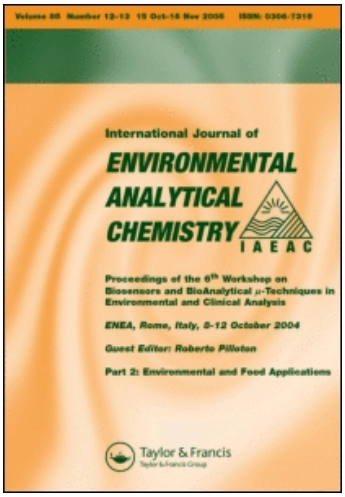

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Petroleum and Chlorinated Hydrocarbon Analysis in Support of *In Vitro* Studies of Natural Anaerobic and Aerobic Microbial Degradation of Xenobiotics in Contaminated Groundwater and Soil

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PETROLEUM AND CHLORINATED HYDROCARBON ANALYSIS IN SUPPORT OF *IN VITRO* STUDIES OF NATURAL ANAEROBIC AND AEROBIC MICROBIAL DEGRADATION OF XENOBIOTICS IN CONTAMINATED GROUNDWATER AND SOIL

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This program investigated *in situ* biological degradation as a remedial treatment technique at a United States Air Force Base in Texas. Soil and groundwater samples from the site were characterized regarding microbial populations and presence or absence of priority pollutant xenobiotics and petroleum hydrocarbon components. Time-series analyses of replicate nutrient-amended microcosms after approximately 1, 25, 50 and 100 days permitted aerobic and anaerobic degradation rate evaluation. Microcosm integrity was demonstrated throughout the experiment by observing appropriate dissolved oxygen, pH, oxidation/reduction potential, and sterile/viable conditions. Gas chromatographic analysis and ratios of straight-chain and isoprenoid components clearly demonstrated that aerobic conditions were preferred for degradation of aliphatic and aromatic petroleum hydrocarbons. Electron capture gas chromatographic analyses and purge-and-trap gas chromatographic/mass spectrometric analyses of lower molecular weight chlorinated species demonstrated that anaerobic conditions were required to promote significant degradation of these components. Some evidence of lower molecular weight chlorinated species generation due to microbial and abiotic processes was also observed.

KEY WORDS: Xenobiotics, petroleum hydrocarbons, anaerobic/aerobic, microbial degradation.

INTRODUCTION

The overall objective of this study was to investigate the feasibility of *in situ* biological degradation as a remedial treatment technique at a contaminated waste site on a United States Air Force Base. The site had been previously used as a disposal pit for chromium electroplating sludges and wastes from 1940 to 1955. The pit was closed with gravel and fill material when the chromium plating operation moved. A clay liner was installed over the fill material in the early 1960s and was subsequently ruptured during local excavation. The site was used again as a chemical disposal pit until 1966 and received chlorinated hydrocarbon cleaning compounds, cresols, acid sludges, and cyanide wastes.[1] Preliminary levels of organic and inorganic contaminants are given in Table 1.

Table 1 Representative contaminant concentrations, ppm

	Tetrachloro-ethylene	*Trichloro-ethylene*	*1,1,1-Trichloro ethane*	*Trans 1,2-Dichloro-ethylene*	*Chloro-benzene*	*Dichloro-benzenes*	*Alkyl-benzenes*	*Toluene*	*Ketones*	*Hydrocarbons*	
										n-Alkanes	*Branched*
Soil											
Borehole 1	0.001	0.01	0.10	—	0.093	—	0.005	0.038	0.037	40	158
Borehole 2	—	0.016	0.97	—	—	0.15	—	0.005	0.30	—	—
Borehole 3	0.022	—	—	—	—	—	—	—	1.0	—	0.009
Borehole 4	—	0.91	—	—	—	0.16	0.020	0.022	2.2	58	0.11
Borehole 5	0.019	0.082	—	—	0.012	38	78	0.13	26	454	456
Groundwater											
Well: AA	—	—	0.076	7.7	—	0.26	50	—	<1.0	1145	2450
Well: BB	0.13	0.12	0.12	0.13	—	0.005	—	—	<1.0	—	—
Well: CC	15	6.7	—	2.2	—	<0.001	—	—	<1.0	—	—

Because the site is surrounded on three sides by drainage ditches and a creek, and since xenobiotics found on the site had been detected in water samples from the creek,[1] investigation of remedial techniques to clean up the site were warranted. Due to the exorbitant cost of removal or incineration, biodegradation was considered as an alternative. Program elements included geological site investigation, determination of the nature and extent of contamination, a microbial investigation of the site, the laboratory biodegradation study, and final field implementation of a treatment system.

The primary focus of this paper is the laboratory program which consisted of soil and groundwater collection, preliminary chemical analysis of blended samples for homogeneity, aerobic and anaerobic microcosm preparation, microcosm sacrifice and chemical analysis, and data reduction and interpretation.

Extensive literature review indicated that aerobic degradation was most effective at removing aliphatic and aromatic hydrocarbons,[2–4] while anaerobic conditions were required for significant chlorinated solvent degradation.[5–9] Two means of oxygenating the aerobic systems were considered: stabilized hydrogen peroxide and 100% oxygen gas.[10,11]

The wide variety in type and level of contaminants suggested that stimulation of native microorganisms had a higher probability of success than the introduction of foreign organisms to significantly degrade the compounds at the site. At the time this study was performed, cultures developed specifically to degrade environmental pollutants were not readily available, so attempts at bioaugmentation were not investigated. Acridine orange-direct cell counts ranged from 7.6×10^6 to 1.68×10^8 green fluorescent cells/wet gram, while viable cell counts ranged from 1.0×10^2 to 7.1×10^6 colony forming units/wet gram sample using dilution-spread procedures[12–14] on seven media. Viable counts on all seven media indicated a diverse or highly adaptive microbial population capable of metabolizing (or co-metabolizing) a large variety of substrates, and suggested that the limiting factor to successful site remediation would not be microbiological population dynamics.

Several methods of preparing sterile control microcosms were considered. Autoclaving and chemical sterilization were discarded as options since they would radically alter the chemical nature of the microcosms.[15] Exposure of the microcosm contents to ethylene oxide, aside from being highly toxic to laboratory personnel, would be difficult to perform on samples of this type without releasing volatile compounds from the slurries. Filtration would remove the soil phase along with *in situ* microbes. Radiation sterilization was finally selected as the most efficient method that was least harmful overall to the microcosm contents. The controls were sterilized by gamma-irradiating intact microcosms with a multi-position cobalt-60 source for a total of one hour.

EXPERIMENTAL DESIGN

Soil samples were collected from six borings adjacent to boreholes that had previously been determined to contain high xenobiotic concentrations [tetrachloroethylene (perchloroethylene, PCE), trichloroethylene (TCE), trans-1,2-dichloroethylene (trans-1,2-DCE), 1,1-dichloroethylene (1,1-DCE), 1,1-

dichloroethane (1,1-DCA), and straight-chain hydrocarbons (n-alkanes)]. The split-spoon sampler was cleaned and sterilized between samples by soap-and-water washing and steam-cleaning. Once the cores were above the surface, the sampler ends were capped with hands wearing alcohol-decontaminated gloves to protect anaerobic organisms from oxygen toxicity while carrying the corer from the borehole to the mobile laboratory. Soil was packaged for shipment in a glove bag under nitrogen in sterile 1-liter glass jars sealed with duct tape inside 1-gallon paint cans and shipped in the dark at 4 °C to minimize microbial activity. Groundwater samples were obtained by teflon bailer and were also shipped in the dark at 4 °C.

At the laboratory, the soil cores were split longitudinally in a nitrogen-filled glovebag, and all samples were divided into two lots, one for aerobic microcosms and the other for anaerobic microcosms. Further preparation was handled similarly except that manipulation of anaerobic microcosms continued in the nitrogen-filled glovebags while aerobic microcosms were assembled in an air-filled glovebag. Each soil type was homogenized with groundwater collected from the site to provide all microcosms with the same substrate profile and bacterial population.

Aerobic biodegradation testing was performed using either pure oxygen or hydrogen peroxide (H_2O_2) as oxygen sources. Due to the limited quantity of soil and groundwater available, sterilized controls for both aerobic treatments could not be performed. Sterile H_2O_2 controls were prepared since this treatment was presumed more likely to produce abiotic chemical changes in the microcosms.

Microcosm size was dictated by the gas chromatograph/mass spectrometer (GC/MS) sampling size requirements. Each aerobic microcosm consisted of a 200 ml, 10% soil/groundwater slurry in a 240 ml amber narrow-mouth bottle, capped with a silicon/teflon-lined screw cap. The headspace was minimized to prevent loss of volatiles. All aerobic treatments were amended with the following mineral nutrient media (g/l except where noted):[16] NH_4Cl, 5.0; KH_2PO_4, 3.4; Na_2HPO_4, 6.6; $MgSO_4.7H_2O$, 0.2; $CaCl_2.2H_2O$, 0.01; $FeCl_3$, 0.01; H_3BO_4, 1.176 ug/l; $MnSO_4$, 1.0; $ZnSO_4$, 7.0; $CuSO_4$, 5.0; $NaMoO_4$, 1.0; adjusted to pH 7.

Peroxide-treated microcosms received stabilized H_2O_2 (Restore 105™, FMC Corporation) injected through the septa. To degrade the 50 ppm hydrocarbons present in each microcosm, an estimated 300 ppm H_2O_2 was required. To avoid the addition of toxic concentrations, only 100 ppm H_2O_2 was added to each replicate at any given time. Microcosms were dosed with 100 ppm H_2O_2 initially, and again on day 20 and on day 40. Microcosms receiving oxygen treatment were blanketed with 100% oxygen in the 40 ml headspace prior to capping. They were amended with additional 100% O_2, injected through the septa, on days 20 and 40.

Anaerobic microcosm construction was similar to the aerobic systems with the following exceptions. Microcosms were filled to the top with the soil/groundwater slurry, and cappd with solid silicon/teflon-lined caps. They were amended with a standard anaerobic mineral nutrient media as follows (mg/l)[17]: K_2HPO_4, 350; KH_2PO_4, 270; NH_4Cl, 530; $CaCl.2H_2O$, 75; $MgCl.6H_2O$, 100; $FeCl_2.4H_2O$, 20; $MnCl_2.4H_2O$, 0.5; H_3BO_3, 0.05; $ZnCl_2$, 0.05; $CuCl_2$, 0.03; $NaMo_4.2H_2O$, 0.01; $CoCl_2.6H_2O$, 0.5; $NiCl_2.6H_2O$, 0.05; Na_2SeO_3, 0.05; $NaHCO_3$, 1200. To enhance

co-metabolism and anaerobic degradation, 500 mg/l acetate was provided as a primary substrate and 500 mg/l sodium sulfide was added as a reducing agent.

Aerobic microcosms were incubated at room temperature (19–22 °C) and were agitated by hand once a day up to day 50 to enhance aeration. The day 100 microcosms were subjected to continuous, shaker-table agitation from day 50 until sacrifice. All anaerobic systems were also incubated at room temperature but were left undisturbed until sacrifice, to minimize the opportunity for ambient air to leak through the microcosm cap, compromising anaerobicity, and in the event of cap leakage, to maintain the most reduced conditions possible at the bottom of these bottles.

Triplicate aerobic systems were then sacrificed after 1, 24, 49 and 100 days, while triplicate anaerobic microcosms were sacrificed after 1, 25, 50 and 101 days incubation. System reagent blanks were obtained by sacrificing and analyzing empty containers at day 1, 25, 50 and 100. At the time of sacrifice, the samples were subjected to volatile organic hydrocarbon analyses, extraction, and subsequent analysis by flame ionization detector-gas chromatography (FID-GC), electron capture detector-gas chromatography (ECD-GC), or gas chromatography/mass spectroscopy (GC/MS), as depicted in Figure 1 and described below.

At the time of sacrifice, each microcosm was agitated to ensure homogeneity and an aliquot was removed for volatile organics analysis (VOA) by GC/MS. A 1 ml aliquot of the slurry was used to determine microbial viability by simple spread plating. The remainder of each mirocosm was then centrifuged in its bottle, and the aqueous phase was decanted and extracted at pH 12 and 2 for separate analyses of extractable hydrocarbons by FID-GC and GC/MS. In addition, unextracted aqueous samples were examined for oxidation-reduction potential, temperature, dissolved oxygen, pH and viability (Table 2).

After removing a portion for percent moisture determination, the remaining soil was processed for analysis of extractable priority pollutants and aliphatic and aromatic hydrocarbons by FID-GC and GC/MS, and analysis of intermediate and higher molecular weight chlorinated hydrocarbons, such as PCBs and pesticides, by ECD-GC. These compounds were extracted from the soil matrix by sequential pulsed sonication in methylene chloride (DCM), and then the combined extracts were concentrated to 1000 ul.

Five hundred microliters of this concentrated extract were solvent exchanged to hexane and separated into three fractions by silica gel column chromatography. The hexane fraction (F1) was concentrated to 500 ul, aliquots were run through FID-GC, and data were collected on cassette tape and reduced by computer[18,19] to determine the identities and concentrations of soil aliphatic compounds. The 1:1 hexane:benzene fraction (F2) was concentrated to 250 ul, and processed similarly to F1, to determine soil aromatic compounds. The 1:1 DCM:methanol fraction (F3) was concentrated to 1000 ul, with aliquots run on FID-GC, and data-reduced manually to determine soil polar compounds. The other half of the DCM extract was analyzed on FID-GC, and GC/MS as needed, to assist in the identification of soil base/neutral/acid components.

Qualitative observation of time-series chromatograms was performed to determine if time-series degradation of n-alkanes was occurring. Raw and statistical

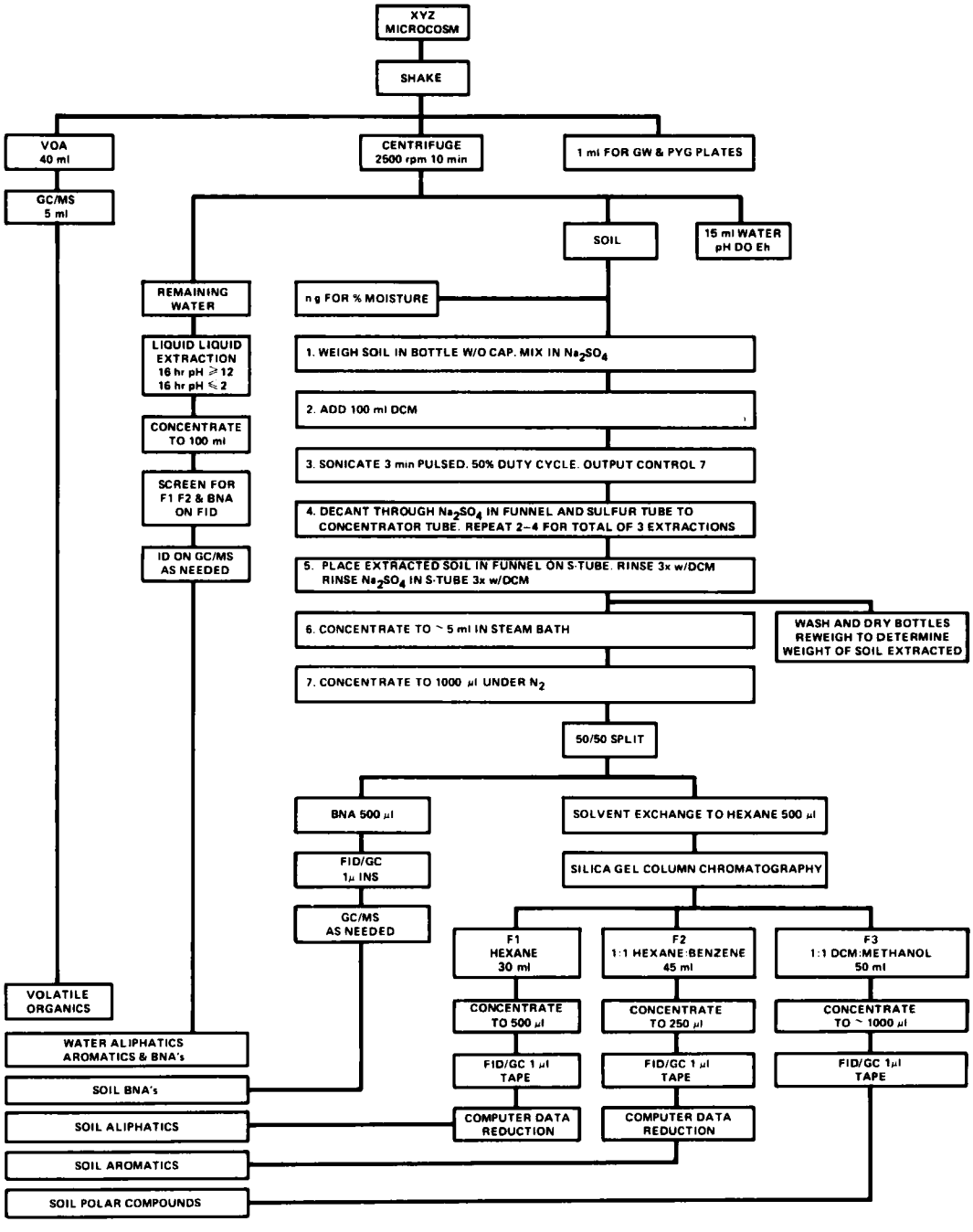

Figure 1 Bioreclamation flow diagram of analyses.

data for these analyses were tabulated and plotted for verification and interpretation.

RESULTS AND DISCUSSION

The results will be divided according to class of compound considered (aromatic/aliphatic hydrocarbon vs. chlorinated hydrocarbon) and microcosm conditions (aerobic vs. anaerobic).

Aromatic and Aliphatic Petroleum Hydrocarbon Degradation

The day 1 H_2O_2-amended microcosms (Figure 2A) were characterized by the presence of lower and intermediate molecular weight n-alkanes and an unresolved complex mixture (UCM) between nC-18 and nC-25. From day 24 through 100, degradation of the straight chain n-alkanes is readily apparent by individual peak height reductions and ultimately the predominance of the isoprenoid components, pristane and phytane, can be observed in the chromatographic profile near carbon numbers 17 and 18, respectively. With continued microbial degradation, the UCM becomes a much more significant portion of the chromatograms compared to the resolved components, as observed in Figure 2A to 2D. In the oxygen treated microcosms (Figures 2I to 2L), as in the peroxide treated system, almost all resolvable n-alkanes were removed from the soil extracts over the 100 day period. Hydrocarbon degradation has been detected in systems incorporating as little as 2–3 ppm oxygen,[20] below the levels measured in all of our aerobic microcosms, as shown in Table 2. Raymond *et al.*[2] observed aerobic microbial degradation of all classes of compounds in oil-contaminated soil, with more polar types degrading more slowly, suggesting an explanation for the continued presence of isoprenoid compounds after 100 days of incubation. Klug and Markovetz[21] noted that terminal oxidation of n-alkanes involves the participation of molecular oxygen with the formation of a primary alcohol as the first stable intermediate.

The chromatograms from the aerobic sterilized control samples shown in Figure 2E to 2H indicate very little n-alkane degradation during the first 50 days of the experiment. At that time, limited degradation was observed to occur (Figure 2H): possibly due to the continuous agitation introduced by the shaker table, or abiotic effects of the H_2O_2 present. Lack of hydrocarbon degradation in the sterilized controls in the first 50 days is further evidenced in Figure 3, where the time-series change in the ratio of the unresolved complex mixture to total resolved hydrocarbons is presented graphically. The increases in the peroxide- and oxygen-treated microcosm are significantly greater than those of the anaerobic system, or either sterile control type. The ratio of unresolved to resolved components for the anaerobic system and its sterile control remain between 5 and 20 for the 100 day period, while the non-sterile aerobic microcosms increased to values over 80, indicating that newly formed polar components which contribute to the UCM make up a greater proportion of the chromatogram relative to any resolvable n-alkanes present.

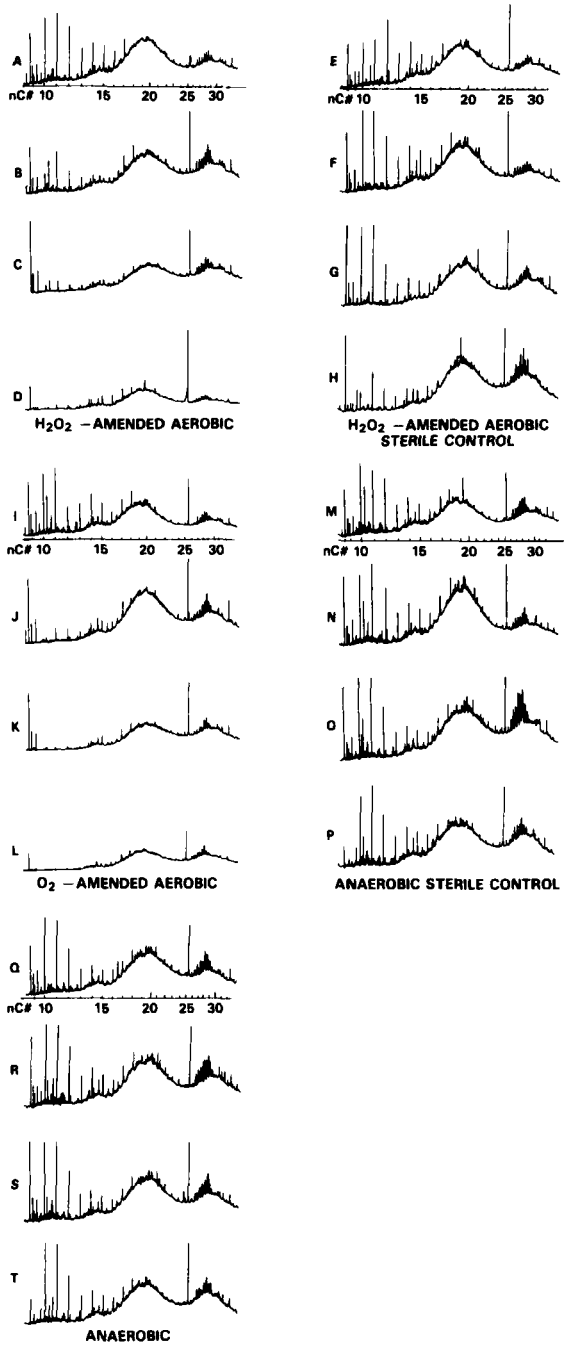

Figure 2 FID-GC chromatographic profiles of microcosm soil extracts. Day 1 = A,E,I,M,Q; Day 24 = B,F,J; Day 25 = N,R; Day 49 = C,G,K; Day 50 = O,S; Day 100 = D,H,L; Day 101 = P,T.

Table 2 Microcosm conditions at time of sacrifice

Microcosm type[a]	*Day sacrificed*	*REDOX mV*	*Temp °C*	*DO, ppm*	*pH*	*H_2O_2, ppm*	*Viability*
H	1		21.6	3.2	7.45		+
H	24	165	21.2	4.7	7.37		+
H	49		19.3	5.6	7.26		+
H	100	158	21.0	3.6	7.33	15.8	+
HS	1		22.5	3.2	7.58		−
HS	24	163	22.0	6.6	7.37		−
HS	49		19.3	9.0	7.27		−
HS	100	162	22.0	6.7	7.40	10.7	−
O	1		22.2	3.6	7.33		+
O	24	164	22.3	10.4	7.37		+
O	49		19.0	>15	7.23		+
O	100	164	22.0	13.2	7.36	5.3	+
A	1		23.3	2.7	8.04		+
A	25	−151	25.0	1.5	7.31		+
A	50		20.0	3.7	6.79		+
A	101	−148	21.7	3.3	7.55		+
AS	1		23.0	3.0	7.92		−
AS	25	−48	25.0	1.4	7.50		−
AS	50		20.0	5.6	7.04		−
AS	101	−55	21.0	5.3	7.98		−

[a]Microcosm types: H = H_2O_2-amended aerobic; HS = H_2O_2-amended aerobic sterile control; O = O_2-amended aerobic; A = Anaerobic; AS = anaerobic sterile control.

Figures 2M through 2T present selected chromatographic profiles from the non-sterile and sterile anaerobic microcosms. In both cases, very similar n-alkane profiles are observed with little or no evidence of any degradation of petroleum components, as was anticipated from the literature.

The ratios of odd to even n-alkanes, presented in Figure 4, indicate a sharp increase in the odd carbon predominance in both non-sterile aerobic systems over the first 25 days. At this point, the oxygen treated microcosms maintained this increasing trend, suggesting continued microbial growth. A hypothesis for this condition is that the increase in odd hydrocarbon number compounds with time in the presence of active populations of microbes represents the generation of predominantly odd-numbered or branched (isoprenoid) compounds by the biological populations present. Odd-carbon number hydrocarbon generation has been noted in several marine species of bacteria and phytoplankton.[19,22,23] The odd to even ratio for the other systems remained essentially constant; the observed variation over time was negligible relative to that observed for the oxygenated treatment, suggesting that odd-numbered compound generation was not occurring in these systems.

This theory is further supported in Figure 5, where the ratio of the isoprenoid pristane to nC-17 is plotted against time. Both the H_2O_2 and oxygen treated systems showed an increase in pristane during the first 25 days of incubation, while all sterile controls remained relatively constant. One or more microbial

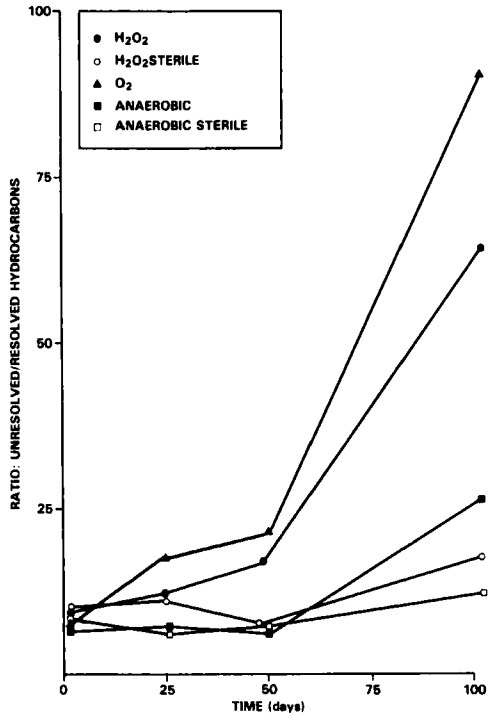

Figure 3 Ratio of unresolved/resolved hydrocarbons versus time.

groups of the consortia present either alter their metabolism or die off such that other microbes can then begin degradation of branched compounds, a less favored process, as inferred by Raymond.[2] The rapid decrease of pristane vs. nC-17 in the oxygenated systems after 25 days supports this theory in that, as noted in Figure 2J, n-alkane levels had already dropped significantly and the organisms were degrading a new carbon source, e.g., pristane. Since pristane: nC-17 levels never dropped in the H_2O_2 systems, it appears that the microbes in these microcosms were either degrading hydrocarbons at a slower rate (Figure 2A to 2D), continuing to generate pristane, or they never began to degrade branched alkanes in the observed time period. Alternatively, the microbes in the O_2-treated microcosms may have simply continued to generate predominantly odd-carbon numbered n-alkanes (e.g., nC-17) in the process of cell growth.

Chlorinated Hydrocarbon Compound Degradation

As discussed earlier, aliquots of the sample extracts sacrificed at day 1, day 24, and day 50 were also subjected to electron capture detector gas chromatographic (ECD-GC) analyses. Unfortunately, unlike the case for the petroleum type components, several materials in the solvent systems used for sample extractions interferred with the more sensitive ECD. Several of these lower molecular weight component peaks ($RT \leqq 12$) are shown in ECD-GC chromatographic profiles in Figure 6. The presence of these components in the pesticide-quality solvents used

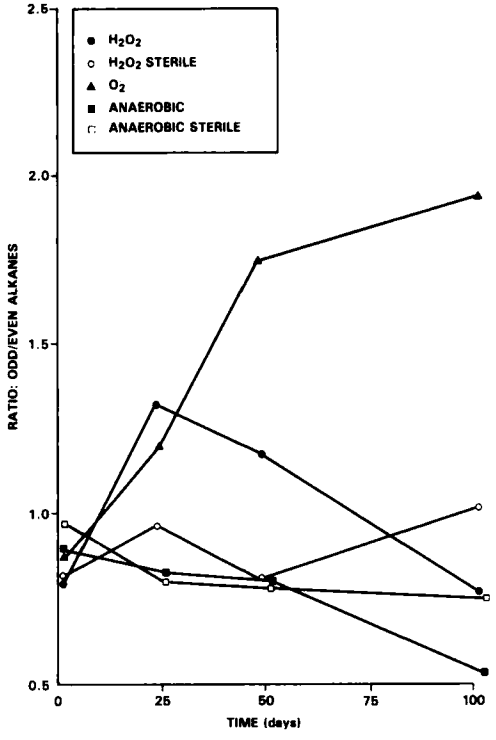

Figure 4 Ratio of odd/even alkanes versus time.

for sample extraction, plus the high levels of petroleum hydrocarbons present made analysis of ECD-sensitive species extremely difficult.

Specifically, the high levels of aliphatic hydrocarbons, which could be detected at levels three orders of magnitude higher by flame ionization detector, tended to introduce negative peaks with the ECD causing integration problems, and a large number of background components could not be resolved. Secondly, the resolved peaks which were present did not correlate with the retention times of pesticide or PCB standards, confounding peak identifications. Finally, the addition of sodium sulfide to ensure reducing conditions in the anaerobic microcosms caused significant interference problems with the ECD; another reducing agent would be recommended for further studies.

With these and other background interference problems, identification of specific components and determinations of rates of degradation for higher molecular weight chlorinated organics by ECD-GC proved to be fruitless. Further, when GC/MS analyses were attempted on these extracts at these concentrations, higher molecular weight chlorinated species were overwhelmed by petroleum hydrocarbon components that were present at levels several orders of magnitude higher. For these reasons, additional ECD-GC analyses of the soil extracts were not pursued. Instead, microbial degradation of chlorinated organics occurring in the microcosms was monitored by analysis of purgable lower molecular weight halogenated solvents by purge-and-trap GC/MS. Table 3 and Figures 7–9 present

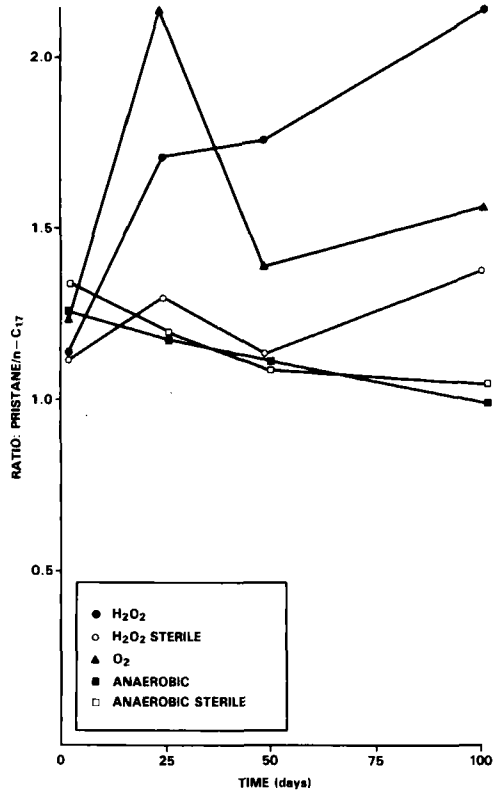

Figure 5 Ratio of pristane/nC-17 versus time.

means derived from triplicate determinations obtained over the 100 days of the study.

The time-series concentrations for PCE and TCE in Figure 7 show rapid degradation under anaerobic conditions. Within 50 days, all PCE and TCE were removed from these systems and remained essentially absent from those microcosms through day 100. Kleopfer *et al.*[24] suggested that experiments that monitor the loss of a compound and attribute it solely to degradation are potentially suspect if care is not taken to account for volatilization and adsorption losses. A better means of detecting biodegradation is to monitor the formation of product compounds relative to sterile (abiotic) controls. As degradation of PCE and TCE occurred over the first 50 days, a concomitant increase in trans-1,2-DCE concentration was observed, which levelled off at the same time as PCE and TCE levels dropped below detection limits. After Day 50, trans-1,2-DCE levels under anaerobic conditions began dropping with corresponding increases in 1,1-DCE and 1,1-DCA (Table 3) levels. Parsons *et al.* have demonstrated reductive dechlorination of PCE and TCE, dichlorethylene (DCE, both cis- and trans-isomers), and vinyl chloride (VC) in Florida muck/surface water microcosms,[8] while Bouwer and

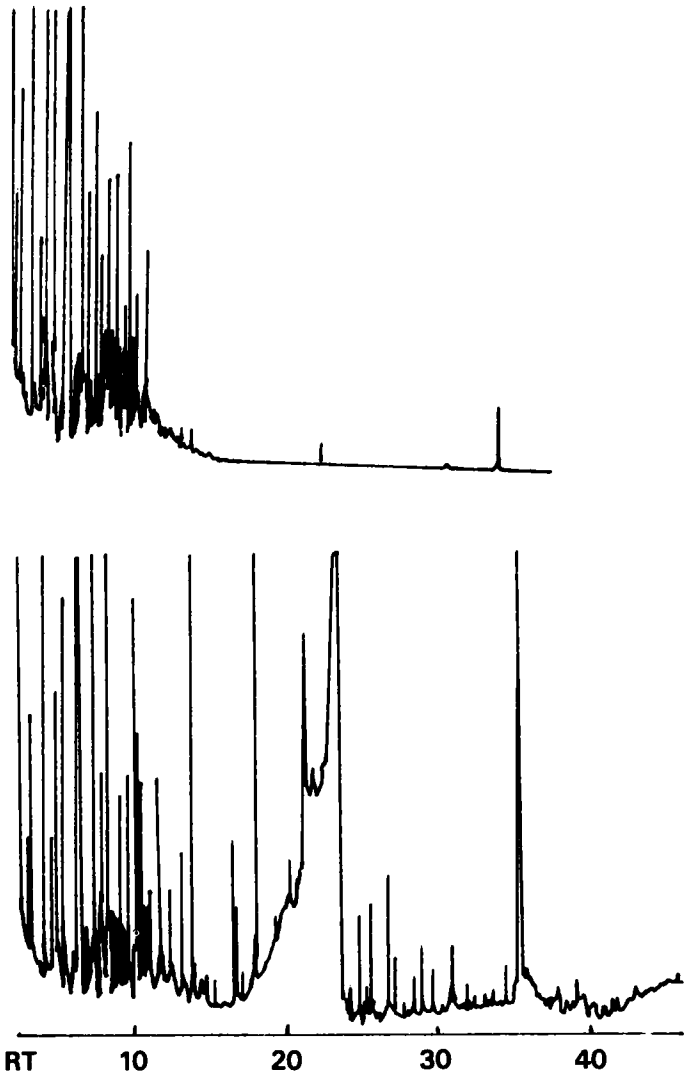

Figure 6 Capillary ECD-GC chromatographic profiles of soil extracts from Day 50 microcosms. A: method blanks; B: anaerobic. RT = retention time, min.

McCarty have observed transformation of PCE to TCE in batch bacterial cultures and in a continuous-flow column study.[5]

Vogel and McCarty observed transformation of PCE and TCE to DCE to VC and proposed that mineralization of PCE to carbon dioxide may involve further degradation of VC or the utilization of a different metabolic pathway than the formation of VC from PCE.[9] Barrio–Lage *et al.* compared the ability of various subsurface materials to biologically transform TCE to cis-1,2-dichloroethylene, and noted a variety of depletion times depending on the microbial consortia present in each material.[5] Bouwer *et al.* detected neither aerobic nor anaerobic degradation

Table 3 Statistical analysis of VOA sample results

Microcosm type	*H*	*H*	*H*	*H*	*O*	*O*	*O*	*O*	*A*	*A*	*A*
Sample Day	*1*	*24*	*49*	*100*	*1*	*24*	*49*	*100*	*1*	*25*	*50*
DCM, ppb	53±36	7±58[a]	[a]	14±26	38±28	3±154	4±18	16±18	34±9	[a]	[a]
Acetone	303±30	[a]	[a]	[a]	376±10	[a]	[a]	[a]	447±1	[a]	[a]
1,1-DCE	12±21	3±51	[a]	14±29	9±28	[a]	[a]	17±12	9±28	[a]	[a]
1,1-DCA	25±6	24±3	[a]	[a]	24±16	20±53	[a]	[a]	28±13	[a]	[a]
trans-1,2-DCE	3128±5	1801±2	6576±7	3304±57	3007±8	3100±61	3422±27	4425±7	3266±6	4955±14	4830±6
1,1,1-DCA	26±18	35±97	30±55	21±24	22±18	15±47	19±46	39±33	26±11	6±25	8±19
TCE	421±9	466±4	1309±24	677±16	358±9	563±69	764±13	812±8	367±3	218±64	[a]
PCE	716±12	286±4	1577±7	514±13	630±12	533±79	1006±24	631±8	665±0.4	363±34	[a]
Benzene	3±0	1±0	2±35	3±14	2±29	1±0	[a]	2±0	2±29	1±0	1±0
VC	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]
Chlorobenzene	8±0	9±36	[a]	4±114	7±30	[a]	[a]	[a]	11±14	21±24	18±22

Data reported as mean (ppb) ± coefficient of variation (CV %)

[a]None detected

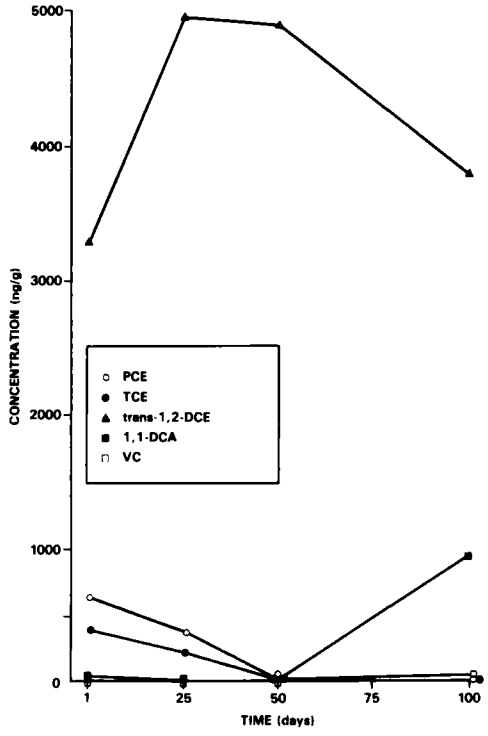

Figure 7 Time-series chlorinated hydrocarbon concentrations (ng/g) in anaerobic microcosms over 100 Day incubation period.

of PCE or TCE by a methanogenic mixed culture grown in a laboratory-scale digester fed waste-activated sludge.[7]

These studies indicate that the by-products of biodegradation are directly related to the microbial consortium present. Certain combinations produce VC while others appear to yield CO_2, each passing through more than one possible metabolic pathway to attain their respective product. Our assemblage seems, at least in part, to include anaerobic VC producers, but through trans-1,2-DCE, rather than the cis- isomer. The presence of CO_2-generating species could not be verified, since we did not analyze for this product.

Coefficients of variation (CV) for the replicate determinations of 1,1-DCA were in the 2 to 14% range. Triplicate analyses for 1,1-DCA in day 100 anaerobic systems yielded a mean concentration of 930 ng/g dry weight with a CV of only 3%. Levels for this compound at both day 25 and day 50 were below instrumental detection limits. Sterile anaerobic controls showed virtually no variation in the levels of all five of these chlorinated compounds over the entire 100 day experimental period.

It seems unlikely that 1,1-DCE or 1,1-DCA are products of trans-1,2-DCE biodegradation, since reductive dechlorination would proceed in removing individual chloride ions from the former, yielding VC directly. There is no reason to

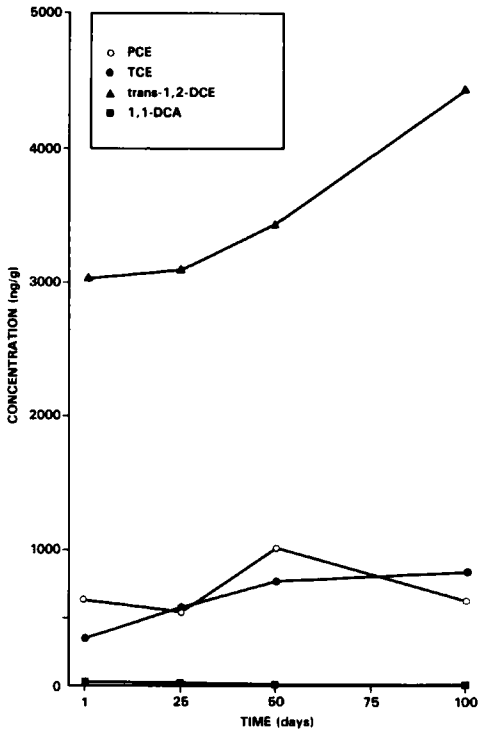

Figure 8 Time-series chlorinated hydrocarbon concentrations (ng/g) in oxygenated aerobic microcosms over 100 Day incubation period.

assume that breaking the double bond at this point is favored over dehalogenation if it was not favored during the transformation of PCE to TCE or TCE to trans-1,2-DCE. It is more probable that 1,1-DCE and 1,1-DCA are degradation products of more complex species not detected by GC/MS in the earlier stages of the study.

Degradation to VC was observed in two of the day 100 anaerobic systems. This product shows that extreme care must be taken in dealing with unknown native organisms, such that compounds at least as toxic[26,27] than those originally present *in situ* are not generated. Specialized microbial consortia are now commercially available that will only partially degrade these kinds of compounds without forming toxics such as VC,[28] perhaps utilizing the CO_2-generating species mentioned above.

The position of chlorine substitution also appears to determine which compounds are susceptible to anaerobic degradation. In particular, trans-1,2-DCE remained in the microcosms for the 100 day period at concentrations that were two orders of magnitude higher than the concentrations of the 1,1-DCE isomer. However, analysis of anaerobic samples past day 100 may have indicated a continued microbial reduction in trans-1,2-DCE concentration with corresponding

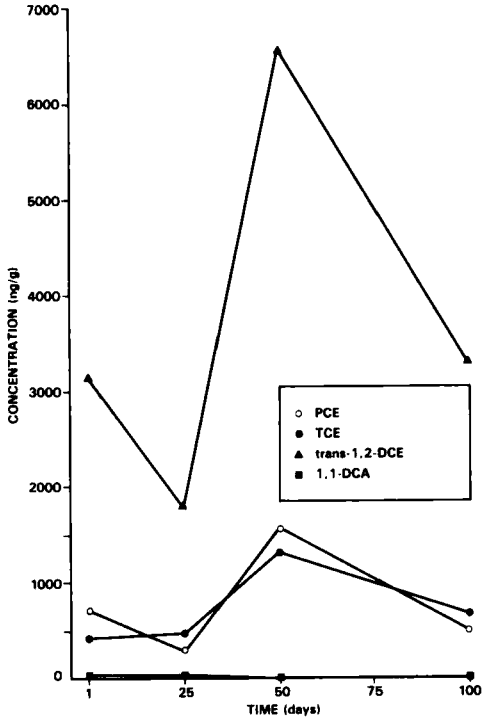

Figure 9 Time-series chlorinated hydrocarbon concentrations (ng/g) in hydrogen peroxide amended microcosms over 100 Day incubation period.

increases in other degradation products.[25] Alternatively, trans-1,2-DCE may be an end product in this system with no pathways available for further biodegradation.

The data for aerobic systems do not suggest that any degree of chlorinated hydrocarbon degradation occurred over the experimental time period. The O_2-amended system (Figure 8) shows a general *increase* in lower molecular weight chlorinated levels, while the H_2O_2-amended microcosms (Figure 9) show wide fluctuations in the levels of these compounds that are difficult if not impossible to interpret.

Data obtained for chlorobenzene were somewhat sporadic, due in part to the low concentrations present in the microcosms. However, in general, they suggest more rapid and thorough removal of this compound under oxygenated treatments as opposed to anaerobic conditions. Other studies of substituted aromatic compounds have demonstrated the requirement of molecular oxygen for biotransformation and ring cleavage.[29,30] The basic requirement for aromatic ring cleavage under anoxic conditions appears to be the presence of oxygen on the ring, which is not present on chlorinated benzenes.[6]

Aerobic incubation effected rapid degradation of 1,1-DCE in the first 25 days followed by a gradual increase to above initial levels by day 100, indicating further evidence of either the action of facultative anaerobic microbes degrading a non-resolvable precursor, or some abiotic process occurring here. On the other hand,

since even the elevated levels at day 100 are barely above detection limits (14 to 17 ng/g), these fluctuations may be insignificant.

CONCLUSIONS

In the execution of this program, *in vitro* aerobic and anaerobic microcosms (and associated sterilized controls) were successfully prepared and maintained in the laboratory for over 100 days to allow determination of the rates of degradation of numerous xenobiotic pollutants by native microbial populations. A chemical analysis scheme was developed for the quantitative determination of petroleum and chlorinated hydrocarbon components from common microcosms, while geochemical and microbiological techniques were employed to allow characterization of redox state, dissolved oxygen, pH, and viability of the microbial populations present in each microcosm. Field sampling strategies succeeded in maintaining the viability of both anaerobic and aerobic systems, providing healthy microbial consortia for the initiation of the laboratory program that demonstrated degradation of aliphatic and aromatic hydrocarbons under aerobic conditions and chlorinated hydrocarbons under anaerobic conditions.

From the chromatographic profiles, rate data, and ratios of individual hydrocarbon components, it is clearly demonstrated that microbial degradation of petroleum hydrocarbon components occurred similarly in H_2O_2- and O_2-amended microcosms. The aerobic sterile controls showed significantly less degradation than the non-sterile counterparts; however, over time some abiotic degradation of the aliphatic compounds may have been initiated by the peroxide present. The anaerobic systems showed essentially no significant aliphatic hydrocarbon degradation over the 100 day experimental period.

The change in appearance of the chromatographic profiles as the experiment proceeded showed that aerobic microbial degradation could be observed visually. Clear distinctions between aerobic, anaerobic, and sterile controls could be determined on inspection of chromatograms, thereby providing information on the amount of additional time required for various aspects of the study. For example, as a result of detailed examination of the Day 49/50 data, the decision was made to continue the experiment to Day 100.

This extension of the experimental program provided a longer term profile of relative component levels in the microcosms, and allowed for the demonstrated production of several lower molecular weight chlorinated species from anaerobic degradation of tetrachloroethylene. Rates of biodegradation may be calculated from statistical analysis of hydrocarbon component concentrations. In general, intermediate hydrocarbon metabolites could not be identified because they were probably polar compounds which yield few chromatographically resolved peaks when analyzed by capillary gas chromatography. Further research is warranted to investigate metabolic pathways to CO_2 and the roles that 1,1-DCE and 1,1-DCA play in the biodegradative scenario.

The decision to selectively remove chlorinated species versus aromatic and aliphatic petroleum hydrocarbon species must be based on site priorities and estimates of the potential toxicity of each compound. Nevertheless, a clear choice

is available in controlling the groundwater or soil conditions to stimulate degradation of one compound class over the other. While anaerobic biodegradation appears viable for the volatile halogenated organics, these compounds might also be removed if air stripping was used to facilitate aerobic degradation, and at the same time, minimize the potential of toxic endproduct production.

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