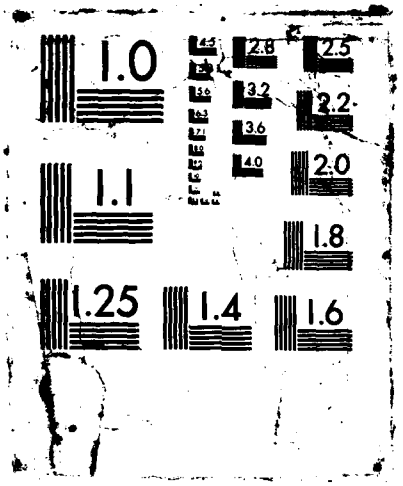
AD-A192 743 BIODEGRADATION OF JP-5 AVIATION FUEL BY SUBSURFACE MICROBIAL COMMUNITIES(U) NAVAL CIVIL ENGINEERING LAB PORT HUENEME CA C M SWINDOLL 1988 1/1

UNCLASSIFIED F/G 24/4 NL


1.0
1.1
1.25
1.4
1.6
1.8
2.0
2.2
2.5
2.8
3.2
3.6
4.0

(4)

Progress Report

for

the Office of Naval Technology Postdoctoral Fellowship Program

Administered by American Society for Engineering Education

Biodegradation of JP-5 Aviation Fuel by Subsurface Microbial Communities

for the period

January 1, 1987 to March 15, 1987

by

Dr. Charles Michael Swindoll
Naval Civil Engineering Laboratory
Code L71
Port Hueneme, CA 93043

BIODEGRADATION OF JP-5 AVIATION FUEL

BY SUBSURFACE MICROBIAL COMMUNITIES

This document summarizes the progress of my research during my fellowship, covering the period July 1, 1987 to March 15, 1988. During this period, my research was direct toward the characterization of microbes from soil samples collected at NAS Patuxent River, Maryland. The following manuscript summarizes this research, and will be presented by Dr. Susan Landon-Arnold at a conference this spring.

Accession For	
NTIS GRA&I	☑
DTIC TAB	☐
Unannounced	☐
Justification	
By per NP	
Distribution/	
Availability Codes	
Dist	Avail and/or Special
A-1	

Characterization of Indigenous Microbial Degraders of Aviation Fuel Components from a Contaminated Site.

C.M. Swindoll, S. Landon-Arnold, and D.I. Flynn. Environmental Protection, Naval Civil Engineering Laboratory, Port Hueneme, CA 93043.

ABSTRACT

Leakage of the aviation fuels JP-5, JP-4 and AVGAS from storage tanks has resulted in a severe environmental insult to a Naval fuel farm and adjacent areas. As part of the reclamation effort, the indigenous microorganisms are being characterized. This information will be used to optimize the bioreclamation of the site. Approximately 60 aerobic microorganisms, including more than 40 bacteria, 6 actinomycetes and 10 fungi have been isolated from soil contaminated with aviation fuels and adjacent non-contaminated soil. All isolated bacteria were able to grow on JP-5 as their sole carbon source. Most of the bacteria from the contaminated sites were small, gram negative rods, while most bacteria from the non-contaminated site were gram positive rods. All of these microorganisms would be expected to contribute to the bioremediation of the contaminated site.

INTRODUCTION

Approximately 95% of the U.S. freshwater supply is contained in subsurface environments (12). Society's increasing reliance on ground water for domestic, agricultural and industrial usages has made quality of ground water one of the primary environmental concerns of the 1980's. Contamination of ground water by chemicals which may be hazardous to public health has become an all-too-common problem. Ground water supplies are known to contain levels of chemicals that are suspected to be toxic, teratogenic and carcinogenic.

Historically, due to their widespread and large-scale uses, petroleum fuels have generally been viewed as environmentally acceptable. However, in recent years there has been an increased interest in a more careful examination of the environmental implications of petroleum products and uses. Petroleum fuels are complex mixtures of aliphatic and aromatic hydrocarbons. The major components of refined petroleum fuels are parafins, olefins, naphthenes, aromatics and alcohols (11). Product specifications for aviation fuels address only gross properties, such as density, thermal stability, and maximum percent aromatic: therefore, considerable variability can exist in the component composition of fuels from different sources. Specific data on the composition of JP-5, the primary jet fuel of the U.S. Navy, are not available; however, the jet fuel used by the U.S. Air Force, JP-4, contains over 300 different hydrocarbons (5).

Underground storage tanks (USTs), which have been used for decades for the storage of petroleum fuels and chemicals, are major sources of subsurface contamination. Approximately 50,000 USTs are owned by the Navy, of which a majority are suspected to be leaking (14). It has been estimated that as many as 100,000 buried gasoline tanks in the United States were leaking as of 1985 (6), and as many as 350,000 USTs will leak by the end of this decade (16).

Contamination of subsurface soils and ground water with petroleum hydrocarbons can be a complex phenomenon. The movement of the fuel in subsurface soil is initially controlled by gravitational forces, viscosity of the liquid, and permeability of the soil (8). Subsurface fuel spills can exist in four phases: (a) free product phase - refined petroleum fuels are less dense than water; therefore fuel floats on the water surface; (b) adsorbed phase - components attach to the soil due to physical and chemical interactions; (c) dissolved phase - soluble components dissolve into ground water; and (d) vapor phase - volatile components diffuse as vapors in the unsaturated zone. The actual phase distribution of fuel components at any given location will be dependent upon the physico-

chemical properties of the contaminants, as well as hydrogeological and geochemical properties of the site.

The clean-up of subsurface contamination can be extremely difficult due to the inaccessability of the sites. Conventional reclamation techniques, such as incineration, chemical oxidation, air-stripping, or impoundment are expensive, and often are not completely effective. Due to the large number of contaminated sites, there exists a need for less expensive reclamation technologies. Bioreclamation offers great promise as a cost-effective reclamation strategy. Although the actual cost of restoration will be site-specific, biological treatment costs approximately one order of magnitude less than conventional restoration technologies (21). In situ bioreclamation uses indigenous, subsurface microbial communities to metabolize organic contaminants into CO2 and H2O. The feasibility of in situ bioreclamation depends upon numerous factors including the presence of a microbial community with the requisite enzymes for contaminant metabolism, and the availability of essential nutrients and electron acceptors.

The presence of diverse and abundant subsurface microbial populations has been established (10, 19). It has been shown in laboratory studies that subsurface microbial communities are responsible for the biodegradation of a wide range of environmentally important compounds (1,17,18). Heterotrophic microorganisms can metabolize most petroleum hydrocarbons to carbon dioxide, water, and other harmless compounds (13), and in the process the microorganisms acquire energy and materials for growth. During aerobic biodegradation, the carbon source is broken down by a series of enzyme-mediated reactions in which oxygen serves as an external electron acceptor. With anaerobic biodegradation sulfates, nitrates, carbon dioxide and other compounds containing oxygen can serve as the external electron acceptor. Aerobic biodegradation of petroleum hydrocarbons is much more rapid than anaerobic biodegradation, and thus is preferred for bioremediation.

The Environmental Protection Division of the Naval Civil Engineering Laboratory has the task to develop and execute a project plan to remove fuel contamination from soil and water at a fuel farm of a Naval facility. It is believed that the pollution at this site is derived from the chronic leakage of undetermined amounts of JP-5, JP-4 and AVGAS from underground pipelines. In addition, several large concrete USTs may be contributors to the contamination problem. Preliminary studies indicate that the area of contamination covers a surface area of approximately 10 acres, to the depth of the saturated zone, which varies from 7 to 10 m. Because of the large volume of soil contaminated with fuel, *in situ* biorestoration technologies are being investigated as potential cost-effective remedies.

The research described in this report is the first stage of our investigation, in which microorganisms indigenous to the contaminated field site were isolated, and the ability of isolated microorganisms to metabolize JP-5 and select individual JP-5 components is being examined. Individual components chosen for this investigation are representatives of the major chemical groups comprising petroleum fuels: alkanes, cycloalkanes, alkenes, alkyl benzenes, and polynuclear aromatic hydrocarbons.

MATERIALS AND METHODS

Site Description

The contaminated site is composed of 9 - 13 m, well graded to unsorted fluvial fine sand to gravel, underlain by 0 - 0.3 m peat. The upper stratum lies directly on Miocene marine grey silt and clay, which confines the contamination to the upper stratum and forces the free product to the surface along the bluff of an adjacent creek. The site is partly covered by natural vegetation, consisting of rhododendron and deciduous forest.

Sample Collection and Preparation

Soil was collected using aseptic sampling techniques from areas of the fuel farm which were

contaminated with aviation fuel, and from adjacent non-contaminated areas. Soil samples were taken from three locations. At Site A, fuel seeps to the surface along the embankment of a small creek. Site A samples were taken with a small trenching shovel from a depth of approximately 0.3 m. The sample consisted of coarse sand and gravel, and contained a small amount of peat. Site B samples were taken during the installation of a monitoring well, from a depth of 5.5 to 6 m. Samples from Site B were contaminated with fuel, and consisted of medium- and coarse-grained sand. Site C samples were collected from a non-contaminated area from a depth of approximately 0.3 m. Gas chromatographic analysis confirmed that Site C was free from petroleum hydrocarbon contaminants (3). Soil samples were transported on ice to the laboratory in 4 L polyethylene containers. Samples were processed within 48-72 h of collection. Chemical characterization of sites A and C are given in Table 1.

Table 1. Chemical characterization of soil samples from sites A and C (3).

Parameter	Site A	Site C
pH	5.6	4.7
Phosphorus (ug/g)	14.0	6.0
Total Nitrogen (%)	0.2	0.84
Iron (ug/g)	419	429
Cation Exchange Capacity (meq/ 100 g)	3	10
Organic Matter (%)	4.6	2.1

Culture and Isolation

Soil (10 g) was placed in a blender with 0.1% sodium pyrophosphate (100 ml; pH 7), and mixed twice for 30 sec, as described elsewhere (4). A 10-fold

dilution series was prepared from the soil slurry in 0.1% sodium pyrophosphate. Aliquots of the dilution series were then pipetted and spread onto plates containing approximately 20 ml media, and inverted after 30 min. The composition of the five growth media used in this investigation were:

(a) LA - modified Luria Agar: agar (BBL; 20 g/L), tryptone (0.5 g/L), dextrose (0.25 g/L), NaCl (0.25 g/L), yeast extract (0.25 g/L), pH 7;

(b) ACT - Actinomycete Isolation Agar (Difco; 22 g/L), glycerol (5 g/L), pH 8;

(c) YMA - Yeast Mannitol Agar: YM Broth (Difco; 21 g/L), granulated agar (BBL; 20 g/l), pH 4;

(d) BHA - minimal salts agar, Bushnell-Haas Agar (Difco; 23 g/L), pH 6.6 ; and

(e) BHA & JP-5 - Bushnell-Haas Agar (23 g/L) with 100 ul JP-5/plate.

Each culture treatment was done in triplicate, and stored in the dark at room temperature (20-22 C). Plates for the isolation of anaerobic microorganisms were incubated in Gas-Pack jars (BBL) in the dark at room temperature. All plates were incubated for 1 week, and plates with 30 - 300 colony-forming-units (CFU) were counted.

Aerobic microorganisms which grew on the five media were isolated for characterization by culturing individual colonies on BHA, BHA & JP5, and ACT media. Characterization included Gram stain reaction, cell and colony morphology, colony color, and cell size (7).

Replica Plating

In an attempt to culture and isolate as many potential JP-5 degraders as possible from the study sites, a replica plating procedure (15) was used. Culture plates which had between 30 and 100 well-defined, non-overlapping CFU were chosen as master plates for replication. Replication was done in

duplicate onto the media LA, ACT, BHA, or BHA & JP5. Positive controls contained the same media as the master plates; negative controls contained the minimal salts medium (BHA) with no additional carbon source. Plates were incubated at room temperature for 1 week. then CFU were counted.

Component Utilization

The metabolic abilities of the isolated microbes were examined using BHA medium with JP-5 or fuel components as the only carbon source. Pure cultures of isolated microorganisms were established on LA medium. and then replicated onto BHA medium. Each treatment was done in triplicate, with both positive (with glucose as carbon source) and negative (no carbon source) controls. After inoculation. 100 ul JP-5 or fuel component was pipetted onto a 25mm glass fiber filter (Gelman), which was placed in the lid of the plate. The plates were then inverted and placed in a desiccator which contained a beaker with 25 ml of the fuel component. The plates were incubated at room temperature (20-22 C). CFU were counted after a one week incubation. Fuel components included in this part of the investigation were: naphthalene. o-xylene. benzene, toluene, n-octane, cyclohexane and cyclonexene.

RESULTS

The number of CFU resulting from inoculation of the soil samples onto the different media are given in Table 2. CFU were greatest in soil A and C, (approximately 10E6/g) and approximately one order of magnitude less in soil B.

Attempts to culture and enumerate anaerobic microorganisms were largely unsuccessful, with no more than 2 CFU growing on any of the five media used. For many of the plates there was no growth during a 10 day incubation. Due to the difficulty of maintaining anaerobic conditions for culturing anaerobic microorganisms. no further attempts were made to characterize these microorganisms.

Approximately 60 aerobic microorganisms were isolated from the five media, including 10 fungi, 6 actinomycetes, and more than 40 bacteria. Of these bacteria, 12 from the contaminated sites and 13 from the non-contaminated site were chosen for further examination. Most of the bacteria from the contaminated sites were small, gram negative, rods, while most of the bacteria from the non-contaminated site were gram positive bacilli. The description of these aerobic bacteria are given in Table 3.

Table 2. Mean numbers of colony-forming-units per gram soil, growing on different media, from three different soil samples.

	Soil Sample **		
	A	B	C
Media			
LA	4.5 x 10E6	6.3 x 10E6	3.6 x 10E6
YMA	4.0 x 10E1	5.7 x 10E1	1.3 x 10E5
ACT	1.0 x 10E5	5.7 x 10E6	1.9 x 10E6
BHA	6.2 x 10E5	7.6 x 10E6	3.4 x 10E6
BHA & JP5	6.7 x 10E5	6.6 x 10E6	2.5 x 10E6

** Soil samples: (A) contaminated soil; (B) contaminated soil, core; (C) non-contaminated soil.

The ability of pure cultures of isolated microorganisms to grow with JP-5 or individual JP-5 components as the sole carbon source is shown in Table 4. All microorganisms isolated from Sites A (contaminated) and C (non-contaminated) grew on plates amended with JP-5. On plates amended with n-octane, 67% of Site A and 100% of Site C microbes were able to grow. Bacteria from Site A were unable to grow on benzene, toluene or cyclohexene. Only one Site A organism grew on naphthalene or o-xylene. Eleven of

the 22 isolated microorganisms grew on the negative control plates.

Table 3. Cell and colony descriptions of bacteria isolated from fuel-contaminated (sites A and B) and non-contaminated (Site C) soils.

Bacteria	Gram Stain	Cell Size (um)	Colony Morph C-M-E-T*	Cell Morph	Colony Color **
A-01	-	1.6 x .82	6-3-2-3	bacillus	T
A-02	+	1.1 x .55	4-1-3-1	bacillus	T
A-03	-	2.2 x .82	1-1-3-3	bacillus	T
A-04	-	2.2 x .55	4-1-3-1	bacillus	T
A-05	-	2.2 x .55	4-1-3-1	bacillus	T
A-06	-	1.4 x .55	5-1-1-1	bacillus	Y,B
A-07	-	1.4 x .55	1-1-3-1	bacillus	Y,W
B-01	-	2.2 x .74	1-3-2-2	bacillus	T
C-01	+/-	1.4 x .55	1-1-3-1	bacillus	Y
C-02	-	1.7 x .36	1-1-3-1	bacillus	Y
C-03	+	3.3 x 1.1	3-2-5-3	bacillus	T
C-04	+	2.8 x .74	4-4-4-2	bacillus	W
C-05	+/-	3.9 x .83	3-2-5-2	bacillus	W
C-06	+	2.8 x 1.1	3-1-2-1	bacillus	W
C-07	+	1.1 x .55	1-2-2-1	pleomorph	T
C-08	+/-	variable	1-1-3-3	unknown	T
C-09	+	1.1 x .55	1-1-3-3	pleomorph	T
C-10	+	2.8 x 1.1	5-3-2-2	bacillus	G
C-11	+	1.7 x .83	1-3-2-1	bacillus	G
C-12	+	1.4 x .28	3-1-3-1	bacillus	T
C-13	+	3.3 x .55	1-3-2-2	bacillus	T
C-14	+	.55 - 1.1	1-3-2-2	coccus	T
C-15	+/-	variable	1-2-2-2	unknown	C

* Colony Morphology Codes:
C = configurations, 1 = round, 2 = rhizoid, 3 = round, raised margin, 4 = L-form, 5 = concentric, 6 = irregular, spreading;
M = margins, 1 = smooth, 2 = wavy, 3 = lobate, 4 = threadlike;

E = elevations, 1 = crateriform, 2 = raised,
3 = convex, 4 = hilly, 5 = umbonate;
T = texture, 1 = moist, 2 = dry, 3 = mucoid
** Color Codes: B = brown, C = cream, G = grey,
T = tan, W = white, Y = yellow,

Table 4. Ability of bacteria isolates to utilize JP-5 or fuel components (+ utilizer; - non-utilizer).

	Carbon Source *									
	A	B	C	D	E	F	G	H	I	J
Bacteria**										
A-01	-	-	-	-	-	-	-	+	+	+
A-02	+	-	-	-	-	-	-	+	-	+
A-03	+	-	-	-	-	-	-	+	-	+
A-04	-	+	-	-	-	-	-	+	-	+
A-05	+	+	-	-	-	-	-	+	-	+
A-06	+	+	-	-	-	-	-	+	-	+
A-07	+	+	-	-	-	-	+	+	-	+
C-01	+	+	+	-	-	+	+	+	+	+
C-02	+	+	-	-	-	+	+	+	-	+
C-03	+	+	+	+	+	+	+	+	+	+
C-04	+	-	-	-	-	-	-	+	-	+
C-05	+	+	+	-	+	+	+	+	+	+
C-06	+	-	-	-	+	+	+	+	-	+
C-07	+	+	+	+	+	+	+	+	+	+
C-08	+	+	+	-	-	+	+	+	+	+
C-09	+	+	+	-	+	+	+	+	+	+
C-10	+	+	+	-	+	+	+	+	+	+
C-11	+	-	-	-	+	+	+	+	-	+
C-12	+	+	+	+	+	+	+	+	+	+
C-13	+	+	+	+	+	+	+	+	+	+
C-14	+	-	-	-	-	-	-	+	-	+
C-15	-	+	-	-	-	+	+	+	+	+

* Carbon source: (A) n-octane; (B) cyclonexane: (C) cyclohexene; (D) benzene; (E) toluene; (F) o-xylene: (G) naphthalene; (H) JP-5 (I) negative control: (J)positive control

** Bacterial isolates: "A" from site A; "C" from site C.

DISCUSSION

The numbers of microorganisms from the 3 soils, as determined by CFU, were approximately 10E5 - 10E6 /g soil. These numbers are similar to those reported for other subsurface sites (20). The enumeration of aerobic microorganisms in this investigation relied on standard microbial culturing techniques (9). It is known that only a fraction, perhaps 1-10%, of the microbes in soil can be cultured and detected on growth media (2). In addition, the isolation of anaerobic microorganisms was largely unsuccessful due to our procedure which did not maintain anaerobic conditions. Therefore, the numbers of microorganisms given in Table 2 are probably underestimates of the abundance of indigenous microbes.

It is apparent from the data in Table 2 that the numbers of CFU are approximately the same for the contaminated and non-contaminated soils. YMA proved to be ineffective as a growth medium (< 10E2 CFU/g) for the core and the seep samples. This is not unexpected because it is well known that many microbes are unable to grow on media in the laboratory (2). YMA selects for fungi which grow better under acidic conditions. Since fungi from the non-contaminated soil were able to grow on YMA while those from fuel-contaminated soil were not, it is probable that the release of fuel into the soil has resulted in a community shift which significantly reduced the numbers of microorganisms capable of utilizing YMA. As shown in Table 1, the pH at Site C was lower than Site A. It is probable that the petroleum has altered the pH, thus selected for non-acidiphilic microorganims.

Organisms isolated from Site A were chosen for further characterization based on their ability to use JP-5 as a sole carbon source. The predominance of gram negative bacilli from the contaminated site is not unexpected since several of these gram negative rods

have been identified as Pseudomonas spp. and pseudomonads are capable of utilizing a wide variety of carbon sources (7). Organisms C-04, C-06 and C-11 were the only gram positive bacilli able to utilize JP-5 as a sole carbon source while exhibiting no growth on the negative control. These organisms are spore formers, and two have been identified as Bacillus spp. Studies are continuing to further characterize the metabolic abilities of the isolated microorganisms.

The replica plating procedure was used in order to isolate microorganisms which could grow with JP-5 as their sole carbon source. Growth of the microbes under these conditions would indicate that the microorganisms possess the requisite enzymes for utilization of the volatile carbon source. Growth on the positive control plates, which contained nutrients for optimum growth, was unexpectedly low. In many cases growth on the positive controls was less than 50%. Negative control plates, which did not contain any additional carbon source, should not have allowed growth. However, negative control plates consistently exibited growth, indicating that the bacteria obtained carbon from the agar. Growth of different CFU was lowest on plates amended with JP-5, even though the component utilization data (Table 4) showed that all isolates could grow on JP-5. These results indicate that replica plating may not be a suitable procedure for microbial isolation, and illustrates the problems associated with the isolation of indigenous microorganims.

Nine of 13 bacteria isolated from the non-contaminated soil (Site C) were able to grow on the negative control plates. Due to growth on the negative control plates it is not possible with these data to determine whether these microbes are able to utilze JP-5 components as a carbon source. Other experiments are in progress to provide this information. All bacteria isolated from contaminated soil were able to utilize JP-5; 5 used n-octane; 4 used cyclohexane; and only 1 used naphthalene. None of these bacteria could use benzene, toluene, o-xylene or cyclonexene. These data suggest that the contamination of the soil has resulted in the growth of a microbial community which

readily utilizes aliphatic hydrocarbons as a carbon source, yet are unable to metabolize aromatic compounds. The growth of this community in the soil would be expected to reduce the concentration of straight chain compounds, resulting in the accumulation of the more toxic aromatic fraction. It is known that the behavior of a chemical in combination with other chemicals may be significantly different from that of a single compound (18). When dealing with environments contaminated with petroleum fuels, such as aviation fuels that may contain over 300 compounds (5), making predictions concerning the fate of a single compound exceedingly difficult.

In summary, microorganisms capable of growing on fuel hydrocarbons were found in all three soil examined. Thus, there is a potential for promoting the in situ biodegradation of these contaminants by stimulating the growth of the indigenous microflora. However, the data show that the isolated microorganisms were not able to utilize all the petroluem hydrocarbons tested. At the contaminated site, there could be selective biodegradation of more easily degraded components or preferred substates, resulting in an accumulation of the less biodegradable components in the soil. The fate of the petroleum hydrocarbons could also be affected by other processes not examined in this investigation, such as cometabolism or biodegradation requiring consortia of microorganisms. Research is continuing in order to address these processes.

ACKNOWLEDGMENTS

Thanks to Ron Hoeppel for reviewing the manuscript.

REFERENCES

1. Aelion, C.M., Swindoll, C.M., Pfaender, F.K., 1987. "Adaptation to and Biodegradation of Xenobiotic Compounds by Microbial Communities from a Pristine Aquifer", Appl. Environ. Microbiol. 53:2212-2217.
2. Alexander, M., 1977. Introduction to Soil

Microbiology, 2nd ed. Wiley, New York.
3. Arthur, M., 1987, Written Communication, Battelle Columbus OH
4. Balkwill, D.L. and Ghiorse, W.C, 1985, "Characterization of Subsurface Bacteria Associated with Two Shallow Aquifers in Oklahoma", Appl. Environ. Microbiol., 50:580-588.
5. Bishop, E.C., MacNaughton, M., DeTreville, R.P. and Drawbaugh, R.B., 1983, "Rationale for a threshold limit value for JP-4/Jet B wide cut aviation turbine fuel", USAF OEHL Report 83-128EH111DGA.
6. Brown, R.A., Norris, R.D. and Brubaker, G.R., 1985,"Aquifer Restoration with Enhanced Bioreclamation", Brochure - FMC Aquifer Remediation Systems, FMC Corporation, Inc. Chicago, Il.
7. Buchanan, R.E. and Gibbons, N.E., Eds., 1974, Bergey's Manual of Determinative Bacteriology, 8th ed., Baltimore, Williams and Wilkins.
8. Davis, J.B. et al., 1972, "The Migration of Petroleum Products in Soil and Groundwater: Principles and Countermeasures", American Petroleum Institute Pub. # 4149, Washington, D.C.
9. Gerhardt, P., Murray, R.G.E., Costilow, R.N., Nester, E.W., Wood, W.A., Krieg, N.R., and Phillips, G.B., Eds., 1981, Manual of Methods for General Bacteriology, American Society for Microbiolgy, Washington, D.C.
10. Ghiorse, W.C. and Balkwill, D.L., 1983, "Enumeration and Morphological Characterization of Bacteria Indigenous to Subsurface Environments", Dev. Ind. Microbiol. 24:213-224.
11. Hancock, E.G., 1985, "The Manufacture of Gasoline and the Chemistry of its Components", Technology of Gasoline, edited by E.G. Hancock, Blackwell Scientific Publications, London, pp. 20-56.
12. Josephson, J., 1980, "Safeguards for ground water", Environ. Sci. Technol. 14:39-44.
13. Marshall, T.R. and DeVinney, J.S., 1986, "Methods for Enumeration of Microorganisms in Petroleum Land Treatment Soils", Hazardous Waste & Hazardous Materials 3:175-181.
14. Niaki, S., Pollock, C.R., Medlin, W.E., Shealy S., Broscious, J.A., 1987, "Remedial Measures Initiation Decision Report (IDR) Literature

Review", IT Corp., Monroeville, PA
15. Shiaris, M.P. and Cooney, J.J., 1983, "Replica Plating Method for Estimating Phenanthrene-Utilizing and Phenanthrene-Cometabolizing Micro-organisms", Appl. Environ. Microbiol. 45:706-710.
16. Sun, M., 1986, "Ground Water Ills: Many Diagnoses. Few Remedies", Science 232:1490-1493.
17. Swindoll, C.M., Aelion, C.M., Dobbins, D.C., Jiang, O., Long, S.C., Pfaender, F.K., 1988, "Aerobic Biodegradation of Natural and Xenobiotic Organic Compounds by Subsurface Microbial Communities", Environ. Toxicol. Chem. (In Press).
18. Swindoll, C.M., Aelion, C.M., and Pfaender, F.K., 1988, "Influence of Inorganic and Organic Nutrients on Aerobic Biodegradation and on the Adaptation Response of Subsurface Microbial Communities",Appl.Environ. Microbiol.54:212-217.
19. White, D.C., Smith, G.A., Gehron, M.J., Parker, J.H., Findlay, R.H., Matz, R.F., and Fredrickson, H.L., 1983, "The Groundwater Aquifer Microbiota: Biomass, Community Structure and Nutritional Status", Dev. Ind. Microbiol. 24:201-211.
20. Wilson,J.T., McNabb, J.F., Balkwill, D.L. and Ghiorse, W.C., 1983, "Enumeration and Characterization of Bacteria Indigenous to a Shallow Water-Table Aquifer" Ground Water 21:134-142.
21. Zitrides, T., 1983, "Biodecontamination of Spill Sites", Poll. Engn. 15:26-27.

END

DATE

FILMED

6-1988

DTIC