradient of the landfill. Usually, a group of three

samples within a vertical distance of 1.5 m was collected from each of the 13 locations, presented as dots in Fig. 2 and the following figures.

Groundwater was sampled by nitrogen pressure through a Teflon tube lowered into a well (iron pipe, diameter of 2.0 cm) as described in Lyngkilde and Christensen (1992a). Groundwater for bioassay studies was collected into 0.5 l pre-sterilized and nitrogen flushed serum bottles, which were filled to capacity and tightly capped with butyl rubber stoppers.

The sediment samples were collected with a piston sampler (Starr and Ingleton, 1992). The cores were kept at 10–15°C for a maximum of 5 days, cut into 23 cm long sections and transferred to an anaerobic glove box (Coy). To prevent contamination of the sediment sample, the outer 0.5 cm of the sediment core, which had been in contact with the core barrel, was pared off using a paring device modified after Wilson et al. (1983).

2.3. Analysis of geochemical parameters

Samples for water analysis were preserved and analysed as described in Lyngkilde and Christensen (1992a) and Bjerg et al. (1995), except for sulfate, which was determined by ion chromatography.

The sediment geochemistry was investigated in terms of solid organic carbon (Heron et al., 1994) oxidation capacity related to iron oxides and reduced species of iron and sulfur as described in Heron et al. (1997).

2.4. Bioassays

For the bioassay experiments, subsamples of sediment were transferred into the glove box to serum bottles (50 ml and 100 ml) and sealed by 1 cm thick butyl rubber stoppers. The head space of the bottles (consisting of nitrogen with up to 5% hydrogen from the anaerobic atmosphere in the glove box) was replaced immediately with N_2–CO_2 (80–20%). Sediment was mixed with corresponding samples of groundwater in a ratio of 2 g wet weight (ww) per 3 ml of water, except for the sulfate reduction assays where the ratio was 2 g (ww) per ml of groundwater. All the bioassays were incubated in the dark at 10°C.

Methane production was measured by gas chromatography as accumulation of methane in the head space of the bottle.

Sulfate reduction was determined as production of $H_2{}^{35}S$ after addition of a trace amount of $^{35}SO_4^{2-}$ (final concentration: 8.9 MBq/l or $1.6 \cdot 10^{-8}$ g $^{35}SO_4^{2-}$/l). For each sample, four replicates of sediment suspensions were incubated. After 5, 10, 15, and 20 days respectively, one replicate was harvested. Sulfate reduction was terminated by injecting 1 ml of 20% (weight/volume) zinc acetate solution before freezing the bioassay. For analysis, the sample was attacked by a slightly modified single-step chromium reduction method (Fossing and Jørgensen, 1989). By this method the two pools of ^{35}S reduced during incubation were determined: AVS (Acid Volatile Sulfide) mainly consisting of monosulfides, and the CRS (Chromium Reducible Sulfide) mainly consisting of pyrite, but also elemental sulfur, S^0. After the sample was degassed with

N_2, 50 ml of 1 M Cr(II) and 12 ml of 12 N HCl were added. The suspension was boiled for 2 h and purged by nitrogen to transport the evolved $H_2{}^{35}S$ into zinc acetate traps. The $Zn^{35}S$ was measured by scintillation counting.

Iron and manganese reduction were detected by accumulation of Fe(II) or dissolved manganese. Since it was observed that a substantial part of the produced Fe(II) was associated with the suspended fine particles (e.g. ion exchanged or precipitated) as also described by Albrechtsen et al. (1995), Fe(II) was extracted before quantification. Subsamples of the suspension were collected through syringes with needles (diameter of 0.6 mm) and Fe(II) was extracted by 0.5 M HCl for 1 h and measured by the ferrozine method (Stookey, 1970) after filtration (0.45 μm Minisart SRP 15, Sartorius). Mn(II) was measured by atomic absorption spectroscopy after filtration.

Denitrification was quantified by the acetylene blockage technique by injection of acetylene (generated from calcium carbide) into the bottle to a concentration of 10% acetylene in the head space (Knowles, 1982; Tiedje, 1988). The accumulated N_2O was sampled from the head space and quantified by gas chromatography.

From each of the 13 sample locations, bioassays were performed in triplicates in one of the three sampling depths per location, except for the resource demanding sulfate reduction bioassay. For each of all of the tested redox processes, one bioassay per location served as sterile control. Autoclaved samples supplemented with $HgCl_2$ (final conc. 1 mg/l) served as controls for methane production and sulfate reduction, whereas samples were autoclaved and supplemented with 6.4 g/l chloroform (near saturation) to inhibit microbial activity of iron and manganese reduction and denitrification.

The rates of the different redox processes in the bioassays were estimated by linear regression of the developments of the end products over time and quantified in mole per gram dry weight of sediment per day. For methane production, iron reduction, and manganese reduction the rates were estimated on the basis of at least 10 sampling points (within 120 days). Denitrification was estimated on basis of at least five sampling points within 6 days (the acetylene blockage has a limited durability). Sulfate reduction rates were determined on basis of 4 sampling points within 20 days and the rates were calculated according to Jørgensen (1978) based on the accumulated $H_2{}^{35}S$ accounting for isotopic fractionation due to the preference of SO_4^{2-} to $^{35}SO_4^{2-}$.

3. Results

The composition of the groundwater samples obtained in the present study (37 samples, May 1994–March 1995) compared well with a previous study (285 samples, autumn 1992, published by Bjerg et al., 1995), indicating that the redox conditions in the plume determined by the composition of the different dissolved redox sensitive components are fairly stable with time. Accordingly, the composition of groundwater samples determined in this study is not further reported but references are made to results presented by Bjerg et al. (1995).

Examples of the results obtained in the bioassays are shown for the different redox processes in Fig. 1, while the rates of the microbial redox processes estimated from the

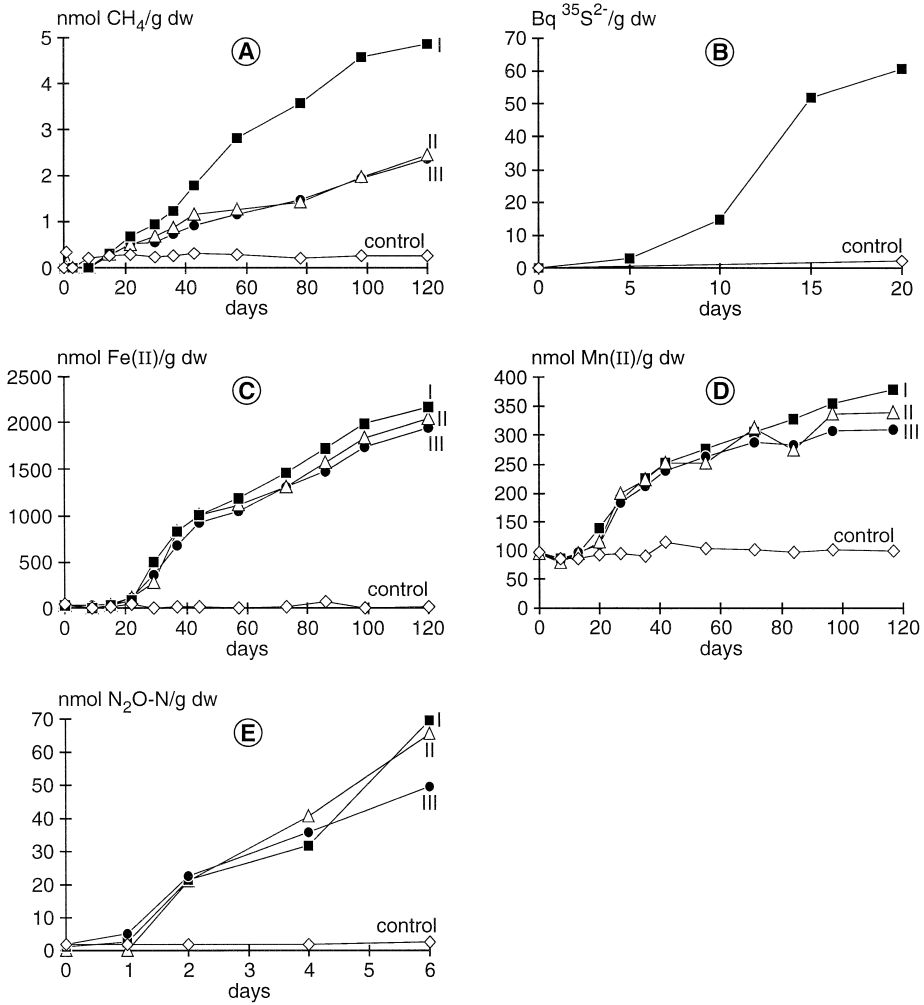

Fig. 1. Triplicate curves (I, II, III) and curves from sterilized controls of concentrations of CH_4 (A), Fe(II) (C), Mn(II) (D) and N_2O–N (E) over time in bioassays from selected samples: 36 m from the landfill, 5.3 m.b.s. (A), 170 m from the landfill, 7.2 m.b.s. (C and D) and 113 m from the landfill, 5.5 m.b.s. (E). Production of $H_2{}^{35}S$ over time in a bioassay and in a sterilized control from a selected sample 78 m from the landfill, 5.5 m.b.s. (B).

bioassays are presented below for the individual processes in the transect. All the redox processes observed were microbial since none of the controls showed abiotic activity.

3.1. Methane production

The bioassays showed that methane production took place within the first 80 m of the plume at rates of 0.003–0.055 nmol CH_4/g dry weight/day (g dw/day) (Fig. 2),

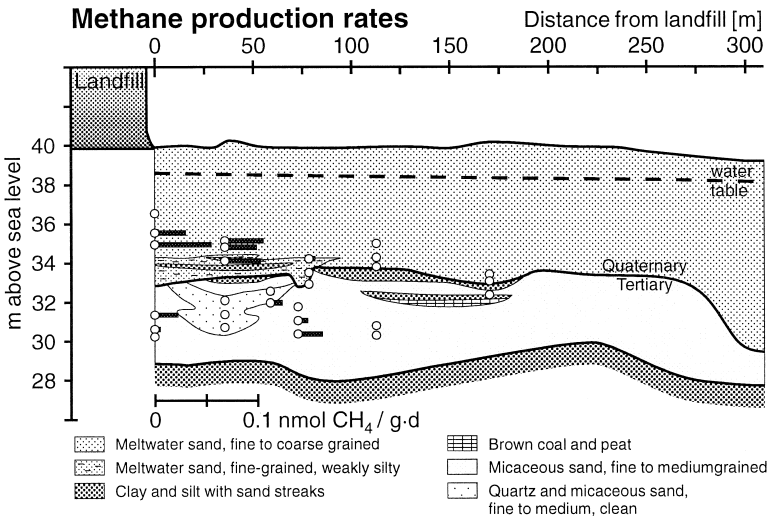

Fig. 2. Methane production rates in nmol CH_4 /g dw day estimated from bioassays of adjacent sediment and groundwater (white dots).

whereas no methane production was detected in locations at 113 and 170 m from the landfill. The methane production rates were higher in the Quaternary layer than in the Tertiary sand layer and the maximum rate was observed at the border of the landfill (4.8 m below surface). Between triplicates of methane producing bioassays the relative standard deviation on the rate estimate ranged from 9 to 37% indicating some variation related to the experimental setup as also indicated in Fig. 1A.

The methane production close to the landfill, as observed in the bioassays, was in accordance with the presence of high levels of dissolved methane (250–2700 μmol/l) within the same area of the transect (see Bjerg et al., 1995). Dissolved methane (50–250 μmol/l) was also observed in the plume at greater distances than 80 m, but since no bioassays showed methane production at these distances, the concentrations observed by Bjerg et al. (1995) may be due to migration of methane.

3.2. Sulfate reduction

Sulfate reduction was observed in almost all samples within the first 80 m of the transect (Fig. 3) at rates of 0.01–1.3 nmol SO_4^{2-}/g dw/day. At greater distances from the landfill, at locations 113 m and 170 m from the landfill, a few samples showed remarkably high sulfate reduction rates, with a maximum rate of 1.8 nmol SO_4^{2-}/g/dw day at 113 m from the landfill. These latter samples were from the silt and clay layers. The sulfate reduction rates were within the same range as observed in an uncontaminated shallow aquifer of young aeolian sands (Jakobsen and Postma, 1994).

The sulfate reduction, as observed in the bioassays, representing the first 80 m of the transect corresponded well with the distribution of dissolved sulfide (see Bjerg et al., 1995) and also with the finding of reduced S-species on the sediment. Reduced solid

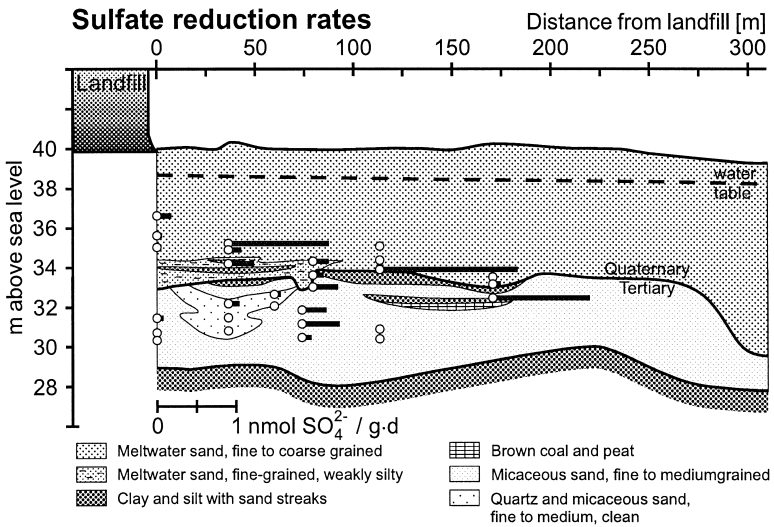

Fig. 3. Sulfate reduction rates in nmol SO_4^{2-}/g dw day estimated from bioassays of adjacent sediment and groundwater (white dots).

S-species (monosulfides, pyrite and S^0) were detected within the first 60 m from the landfill although in low concentrations (less than 1.6 μmol S/g dw or 50 μg S/g dw; see Heron et al., 1998). The pyrite fraction made up less than 0.3 μmol S/g dw with the highest concentrations in the intermediate silt/clay layer 5 to 7 m below ground surface.

3.3. Iron reduction

The bioassays showed iron reduction only in a few locations in the leachate plume. A few samples from the Quaternary layer, close to the landfill, showed iron reduction at low rates of approximately 5 nmol Fe(II)/g dw/day (Fig. 4). The iron reduction rates were 7–12 nmol Fe(II)/g dw/day in two samples collected in the Tertiary layer 73 m from the landfill and finally iron reduction was observed much further out in the plume at 170 m from the landfill in the pond deposits, where the highest rate was observed (19 nmol Fe(II)/g dw/day). The iron reduction rates were in the same range as previously reported for a leachate polluted aquifer at the Vejen landfill (Albrechtsen et al., 1995).

The fact that only few bioassays showed iron reduction may partly be due to a high detection limit in certain parts of the transect. High background levels of dissolved Fe(II), often higher than 1800 μmol/l (100 mg/l), made it difficult to detect small increases in Fe(II) over time and to distinguish small increases in Fe(II) from analytical variations, resulting in relatively high detection limits for iron reduction (approximately 5 nmol Fe(II)/g dw/day). The relative standard deviation between triplicates of iron reduction measurements in the sections of the plume with high Fe(II) background levels and low rates was more than 100%. In other sections of the plume with higher iron reduction rates and lower Fe(II) background levels, e.g. at the distance 170 m from the landfill (see Fig. 2), the standard deviation was only 4–18%.

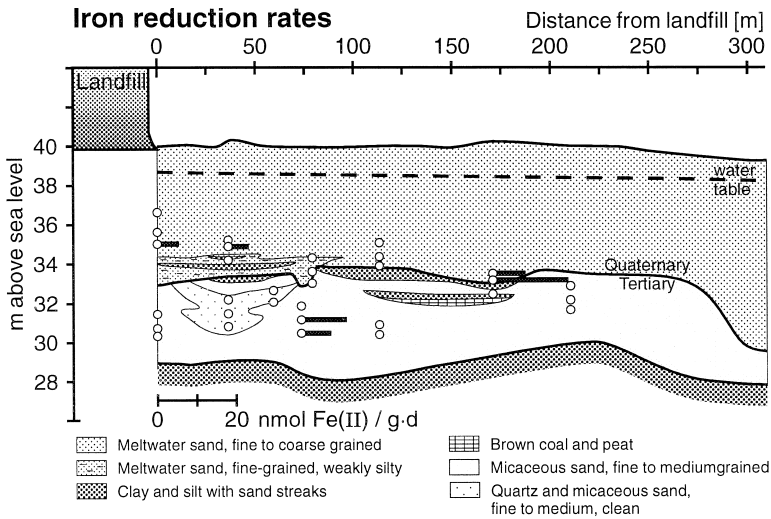

Fig. 4. Iron reduction rates in nmol Fe(II)/g dw day estimated from bioassays of adjacent sediment and groundwater (white dots).

In the lower Tertiary part of the transect, the contents of dissolved Fe(II) (Bjerg et al., 1995) and of sediment bound Fe(II) (Heron et al., 1998) were high for the first 80 m of the transect, but only the bioassays located at a distance of 73 m from the landfill showed significant iron reduction. This indicated that iron reduction was low close to the landfill (too low to be detected with the current methods) and that the elevated concentrations of reduced iron close to the landfill to some extent may be the result of redox activity in the past or further upstream. Close to the landfill, the oxidation capacity related to Fe(III) associated with the sediment was very low, while elevated capacities of Fe(III) were found in the area of the transect with the bioassays showing significant iron reduction (73 m) (Heron et al., 1998).

In the pond deposits, 170 m from the landfill, where the highest rate of iron reduction was observed, also the oxidation capacity related to Fe(III) was very high (Heron et al., 1998). The central sample from this location was red-coloured, fine grained sand, supposedly coated by Fe(III)-oxides, and the significantly highest rate of iron reduction detected in this sample might be due to higher content of Fe(III)-oxides. The observations of iron reducing conditions in this part of the transect were supported by elevated concentrations of solid Fe(II) (Heron et al., 1998).

3.4. Manganese reduction

The bioassays showed substantial manganese reduction in samples from locations at distances of 113 and 170 m from the landfill (Fig. 5) with rates of maximum 2.4 nmol Mn(II)/g dw/day. The relative standard deviation between triplicates of bioassays

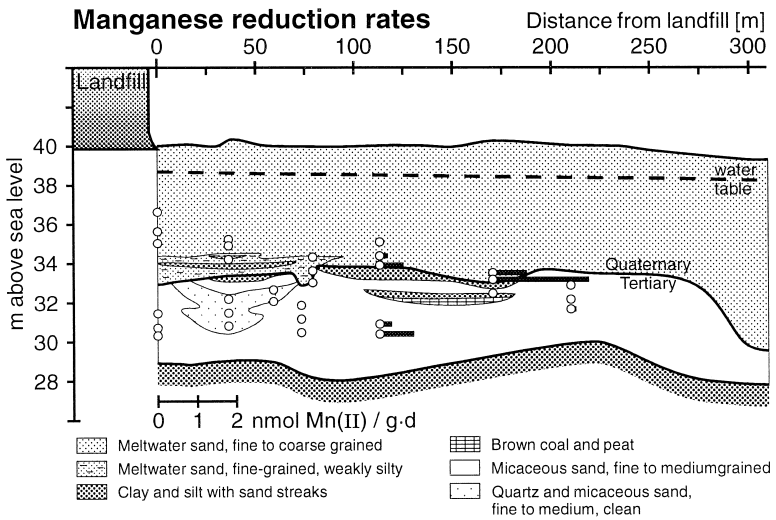

Fig. 5. Manganese reduction rates in nmol Mn(II)/g dw day estimated from bioassays of adjacent sediment and groundwater (white dots).

showing manganese reduction varied between 6 and 42%, indicating some variation in the experimental setup also for manganese reduction.

The location at 113 m from the landfill showing substantial manganese reduction by the bioassays were sampled in the area of the transect with highly increased concentrations of dissolved Mn(II) (Bjerg et al., 1995). Only the bioassays showing manganese reduction at a distance of 170 m did not correspond with higher concentrations of dissolved Mn(II), but these samples belonged to the silty deposits in the pond.

3.5. Denitrification

The bioassays showed substantial denitrification in samples from locations at distances 113, 170 and 210 m from the landfill (Fig. 6A) generating N_2O at rates of 0.2–37 nmol N_2O–N/g dw/day. Between triplicates of bioassays showing denitrification, the standard deviation of the denitrification rate was 9–27%. The deepest located, clayish sample at the distance of 170 m from the landfill showed a remarkable high denitrification rate of 37 nmol N_2O–N/g dw/day, four times higher than the second highest rate observed. The denitrification rates observed were of the same level as observed in pristine aquifers (Francis et al., 1989; Smith and Duff, 1988) and in a landfill leachate polluted aquifer at the Vejen landfill, Denmark (Nielsen et al., 1995).

The bioassays showing denitrification were all located in the area of the plume with high nitrate concentrations (100–370 μmol NO_3^-–N/l, Bjerg et al., 1995), and substantial concentrations of the nitrogen species N_2O (Fig. 6B) and nitrite (not shown). At a distance of 305 m from the landfill, nitrate was also present, although at lower concentrations (< 70 μmol/l, Bjerg et al., 1995), but the bioassays did not reveal any

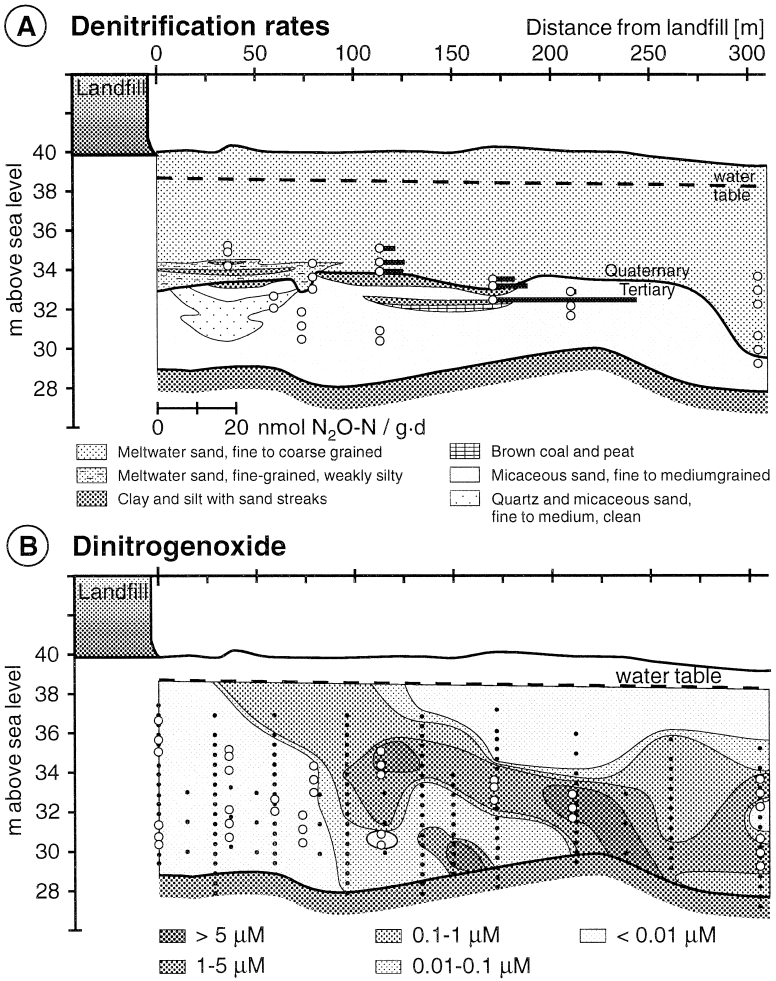

Fig. 6. Denitrification rates in nmol N_2O–N/g dw day (A) estimated from bioassays of adjacent sediment and groundwater (white dots). Distribution of N_2O in μmol N_2O–N/l (B), black dots indicate samples analysed in a prior study (Bjerg et al., 1995).

active denitrification, probably because also oxygen is present in this area of the transect (Bjerg et al., 1995).

4. Discussion

4.1. Simultaneous redox processes

All of the investigated anaerobic redox processes were observed in at least some of the unamended bioassays and they were all microbially mediated, since no signs of

abiotic redox processes were observed in the controls. However, many of the processes seemed to occur simultaneously in the same sample. Within the first 80 m from the landfill, methane production and sulfate reduction occurred simultaneously in almost all samples, both at significant rates. Methane production may be depressed by sulfate reduction (Beeman and Suflita, 1987), since this process is thermodynamically more favourable, but in our investigation variations between samples in the sulfate reduction rates or sulfate concentrations were not reflected in the methane production rates. The sulfate concentrations (< 2500 μmol SO_4^{2-}/l; < 100 mg SO_4^{2-}–S/l) were probably too low to inhibit the methane production. This has been indicated also in another investigation of a landfill leachate polluted aquifer (Beeman and Suflita, 1987), where methane production was inhibited in the section of the aquifer with up to 537 mg SO_4^{2-}–S/l, but not in another section of the aquifer with less than 230 mg SO_4^{2-}–S/l.

Manganese was reduced in some of the samples with denitrification, and slow iron reduction was observed in samples (e.g. 73 m from the landfill) with methane production and sulfate reduction. Such simultaneous redox processes have also been observed in various laboratory and field studies (Lovley, 1991), and even though iron reduction in most cases is the energetically most favourable of these processes and will outcompete the other processes by maintaining low concentrations of electron donors, this will not be the case if other factors, e.g. availability of iron oxides, are limiting the iron reduction (Lovley, 1991). In our samples, the lack of available Fe(III)-oxides in the sediment initially was considered to be a limiting factor for the iron reduction, but amendments with amorphous iron hydroxides only stimulated the iron reduction when an electron donor (acetate) also was added to the bioassay (data not shown). This indicates that the availability of the electron donor might be an important controlling factor in the leachate plume studied. This was surprising since relatively high concentrations of dissolved organic carbon (4000–12 000 μmol C/l or 50–150 mg C/l) were found in the aquifer close to the landfill (Bjerg et al., 1995), but the organic carbon present may be relatively recalcitrant under the actual redox conditions.

In addition to concentration level and availability of the electron acceptor or donor, other parameters such as pH or presence of micro niches in the samples may also contribute to the very complex pattern of redox processes with several simultaneous redox processes. However, in most cases one electron acceptor seemed to be dominant in each sample when calculated on the basis of electron equivalents or of the carbon equivalents transformed, as discussed later.

4.2. Microbial redox processes related to the geological heterogeneities and to the leachate plume

The redox processes in the aquifer varied highly in the transect studied both with respect to types of redox processes taking place and corresponding redox rates. Since the pristine aquifer in general was aerobic outside the plume, most of the anaerobic redox processes observed in the aquifer are believed to be induced by the continuous migration of leachate from the landfill. However, in a few cases also the geological heterogeneities seemed to have influenced the redox processes. The samples collected from the silt and clay layers were rich in organic matter (about 200 μmol C/g dw compared to less than

20 μmol C/g dw in the surrounding sandy samples, Heron et al., 1998) and diffusion of dissolved electron acceptors may be limited by the texture of the sediment. This may have allowed the silt/clay layers to develop micro niches with other availabilities of electron acceptors and electron donors than in the surrounding sediment. This is most likely the case for one sample collected at 170 m (depth 6.9 meter below surface (m.b.s)) from the landfill, showing simultaneous sulfate reduction, iron reduction and manganese reduction in addition to denitrification and for one sample at 113 m (depth 6.2 m.b.s.) showing simultaneous sulfate reduction, manganese reduction and denitrification. Sulfate and iron reduction probably occurred in these samples independently of the leachate pollution.

Specific features were observed in the Tertiary sand. In the deep (31 to 33 m above sea level) samples collected 35 to 60 m from the landfill very low rates of the microbial redox processes were observed. Currently it cannot be judged whether this low activity is of geological reasons or simply related to the fact that the electron acceptors are depleted as a result of previous activity (e.g. low content of sulfate and iron oxides, Bjerg et al., 1995; Heron et al., 1998). The reactivity of the dissolved organic matter may also be low in this part of the aquifer.

4.3. Significance of redox processes in the plume

In order to evaluate the significance of the various anaerobic redox processes quantified by the bioassays, we converted, as shown in Table 2, the measured redox rates to carbon conversion rates assuming a coupling of the electron-accepting processes to oxidation of organic carbon (oxidation level zero) to carbon dioxide. The aerobic samples (305 m) as well as the samples strongly affected by the geological heterogeneities were excluded (1 sample at 113 m distance at depth 6.2 m, 3 samples at 170 m distance). The detection limits of the various redox rates determined by the bioassays were also converted to carbon conversion rates as shown in Table 2.

The conversions presented in Table 2 show that at the strongly reduced conditions close to the landfill (including the samples obtained at 78 m), the redox rates were fairly low, typically converting of the order of 10 g C/m^3 of aquifer per year (corresponds approximately to 500 nmol C/g dw/yr). In a few samples (e.g. 0 m at 9.5 m depth, 36 m at 9.4 m depth) the redox rates all were below the detection limits. However, most of the samples showed significant rates of more than one redox process, and in all these samples, a single redox process dominated accounting for at least 70% of the carbon conversion. This picture may be partly affected by the fact that the detection limit of the iron reduction rate was very high (400 nmol C/g dw/yr), implying that undetected iron reduction, which means at rates less than the detection limit, may have taken place simultaneously with for example methanogenesis or sulfate reduction without being reported in Table 2.

The carbon conversion rates estimated from the bioassays can be compared in general terms with the observations on the distribution of dissolved carbon in the plume as reported by Bjerg et al. (1995). Close to the landfill, where the bioassays showed very little carbon conversion, in particular in the Tertiary part of the plume, Bjerg et al. (1995) found rather constant concentrations of dissolved organic carbon within the first

Table 2
Estimated carbon oxidation rates (nmol C/g dw yr) calculated by simple stoichiometry[a] from the laboratory rate measurements of the redox processes

Sample		Estimated carbon conversion rates (nmol C/g dw/yr), based on rates of redox processes measured in laboratory bioassays					
Distance from landfill	Depth (m.b.s.)	Methano-genesis	Sulfate-reduction	Fe(III)-reduction	Mn(IV)-reduction	Denitrification	Sum
0 m	3.2	<[b]	135	<	<	nm[c]	135
	4.2	20	<	<	<	nm	20
	4.8	40	<	505	<	nm	445
	8.4	15	55	<	<	nm	70
	9.1	4	<	<	<	nm	4
	9.5	<	<	<	<	nm	<
36 m	4.9	30	960	<	<	<	990
	5.3	25	120	475	<	<	620
	6.0	25	240	<	<	<	265
	8.0	<	105	<	<	nm	105
	8.7	3	<	<	<	nm	3
	9.4	<	<	<	<	nm	<
59 m	7.2	<	55	<	<	<	55
	7.8	9	<	<	<	<	9
73 m	8.0	<	230	<	<	<	230
	8.7	7	355	1055	<	<	1420
	9.4	15	85	630	<	<	730
78 m	5.5	2	150	<	<	<	152
	6.2	<	100	<	<	<	100
	6.9	<	235	<	<	<	235
113 m	4.8	<	<	<	<	3655	3655
	5.5	<	<	<	35	5830	5865
	9.0	<	<	<	55	<	55
	9.7	<	<	<	155	<	155
210 m	7.0	nm	nm	<	<	1305	1305
	7.7	nm	nm	<	<	140	140
	8.4	nm	nm	<	20	375	395
Detection limits		1	50[d]	400	20	140	

40 m from the landfill. Assuming that the carbon conversion rate (730 nmol C/g dw yr) measured at 73 m from landfill in the Tertiary part of the plume is representative for the Tertiary layer 70–80 m from the landfill, the rate determined in the bioassays can be compared to a corresponding removal rate in the plume. The decrease in the dissolved organic carbon concentration over a 10 m distance (70 m–80 m) is about 30 mg C/l (approximately 50 mg C/l to 20 mg C/l, according to Bjerg et al. (1995)) and corresponds to a retention time of 1 yr (pore flow velocity of 10 m/yr) yielding an estimated removal rate of 500 nmol C/g dw/yr (when assuming a dry bulk density of the aquifer material of 1.7 kg/l). This removal is comparable to 730 nmol C/g dw/yr determined in the bioassays (actually 230–1420 nmol C/g dw/yr, Table 2). However, further out in the plume (from 113 m) the carbon conversion rates from the bioassays exceed the actual removal of dissolved organic carbon from the groundwater by a factor of about 15 and would correspond to complete removal of all dissolved as well as sediment bound organic carbon within two years (data not shown). These calculations suggest that other electron donors than organic carbon may be involved in the redox processes under manganese reducing and nitrate reducing conditions. Ammonia, which disappeared from the groundwater in this part of the plume (Bjerg et al., 1995) may play a role here, but more studies are needed to evaluate this hypothesis.

4.4. Assignment of redox conditions

Table 3 summarizes the ongoing redox processes in the various bioassays, as presented in Table 2, for comparison with the redox status assigned on the basis of the groundwater composition according to the criteria presented by Bjerg et al. (1995). Table 3 also shows the redox conditions determined by Bjerg et al. (1995) on the basis of many groundwater samples for the same area of the plume, where the bioassay samples have been taken. For the nitrate reducing and manganese reducing conditions consistency is observed in redox level determined by the bioassays and by the groundwater composition. At the strongly reducing conditions with low redox rates, the picture is less consistent. The groundwater composition indicated that iron reduction dominated in much more samples that identified by the bioassays. However, this is probably related to the high detection limit for the bioassay for iron reduction as previously discussed. The samples implying the apparent inconsistency all had carbon conversion rates related to sulfate reduction that were lower than the detection limit for iron reduction. The unamended bioassays and the composition of the groundwater are two very different approaches to characterize the redox environment in a polluted aquifer. The bioassays allow for measuring the rates of all individual redox processes on a small sediment sample as long as the rates are significantly higher than the detection limits. The bioassays thus reflect the actual redox processes in the small sediment

Notes to Table 2:

[a] Carbon is assumed to start at redox status 0 and be converted to CH_4 or oxidized to CO_2 by the generation of the reduced species S(II), Fe(II), Mn(II) and N_2 respectively.

[b] <: below detection limit of the bioassay.

[c] nm: not measured.

[d] Based on sulfate concentrations of 0.3–43 mg/l.

Table 3
Proposed dominating redox process based on the bioassay studies and water chemistry

Sample		Summary of dominating redox process		
Distance from landfill	Depth (m.b.s.)	Based on rates estimated from bioassays[a]	Based on water chemistry of the individual sample[b]	Redox zone identified by Bjerg et al. (1995)[c]
0 m	3.2	SO_4^{2-}-reduction	?	CH_4-production/SO_4^{2-}-reduction
	4.2	CH_4-production	?	CH_4-production/SO_4^{2-}-reduction
	4.8	Fe(III)-reduction	?	CH_4-production/SO_4^{2-}-reduction
	8.4	SO_4^{2-}-reduction	CH_4-production	CH_4-production/SO_4^{2-} reduction
	9.1	CH_4-production	CH_4-production/Fe(III)-reduction	CH_4-production/SO_4^{2-}-reduction
	9.5	?	?	CH_4-production/SO_4^{2-}-reduction
36 m	4.9	SO_4^{2-}-reduction	SO_4^{2-}-reduction	CH_4-production/SO_4^{2-}-reduction
	5.3	Fe(III)-reduction	SO_4^{2-}-reduction	CH_4-production/SO_4^{2-}-reduction
	6.0	SO_4^{2-}-reduction	SO_4^{2-}-reduction	CH_4-production/SO_4^{2-}-reduction
	8.0	SO_4^{2-}-reduction	CH_4-production	CH_4-production/SO_4^{2-}-reduction
	8.7	CH_4-production	CH_4-production	CH_4-production/SO_4^{2-}-reduction
	9.4	?	CH_4-production	CH_4-production/SO_4^{2-}-reduction
59 m	7.2	SO_4^{2-}-reduction	Fe(III)-reduction	Fe(III)-reduction
	7.8	CH_4-production	Fe(III)-reduction	Fe(III)-reduction
73 m	8.0	SO_4^{2-}-reduction	Fe(III)-reduction	Fe(III)-reduction
	8.7	Fe(III)-reduction	Fe(III)-reduction	Fe(III)-reduction
	9.4	Fe(III)-reduction	Fe(III)-reduction	Fe(III)-reduction
78 m	5.5	SO_4^{2-}-reduction	Fe(III)-reduction	Fe(III)-reduction
	6.2	SO_4^{2-}-reduction	Fe(III)-reduction	Fe(III)-reduction
	6.9	SO_4^{2-}-reduction	Fe(III)-reduction	Fe(III)-reduction
113 m	4.8	NO_3^--reduction	NO_3^--reduction	Mn(IV)-reduction/NO_3^--reduction
	5.5	NO_3^--reduction	Mn(IV)-reduction/NO_3^--reduction	Mn(IV)-reduction/NO_3^--reduction
	9.0	Mn(IV)-reduction	Mn(IV)-reduction	Mn(IV)-reduction/NO_3^--reduction
	9.7	Mn(IV)-reduction	Mn(IV)-reduction	Mn(IV)-reduction/NO_3^--reduction
210 m	7.0	NO_3^--reduction	NO_3^--reduction	NO_3^--reduction
	7.7	NO_3^--reduction	NO_3^--reduction	NO_3^--reduction
	8.4	NO_3^--reduction	NO_3^--reduction	NO_3^--reduction

sample and the associated groundwater. In contrast to this the groundwater composition represents the status of the electron acceptors or their conversion products in the water phase. However, the groundwater composition may be affected also by upstream processes due to transport of reaction products (e.g. CH_4, Fe^{2+}). The criteria used to assign a redox status to the groundwater sample (Bjerg et al., 1995) identify only the dominating redox environment that has governed the composition of the groundwater sample and ignores the presence of simultaneous redox processes and their actual rates. The results of this study indicate that characterization of the redox conditions in a polluted aquifer may benefit from doing both bioassays and groundwater characterization. The latter can provide mapping of dominating redox conditions due to its integrating approach and robust criteria, while the bioassays may show that also other redox processes may take place in micro niches. This may be important in relation to the degradation of trace amounts of specific organic chemicals that may degrade only at certain redox conditions.

5. Conclusions

The anaerobic, unamended bioassays containing aquifer sediment and groundwater were able to identify and estimate rates of methane production, sulfate reduction, iron reduction, manganese reduction and denitrification. All the redox processes measured in the bioassays were microbially mediated since all inhibited controls were negative. The unamended bioassays performed on samples from the Grindsted landfill leachate plume prove that all the redox processes, previously deducted from the groundwater composition, actually take place in the plume and that they with respect to the general activity are microbial processes.

The unamended bioassays as a laboratory method are rather labour intensive and require for some of the processes long incubation times (up to three months) to reveal the actual redox process. This makes bioassays less useful for detailed mapping of redox conditions in pollutions plumes from a practical perspective. In the current experiment, where some of the bioassays from the beginning had very high contents of ferrous ions, the detection limit for iron reduction unfortunately was very high and may be the reason for the relative low numbers of samples showing active iron reduction.

Most of the samples showed several simultaneous redox processes, but comparing the actual rates measured in the bioassays by converting all rates to carbon conversion rates, revealed that in most cases a single redox process dominated accounting for more than 70% of the carbon conversion in the sample. Within the first 80 m of the leachate plume, methanogenesis, sulfate reduction and iron reduction were observed, and no clear redox zonation could be related to the redox rates measured in the bioassays. At further

Notes to Table 3:
[a] From Table 2.
[b] Based on the water chemistry data of the individual groundwater samples (data not shown) according to the criteria in Bjerg et al. (1995).
[c] Zones identified from the composition of many groundwater samples from the same area as reported by Bjerg et al. (1995).

distance, manganese reduction and nitrate reduction dominated as was expected since the aquifer outside the plume is mainly aerobic. A few samples far away from the landfill showed strongly reduced redox processes (sulfate reduction and iron reduction), but these samples originated from local silt and clay inhomogeneities. The presence of such inhomogeneities with different redox activities makes it unwarranted to base such bioassays measurements of redox processes on only few samples, since it may lead to erroneous interpretation of the distribution of the important redox processes in the plume.

The dominating redox process determined by the bioassays and by the groundwater composition was identical under manganese reducing and nitrate reducing conditions, but at strongly reduced conditions no consistency was found. This may be related to the fact that the redox processes occur simultaneously at fairly low rates in this part of the plume, that the detection limit for iron reduction by the bioassay was very high, and that the redox conditions evaluated from the groundwater composition averages over a larger volume than the bioassays. However, in contrast to the redox information deducted from the groundwater composition, the bioassays provide estimates of rates and the bioassays clearly showed that more redox processes took place simultaneously in the same sample. This may not be significant in controlling the local redox level, but it may have implications for the degradation of specific chemicals vulnerable to degradation only by specific redox processes.

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