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Microbial Biomass and Activity in Subsurface Sediments from Vejen, Denmark

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Abstract. Subsurface sediment samples were collected from 4 to 31 m below landsurface in glacio-fluvial sediments from the Quaternary period. The samples were described in terms of pH, electrical conductivity, chloride concentration, organic matter content, and grain size distribution. Viable counts of bacteria varied from 0.5 to 1,203 × 10^3 colony forming units/g dry weight (gdw); total numbers of bacteria acridine orange direct counts (AODC) varied from 1.7 to 147 × 10^7 cells/gdw; growth rates (incorporation of [^{3}H]-thymidine) varied from 1.4 to 60.7 × 10^4 cells/(gdw·day); and rate constants for mineralization of ^{14}C-labelled compounds varied from 0.2 to 2.3 × 10^{-3} ml/(dpm·day) for acetate, and from 0 to 2.0 × 10^{-3} ml/(dpm·day) for phenol. Sediment texture influenced the total number of bacteria and potential for mineralization; with increasing content of clay and silt and decreasing content of sand, AODC increased and the mineralization rate declined. Intrinsic permeability calculated from grain size correlated positively with mineralization rate for acetate. Statistical correlation analysis showed high correlations between some of the abiotic parameters, but it was not possible to point out a single abiotic parameter that could explain the variation of size and activity of the microbial population. The microbial data obtained in these geologically young sediments were compared to literature data from older sediments, and this comparison showed that age and type of geological formation might be important for the size and activity of the microbial populations.

Introduction

The existence of an active microbial population in groundwater sediments has been established in recent years, and the ecology of the microorganisms in the

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subsurface is currently under exploration [13]. Microbiological methods from terrestial and aquatic environments have been modified for application in oligotrophic subsurface environments [5, 19, 35], but further modification and optimization are still needed. Microbial populations have been shown in polluted [e.g., 16] as well as unpolluted aquifers [e.g., 19]. Even in very deep sediments (265 meters below surface (mbs)) significant microbial populations have been revealed with no decrease in the number of microorganisms with depth, although the abundance of organisms and the composition of the microbial community varied with depth [3].

Investigations of bacterial strains isolated from samples from different boreholes and different geological formations in the same borehole have revealed morphological and physiological differences, especially the capability to metabolize different substrates, among different strains [11, 20]. This indicates the existence of different ecological niches in the subsurface environment. Another approach has been to show correlations between different abiotic parameters and occurrence or activity of microbial populations. The density of microbial populations (AODC, viable counts) has been found to correlate with sediment texture in the subsurface [7, 32, 33]. In addition, microbial activity, estimated from mineralization of acetate, phenol, and hydroxybenzoate, correlated with the content of clay [5, 10, 17, 24]. In all the cited investigations the content of clay and silt or sand were used as the only measure of the grain size distribution, and in some cases samples with high clay content represented thick layers of low permeable geological formations. Apart from the texture, differences in the age of the sediment and in the environment at the time of sedimentation might influence the microbial activity measured today.

The present investigation was part of a larger hydrogeological study on the transport and degradation of leachate from Vejen Municipal Landfill and was the first microbial survey of deeper subsurface sediments in Danish geological formations. The purpose of this study was to compare different methods for estimation of microbial biomass and activity in the subsurface environment and to examine which physical and chemical parameters are important in regard to the occurrence and activity of microbial populations.

Materials and Methods

Study Site and Sampling

The study site was located near the city of Vejen in the western part of Denmark (Fig. 1) and consisted of glacigenic and freshwater sediments from different parts of the Quaternary period. The investigated sediment profile of 35 m (Fig. 2) represented two main reservoirs: the lower from the Saalian glacial period (ca. 240,000–95,000 years before present (BP)) and the upper from the Weichsilian glacial period (ca. 70,000–10,000 years BP). The Saalian beds were created by glacial deposits, first by alternating layers of gravel, sand, and moraine clay (31–35 mbs), and later by moraine clay (22–31 mbs). On top of these two layers, clay has been deposited in a meltwater lake (17–22 mbs). The deposits from the Weichsilian period (2–17 mbs) are more sandy and consist of meltwater sand with clay and silt inhomogenities [14].

The drilling of the borehole to 35 mbs (Danish Geological Survey file No. 132.1039 (B 203)) was executed by the Danish Geotechnical Institute as a dry, cased, rotating 8 inch drilling without use of drilling mud. To a limited extent, groundwater (from the same geological formation) was

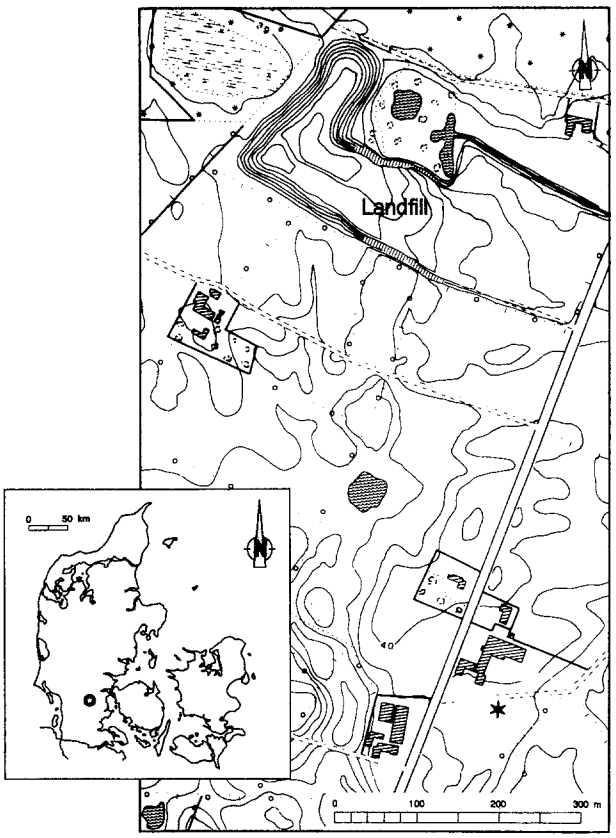

Fig. 1. Map of Denmark showing the location of the study site.

added to the hole during the drilling. To prevent contamination by unwanted microorganisms undisturbed sandy sediment samples were collected by pressing a corer with an inner PVC-plastic liner down into the sediment through the bottom of the borehole. Each sample was 1 m long with a diameter of 10 cm. Clay samples were collected by the same technique in stainless steel tubes, 1 m long and 7 cm in diameter. The sediment cores were transported and stored at low temperature (below 10°C during transport and at 2°C during storage), and they reached the laboratory within 3–4 days. As the ambient temperature of groundwater in Denmark is 10°C, this cooling was considered satisfying. After the PVC-liner was cut open (sandy sediment samples) or the cores were pressed out of the stainless steel tubes with hydraulic pressure (clay samples), the outer part of the core material, which might have been contaminated during sampling, was removed by sterile spoons, and from the core center, 11 subsamples were collected aseptically into sterile glass bottles. The subsamples for microbiological tests were handled within 24 hours.

Chemical and Physical Analysis

Electrical conductivity, pH, and chloride concentration were measured in sediment slurries (10 g wet weight (gww) and 25 ml distilled water shaken for 2 hours (200 rpm)). The grain size analyses were executed at Danish Geological Investigations (B. Larsen, personal communication). The intrinsic permeability (k) was calculated by the formula: $k = 3{,}500 \cdot (d_{50})^{1.65}$, where d_{50} is the 50% quantile of the grain size [29]. The content of organic matter was estimated by a modification of the method of Mebius [23]; 5 or 10 gww of sediment was digested by 9.5 ml 0.04 N $K_2Cr_2O_7$ in 60% (w/w) H_2SO_4 (140°C, 1 hour). The residual dichromate was determined by titration with 0.1

N $Fe(NH_4)_2(SO_4)_2$ and converted to organic carbon, assuming an oxidation number of organic carbon of zero.

Isotopes

The isotopes used were methyl-[^{3}H]-thymidine (2.89 TBq/mmol, 11.7 GBq/mg, radiochemical purity: 98.4%), sodium-[1-^{14}C]-acetate (1.96 GBq/mmol, 23.3 MBq/mg, 1.3 nmol/50 µl, radiochemical purity: 99.1%) and [U-^{14}C]-phenol (4.07 GBq/mmol, 41.4 MBq/mg, 0.9 nmol/50 µl, radiochemical purity: 97.8%), all from Amersham Inc. The radioactivity was quantified by scintillation counting (LKB Wallac 1209 Rackbeta) and converted to dpm according to the internal standard addition method (Internal Standard Kit (glycerol-tri[1-^{14}C]-palmitate) or ([6,6'(n)-^{3}H]sucrose), Pharmacia). Unless specified otherwise, 5 ml Optiphase 'HiSafe II' (LKB-Wallac) was used as scintillation liquid.

Acridine Orange Direct Count (AODC)

Sediment, 1.0 gww, fixed by phosphate buffered formaldehyde (final concentration: 1%) and diluted with 1 ml Winogradsky salt solution [37] (250 mg/liter K_2HPO_4, 125 mg/liter $MgSO_4 \cdot 7H_2O$, 125 mg/liter NaCl, 2.5 mg/liter $FeSO_2 \cdot 7H_2O$, 2.5 mg/liter $MnSO_4 \cdot 7H_2O$) was sonicated (4 × 10 sec with a MSE, Ultrasonic Disintegrator 100 W, equipped with a 3 mm titanium microprobe tip). Dilutions of the suspensions were stained with acridine orange (final concentration: 10 µg/ml), filtered onto a black 0.2-µm Nucleopore filter, and mounted in immersion oil (Olympus UVFL, SI). The bacteria were counted using an epifluorescence microscope (Olympus BH-2) equipped with dichroic mirrors (DM500), barrier filters (O-515), exciter filter B (BP-490), and an achromatic (Splan) 100×/1.15 oil objective. All liquids were filtered (0.2 µm) before use. On each filter (triplicate samples) 30 fields were counted; on filters with high numbers of bacteria a maximum of 200 bacteria were counted, which gave a sufficient representation of the variance.

Plate Counts

The medium used was a PYGV-agar modified after [19], containing 0.25 g/liter peptone 190 (Gibco), 0.25 g/liter yeast extract (Difco), 0.25 g/liter glucose, 200 ml/liter Winogradsky salt solution, and 5 ml/liter vitamin solution. Vitamin solution (in mg/liter): d-biotin, 2; folic acid, 2; pyridoxine-HCl, 10; riboflavin, 5; thiamine-HCl, 5; nicotine amide, 5; calcium pantothenate, 5; B_{12}, 5; p-amino-benzo acid, 5. The plates, triplicates from each tenfold dilution, were incubated in the dark for 3 weeks at 10°C before enumeration, and the weighted average for two or three dilutions was calculated.

Growth Rate Measurements

Incorporation rates of thymidine into bacterial DNA and protein were measured by incubation of a slurry of sediment (2.0 gww) and distilled water (1.0 ml) with 202 nM methyl-[^{3}H]-thymidine for 16 hours at 10°C. During the incubation period the incorporation rate of ^{3}H into DNA and protein was constant. The incubation was terminated by addition of buffered formaldehyde (final concentration: 1%). The sample was centrifuged for 20 min at 4,330 × *g*, and the macromolecules were extracted for 12 hours at 25°C by addition of 5 ml 0.3 N NaOH, 25 mM EDTA, and 0.1% SDS to the pellet (modified after Thorn and Ventullo [35]). After centrifugation (4,330 × *g* for 5 min) the supernatant was chilled to 0°C, 50% TCA was added to a final concentration of 5%, thymidine was added to a final concentration of 17 µg/ml, and the sample was acidified to pH <2 with HCl. After 60 minutes on ice, centrifugation (25,000 × *g* for 10 min), and washing of the

pellet with cold 5% TCA, the DNA was hydrolyzed by boiling in 3 ml 5% TCA for 30 min. Following centrifugation (2,500 × *g* for 6 min), the radioactivity in 1 ml of supernatant (hydrolyzed DNA) was counted. The protein was hydrolyzed by resuspending the pellet in 3 ml of 0.3 N NaOH and boiling for 1 hour. After centrifugation (2,500 × *g* for 6 min) the radioactivity in protein (1 ml) was counted. The growth rates were calculated using a conversion factor of 2×10^{18} cells/mol thymidine incorporated in DNA, derived in aquatic environments [34] and used in subsurface sediments [35]. Isotope dilution was not evaluated.

Aerobic Heterotrophic Activity Measurements

Uptake and respiration rates of ^{14}C-acetate and ^{14}C-phenol were estimated at 10°C by incubation of a suspension of sediment (10.0 gww) and distilled water (100 ml) in 125.5 ml infusion bottles with a 3-mm-thick rubber septum. Stock solutions (50 µl) of the isotopes were added to the suspension. When sampling, 5 ml of suspension was transferred to a double vial system consisting of a 20-ml glass vial containing 1 ml 1 N HCl and a 6-ml plastic inner vial with 2.5 ml 0.5 N KOH. The evolved CO_2 from the subsample was trapped in the base. After at least 24 hours the minivial was removed, and 2.5 ml scintillation liquid (Lumagel, Lumac) was added. The acidified subsamples were filtered (Minisart SRP15, 0.2 µm, Sartorius), and the radioactivity in 750 µl of the filtered sample was counted. All experiments were in duplicate or triplicate, and as a control a triplicate sample was killed by addition of formaldehyde (final concentration: 2%).

Modelling and Statistics

The ^{14}C-substrate uptake was fitted to a logistic model [31] by the Marquardt algorithm in the statistical package STATGRAPHICS. The substrate concentration (S) at the time (t) was given by:

$$S = \frac{S_0 + X_0}{1 + (X_0/S_0)\cdot\exp[k_4\cdot(S_0 + X_0)\cdot t]}$$

The model provided three parameter estimates: S_0, initial substrate concentration; X_0, amount of substrate required to produce a population density equal to B_0 (the initial biomass), with $X_0 = B_0/Y$ (yield); and k_4, rate constant, derived from $k_4 = \mu_{max}/K_s$. Data of S_0 and X_0 are not shown.

Because of a wide range of the measured values (sometimes severalfold), a nonparametric test (Spearman rank correlation coefficient (r_s) [30]) was chosen to test the correlation among the different parameters. The correlation coefficients were interpreted as follows (the statistical probability for the actual sample size in parentheses): r_s = 0.4–0.6 (77.7–94.9%), moderate correlation; 0.6–0.8 (95.0–99.7%), high correlation; 0.8–1.0 (99.8–100.0%), very high correlation, as suggested by Darlington [8] for similar (Pearson) correlation coefficients.

Results

Physical and Chemical Parameters

Eight of the 11 samples were taken in the upper unconfined aquifer, 2–16 mbs, two in the clay layer, 17–31 mbs, and one in the lower confined aquifer, deeper than 31 mbs (Fig. 2). The samples represented sediments with both coarse and fine texture, and well-sorted as well as poorly sorted sediments, which lead to a wide range of values for calculated permeability, from 6×10^{-4} m/sec in the most coarse sample (31.3 mbs) to 1.3×10^{-7} m/sec in the glacial clay (20.4 mbs) (Table 1).

Table 1. Physical and chemical parameters

Depth (mbs)	Texture[a] (silt/sand/gravel in %)	Permeability[b] (10^{-5} m/sec)	Organic matter (mg C/kgdw)	pH	Electrical conductivity (μS/cm)	$[Cl^-]$[c] (mg/liter)
4.5	2.1/97.9/0	12.09	308 ± 60	7.2	57	304
6.0	2.8/97.0/0.1	7.54	166 ± 25	6.9	56	333
6.7	1.6/98.4/0	40.10	189 ± 79	6.8	45	426
7.2	2.5/84.6/12.9	47.20	83 ± 20	6.8	47	399
9.8	2.3/97.6/0	9.31	237 ± 16	7.2	191	364
11.1	58.8/40.9/0	1.38	1,666 ± 376	7.9	99	277
12.0	26.3/54.7/19.1	3.82	264 ± 184	7.8	121	113
16.7	1.0/97.6/1.5	21.43	443 ± 9	8.0	149	214
20.4	99.7/0.3/0	0.01	6,223 ± 355	8.0	275	305
27.5	50.2/47.7/2.1	1.68	3,327 ± 188	8.1	284	322
31.3	3.1/78.8/18.2	60.32	577 ± 46	8.1	155	217

[a] Silt (incl. clay); <63 μm; sand, 63–2,000 μm; gravel, >2 mm
[b] $k = 3{,}500 \cdot (d_{50})^{1.65}$ [29]
[c] $[Cl^-]$ calculated for pore water

The chemical analysis showed a pH in the neutral range from 6.8 to 8.1 (Table 1). The content of organic matter varied from 83 to 6,223 mg C/kg dry weight (kgdw), with a maximum in the clay. The electrical conductivity ranged from 45–284 μS/cm, with the lowest values in the upper part of the core. The chloride concentration in the slurry was low (8–22 mg/liter). However, when recalculated to give concentration in the pore water only, it reached from 113 to 426 mg Cl/liter, with an increase in the upper part of the aquifer and a peak in the samples from 6.7 and 7.2 mbs. These values are high compared with

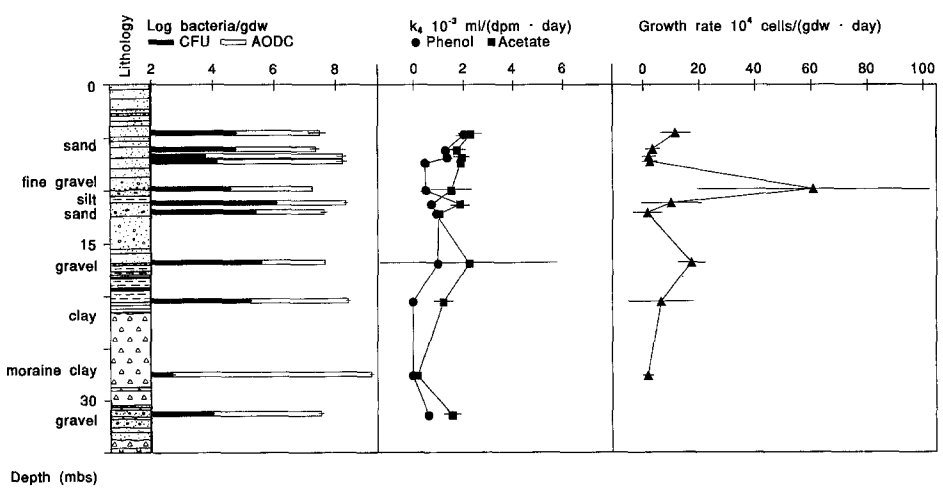

Fig. 2. Sediment profile showing the lithology and viable counts (CFU), total counts (AODC), rate constants (k_4) for uptake of ^{14}C-acetate and ^{14}C-phenol, and the growth rates in the samples. The growth rates were calculated from incorporation of ^{3}H-thymidine into DNA. The bars show ±SD (±SEM for CFU). In some cases the bars are hidden behind the symbols. The lithology is from [14].

Table 2. Values derived from the microbiological data, showing the ratio of active bacteria, the ratio of bacterial carbon to total organic carbon (C_{bact}/C_{org}), and the distribution of ^3H incorporated into DNA and protein in percent of the total incorporation (DNA plus protein)

Depth (mbs)	CFU/AODC (%)	C_{bact}/C_{org} (%)	^3H-DNA/ ^3H-protein (%)
4.5	0.187	0.87	89.2/10.8
6.0	0.252	1.16	39.5/60.5
6.7	0.003	7.55	76.9/23.1
7.2	0.009	17.07	60.0/40.0
9.8	0.222	0.61	61.8/38.2
11.1	0.568	1.09	68.1/31.9
12.0	0.578	1.35	50.0/50.0
16.7	0.867	0.87	86.9/13.1
20.4	0.069	0.36	88.9/11.1
27.5	<0.001	3.80	75.0/25.0
31.3	0.031	0.53	ND[a]

[a] ND, not determined

bulk water from a well 3.5 m away (Danish Geological Survey file No.132.990 (T21)), which had a background level of 22–25 mg Cl/liter and also showed a peak (73–115 mg Cl/liter) in the upper aquifer at 6–8 mbs [18].

The redox potentials of the actual samples were not measured. However, in water samples from the nearby well, T21, oxygen was measured down to 17 mbs, whereas no oxygen was measured from the top of the glacial clay (17 mbs) and down to the bottom of the drilling (24 mbs) [18]. Two of the samples (20.4 and 27.5 mbs) were probably anaerobic, but incubation under reduced oxygen tensions was not attempted.

Microbial Parameters

The total number of bacteria (AODC) ranged from 1.7 to 147 $\times$ 10^7 cells/gdw; the numbers of aerobic heterotrophic bacteria (viable counts), ranging from 0.5 to 1,203 $\times$ 10^3 colony-forming units (CFU)/gdw, showed larger variations (Fig. 2). The highest number of CFU was found in the lower part of the upper aquifer (11.1–16.7 mbs), whereas the highest total number of bacteria was found in samples with high clay content (20.4 and 27.5 mbs). The ratio of active bacteria (CFU/AODC) ranged from <0.001% to 0.9% (Table 2).

The incorporation of ^3H into DNA and protein was measured, and bacterial growth rates were calculated in all depths, except 31.3 mbs. The growth rate varied from 1.7 to 60.7 $\times$ 10^4 cells/(gdw·day) with a peak in 9.8 mbs (Fig. 2). Like Thorn and Ventullo [35], we did not measure the incorporation of ^3H into RNA. The incorporation of ^3H into DNA relative to the total incorporation (DNA plus protein) varied between 40% and 89% (Table 2).

The heterotrophic activity was measured by mineralization of acetate and phenol. All samples showed significant mineralization, except two samples with

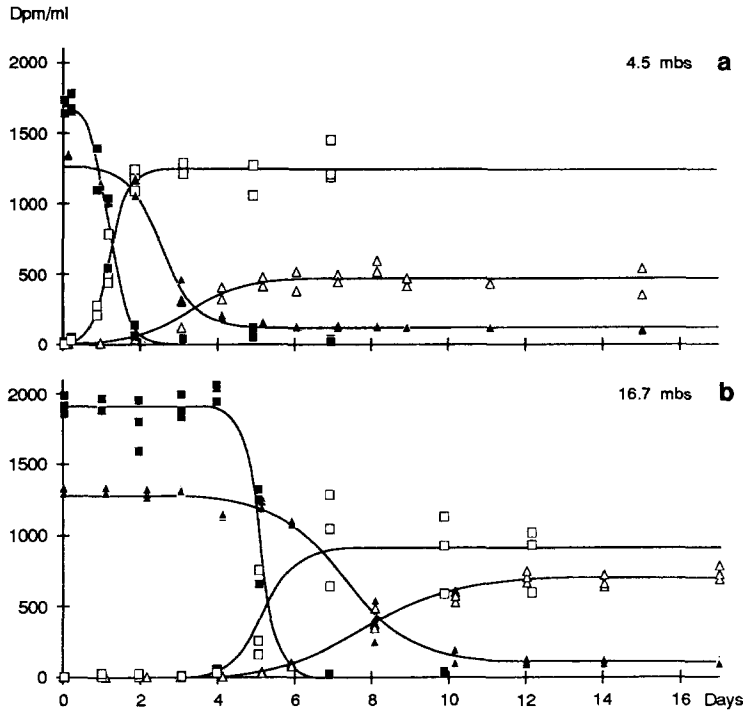

Fig. 3. Examples of ^{14}C-acetate (■) and ^{14}C-phenol (▲) removal plus evolution of $^{14}CO_2$ from acetate (□) and phenol (△) in sediment samples. The curves are fitted by a logistic model [31]. The samples were from two depths, **a** and **b**, and incubated under aerobic conditions at 10°C.

high clay content (20.4 and 27.5 mbs), where no phenol mineralization was observed during more than 60 days of incubation. In these two samples very low acetate mineralization was observed with the replicates showing large variations. The acidified filtered sample was considered as unaltered ^{14}C-substrate, although it might have contained some metabolites. In acetate mineralization experiments, this fraction was depleted, which shows complete mineralization, whereas in phenol mineralization experiments a minor part (3–13%) of this fraction remained. The reason for this remaining fraction is unknown as pathways for phenol degradation under aerobic conditions generally lead to complete mineralization [4]. The explanation is probably that some phenol was adsorbed and therefore inaccessible to the bacteria, but released during the acidification. Mineralization was verified by concomitant $^{14}CO_2$-evolution (Fig. 3a and 3b). The uptake of ^{14}C-substrate showed a low uptake rate in the beginning and at the end of the time space, with a higher intermediate rate, indicating a logistic curve. It seems realistic to assume that the necessary condition of $S_0 \ll K_s$ for a logistic model [31] was fulfilled for the radioactive tracers in the experiments. The model was fitted to the substrate removal data, even though Simkins and Alexander [31] point out the inapplicability of the model to fit data from populations requiring significant induction before the onset of mineralization. When the remaining fraction of phenol was defined

as total depletion, the model showed significant correlation with the removal of acetate and phenol in all the samples. The rate constant k_4 varied between 0.2 and 2.3 $\times$ 10^{-3} ml/(dpm·day) for uptake of ^{14}C-acetate and between 0 and 2.0 $\times$ 10^{-3} ml/(dpm·day) for uptake of ^{14}C-phenol (Fig. 2).

Degradation potential of ^{14}C-m-xylene was tested (data not shown), and in only one of the samples was any significant substrate removal observed. In the sample from 20.4 mbs, xylene was removed, probably by adsorption to the clay.

To examine the relationship between physical/chemical and microbial parameters, a range of correlations was tested (Table 3). We observed moderate to high correlation among the abiotic parameters: with increasing content of sand (63–2,000 μm), there was a decrease in pH (94.4%), content of organic matter (96.7%) and electrical conductivity (91.2%). We found a moderate to high negative correlation between electrical conductivity in the slurries and the rate constants (93.4% for acetate and 97.0% for phenol) and the amount of phenol required to sustain the population of phenol mineralizing bacteria: $X_{0\text{-phenol}}$ (83.6%). Organic matter showed moderate to very high negative correlation with rates of acetate mineralization ($k_{4\text{-acetate}}$) and $X_{0\text{-phenol}}$. Increasing rates of acetate mineralization ($k_{4\text{-acetate}}$) highly correlated with increasing permeability (97.1%) and content of sand (99.1%). The amount of acetate required to sustain the population of acetate mineralizing organisms ($X_{0\text{-acetate}}$) showed a high negative correlation with growth rates (98.7%). AODC correlated moderately with increasing amount of clay and silt content (86.7%) and with decreasing amount of sand (92.7%). Correlations with depth were also observed, but this was probably due to the clay samples being the second and third deepest samples.

AODC correlated moderately with increasing amount of organic matter (92.8%), whereas the number of viable counts showed a high correlation with decreasing chloride concentration in the pore water (96.1%). When assuming a biomass of 1.72 $\times$ 10^{-13} gdw/bacteria [2] and 0.5 g C/g organic matter, the ratio between bacterial carbon (AODC) and organic carbon was 0.4–17.1%, with a mean of 3.2% (Table 2).

Discussion

Microbial Investigations

The viable counts, the incorporation of thymidine, and the heterotrophic activity revealed living and active bacteria in all samples (Fig. 2). The ratio of active bacteria (CFU/AODC) (Table 2) as well as the viable counts and the total number of bacteria (Fig. 2) are within the range of values reported from subsurface sediments [3, 7, 33, 36]. The very small percentage of active bacteria indicates that a large part of the total amount of bacteria was inactive or unculturable, especially in the two glacial clay samples (20.4 and 27.5 mbs). These two samples may have contained obligate anaerobic bacteria that would not have been detected by our methods.

There have been few reports of tritiated thymidine incorporation into sub-

Table 3. Correlation matrix showing the possibility of correlation (in percentages) between paired variables. The correlation is based on Spearman rank correlation coefficient (r_s), and the sign indicates whether the correlation is positive or negative[a]

	$k_{4\text{-phenol}}$	$k_{4\text{-acetate}}$	$X_{0\text{-phenol}}$	$X_{0\text{-acetate}}$	Growth rate	CFU/ AODC	AODC	CFU	Organic matter	$[Cl^-]_{porewater}$	pH	Electrical conductivity	Permeability	Sand	Clay + silt
Depth	96.4	92.5	85.5	42.6	37.3	21.0	83.0	12.7	98.7	86.7	>99.9	99.6	36.9	96.7	88.3
	−	−	−	−	−	−	+	−	+	−	+	+	−	−	+
Clay + silt	71.6	93.3	75.5	33.0	75.3	21.0	86.7	31.1	94.4	49.8	86.5	85.8	98.5	>99.9	
	−	−	−	−	−	−	+	+	+	−	+	+	−	−	
Sand	85.1	99.1	80.2	4.2	54.5	2.1	92.7	52.6	96.7	71.0	94.4	91.2	97.2		
	+	+	+	+	+	−	−	−	−	+	−	−	+		
Permeability	27.1	97.1	91.2	12.7	19.7	51.6	77.7	81.0	91.7	40.7	63.3	83.0			
	+	+	+	+	+	−	−	−	−	+	−	−			
Electrical conductivity	97.0	93.4	83.6	44.5	34.9	4.2	46.3	2.1	99.3	71.5	>99.9				
	−	−	−	−	+	−	+	−	+	−	+				
pH	80.4	89.5	95.2	34.2	19.8	12.7	67.8	21.1	>99.9	96.1					
	−	−	−	−	+	−	+	+	+	−					
$[Cl^-]_{porewater}$	38.0	41.5	57.6	2.1	19.7	96.5	8.5	96.1	89.0						
	−	+	+	−	−	−	+	−	−						
Organic matter	57.6	89.3	>99.9	48.1	27.4	4.2	92.8	48.1							
	−	−	−	−	+	−	+	+							
CFU	73.6	4.6	34.1	72.8	72.4	>99.9	14.7								
	+	−	−	−	+	+	−								
AODC	69.6	83.2	99.2	18.9	77.1	77.7									
	−	−	−	−	−	−									
CFU/AODC	77.2	13.7	27.1	49.8	62.4										
	+	+	+	−	+										
Growth rate	37.4	77.7	17.2	98.7											
	+	+	+	−											
$X_{0\text{-acetate}}$	49.9	50.2	35.1												
	+	−	+												
$X_{0\text{-phenol}}$	20.0	69.4													
	+	+													
$k_{4\text{-acetate}}$	80.0														
	+														

[a] 77.7–94.9%, moderate correlation; 95.0–99.7%, high correlation; 99.8–100%, very high correlation

surface sediment bacteria [24, 25, 35]. Only Thorn and Ventullo [35] have calculated growth rates. Whereas the total number of bacteria reported here and by Thorn and Ventullo [35] was roughly within the same range, we estimated growth rates from 1.4 to 60.7 $\times$ 10^4 cells/(gdw·day), which were 1–73 times lower than those reported by Thorn and Ventullo [35]. Only a part of this difference could be explained by the higher incubation temperature in the study of Thorn and Ventullo (16°C) [35]. More probably this indicates that bacteria in the sediments from Vejen had lower growth rates or lower capability of thymidine incorporation than those in the sediments studied by Thorn and Ventullo [35].

We recovered ^3H from DNA and protein separately and found the majority of the recovered ^3H (40–89%) in the DNA (Table 2). This is in accordance with the results of Thorn and Ventullo [35] and shows that thymidine is not exclusively used for DNA synthesis in subsurface sediments. Other workers have shown that nutritionally stressed bacteria incorporate a larger fraction of ^3H-thymidine into protein than into DNA [15, 27]. Davis [9] showed that nutrient-starved, dormant marine bacteria were able to assimilate thymidine without subsequent cell-division. Compared to seawater [12] and freshwater [26] bacteria, the relative incorporation of 3H-thymidine into DNA was low in groundwater sediment bacteria. This confirms the oligotrophic state of the groundwater sediment.

Acetate and phenol were chosen as model compounds for mineralization of organic matter. Phenol was chosen because phenolic structures are expected to be common in the refractory humic substances usually found in aquifers and also as a representative of contaminants. The rate constants of acetate and phenol removal had different numerical values, with $k_{4\text{-phenol}}$ being lower than $k_{4\text{-acetate}}$, but showed the same variation over depth (Fig. 2) and a moderately positive correlation (80.0%) (Table 3). The low heterotrophic activity observed in the glacial clay layer was in accordance with a low flux of nutrients expected from the low calculated intrinsic permeability of the sediments.

Not all bacteria isolated from subsurface sediment are able to grow on acetate. Indeed, Fredrickson et al. [11] showed that 8% to 63% of bacteria isolated from deep Cretaceous sediments could grow on acetate. Thus the low acetate and phenol mineralization rates may reflect the activity of only a part of the total population. The moderate, or lack of, correlations between rates of phenol and acetate mineralization and both viable counts and growth rates support this. Acetate- and phenol-utilizing bacteria may be, at least in part, a different population than populations measured by the other methods.

The observed variation in activity measurements (metabolic activity, ($k_{4\text{-acetate}}$ and $k_{4\text{-phenol}}$) and cell division per time) down through the profile reflects the potential activity of bacteria in the sediments. Because of the possibility that the sampling and sample-handling might have stimulated microbial activity or growth, the reported rates may not be equal to the actual rates in situ, but are used as a means for comparing different samples. In situ activity is difficult to estimate. However, Chapelle and Lovley [6] estimated rates of CO_2 production in laboratory incubations of deep coastal plain sediments amended with ^{14}C-acetate. These were shown to overestimate in situ rates obtained by geochemical modelling from some, but not all, sites.

The Influence of Abiotic Parameters

The influence of leachate was evaluated, as the sampling site was located 500 m downstream from an old municipal landfill. From observations of the leachate plume closer to the landfill, the dissolved organic carbon in the leachate was reduced to the background level (2–3 mg C/liter) approximately 100 m before the water reached the borehole (B 203). Also, the leachate was restricted to the upper aquifer, above the confining clay layer [21]. The chloride concentration per volume of pore water showed a peak in the depth profile at 6–8 mbs, but high chloride concentrations were also observed in samples from deeper parts of the borehole (Table 1). This increase in chloride concentration in the upper aquifer could indicate either good contact with the surfaces, with input of chloride from precipitation and fertilization (P.L. Bjerg, personal communication), or influence of leachate from the landfill [22]. However, the concentration of chloride in the sediment slurry (before converting to porewater concentrations) and the concentration of organic matter in the sediment did not reveal any influence. Based on these observations and the delineation of the leachate [21], we consider the layer from 6 to 8 mbs in the upper aquifer to be slightly influenced by inorganic ions from landfill leachate, but without any influence of organic matter. The lack of ability to degrade ^{14}C-m-xylene further supported that the aquifer was not influenced by leachate.

The total number of bacteria (AODC) correlated moderately with increasing amount of clay and silt content and with decreasing amount of sand (Table 3). Other investigators have also found the highest total number of bacteria (AODC) in samples with a high content of clay and silt [7] and in the clay and silt fraction of size-fractionated sediment [16]. In contrast, measurements of microbial population density (AODC, viable count) have shown positive correlations with sand content and moderate negative correlations with clay content in other subsurface systems [10, 32, 33]. We, Chapelle et al. [7], and Harvey et al. [16] enumerated bacteria on a filter, whereas the other investigators embedded the sediment sample in an agar smear. The amount of sediment it is possible to filter onto a filter decreases as the clay content increases. In contrast, when making an agar smear, the same amount of sediment can be mounted on the slide, potentially increasing shading in clayey samples.

A reason for the disparity in the relation between bacterial numbers and sediment texture could be differences in the microbial populations and in the geological formations. The cited investigations all represent much older (100–1 million years BP) sediments of very different origin compared to this investigation of younger (200,000–10,000 years BP) glacial deposits. The contents of clay, silt, and organic matter reflect the depositing conditions, and the correlation between these parameters and AODC gives some evidence for the importance of the depositing conditions for the present microbiology in subsurface sediments. Compared to an organic rich deltic and marine depositing environment with higher temperature and varying redox conditions [7, 28, 33], only poor microbial activity would be expected during glacial depositing under aerobic conditions and at a lower temperature. The differences in geology and age of the deposits could explain the observed disparity of the effect of texture, as different geological formations contain distinct types of bacteria [3, 20].

Sediment texture, especially clay content, seems to be important for the occurrence and activity of bacteria. As neither grain size nor clay itself necessarily affect the microbial population, the permeability, which represents the potential water flow, was calculated from grain size distribution. Permeability showed high and moderate positive correlation with $k_{4\text{-acetate}}$ and $X_{0\text{-phenol}}$ and moderate negative correlation with CFU. The increased permeability makes a higher flux of substrate possible and could explain the increased metabolic activity, but not a decrease in total viable biomass.

Despite the much smaller content of bacteria and organic matter in subsurface sediments compared to arable soil, the ratios between bacterial carbon (AODC) and total organic carbon (0.4–17.1%, mean, 3.2%) (Table 2), were very close to the mean ratios of 2.3–2.9% reported by Anderson and Domsch [1]. It seems as if the content of organic matter controls the total number of bacteria, whether this is due to the substrate concentration per se or reflects the sedimentation conditions during the deposition of the geological formation.

The correlations between the rate constants of acetate and phenol removal on one side and texture and depth on the other are in accordance with Hicks and Frederickson [17] and Phelps et al. [24]. In the cited studies, pH was low (<5) in the clay samples. The apparent effect of clay content could be caused by pH as the mineralization of acetate and phenol was positively correlated with pH, ranging from 3.9 to 8.0 [17]. We found a moderate to high negative correlation between pH and the rate constants for both acetate (89.5%) and phenol (80.4%), as well as the amount of phenol required to sustain the population of phenol degrading bacteria (95.2%) in the neutral pH-range (6.8–8.1), and in this pH-range other parameters might be more important. The moderate to high negative correlation between electrical conductivity in the slurries and the rate constants indicates that the degradation is sensitive to increased concentration of solutes or solutes extractable from sediment by distilled water.

Conclusion

The four different methods used to estimate the microbial activity in aquifer sediments (acetate and phenol mineralization, [^{3}H]-thymidine incorporation, and viable counts) gave different results with only moderate intercorrelation. Thus, it is important to estimate the activity of microbial populations in aquifer sediments by more than one method to achieve a more precise characterization of the environment.

The sediment texture is important for the microbial populations: with an increase in content of clay and silt, the total number of bacteria (AODC) increased and mineralization rate of acetate decreased. As clay and silt content per se probably does not control the microbial biomass, the influence of permeability was evaluated as a more integrated expression of the texture, and the acetate mineralization rate ($k_{4\text{-acetate}}$) increased with the increase in permeability. The covariance observed between abiotic parameters makes it impossible to point out a single abiotic parameter able to explain the observed variation in microbial biomass and activity. Other investigations have revealed

conflicting relationships, which probably are caused by differences in the age and type of the studied geological formations.

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