Microb Ecol (1990) 20:297–313

MICROBIAL ECOLOGY

Spatial Distribution of Microbial Biomass, Activity, Community Structure, and the Biodegradation of Linear Alkylbenzene Sulfonate (LAS) and Linear Alcohol Ethoxylate (LAE) in the Subsurface

Thomas W. Federle,[1] Roy M. Ventullo,[2] and David C. White[3]

[1]Environmental Safety Department, Procter and Gamble Company, Ivorydale Technical Center, Cincinnati, Ohio 45217, USA; [2]Department of Biology, University of Dayton, Dayton, Ohio 45469, USA; and [3]Institute for Applied Microbiology, University of Tennessee, Knoxville, Tennessee 37932, USA

Received: May 29, 1990; Revised: September 4, 1990

Abstract. The vertical distribution of microbial biomass, activity, community structure and the mineralization of xenobiotic chemicals was examined in two soil profiles in northern Wisconsin. One profile was impacted by infiltrating wastewater from a laundromat, while the other served as a control. An unconfined aquifer was present 14 meters below the surface at both sites. Biomass and community structure were determined by acridine orange direct counts and measuring concentrations of phospholipid-derived fatty acids (PLFA). Microbial activity was estimated by measuring fluorescein diacetate (FDA) hydrolysis, thymidine incorporation into DNA, and mixed amino acid (MAA) mineralization. Mineralization kinetics of linear alkylbenzene sulfonate (LAS) and linear alcohol ethoxylate (LAE) were determined at each depth. Except for MAA mineralization rates, measures of microbial biomass and activity exhibited similar patterns with depth. PLFA concentration and rates of FDA hydrolysis and thymidine incorporation decreased 10–100 fold below 3 m and then exhibited little variation with depth. Fungal fatty acid markers were found at all depths and represented from 1 to 15% of the total PLFAs. The relative proportion of tuberculostearic acid (TBS), an actinomycete marker, declined with depth and was not detected in the saturated zone. The profile impacted by wastewater exhibited higher levels of PLFA but a lower proportion of TBS than the control profile. This profile also exhibited faster rates of FDA hydrolysis and amino acid mineralization at most depths. LAS was mineralized in the upper 2 m of the vadose zone and in the saturated zone of both profiles. Little or no LAS biodegradation occurred at depths between 2 and 14 m. LAE was mineralized at all depths in both profiles, and the mineralization rate exhibited a similar pattern with depth as biomass and activity measurements. In general, biomass and biodegradative activities were much lower in groundwater than in soil samples obtained from the same depth.

Introduction

The presence of synthetic organic chemicals in groundwater has stimulated interest in the microbial ecology of subsurface environments and the biodegradative capabilities of indigenous subsurface microorganisms. Many studies [1, 2, 4, 6, 8–10, 12, 14, 16, 18–21, 23, 25, 26] have demonstrated the existence of metabolically active microbes in groundwater and subsurface soils, and several [7, 9, 20, 27] have shown that subsurface communities, particularly in contaminated sites, degrade xenobiotic chemicals. It is common that subsurface environments are impacted by infiltrating wastewater. Few investigations have examined the effects of wastewater infiltration on biological activity in the subsurface or have attempted to relate specific biodegradative processes with the distribution and composition of subsurface communities.

Surfactants are among the most widely utilized synthetic organic chemicals [24]. They have many industrial uses and are ingredients in a variety of consumer products including detergents and household cleaning agents. The most heavily utilized synthetic anionic surfactant is linear alkylbenzene sulfonate (LAS). Approximately 987 thousand metric tons of LAS are consumed annually in the United States, Western Europe and Japan. The most commonly used nonionic surfactants are the linear alcohol ethoxylates (LAE) with annual consumption of 467 thousand metric tons. As a consequence of their widespread usage and volume, surfactants occur in domestic and industrial wastewaters. Generally, they are well removed by biodegradation and sorption in public or private treatment systems, but reduced concentrations may remain in treated effluents. Typically effluents are discharged to rivers or streams, but occasionally may be discharged to leach fields or seepage pits. These latter disposal practices result in the entry of surfactants into subsurface environments, where their fate will depend upon the biodegradative activities of the indigenous microbes.

The mineralization of LAS and LAE was determined in subsurface soil and groundwater at two sites. Both sites were characterized by sandy soil, and mineralization in each soil profile was evaluated as a function of depth. One profile had been impacted by infiltrating wastewater from a rural laundromat since 1962; the other was not affected by wastewater. Groundwater was obtained from two monitoring wells, upgradient and downgradient from the leach field. The vertical distribution of microbial biomass and activity was determined in the soil profiles. Biomass was estimated by measuring the concentration of phospholipid derived fatty acids and by acridine orange direct counts of bacteria. The distribution of fungi and actinomycetes was determined by measuring relative concentrations of fatty acids diagnostic for these groups. Microbial activity was estimated by measuring rates of fluorescein diacetate (FDA) hydrolysis, thymidine incorporation into DNA and amino acid mineralization.

Materials and Methods

Chemicals

[U-^{14}C ring] sodium tridecylbenzene sulfonate (LAS) with a specific activity of 8.69 mCi/mmol was obtained from New England Nuclear (Boston, MA). Purity was 98% based upon thin-layer

chromatography (TLC) on silica gel G with chloroform/methanol/water/formic acid (80/25/3/1). [U-^{14}C ethoxylate] linear alcohol ethoxylate (LAE) was obtained from Amersham. Its specific activity was 4.34 mCi/mmol and it consisted of 70% $CH_3(CH_2)_{10}CH_2(OCH_2CH_2)_9OH$ and 30% $CH_3(CH_2)_{10}CH_2(OCH_2CH_2)_8OH$ based upon gas chromatography. [U-^{14}C]L-amino acid mixture (specific activity 50 mCi/mmol) was obtained from ICN (Irvine, CA) with a purity >98%. [Methyl-^{3}H] thymidine with a specific activity >62 mCi/mmol was obtained from ICN Pharmaceuticals Inc. (Irvine, CA).

RPI Scintillator scintillation cocktail was purchased from Research Products International (Mount Prospect, IL). Cycloheximide, chloramphenicol, tetracycline, acridine orange, fluorescein diacetate, thymidine and DNA were obtained from Sigma (St. Louis, MO). Fatty acid standards were obtained from Alltech Associates (Deerfield, IL). Highest purity GC^2 chloroform, methanol, acetone and hexane were obtained from Burdick and Jackson (Muskegon, MI).

Sampling

Soil cores were obtained from two sites in north central Wisconsin, near the town of Summit Lake. One core (Leach Field) was drilled in an area intersected by buried perforated pipes, which transport wastewater from a rural laundromat. The other core (Control) was drilled approximately 30 m to the northeast in an area not influenced by wastewater. Aseptic samples were obtained at various depths using a modification of procedures described by Dunlap et al. [5] and Wilson et al. [26]. A bore hole was drilled with a 10.2 cm diameter hollow stem auger to the desired depth. A coring device then was driven into the soil. The corer was recovered and in the field the soil was extruded with a hydraulic jack. Approximately 5 cm was extruded and removed with a sterile spatula to produce an uncontaminated face. The remaining core was extruded through a flame sterilized paring device which removed the outer 1 cm of core material. The inner core material was placed into sterile jars and stored at 4°C. Redox potential was measured with a platinum electrode in the field and E_H (normalized to the hydrogen electrode) ranged between 339 and 474 mV. Physical chemical characteristics of the soil were determined using standard procedures [22] and are listed in Table 1. The depth to the water table was approximately 14 m.

Groundwater was obtained aseptically from existing monitoring wells located approximately 4 m from the coring sites. The wells were downgradient and upgradient from the Leach Field and a nearby wastewater treatment pond.

Bacterial Counts

Bacterial numbers in soil samples were determined using the direct count procedure of Ghiorse and Balkwill [10]. Soil was mixed with sodium pyrophosphate (0.1%) and an aliquot of this suspension was combined with molten agar (1%) then smeared onto a slide. The smear was stained with acridine orange (0.01%) and 30 to 60 fields were counted using epifluorescence microscopy. Bacterial numbers in groundwater samples were determined using the method of Hobbie et al. [13].

Phospholipid Analysis

Soil samples (40 g) were extracted with methanol, chloroform and buffer (0.01 M monosodium phosphate; 0.53 mM disodium EDTA) using the procedure of Federle et al. [8]. The recovered lipids were separated into neutral, glyco- and phospholipid fractions by column chromatography using silicic acid. The phospholipid fraction was subjected to mild alkaline hydrolysis and the resulting fatty acid methyl esters were purified by thin layer chromatography and analyzed by gas chromatography or combined gas chromatography/mass spectrometry. Detailed descriptions of the lipid fractionation, purification and analysis are contained in Guckert et al. [11].

Table 1. Characteristics of Control site and Leach Field soils as a function of depth

Location depth (m)	pH	AEC[a] (mg/kg)	CEC[b] (meq/100 g)	Inorg. N[c] (mg/kg)	Total P (mg/kg)	TOC[d] (mg/kg)	% Composition		
							Sand	Silt	Clay
Control site									
0.5 m	6.2	96	8.1	59	1,250	1,080	52.9	43.8	3.2
1.7	6.9	28	1.3	60	1,250	1,180	78.5	18.4	3.1
2.4	7.2	66	1.3	47	578	563	96.8	2.3	0.9
5.5	7.7	22	0.7	41	964	741	90.9	8.5	0.6
15.4	8.2	26	0.9	48	540	843	98.9	1.1	0
17.7	6.3	11	0.3	39	636	616	90.8	9.1	0.1
Leach field									
0.5 m	6.3	26	7.6	51	869	3,100	78.6	18.1	3.3
1.8	6.3	32	9.1	47	1,003	4,550	82.5	16.0	2.3
3.3	7.0	24	1.9	37	370	1,260	97.3	2.1	0.6
4.9	6.7	28	1.7	47	337	599	96.8	2.7	0.5
7.9	7.0	74	4.7	52	1,074	783	86.5	9.5	4.0
12.5	7.1	86	2.4	53	698	747	97.5	2.4	0.1
18.3	7.4	44	1.5	63	630	724	96.2	2.8	1.0

[a] Anion exchange capacity Cl^-
[b] Cation exchange capacity
[c] Inorganic nitrogen = $NH_4^+ + NO_2^- + NO_3^-$
[d] Total organic carbon

FDA Hydrolysis

Microbial activity was estimated by measuring the rate at which fluorescein diacetate (FDA) was hydrolyzed using a modification of the procedure of Federle et al. [8]. FDA is hydrolyzed by a wide range of enzymes including lipases, proteases and esterases. Replicate soil samples (50 g) were incubated with 100 ml sterile 60 mM phosphate buffer (pH 7.6) amended with 2 mg each of FDA, cycloheximide, chloramphenicol and tetracycline on a rotary shaker. Periodically, subsamples (5 ml) of the suspension were removed and mixed with 10 ml acetone to terminate the reaction. The subsamples were centrifuged (5,000 × *g*; 5 min) and the absorbance of the supernatant determined at 490 nm. Autoclaved samples treated as above were used as abiotic controls. Rates of FDA hydrolysis were estimated by linear regression of increased absorbance versus time and expressed as nmol hydrolyzed/hour/g dry wt soil.

Thymidine Incorporation

Thymidine incorporation into DNA was measured using methods described in Thorn and Ventullo [21]. Soil samples (3 g) were incubated in 2 ml water amended with 250 pmoles [^{3}H-methyl] thymidine in the dark for 3–5 hours. The reactions were terminated and DNA extracted by addition of NaOH, EDTA and sodium dodecyl sulfate to final added concentrations of 0.3 M, 25 mM and 0.1%, respectively. DNA was extracted and RNA hydrolyzed in this mixture for 12 hours at 25°C. The samples were centrifuged and the supernatants were quantitatively recovered, neutralized with HCl and amended with thymidine (17 μg/ml). Trichloracetic acid was added to a final added concentration of 5%, and the samples were chilled on ice for 45 min. TCA insoluble material was collected on Millipore HA filters and the filters were subjected to acid hydrolysis (1 M HCl; 30 min; 95°C) to hydrolyze DNA. Radioactivity in the hydrolysate was determined by liquid scintillation counting (LSC).

Mineralization Assays

Soil (15 g) was aseptically transferred into triplicate serum bottles (60 ml), adjusted to 20–25% moisture content with sterile water containing approximately 750 ng of radiolabeled test compound and mixed with a sterile applicator stick. Groundwater samples (10 ml) were likewise aseptically transferred to sterile serum bottles and amended with 50 ng/ml of the test chemicals. The bottles were sealed with rubber stoppers equipped with plastic wells containing a fluted filter paper soaked with 0.2 ml of 1.5 N KOH to trap the evolved $^{14}CO_2$. They were incubated under static conditions at 24°C. Periodically the filter papers were removed and replaced with fresh filters. Typically, filters were exchanged after 4 hours, 8 hours and 1, 2, 4, 8, 16, 32, and 64 days. The recovered filters were placed in scintillation vials with RPI scintillator and radioactivity was determined by LSC. At the termination of the incubations, residual $^{14}CO_2$ was recovered by acidifying the samples with 6 N HCl. Data were expressed as the cumulative percentage of the radiolabeled chemicals recovered as $^{14}CO_2$. The data were corrected utilizing abiotic controls amended with 1% formalin and fitted to a first-order production equation [15]:

$$y = a(1\text{-}e^{-kt}) \quad (1)$$

where y = percent recovered as $^{14}CO_2$, a = upper asymptote (total % recovered), k = first-order rate constant and t = time (days). The constants (k and a) where estimated from the data by nonlinear regression using the NONLIN program of SYSTAT (Systat, Inc., Evanston, IL). The quality of the fit was evaluated by examining the residual sum of squares and the standard errors of the estimates.

Results

Subsurface Soil

Table 1 shows characteristics of the soil as a function of depth in the two profiles. Cation exchange capacity (CEC), total organic carbon (TOC), silt content and clay content decreased with increasing depth in both profiles. The upper 2 m in the Leach Field profile had 3 to 4 times higher levels of TOC than comparable depths in the Control profile. Figure 1 shows bacterial counts (AODC) and the concentration of phospholipid fatty acids (PLFA) in the two profiles as a function of depth. Bacterial numbers were highest at 0.5 m, declined sharply below this depth and were not significantly different in the two profiles. Bacterial counts in the saturated zone (below 14.5 m) were comparable to those in the immediately overlying vadose zone (4–14 m). In contrast, PLFA concentration has highest at 1.7 m and differed as a function of profile. In the Control profile, PLFA concentration declined 10-fold below 1.7 m; in the Leach Field profile, it declined nearly 100-fold below 3.3 m. There were 10 to 100 times higher levels of PLFA in the top 3.3 m of the Leach Field profile compared to similar depths in the Control profile. Below this depth, PLFA concentration was similar in both profiles with one exception. The sample from the saturated zone in the Leach Field (18.4 m) had one-third the PLFA level of similar depths in the Control site. PLFA concentration was positively correlated with TOC ($r = 0.93$; $P \leq 0.01$) and negatively correlated with depth ($r = -0.63$; $P \leq 0.05$). AODC and PLFA concentration were not significantly correlated ($r = 0.42$).

Microbial activity (Fig. 2), estimated as the rates of fluorescein diacetate (FDA) hydrolysis and thymidine incorporation into DNA, exhibited the same

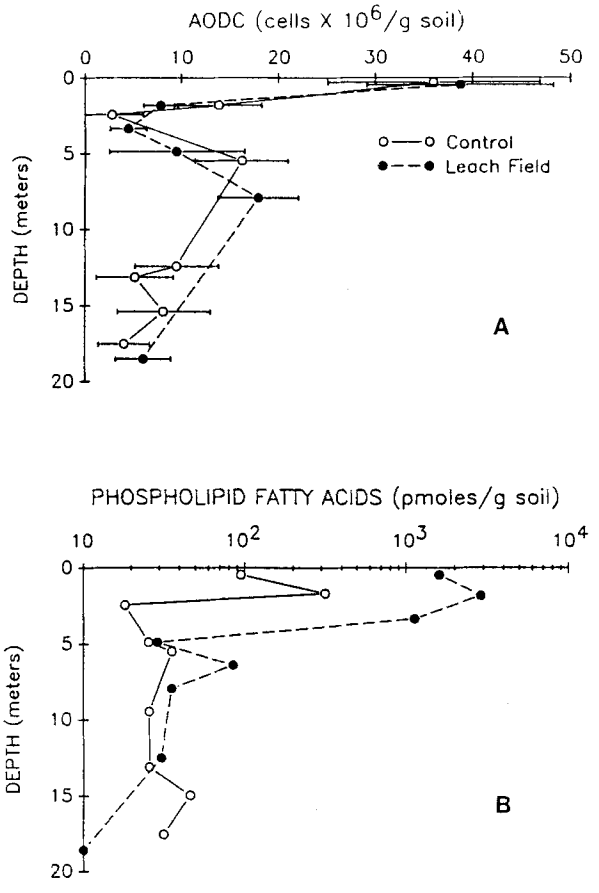

Fig. 1. Bacterial direct counts **(A)** and phospholipid fatty acid concentration **(B)** as a function of depth in subsurface soil profiles.

general pattern with depth as bacterial numbers and PLFA concentration. FDA hydrolysis was positively correlated with cation exchange capacity ($r = 0.62$; $P \leq 0.05$) and TOC ($r = 0.71$; $P \leq 0.01$) but negatively correlated with depth ($r = -0.87$; $P \leq 0.01$). Thymidine incorporation correlated with FDA hydrolysis ($r = 0.85$; $P \leq 0.01$) and likewise correlated positively with TOC ($r = 0.74$; $P \leq 0.01$) and negatively with depth ($r = -0.66$; $P \leq 0.05$). FDA hydrolysis and thymidine incorporation were most rapid in the 0.5 and 1.7–1.8 m samples. FDA hydrolysis declined nearly 100-fold and thymidine incorporation decreased 10 to 100 fold below 2 meters. Rates of FDA hydrolysis in the Leach Field were on average twice those in the Control site. Only between 2.4 and 4 m were the rates of thymidine incorporation significantly higher in the Leach Field profile. FDA hydrolysis correlated positively with AODC ($r = 0.56$; $P \leq 0.05$) and PLFA concentration ($r = 0.74$; $P \leq 0.01$); thymidine incorporation likewise correlated with AODC ($r = 0.63$; $P \leq 0.05$) and PLFA ($r = 0.75$; $P \leq 0.01$).

Table 2 shows the relative abundance of PLFA at selected depths in the Leach Field site. A total of 52 fatty acids was identified in the samples. Fatty

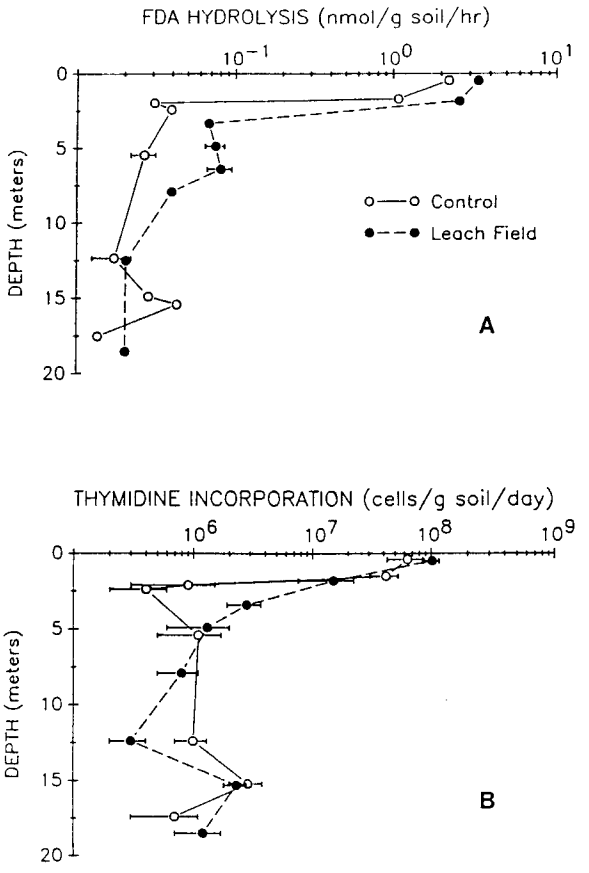

Fig. 2. Rates of fluorescein diacetate (FDA) hydrolysis (**A**) and thymidine incorporation into DNA (**B**) as a function of depth in subsurface soil profiles.

acids are designated as the number of carbon atoms: number of double bonds and position of the double bond closest to the aliphatic (ω) end of the molecule. The prefixes, i and a, refer to iso and anteiso branching, respectively; br refers to methyl branching at an unconfirmed location; and cyc indicates cyclopropane substitution. Other methyl branched fatty acids are designated based upon the methyl groups position relative to the carboxylic acid (e.g., 10-methyl 18:0). The greatest number and diversity of fatty acids were found at depths above 2 meters, where the most abundant fatty acids were 16:1ω7, 16:0, 18:1ω9, 18:1ω7 and cyc19:0. Below this depth in the vadose zone, the fatty acid profile was dominated by 16:1ω7, 16:0, cyc17:0/17:1ω6, and 18:1ω7. In the sample from the saturated zone, 16:0, 18:1ω9, 18:1ω7 and 18:0 were most abundant. A fatty acid unique to photosynthetic organisms, 16:1ω13t, was not detected in the samples indicating that PLFA were derived from soil microbes rather than plant debris. Figure 3 shows relative abundance of fatty acids unique to fungi (18:2ω6 and 18:3ω6) and tuberculostearic acid (10-methyl 18:0), a marker for actinomycetes, as a function of depth in the two profiles. In the control profile, fungal markers accounted for 1 to 7% of the total fatty acids; in the Leach Field profile, they accounted for 2 to 15% of the total. The relative

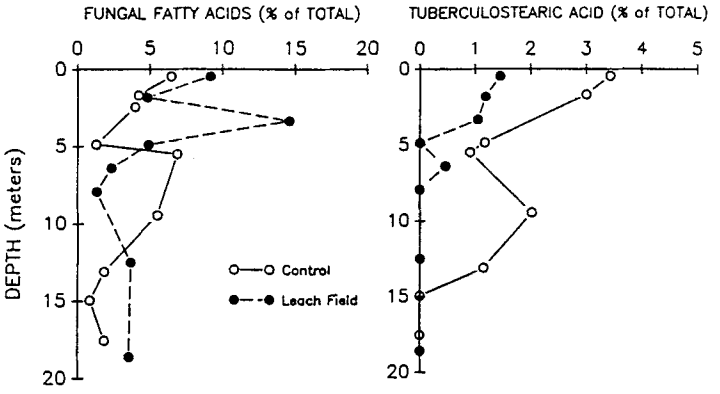

Fig. 3. Relative abundance of fungal fatty acids (18:2ω6 and 18:3ω6) and the actinomycete marker (tuberculostearic acid) as a function of depth in subsurface soil profiles.

proportion of fungal markers was greatest at 3.3 m in the Leach Field but did not exhibit a consistent pattern as a function of depth or site. Tuberculostearic acid (TBS) accounted for 0 to 3.5% of the total PLFA, and its relative abundance exhibited a decreasing trend with increasing depth. The relative proportion of TBS in the control profile was on average twice that at comparable depths in the Leach Field profile. No TBS was detected below 8 m in the Leach Field profile, while in the Control profile TBS was detected to a depth of 12.4 m. Neither site contained TBS in the saturated zone. Fatty acids characteristic of protozoa (20:3ω6 and 20:4ω6) were detected above 3 m in both profiles, accounting for 0.7 to 2.7% of the total PLFA. While not detected below 3 m in the vadose zone, low levels of these acids were detected in the saturated zone (0.23–1.26%).

Figure 4 shows mineralization of LAS, LAE and mixed amino acids (MAA) as a function of time in soil from three representative depths in the Leach Field profile. Figure 5 contains kinetic parameters describing the mineralization of MAA. Mixed amino acids were mineralized without a lag period in every sample. First-order rates ranged from 0.15 to 0.49 $days^{-1}$, and the corresponding half-lives varied between 4.6 and 1.4 days. Between 20.7 and 42.5% of the carbon in the mixed amino acids was recovered as $^{14}CO_2$. In the control profile, the rate of amino acid mineralization exhibited a marked decrease below 3 m. In the Leach Field, the rate increased below 2 m. Below 3 m, rates of MAA mineralization in the Leach Field were approximately twice those in the Control profile. The rate of MAA mineralization did not correlate with any soil characteristic or measure of microbial biomass or activity. The recovery of $^{14}CO_2$ from MAA correlated positively with depth ($r = 0.78$; $P \leq 0.01$), pH ($r = 0.61$; $P \leq 0.05$), and sand content but correlated negatively with with cation exchange capacity ($r = -0.67$; $P \leq 0.05$), silt content ($r = -0.75$; $P \leq 0.01$) and clay content ($r = -0.76$; $P \leq 0.01$).

Kinetic parameters describing LAE mineralization are shown in Fig. 6. LAE was mineralized at every depth in both profiles. Short lag periods (≤ 2 days) were associated with samples obtained below 2 m in both profiles. First-order rates varied between 0.018 and 0.500 $days^{-1}$, and the corresponding half-lives

Table 2. Relative abundance (% of total) of phospholipid fatty acids as function of depth in the Leach Field profile

Fatty acid	0.5 m	1.8 m	6.4 m	12.5 m	18.6 m
a13:0	0.2	0	0	0	0
i14:0	0	0.4	0	1.5	0
14:0	0	0.9	0	8.6	0
i15:1	0	0.4	0	0	0
a15:1	0	0.9	0	0	0
i15:0	0.8	5.4	0.4	0.8	0
a15:0	0.7	3.8	0.3	0.3	0
15:1	0	0.1	0	0	0
15:0	0.1	0.8	0.3	0.6	0.9
i16:0	0.9	1.4	0.2	1.5	0
16:1ω9	0.8	1.7	0.4	2.0	1.4
16:1ω7	5.2	9.0	11.5	14.5	4.9
16:1ω7t	0.1	0.3	0.9	2.4	0
16:1ω5	2.3	4.0	0.3	0.3	0
16:0	7.4	12.0	13.7	18.6	20.0
br17:x	0.7	0.4	0	0	0
i17:1	1.9	2.4	0.3	1.1	0
10-methyl 16:0	4.4	4.4	1.0	0.2	0
br17:0	0.9	0.7	0.8	0	0
i17:0	1.2	1.3	0.7	0.8	2.1
a17:0	1.8	0.3	0.9	0.7	3.2
cyc17:0/17:1ω6	5.0	4.8	17.4	4.4	0
17:0	0.3	0.4	1.1	0.6	2.1
10-methyl 17:0	0.8	0.7	0	0	0
br18:0	0.4	0	0	0	0
18:3ω6	0	0.4	0	0.1	1.0
18:x	0.9	0.5	0.9	3.5	0
18:2ω6	9.1	4.4	2.3	3.6	2.5
18:3ω3	1.3	1.6	1.2	3.8	0
18:1ω9	10.1	6.2	2.5	1.6	9.6
18:1ω7	16.2	11.6	27.8	16.6	11.0
18:1ω7t	0.5	0.4	0.9	1.1	0
18:1ω5	1.4	0.9	0.3	0	0
18:0	2.4	1.9	3.6	3.2	19.2
br19:1	1.1	0.6	1.5	1.6	2.0
10-methyl 18:0	1.4	1.2	0.5	0	0
19:1ω12	0.8	0.4	0	0	0
19:1ω8	0	0.2	0	0	4.2
cyc19:0	11.4	6.2	4.5	4.3	0
20:4ω6	0.6	0.6	0	0	0
20:5ω3	0.2	0.3	0	0	0
20:3ω6	0.4	0.1	0	0.2	0
20:2ω3	0.2	0	0	0	0
i20:0	0.3	0	0	0	1.5
20:1ω9	0.3	0.2	0	0	0
20:0	1.9	0.8	2.3	0	0
21:0	0.2	0.3	0	0	0
22:1ω9	0	0.2	0	0	14.0
22:0	1.5	0.6	1.4	0	0
23:0	0.2	0.1	0	0	0
24:0	1.3	0.4	0.9	0	0

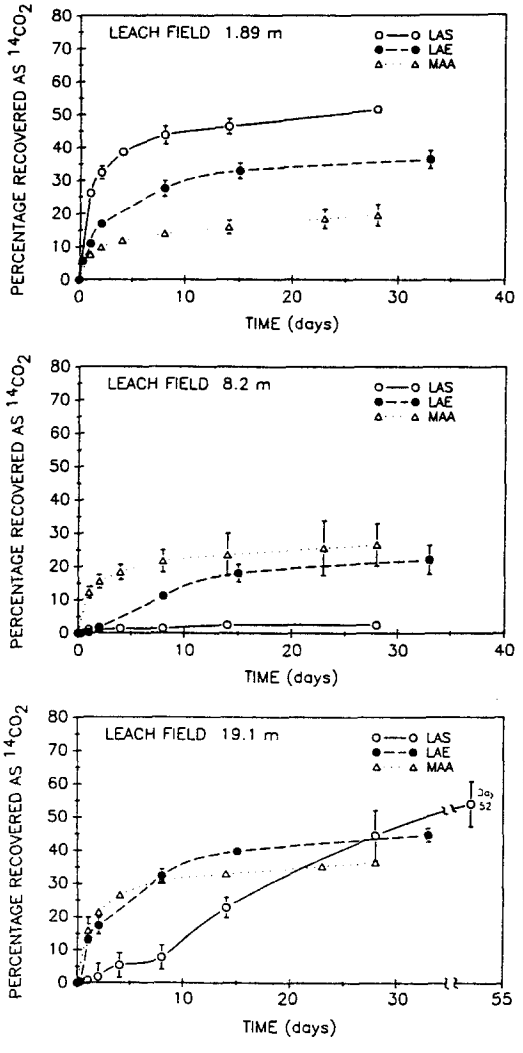

Fig. 4. Mineralization of linear alkylbenzene sulfonate (LAS), linear alcohol ethoxylate (LAE) and mixed amino acids (MAA) as a function of time at three selected depths in the Leach Field soil profile.

ranged from 38.5 and 1.4 days. In both profiles the rate decreased dramatically below 2 meters. At 0.5 m and between 3 and 12 m, LAE mineralization was significantly more rapid in the the Control profile than Leach Field. The rate of LAE mineralization positively correlated with the rate of thymidine incorporation ($r = 0.58$; $P \leq 0.05$), silt content ($r = 0.65$; $P \leq 0.01$), and P content ($r = 0.61$; $P \leq 0.05$). It correlated negatively with sand content ($r = -0.65$; $P \leq 0.01$). The asymptotic yield of $^{14}CO_2$ ranged from 21 to 52.2% and tended to be highest in the uppermost and lowermost samples. This parameter did not correlate with any physicochemical or microbiological characteristic of samples.

Table 3 contains data on the mineralization of LAS. Significant mineralization of LAS occurred above 2 m and in the saturated zone (below 14 m) of

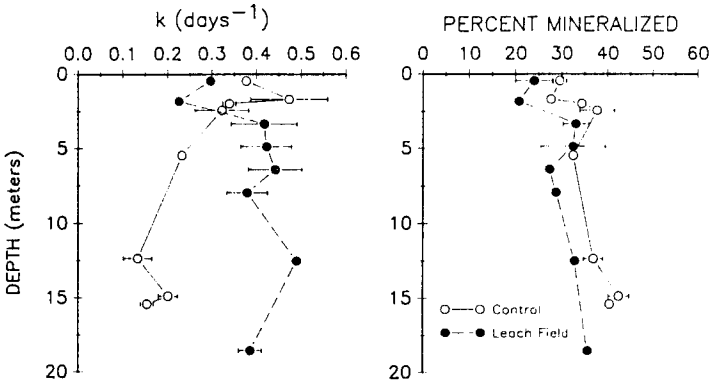

Fig. 5. Kinetic parameters describing the mineralization of mixed amino acids (MAA) as a function of depth in subsurface soil profiles.

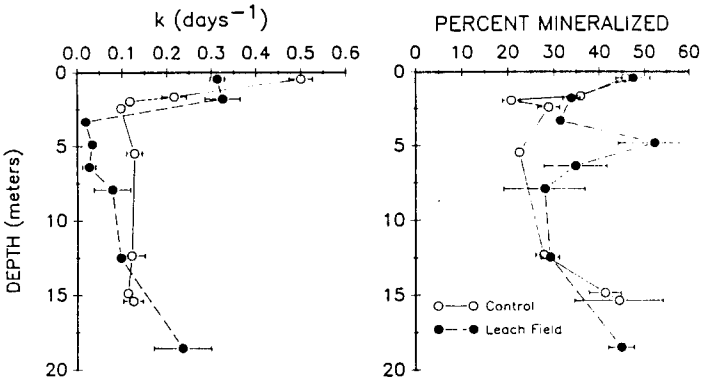

Fig. 6. Kinetic parameters describing the mineralization of linear alcohol ethoxylate (LAE) as a function of depth in subsurface soil profiles.

both profiles. Little or no mineralization was observed in the unsaturated vadose zone (2–14 m) of either profile. First-order rate constants were an order of magnitude lower in the saturated zone compared to upper soil. LAS was mineralized without a lag in the upper soil of both profiles, but mineralization was preceded by lags in the saturated zone. The Leach Field profile was characterized by a faster rate at 1.8 m and shorter lag period in the saturated zone; otherwise LAS mineralization was similar in both profiles.

Groundwater

Groundwater was obtained from two monitoring wells, upgradient and downgradient from the Leach Field and adjacent wastewater pond. LAS, measured as interference limited methylene blue active substances [17], and LAE, detected as cobalt-thiocyanate active substances [3], were not detected in the downgradient well. Of 14 water quality parameters measured, only total al-

Table 3. Parameters describing the mineralization of LAS as a function of depth in two soil profiles

Location depth (m)	% Mineralized[a]	k (days^{-1})[a]	Lag (days)
Control site			
0.5 m	35.8 ± 1.0	0.35 ± 0.19	0
1.7	35.0 ± 2.3	0.35 ± 0.15	0
2.0	1.5 ± 1.9[b]	ND	—
2.4	17.1 ± 8.4[b]	ND	—
5.5	12.1 ± 7.8[b]	ND	—
12.4	4.9 ± 4.1[b]	ND	—
14.8	42.7 ± 2.0	0.032 ± 0.002	5
15.4	37.8 ± 0.1	0.033 ± 0.003	5
Leach field			
0.5 m	49.8 ± 8.4	0.45 ± 0.13	0
1.8	47.8 ± 2.1	0.60 ± 0.13	0
3.3	2.9 ± 2.3[b]	ND	—
4.9	2.1 ± 1.5[b]	ND	—
6.4	11.4 ± 9.9[b]	ND	—
7.9	2.7 ± 1.3[b]	ND	—
12.5	12.2 ± 3.3[b]	ND	—
18.4	63.1 ± 7.1	0.035 ± 0.008	2

[a] Mean ± standard deviation (n = 3)
[b] % recovered as $^{14}CO_2$ after 100 days

kalinity and conductivity were elevated in the downgradient well. Acridine orange direct counts were $3.1 \pm 0.7 \times 10^5$ and $6.0 \pm 2.0 \times 10^4$ cells/ml in the upgradient and downgradient wells, respectively. Figure 7 shows mineralization of LAS, LAE and MAA with time in groundwater. MAA mineralization in water from the upgradient well was more rapid and extensive than in that from the downgradient well. LAS was not mineralized in water from either monitoring well. LAE was mineralized in water from both wells. In the downgradient well, mineralization occurred without a lag; in the upgradient well, the onset of mineralization was preceded by an 8 day lag period. Like MAA, the extent of LAE mineralization was much lower in the downgradient well. In general, mineralization was much slower and less extensive in groundwater than in the soil samples obtained from the same depth.

Discussion

In agreement with other studies, this work shows the presence of active microbial communities in the subsurface and the ability of these organisms to mineralize xenobiotic chemicals. Furthermore, it shows a marked discontinuity in the distribution of active microorganisms in the vadose zone at a depth of 2 to 3 m. Below this depth, rates of thymidine incorporation and FDA hydrolysis as well as PLFA concentration decreased approximately a hundred-fold. Bacterial numbers and rates of LAS and LAE biodegradation likewise exhibited

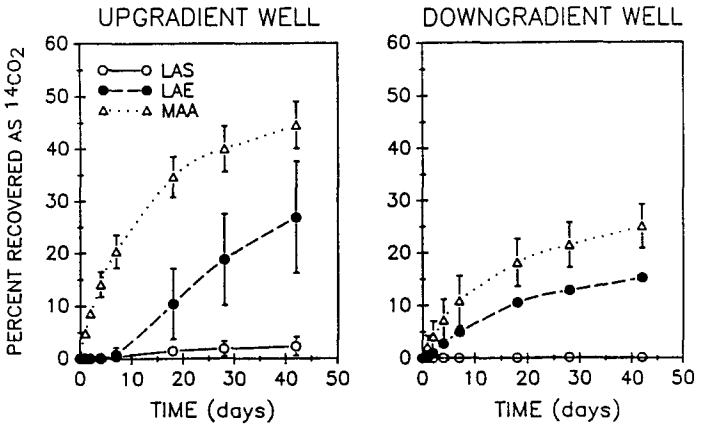

Fig. 7. Mineralization of linear alkylbenzene sulfonate (LAS), linear alcohol ethoxylate (LAE) and mixed amino acids (MAA) as a function of time in groundwater obtained from wells upgradient and downgradient from the Leach Field.

sharp decreases below this level. Infiltrating wastewater did not alter the existence depth of the discontinuity. Sharp declines in microbial parameters have been observed at a similar depth by other investigators. Webster et al. [23] found that ATP concentration declined more than 10-fold below 3 m at the Lula, OK site. At this same location, direct counts, colony forming units, and MPNs for protozoa exhibited sharp declines below 1.5 m [1, 2]. In vertical profiles of a sand aquifer in Germany, colony forming units in soil and groundwater decreased 1–2 orders of magnitude below 4–5 m [14]. The reasons for such a sudden discontinuity are unclear. Although a corresponding decrease in total organic carbon and an increase in sand content were observed, the magnitude of these changes was modest relative to the changes in the microbiological parameters. Changes in the concentration of nitrogen and phosphorus likewise were not consistent with the microbiological changes. However, measures of total carbon and phosphorus do not give an indication of the bioavailability of these nutrients.

In a Pleistocene sand aquifer in Germany, the number of colony forming units cultured from sediment were 10–100 times higher than those cultured from water obtained at the same depth [14]. In a like manner, direct counts in this study were 10 to 100 fold higher in aquifer solids compared to groundwater from the same site and depth. This difference in biomass levels was reflected in much lower levels of biodegradative activity in the groundwater compared to the saturated sediments. In the case of LAS, no biodegradative activity was observed in the groundwater but significant activity was associated with the solids. This finding indicates that measuring biodegradation of a chemical only in groundwater does not give an accurate evaluation of the potential for biodegradation in the subsurface as a whole. Previous work by Thomas et al. [20] similarly concluded that the use of groundwater in lieu of soil to predict biodegradation potential may lead to erroneous conclusions about chemical fate in the subsurface.

In the subsurface soil, the concentration of PLFA was highly correlated with

rates of thymidine incorporation into DNA and FDA hydrolysis. Direct counts correlated weakly with these activity measurements, and the correlation with PLFA concentration was not significant. This suggests that PLFA concentration is a better measure of active biomass in this environment. Previous work has shown little correlation between direct counts and viable counts in the subsurface [4, 10, 26]. In addition, the proportion of active bacteria in the subsurface determined using variations of the combined direct count/INT reduction method is extremely variable [16, 23]. Analysis of the relative abundance of PLFA generates information on the composition of the microbial communities. Overall, the fatty acid profiles in the lower vadose and saturated zones were less complex than those above 3 m. Previous studies have also noted less complex PLFA patterns in the subsurface [6, 19, 25]. Correspondingly, Bone and Balkwill [2] concluded that the subsurface microbial community was less diverse than that at the surface based upon colony types and direct microscopic observations. Tuberculostearic acid (TBS) is an indicator of the presence of actinomycetes and related bacteria [7]. In both profiles, the relative abundance of this fatty acid decreased with depth. The communities in the Control profile contained much higher levels of TBS than those from the profile impacted in infiltrating wastewater. TBS was absent in samples obtained from the saturated zone. Overall, the abundance of TBS seemed to be inversely related to the presence of saturating or near-saturating moisture conditions. Although organisms morphologically similar to *Nocardia* have been observed in groundwater from shallow wells [12], actinomycetes generally have not been isolated from deeper subsurface sediments [4].

Fatty acids derived from fungi were present at every depth and their relative abundance did not exhibit a consistent pattern with depth or profile. These same fatty acids have been detected previously in the subsurface of other sites. Few studies have directly quantified fungi in the subsurface; Hirsch and Rades-Rohkohl [12] have microscopically observed fungal filaments in groundwater. Fatty acids characteristic of protozoa were detected in both profiles above 3 m but not in the vadose zone below this depth. When present, these fatty acids accounted for 0.7 to 2.7% of the total. Low levels of protozoan fatty acids were detected in the saturated zone of both sites. Sinclair and Ghiorse [18] noted a sharp decline in protozoan numbers with depth but detected significant numbers of protozoa in some saturated strata.

Previous work at this site [7] indicated that substituted aromatic compounds were mineralized at every depth without lag periods. Furthermore, mineralization rates for these compounds did not covary with depth, biomass or activity. In the present study, only mixed amino acids were mineralized without lags at every depth. MAA mineralization also had little relationship to depth or microbial biomass and activity. In contrast, the mineralization of LAE and LAS were profoundly affected by depth and exhibited vertical distributions comparable to those for biomass and activity measurements. LAS and LAE were rapidly mineralized in the upper 2.5 m. Below this depth in the vadose zone, LAE mineralization rates decreased dramatically and LAS degradation was no longer observed corresponding with sharp declines in total biomass and activity. It would appear that the same factors controlling the size and activity

of microbial communities were governing the biodegradation of these two compounds.

The effects of wastewater infiltration were highly variable as a function of depth and the microbiological parameter examined. The profile impacted by infiltrating wastewater was characterized by higher PLFA concentration and faster rates of thymidine incorporation and FDA hydrolysis, particularly in the upper 3 m. It likewise had a much higher level of TOC above this depth. Below 3 m, amino acid mineralization was faster and LAE mineralization was slower in the impacted profile compared to the control. Infiltrating wastewater appeared to depress the mineralization of LAE and to stimulate amino acid mineralization at most depths in the soil. In contrast, biomass and the rate and extent of amino acid mineralization were lower in downgradient groundwater than in upgradient groundwater. These differences are not explained by the differences in the water chemistry of these two sites.

Previous work in saturated subsurface sediments [9] revealed that exposure to detergent chemicals resulted in shorter lag periods and more rapid rates of biodegradation of LAS and LAE, presumably as a result of adaptation. In the present study, adaptation was not nearly as important in the unsaturated subsurface environment. In the vadose zone, neither LAS or LAE degradation was significantly stimulated by infiltrating wastewater, but in the saturated zone, a small stimulatory effect of waste water was noted. Despite the patchiness of biodegradative activity in the subsurface, neither LAS or LAE were detected in the groundwater. Thus, the high levels of biodegradative activity in the upper vadose zone and low levels of activity in the saturated zone were sufficient to fully remove these compounds before they could adversely affect water quality in the area. Sorption likely plays an important role in this process by retarding the movement of these compounds through these biologically active zones.

In summary, this work demonstrated i) that there was a marked discontinuity in the vertical distribution of microbial biomass, activity and the biodegradation of LAS and LAE below 2.5 meters in soil profiles, ii) that infiltrating wastewater affected the magnitude of this discontinuity but not its existence or depth, iii) that groundwater had lower bacterial numbers and exhibited less biodegradative activity than soil obtained from the same site and depth, and iv) that despite patchiness in the distribution of biodegradative activity, neither LAE or LAS accumulate in ground water. Furthermore, this work showed the usefulness of PLFA, FDA hydrolysis and thymidine incorporation assays for determining the distribution of microbial communities in subsurface sediments.

Acknowledgments. We are grateful to Glenn Smith, Patti Thorn and Gary Pastwa for their excellent technical assistance. We also acknowledge Jack Saltes of the Wisconsin Department of Natural Resources for his advice and providing access to the site and Bob Prueher and his excellent crews at Wisconsin Test Drilling, Inc. who conducted the sampling.

References

1. Beloin RM, Sinclair JL, Ghiorse WC (1988) Distribution and activity of microorganisms in subsurface sediments of a pristine study site in Oklahoma. Microb Ecol 16:85–98

2. Bone TL, Balkwill DL (1988) Morphological and cultural comparison of microorganisms in surface soil and subsurface sediments at a pristine study site in Oklahoma. Microb Ecol 16: 49–64
3. Boyer SL, Guin KF, Kelley RM, Mausner ML, Robinson HF, Schmitt TM, Stahl CR, Setzkorn EA (1977) Analytical method for nonionic surfactants in laboratory biodegradation and environmental studies. Environ Sci Technol 11:1167–1171
4. Colwell FS (1989) Microbiological comparison of subsurface soil and unsaturated subsurface soil from a semiarid high desert. Applied Environ Microbiol 55:2420–2423
5. Dunlap WJ, McNabb JF, Scalf MR, Cosby RL (1977) Sampling for organic chemicals and microorganisms in the subsurface. EPA-600/2-77-176. US Environmental Protection Agency
6. Federle TW (1986) Microbial distribution in soil—New techniques. In: Megusar F, Gantar M (eds) Perspectives in microbial ecology. Slovene Society for Microbiology, Ljubljana, pp 493–498
7. Federle TW (1988) Mineralization of monosubstituted aromatic compounds in unsaturated and saturated subsurface soils. Can J Microbiol 34:1037–1042
8. Federle TW, Dobbins DC, Thornton-Manning JR, Jones DC (1986) Microbial biomass, activity and community structure in subsurface soils. Ground Water 24:365–374
9. Federle TW, Pastwa GM (1988) Biodegradation of surfactants in saturated subsurface sediments: A field study. Ground Water 26:761–770
10. Ghiorse WC, Balkwill, DL (1983) Enumeration and morphological characterization of bacteria indigenous to subsurface environments. Dev Ind Microbiol 24:213–224
11. Guckert JB, Antworth CB, Nichols PD, White DC (1985) Phospholipid ester-linked fatty acid profiles as reproducible assays for changes in prokaryotic community structure of estuarine sediments. FEMS Microbiol Ecol 31:147–158
12. Hirsch P, Rades-Rohkohl E (1983) Microbial diversity in a groundwater aquifer in northern Germany. Dev Ind Microbiol 24:183–200
13. Hobbie JE, Daley RJ, Jasper S (1977) Use of Nucleopore filters for counting bacteria by fluorescence microscopy. Applied Environ Microbiol 33:1225–1228
14. Kolbel-Boelke J, Anders EM, Nehrkorn A (1988) Microbial communities in saturated groundwater environment II: Diversity of bacterial communities in Pleistocene sand aquifer and their in vitro activities. Microb Ecol 16:31–48
15. Larson RJ (1984) Kinetic and ecological approaches for predicting biodegradation rates of xenobiotic organic chemicals in natural ecosystems. In: Klug MJ, Reddy CJ (eds) Current perspectives in microbial ecology. American Society for Microbiology, Washington, DC, pp 677–686
16. Marxsen J (1988) Investigations into the number of respiring bacteria in groundwater from sandy and gravelly deposits. Microbial Ecol 16:65–72
17. Osburn QW (1986) Analytical methodology for linear alkylbenzene sulfonate in waters and waste. JAOCS 63:257–263
18. Sinclair JL, Ghiorse WC (1987) Distribution of protozoa in subsurface sediments of a pristine groundwater study site in Oklahoma. Applied Environ Microbiol 53:1157–1163
19. Smith GA, Nickels JS, Kerger BD, Davis JD, Collins SP, Wilson JT, McNabb JF, White DC (1986) Quantitative characterization of microbial biomass and community structure in subsurface material: A prokaryotic consortium responsive to organic contamination. Can J Microbiol 32:104–111
20. Thomas JM, Lee MD, Ward CH (1987) Use of ground water in assessment of biodegradation potential in the subsurface. Environ Toxicol Chem 6:607–614
21. Thorn PM, Ventullo RM (1988) Measurement of bacterial growth rates in subsurface sediments. Microb Ecol 16:3–16
22. United States Department of Agriculture (1972) Soil survey laboratory methods and procedures for collecting soil samples. Soil Conservation Service. Soil Survey Investigations Report No 1
23. Webster JJ, Hampton GJ, Wilson JT, Ghiorse WC, Leach FR (1984) Determination of microbial cell number in subsurface samples. Ground Water 22:17–25
24. Werdelmann BW (1984) Tenside in unserer Welte - heute und morgen. Proc World Surfactants Congr II 1:3–21

25. White DC, Smith GA, Gehron MJ, Parker JH, Findlay RH, Martz RF, Fredrickson HL (1983) The groundwater aquifer microbiota: Biomass, community structure and nutritional status. Dev Ind Microbiol 24:201–210
26. Wilson JT, McNabb JF, Balkwill DL, Ghiorse WC (1983) Enumeration and characterization of bacteria indigenous to a shallow water-table aquifer. Ground Water 21:134–142
27. Wilson JT, McNabb JF, Wilson BH, Noonan MJ (1983) Biotransformation of selected organic pollutants in ground water. Dev Ind Microbiol 24:225–233