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Microbial ecology of the subsurface at an abandoned creosote waste site

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SUMMARY

The microbial ecology of pristine, slightly contaminated, and heavily contaminated subsurface materials, and four subsurface materials on the periphery of the plume at an abandoned creosote waste site was investigated. Except for the unsaturated zone of the heavily contaminated material, mineralization of glucose (13.5 ppb) indicated a metabolically active microflora in all subsurface materials. However, mineralization (<40%) of naphthalene, phenanthrene, and 2-methylnaphthalene was observed in contaminated material and material from the periphery of the plume, but not in pristine material. Pentachlorophenol was mineralized in material from the periphery of the plume. Inorganic and organic nutrient amendments and changes in pH and temperature did not increase the extent of mineralization of the aromatic compounds. An array of organic compounds found in creosote were biotransformed in contaminated ground water; however some compounds were still detected after 7 months of incubation. The data suggest that the subsurface microflora in slightly and heavily contaminated subsurface materials and materials from the periphery of the plume has adapted to degrade many compounds found in creosote.

INTRODUCTION

Degradation of organic chemicals at hazardous waste sites by the indigenous microflora is an important process in the removal of contaminants from subsurface material and ground water. Some microorganisms have an innate capacity or acclimate to degrade some chemicals that pollute the

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subsurface. Biotransformation of organic chemicals in pristine aquifer material [2,29,31] suggests an innate capacity for biorestoration. However, some chemicals polluting the subsurface such as toluene, substituted benzenes, and chlorinated hydrocarbons, may be resistant to microbial attack [28,29,31] even though the compounds have been reported to degrade in enrichment cultures [33], soil [14], sea water [25] and a muck-water sample [34].

Acclimation of microorganisms to subsurface pollutants may be required before degradation can occur. Biotransformation of naphthalene, 1-methylnaphthalene, 2-methylnaphthalene, dibenzofuran and fluorene was observed in subsurface material contaminated with creosote; however, these compounds were not degraded in pristine material from the same site [30]. Even in the presence of acclimated organisms, other factors may limit or preclude the biodegradation of subsurface organic pollutants. The lack of sufficient oxygen limits hydrocarbon degradation [2,30]. The addition of oxygen enhanced the biodegradation of several polycyclic aromatic hydrocarbons in ground water contaminated with creosote [13]. Growth of in situ hydrocarbon-degrading microorganisms and subsequent hydrocarbon degradation in ground water contaminated with gasoline was stimulated by adding air and inorganic nutrients [16]. Inorganic nutrients, such as nitrogen and phosphorus, may limit biodegradation of subsurface pollutants when the carbon to inorganic nutrient ratios are too great. On the other hand, anaerobic conditions may be required to degrade halogenated aliphatic compounds [7,8].

Table 1

Core description and characterization

Sample	Contamination regime	Depth (m)	Subsurface material
14	pristine	1.2–1.4	unsaturated
14	pristine	3.6–3.8	unsaturated
14	pristine	3.8–3.9	unsaturated
14	pristine	7.4–7.6	saturated
16	slightly	1.9–2.0	unsaturated
16	slightly	5.7–5.8	unsaturated
16	slightly	7.5–7.6	saturated
15	heavily	2.0–2.2	unsaturated
15	heavily	5.6–5.7	unsaturated
15	heavily	7.6–7.7	saturated
SW2	periphery of plume	6.2–7.0	saturated
SW3	periphery of plume	6.2–7.0	saturated
SW4	periphery of plume	7.3–8.1	saturated
BH10	periphery of plume	6.2–7.0	saturated

The microbial ecology of the subsurface at an abandoned creosoting facility in Conroe, Texas was investigated in the present study. Microbial numbers in subsurface profiles of pristine, slightly contaminated and heavily contaminated materials, and materials from the periphery of the plume were determined. The breadth of microbial adaptation to degrade creosote components and the effect of nutrient amendments on degradation of selected compounds were investigated. The extent of mineralization of selected compounds was determined using ^{14}C-labelled chemicals whereas gas-liquid chromatography was used to determine the breadth of adaptation to compounds found in creosote.

MATERIALS AND METHODS

Environmental samples

Subsurface samples were collected in June, 1983, from three sites at the abandoned United Creosoting Company, Conroe, Texas. Representative subsurface material was obtained using a method developed by the U.S. Environmental Protection Agency (EPA), Ada, OK [29]. Core material was taken from three depths from pristine, slightly contaminated, and heavily contaminated locations (Table 1). At least two subsurface samples were collected from different depths in the unsaturated and one from the saturated zone of each borehole. A comprehensive hydrogeological study [3] of the site indicated that borehole No. 15 is located directly in the creosote waste pit, No. 16 is located south of the pit and in the plume, and No. 14 is southeast of the pit and outside the plume of contamination (Fig. 1). The subsurface materials were used in the experiments after 1 year of storage at 5°C.

Additional subsurface samples were collected in

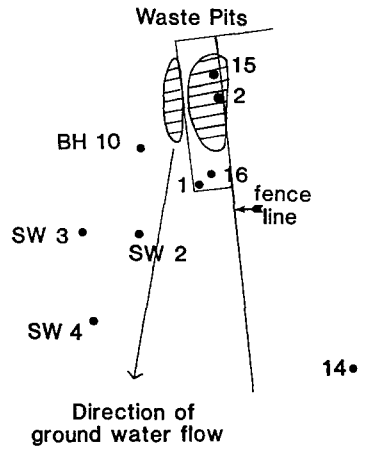

Fig. 1. Locations of boreholes and wells at an abandoned creosote waste site in Conroe, TX.

January, 1985, from four sites at the abandoned creosoting plant. Core material was taken from the saturated zone of boreholes SW2, SW3, SW4 and BH10 (Table 1). These boreholes are located southwest of the waste pits and SW2 and BH10 (Fig. 1) and were predicted by modeling [5] to be on the periphery of the plume at the time of sampling. Data provided by Roy F. Weston, Inc., Houston, TX [27] indicated that ground water from SW2 and SW4 contained 43 and 70 ppb naphthalene, respectively. These subsurface materials were used in experiments within 1 month of their procurement.

Ground water was collected from a monitoring well, well 2, that was located immediately in the waste pit (Fig. 1). Representative ground water was obtained after treating the well screen and the surrounding formation within a 30.5-cm radius with hydrogen peroxide using a previously published method [23]. The hydrogen peroxide solution was prepared by adding a 30% solution of hydrogen peroxide to 15.6 liters (2 well volumes) of ground water collected from well 2. The solution was mixed and then pumped back into the well using teflon tubing. The resulting hydrogen peroxide concentration within a 30.5 cm radius around the well screen was calculated to be 0.5%. Brown et al. [10] reported a 99.9% kill of an *Escherichia coli* culture that had been exposed to 200 ppm (0.02%) hydrogen peroxide for 30 min. The tubing was, hence, disinfected by the hydrogen peroxide solution. A sterile cap was placed on the end of the tubing and the hydrogen peroxide solution was allowed to react with the well casing and formation for 24 h. Ground water then was withdrawn at a rate of 520 ml/h for a second 24 h. After 24 h of continuous pumping (approximately 858 liters), a sample of ground water was collected for biodegradation experiments. The inhibitory effects of the hydrogen peroxide addition were observed until 18 well volumes had been pumped; the details of this method are published elsewhere [23].

Bacterial counts

Bacterial numbers were determined using the spread plate technique and epifluorescence microscopy. Viable counts were determined using three types of solid media: Nutrient Agar (Difco Laboratories, Detroit, MI), pristine ground water agar, and contaminated ground water agar. The pristine and contaminated ground water agars were prepared by adding 2% Noble Agar (Difco), 100 ng/ml peptone, 10 ng/ml yeast extract, and 100 ng/ml glucose to freshly collected ground water from well 14 (pristine) or well 2 (contaminated). The pH of the ground water agars was adjusted to 5.0, the in situ pH of the subsurface soil. Portions of subsurface material (10 g) were shaken mechanically in 95 ml of 0.1% sodium pyrophosphate for 30 min after which the suspension of subsurface material was diluted serially and then plated. The plates were examined for colony formation after 2 weeks of incubation at 24°C, the in situ ground water temperature at the Conroe site. Acridine orange direct counts were performed using the method of Ghiorse and Balkwill [11].

Mineralization studies

Portions (10 g) of wet core material were added aseptically to sterile 25 ml scintillation vials. Then the vials were filled completely with ground water which had been sterilized by autoclaving. The ground water used to fill vials containing subsurface material from sites 14, SW2, SW3, SW4 and BH10 was collected from the borehole from which the core material was obtained. Contaminated ground

water from well No. 2 was used to fill vials containing core material from sites 15 and 16 because wells were not constructed in these boreholes. Vials were filled completely with nonsterile ground water when subsurface solids were not used. Sterile controls and microcosms to account for sorption and volatilization were prepared by adding a 1% solution of sodium azide in ground water to vials with or without autoclaved soil. All vials were sealed with teflon-lined septa and open-top screw caps.

Each vial was injected with 0.5 μl of methanol that contained a specified amount of ^{14}C-labelled naphthalene, phenanthrene, 2-methylnaphthalene, pentachlorophenol (PCP), or glucose. Addition of methanol added 5.9 μg of extra carbon/ml of aquifer material (solids plus ground water). The vials were incubated in the dark at 24°C. Immediately after filling and injecting the vials and after a specified amount of time that was different for each compound, three nonsterile and three sterile vials were sampled to determine the extent of mineralization.

The extent of mineralization was determined by trapping $^{14}CO_2$ using a modified method of Marinucci and Bartha [15]. To purge vials of CO_2, 10 ml of ground water was removed, using a gas-tight syringe, and injected into a second sealed vial containing one drop of concentrated H_2SO_4. One drop of concentrated H_2SO_4 was added to the first vial and then the samples in both vials were bubbled in series for 10 min with CO_2-free air to remove $^{14}CO_2$. The air was purged through a series of three traps. The first trap contained one drop of 1 N HCl, 7 ml of scintillation cocktail (Beckman MP, Beckman Instruments, Irvine, CA) and 3 ml of reagent-grade hexane to remove lipid-soluble compounds and the other two traps contained 5 ml of scintillation cocktail (Beckman MP) and 5 ml of a CO_2-trapping agent (Oxifluor, New England Nuclear, Boston, MA).

In separate experiments, sorption and volatilization of naphthalene, phenanthrene, PCP, and 2-methylnaphthalene were determined at intervals by removing a portion (10 ml) of the water phase from microcosms containing subsurface material and those without subsurface material, respectively. The aqueous portion was added to 10 ml of scintillation cocktail (Beckman MP) and the amount of radioactivity was determined by liquid scintillation counting. Loss of compound as a result of volatilization or sorption was determined by measuring the percent of ^{14}C-label remaining in solution in microcosms with water or with subsurface material and ground water, respectively.

Chemicals

The following radioactive chemicals and their specific activities were purchased from Pathfinder Laboratories, Inc., St. Louis, MO: [1(4,5,8)-^{14}C]naphthalene, 5.0 mCi/mmol; 2-[8-^{14}C]methylnaphthalene, 7.9 mCi/mmol; [UL-^{14}C]pentachlorophenol, 10.6 mCi/mmol; D-[(U)-^{14}C]glucose, 1.9 mCi/mmol; [9-^{14}C]phenanthrene, 14.0 mCi/mmol. All labelled chemicals were at least 98% pure and all unlabelled chemicals were of the highest purity that could be obtained commercially.

Extraction of soil and ground water

Duplicate ground water samples were extracted for organic compounds by concentrating 50–250 ml portions with Sep-Pak C-18 cartridges (Waters Associates, Milford, ME) using the method of Wang et al. [26]. The organic concentrate was eluted with methylene chloride. Duplicate samples of subsurface material were extracted with methylene chloride and methanol using a modified method of Brown et al. [9]. Portions (1 μl) of eluent from the subsurface material or ground water extract were injected into a gas chromatograph (Model 560, Tracor, Austin, TX) equipped with a flame ionization detector. The injector and detector were held at 275°C and 300°C, respectively, and the flow rate of the carrier gas, helium, was 1 ml/min. The samples were injected into a Hewlett Packard ultra-performance silica capillary column with cross-linked 5% phenylmethylsilicone packing that was programmed to hold at 50°C for 4 min and then increase by 10°C per min until reaching 250°C. The organic compounds were identified by comparing retention times to standards and quantified using an SP-4100 integrator (Supelco, Bellefonte, PA).

Table 2

Microbial numbers (No. cells (S.D.) × 10^4/g dry core material) with depth from pristine, slightly and heavily contaminated core material incubated for 2 weeks

Sample	Depth of sample (m)	Direct counts	Medium		
			Nutrient agar	Contaminated ground water agar	Pristine ground water agar
14[a]	3.6–3.8	212 (93)	9.7 (2.3)	6.1 (4.2)	$<10^2$
14	3.8–3.9	227 (91)	$<10^2$	$<10^2$	$<10^2$
14	7.4–7.6	142 (67)	$<10^2$	$<10^2$	$<10^2$
16[b]	1.9–2.0	224 (127)	$<10^2$	$<10^2$	$<10^2$
16	5.7–5.8	129 (57)	230 (11)	218 (13)	137 (27)
16	7.5–7.6	237 (83)	290 (18)	198 (28)	283 (22)
15[c]	2.0–2.2	212 (75)	$<10^2$	$<10^2$	$<10^2$
15	5.6–5.7	86 (42)	$<10^2$	$<10^2$	$<10^2$
15	7.6–7.7	139 (63)	F[d]	F	F

[a] Pristine.
[b] Slightly contaminated.
[c] Heavily contaminated.
[d] Fungal growth.

Treatments for mineralization enhancement

Attempts to increase the extent of mineralization of naphthalene, phenanthrene and 2-methylnaphthalene were made by adding inorganic nutrients with either NH_3 or NO_3^- as the nitrogen source, 10 ng/ml yeast extract, 1 μg/ml acetate, 1 μg/ml humic acid or by adjusting the in situ temperature (24°C) to 34°C and the in situ pH (5.0) to 7.0. Inorganic nutrients were added to ground water to achieve final concentrations per liter of: 40 mg Na_2HPO_4, 20 mg $MgSO_4 \cdot 7H_2O$, 100 mg Na_2CO_3, 1 mg $CaCl_2 \cdot 2H_2O$, 1.2 mg $MnSO_4 \cdot H_2O$, 0.5 mg $FeSO_4 \cdot 7H_2O$, and 100 mg $(NH_4)_2SO_4$ or 100 mg $NaNO_3$ as alternate nitrogen sources.

Statistical analyses

Data analysis was performed using the Statistical Analysis System [17]. The data were analyzed by analysis of variance using the ANOVA subprogram. Significant differences between treatments were determined using the Scheffe test for multiple contrasts ($\alpha = 0.05$).

RESULTS

Microbial counts

Viable counts with depth from cores 14, 15 and 16 were variable (Table 2). Microbial numbers from the saturated zone of core 15 could not be quantified because of abundant growth of what appeared to be a single type of fungus. Only core SW4 yielded viable counts from the cores located on the periphery of the plume (Table 3). Except for pristine core material taken 3.6–3.8 m in depth, viable counts, when present, were recovered on all three media. Direct counts ranged from 8.6×10^5 to 2.4×10^6 cells/g soil in the pristine, slightly and heavily contaminated cores. However, direct counts were about the same as viable counts in the lower unsaturated and saturated zones of borehole 16, which is within the plume of creosote contaminated material. Direct and viable count determinations for these samples were repeated with similar results.

Extraction of ground water and soil

Organic compounds were detected throughout

Table 3

Microbial numbers (No. cells (S.D.) × 10^3/g dry soil) in the saturated zone of core material from the periphery of the plume after 2 weeks of incubation

Sample	Direct counts	Medium		
		Nutrient agar	Contaminated ground water agar	Pristine ground water agar
SW2	12 000 (6 000)	$<10^2$	$<10^2$	$<10^2$
SW3	20 000 (7 000)	$<10^2$	$<10^2$	$<10^2$
SW4	28 000 (16 000)	9.2 (1.6)	10.0 (3.7)	7.2 (0.7)
BH10	13 000 (5 000)	$<10^2$	$<10^2$	$<10^2$

the profile of only the heavily contaminated subsurface material (Table 4). Concentrations of naphthalene (17.3 μg/g) and phenanthrene (18.3 μg/g) and 2-methylnaphthalene (7.7 μg/g) were highest in the uppermost layer of the unsaturated zone of the heavily contaminated material; concentration decreased with depth and only naphthalene was detected in the saturated zone of the borehole. The concentrations of compounds detected were lower than would be expected in subsurface material contaminated with creosote sludge; however, many of the compounds in creosote are volatile and may have been lost during storage. The concentrations of naphthalene, 2-methylnaphthalene and phenanthrene in ground water from well 2 were 0.41, 0.23 and 0.05 μg/ml, respectively. Extraction of ground water collected from cores SW2, SW3, SW4 and BH10 by Roy F. Weston, Inc. [27] indicated that the concentrations of naphthalene in ground water collected from cores SW2 and SW4 were 43 and 70 ng/ml, respectively, whereas none was detected in cores SW3 and BH10.

Extent of mineralization

Glucose (14 ng/g soil) was mineralized to some extent in all cores with depth except for the unsaturated zones of the heavily contaminated material (Table 5). Mineralization of glucose indicates a me-

Table 4

Naphthalene, 2-methylnaphthalene and phenanthrene concentrations (ppm) in ground water and slightly and heavily contaminated core material from the Conroe site with depth

Compound	Ground water	Heavily contaminated material			Slightly contaminated material		
		80–85″	222–226″	301–304″	76–79″	224–230″	294–330″
Naphthalene	0.41	17.3	0.3	0.06	0.27	n.d.[a]	n.d.
2-Methylnaphthalene	0.23	7.7	0.7	n.d.	n.d.	n.d.	n.d.
Phenanthrene	0.05	18.3	1.4	n.d.	n.d.	n.d.	n.d.

[a] Below detection units for naphthalene (0.05 μg/g), 2-methylnaphthalene (0.07 μg/g), or phenanthrene (0.10 μg/g).

Table 5

Mineralization (percent (S.D.)) of selected creosote components and glucose in the saturated and unsaturated zones of pristine, slightly and heavily contaminated core material

Sample	Subsurface[a] material	Depth of sample (m)	Incubation: (days)	Naphthalene 8	Phenanthrene 19	PCP 56	Glucose 55
14[b]	U	1.2–1.4		n.d.[c]	n.d.	0	16.7[d]
14	U	3.6–3.8		0	0	0	20.0[d]
14	U	3.8–3.9		0	0	n.d.	n.d.
14	S	7.4–7.6		0	0	0	12.9[d]
16[e]	U	1.9–2.0		0	17.4 (2.3)	0	23.0 (10.8)
16	U	5.7–5.8		10.1 (1.7)	19.2 (0.4)	0	7.8 (2.4)
16	S	7.5–7.6		31.9 (6.1)	23.1 (1.4)	0	20.1 (6.5)
15[f]	U	2.0–2.2		0	0	0	0
15	U	5.6–5.7		0	0	0	0
15	S	7.6–7.7		25.4 (5.9)	28.5 (1.3)	0	26.2 (9.8)

[a] U, unsaturated; S, saturated.
[b] Pristine.
[c] Not determined.
[d] Two replicates.
[e] Slightly contaminated.
[f] Heavily contaminated.

tabolically active microflora. Although glucose was mineralized in samples of pristine material, naphthalene, phenanthrene, and PCP were not mineralized. Metabolism of glucose but not hydrocarbons indicates that the microflora in this material is not adapted to degrade the more complex compounds. The lack of mineralization of glucose, naphthalene, phenanthrene and PCP in the unsaturated zones of the heavily contaminated core material may be caused by toxic concentrations of creosote sludge. Although the lack of nitrogen and phosphorus could prevent biodegradation of carbonaceous materials, such a nutrient deficiency probably would not totally inhibit glucose metabolism. Both naphthalene and phenanthrene were mineralized in the saturated zone of this core. Mineralization of naph-

Table 6

Mineralization (percent (S.D.)) of selected creosote components and glucose in subsurface material from the periphery of the plume at Conroe

Sample	Incubation: (days)	Naphthalene 3	Phenanthrene 7	2-Methylnaphthalene 7	PCP 56	Glucose 55
SW2		0	0	0	0	23.7 (5.7)
SW3		0	0	0	0	11.5 (10.6)
SW4		35.1 (1.1)	4.4 (4.6)	5.4 (0.7)	20.3 (9.0)	13.6 (7.4)
BH10		0	0	0	0	25.1 (8.0)

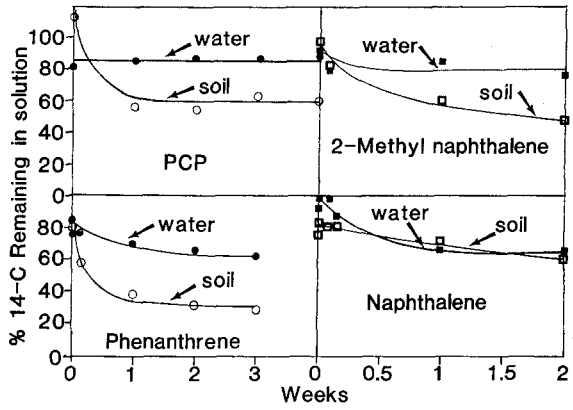

Fig. 2. Percent of ^{14}C-labelled PCP, 2-methylnaphthalene, phenanthrene, and naphthalene remaining in solution in soil and water microcosms incubated for 2–4 weeks.

thalene and phenanthrene was observed in the slightly contaminated material.

Glucose (14 ng/g soil) was mineralized in the saturated zone of all cores collected on the periphery of the plume (Table 6). However, mineralization of naphthalene, phenanthrene, 2-methylnaphthalene, and PCP was detected in core SW4 only.

Sorption and volatilization studies

Sorption data indicate that PCP, phenanthrene, and 2-methylnaphthalene were greatly sorbed by the subsurface material, whereas naphthalene was sorbed to a lesser extent (Fig. 2). There was no substantial loss of PCP by volatilization although about 15% of the added phenanthrene in water was lost after 21 days. About 35% of the naphthalene was lost in 14 days from water samples whereas less of the compound was lost from soil microcosms. The controls clearly demonstrate that the absence of mineralization in some samples was due to the absence of microbial activity rather than loss of the compounds by volatilization and/or sorption.

Enhancement of mineralization

There was no stimulation of the extent of naphthalene and 2-methylnaphthalene mineralization ($\alpha = 0.05$) from the addition of nutrients or changes in temperature and pH (Table 7). However, phenanthrene mineralization was enhanced by a 10°C increase in incubation temperature and by the addition of 1 μg/ml humic acid.

Disappearance of creosote compounds in ground water

Ground water collected from well 2 was incubated aerobically in sterile 1-gallon amber bottles for 7 months to determine the biotransformation of 14 organic compounds. These compounds had been identified previously in samples of ground water from well 2 [12]. The concentration of dissolved oxygen in water from well 2 was 2.0 mg/l. Portions of ground water were extracted after 0, 1, 2, 3, 4, 8, 13 and 28 weeks of incubation. Ground water amend-

Table 7

Effect of treatments on mineralization (percent (S.D.)) of selected aromatics in contaminated ground water from the Conroe site after 3 weeks of incubation

Treatment	Naphthalene	Phenanthrene	2-Methylnaphthalene
In situ conditions (24°C, pH 5.0)	28.6 (2.4)	7.3 (0.9)	29.1 (5.1)
Inorganic nutrients, NH_3 as N source	24.0 (1.2)	12.0 (2.0)	26.3 (5.5)
Inorganic nutrients, NO_3^- as N source	24.8 (1.5)	17.4 (2.5)	40.2 (3.7)
34°C	27.1 (0.3)	35.3 (8.2)	32.5 (4.5)
pH 7	26.5 (3.1)	2.6 (1.4)	39.2 (15.4)
Yeast extract (1 μg/ml)	26.6 (0.6)	5.1 (0.9)	35.3 (8.7)
Acetate (1 μg/ml)	23.8 (0.2)	5.0 (0.4)	29.9 (6.7)
Humic acid (1 μg/ml)	23.8 (0.7)	25.8 (8.6)	42.5 (0.7)

Table 8

Biotransformation of selected organic compounds (ng/ml) in ground water contaminated with creosote

Compound	Weeks of incubation							
	0	1	2	3	4	8	13	28
Phenol	n.d.[a]	n.d.	n.d.	n.d.	n.d.	n.d.	13.7, n.d.	34.6
o-Cresol	33.8	n.d.	n.d.	9.0	n.d.	9.0, n.d.	31.3	35.5, n.d.
Borneol	18.7	19.5	8.6	19.2	4.2	14.0	20.4	8.7
Naphthalene	409	7.9	n.d.	n.d.	n.d.	5.0, n.d.	n.d.	4.3, n.d.
2-Methylnaphthalene	231.0	10.0	111	5.4	n.d.	2.0, n.d.	5.9, n.d.	6.1, n.d.
1-Methylnaphthalene	112	27.2	8.7	n.d.	n.d.	n.d.	n.d.	5.1, n.d.
Biphenyl	24.9	28.1	10.8	n.d.	n.d.	n.d.	n.d.	5.5, n.d.
1,2-Dimethylnaphthalene	10.1	14.7	11.5	13.5	5.8	10.0, n.d.	10.4	5.2
Acenaphthalene	80.4	58.0	24.4	16.2	8.1	5.5	7.6	8.6, n.d.
Dibenzofuran	45.3	28.3	n.d.	n.d.	n.d.	n.d.	15.6, n.d.	n.d.
Fluorene	63.1	80.6	55.6	26.1	6.8	10.0	17.4, n.d.	4.1, n.d.
Dibenzothiophene	n.d.	12.9, n.d.[b]	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Phenanthrene	51.0	44.7	17.8	9.3	n.d.	12.0, n.d.	7.1	n.d.
Carbazole	60.0	41.6	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

[a] Not detectable: detection limits (ng/ml); phenol, 5.3; *o*-cresol, 4.7; borneol, 4.2; naphthalene, 3.1; 2-methylnaphthalene, 3.9; 1-methylnaphthalene, 3.2; biphenyl, 3.5; 1,2-dimethylnaphthalene, 3.6; acenaphthalene, 3.9; dibenzofuran, 4.7; fluorene, 4.0; dibenzothiophene, 5.6; phenanthrene, 4.9; carbazole, 13.3.

[b] Compound detected in one replicate only.

Table 9

Average concentration (ng/ml) of selected compounds in contaminated ground water amended with 1% sodium azide

Compound	Weeks of incubation							
	0	1	2	3	4	8	13	28
Phenol	n.d.[a]	n.d.	n.d.	n.d.	n.d.	n.d.	29.7	13.4
o-Cresol	63.4	26.2	36.3	38.3	27.7	31.5	61.9	17.2
Borneol	33.7	28.2	59.7	47.5	32.7	11.5	28.5	12.2
Naphthalene	414	431	425	406	390	427	440	313
2-Methylnaphthalene	229	253	245	243	179	179	221	142
1-Methylnaphthalene	111	128	116	134	85.5	85.0	103	68.0
Biphenyl	27.1	26.7	26.0	26.1	17.6	14.5	28.4	14.4
1,2-Dimethylnaphthalene	14.7	17.6	13.3	14.5	6.6	6.0	17.9	7.5
Acenaphthalene	83.4	89.8	90.5	86.5	57.7	59.5	84.4	45.9
Dibenzofuran	53.1	63.6	64.0	52.0	29.6	38.0	68.7	28.6
Fluorene	90.1	337	70.0	42.9	23.1	31.5	47.6	19.5
Dibenzothiophene	18.7	7.3, n.d.[b]	n.d.	n.d.	n.d.	19.2	n.d.	n.d.
Phenanthrene	69.4	57.0	62.5	55.2	25.1	50.0	55.5	10.1
Carbazole	55.5	107	80.4	n.d.	68.6, n.d.	27.0, n.d.	n.d.	n.d.

[a] Not detectable: detection limits are given in Table 8.

[b] Compound detected in one replicate only.

ed with 1% sodium azide to inhibit microbial activity was extracted simultaneously to determine the abiotic disappearance of the organic compounds. The detection limits of the 14 organic compounds are listed in Table 8. Biotransformation of the organic compounds in well 2 water with and without 1% sodium azide is given in Tables 8 and 9, respectively. The concentrations of the organic compounds are presented as averages of duplicate samples.

Of the 13 organic compounds detected in unamended ground water, most were degraded faster and more completely than those detected in ground water amended with sodium azide. The percentage of 1,2-dimethylnaphthalene and carbazole remaining was about the same in ground water with and without the inhibitor. Phenol was not detected until 8 weeks in the unamended and 13 weeks in amended ground water and may therefore be an intermediate in the degradation sequence of some of the organic compounds in creosote. The concentration of *o*-cresol decreased and then increased in unamended ground water and may also be an intermediate in the degradation of some compounds found in creosote. Some of the loss of the organics in the inhibited sample may have been due to volatilization or sorption to the bottle, or the organisms may have adjusted to the sodium azide or the sodium azide may have been chemically altered with time. After 28 weeks of incubation, nine of the 12 compounds initially present in the unamended ground waters were still detected, which indicated that complete degradation would probably require longer than 6 months.

DISCUSSION

Except for the unsaturated zones of the heavily contaminated core material, the presence of a metabolically active microbial population is indicated in all soils by the ability to mineralize glucose. The unsaturated samples from the heavily contaminated site may have been inhibited by toxic levels of organics that were once present in the creosote sludge. Thomas et al. [22] observed glucose mineralization in uncontaminated but not contaminated aquifer material collected from an oil refinery; further experiments suggested that the contaminated material contained a microbial inhibitor. Mineralization of selected organic compounds in slightly and heavily contaminated but not pristine material suggests that the subsurface microflora must adapt to the creosote before degradation of the organic pollutants can occur. Using samples from the same waste site, Wilson et al. [30] also detected biotransformation of naphthalene, 2-methylnaphthalene and other selected organic pollutants in contaminated but not pristine subsurface material.

In addition, the time required for acclimation to occur may be long. Mineralization of naphthalene, phenanthrene, 2-methylnaphthalene and PCP was detected in only one (SW4) of the four subsurface materials on the periphery of the plume. Although modeling efforts [5] and chemical analyses [27] indicated that the leading edge of the plume had reached these cores, the concentration of organic compounds at the periphery may have been too low or more time may be required to achieve acclimation. Spain and Van Veld [20] reported a threshold concentration of 10 ppb for *p*-nitrophenol below which adaptation did not occur in samples of sediment. Pre-exposure of sediment cores to 0.45 μM *p*-nitrophenol yielded slower mineralization rates than those exposed to higher concentrations [19]. The biotransformation data suggest that the subsurface microflora in the plume has adapted to degrade an array of organic compounds. Of the 14 organic pollutants found in creosote that were investigated, about 60% was removed to a greater extent than those in sodium azide-amended ground water. However, many organic compounds persisted in ground water after 7 months of incubation at concentrations ranging from about 5 to 35 ppb. Thresholds for substrates below which biodegradation does not occur have been suggested [4] and may thwart biorestoration of contaminated aquifers. Borden et al. [6] reported threshold concentrations between 5 and 30 μg/l for naphthalene, 2-methylnaphthalene, fluorene, phenanthrene, and dibenzofuran biotransformation in samples of creosote-contaminated ground water from the Conroe

site, regardless of the initial dissolved oxygen levels (9 or 1.7 mg/l).

Stimulation of phenanthrene mineralization by increased temperature and humic acid amendment may be sorption-related. An increase in temperature and the addition of humic acid may counteract the decrease in degradation of organic compounds often associated with sorption [21]. The low percent mineralization and overall failure to stimulate the extent of mineralization of naphthalene and 2-methylnaphthalene may result from an initial incorporation of the contaminants into biomass with a slow release of CO_2 as the biomass turns over (John T. Wilson, USEPA, Ada, OK, personal communication). In addition, some of the hydrocarbons may have been unavailable for microbial attack because of sorption to aquifer solids and/or volatilization.

Slight variations in total cell counts with depth by the direct count method are consistent with previously published data for pristine subsurface materials [1,11,29] but contrast with that determined in a chalk aquifer [24]. Contaminated subsurface material has been reported to contain a greater total microbial biomass than pristine material from the same site [18]. Our direct counts of pristine and contaminated core materials did not reflect this finding; however, mineralization of selected aromatic hydrocarbons was observed in every contaminated core sample that yielded viable counts with one exception (core 16, 1.9–2.0 m). Similarly, Wilson et al. [32] reported that rates of toluene degradation decreased as ATP content of the aquifer material decreased.

The data suggest that microorganisms in the contaminated zones have adapted to degrade many compounds found in creosote but the conditions required for acclimation to occur are unknown. The concentration of dissolved oxygen may be limiting the extent of mineralization of the hydrocarbons in this subsurface environment. Wilson et al. [30] suggested that the rate of biodegradation of selected organic compounds in contaminated material from the Conroe site is controlled by the supply of dissolved oxygen. Our data suggest that the indigenous microflora may be important in the natural fate of contaminants and in the reclamation of contaminated soil and ground water.

DISCLAIMER

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