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An Investigation Conducted by:
Life Systems, Inc.
Cleveland, Ohio

BIOTECHNOLOGY WORKGROUP FOR DEPARTMENT OF DEFENSE SOIL AND GROUNDWATER DECONTAMINATION APPLICATIONS

ABSTRACT This report contains materials used in and generated by the Department of Defense Biotechnology Workgroup on Soil and Groundwater Decontamination Applications. Various bioremediation techniques for treating soil and water contaminated with sludges, solvents, toxins, acids, bases, and heavy metals were discussed as well as the overall place of biotechnology in Installation Restoration programs. Among the specific applications discussed were: biochemical sensors to determine environmental stress in organisms; in-situ detoxification and biodecontamination of pollutants in soils and waste streams; sequestration, removal, and recovery of metals in waste streams with metal-binding proteins; and the use of vegetation to limit the transport to sequester, and/or to remove contaminants from soil or water.

NAVAL CIVIL ENGINEERING LABORATORY PORT HUENEME CALIFORNIA 93043

METRIC CONVERSION FACTORS

Approximate Conversions to Metric Measures

Symbol	When You Know	Multiply by	To Find	Symbol
		LENGTH		
in	inches	*2.5	centimeters	cm
ft	feet	30	centimeters	cm
yd	yards	0.9	meters	m
mi	miles	1.6	kilometers	km
		AREA		
in^2	square inches	6.5	square centimeters	cm^2
ft^2	square feet	0.09	square meters	m^2
yd^2	square yards	0.8	square meters	m^2
mi^2	square miles	2.6	square kilometers	km^2
	acres	0.4	hectares	ha
		MASS (weight)		
oz	ounces	28	grams	g
lb	pounds	0.45	kilograms	kg
	short tons (2,000 lb)	0.9	tonnes	t
		VOLUME		
tsp	teaspoons	5	milliliters	ml
Tbsp	tablespoons	15	milliliters	ml
fl oz	fluid ounces	30	milliliters	ml
c	cups	0.24	liters	l
pt	pints	0.47	liters	l
qt	quarts	0.95	liters	l
gal	gallons	3.8	liters	l
ft^3	cubic feet	0.03	cubic meters	m^3
yd^3	cubic yards	0.76	cubic meters	m^3
		TEMPERATURE (exact)		
°F	Fahrenheit temperature	5/9 (after subtracting 32)	Celsius temperature	°C

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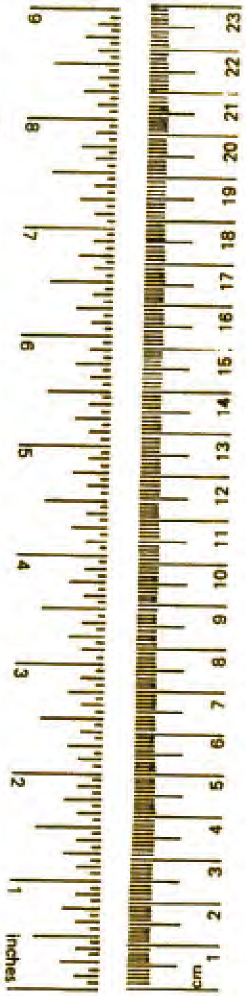
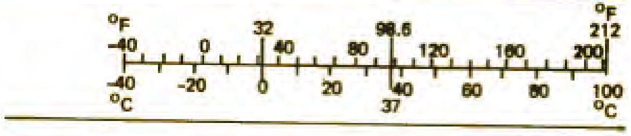
*1 in = 2.54 (exactly). For other exact conversions and more detailed tables, see NBS Misc. Publ. 286, Units of Weights and Measures, Price $2.25, SD Catalog No. C13.10:286.



Approximate Conversions from Metric Measures

Symbol	When You Know	Multiply by	To Find	Symbol
		LENGTH		
mm	millimeters	0.04	inches	in
cm	centimeters	0.4	inches	in
m	meters	3.3	feet	ft
m	meters	1.1	yards	yd
km	kilometers	0.6	miles	mi
		AREA		
cm^2	square centimeters	0.16	square inches	in^2
m^2	square meters	1.2	square yards	yd^2
km^2	square kilometers	0.4	square miles	mi^2
ha	hectares (10,000 m^2)	2.5	acres	
		MASS (weight)		
g	grams	0.035	ounces	oz
kg	kilograms	2.2	pounds	lb
t	tonnes (1,000 kg)	1.1	short tons	
		VOLUME		
ml	milliliters	0.03	fluid ounces	fl oz
l	liters	2.1	pints	pt
l	liters	1.06	quarts	qt
l	liters	0.26	gallons	gal
m^3	cubic meters	35	cubic feet	ft^3
m^3	cubic meters	1.3	cubic yards	yd^3
		TEMPERATURE (exact)		
°C	Celsius temperature	9/5 (then add 32)	Fahrenheit temperature	°F



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Dr. Roy H. Reuter

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
ICAIR
Life Systems, Inc.
24755 Highpoint Road
Cleveland, OH 44122

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FOREWORD

This Workgroup Summary Report was prepared by ICAIR, Life Systems, Inc., under USA CERL Contract No. DACA 88-88D-0018. The USA CERL Technical Contact was Dr. Ed Smith. The NCEL Technical Contacts were Dr. D. B. Chan and Mr. Ron Hoeppel. Dr. Roy Reuter managed this Delivery Order, facilitated the Workgroup and prepared the Summary Report.

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1.0 INTRODUCTION

This report summarizes the recommendations of a Workgroup held February 21-23, 1989 to examine biotechnology applicability (unique or highly significant) to the military environment with particular emphasis on the military's hazardous waste problems. The Workgroup was planned and organized by ICAIR (Interdisciplinary Consulting and Information Research), Life Systems, Inc. (ICAIR) for the U.S. Army Construction Engineering Laboratory (CERL) and the Naval Civil Engineering Laboratory (NCEL). This work was performed under CERL's Indefinite Delivery Order Contract for Research Support in the Areas of Water Quality Management Support. Administrative details for this assignment are:

1. Delivery Order Title: Conduct a Biotechnology Workgroup for Department of Defense (DOD) Soil and Groundwater Decontamination Application.
2. CERL Technical Contact: Dr. Ed Smith.
3. NCEL Technical Contacts: Dr. D. B. Chan and Mr. Ron Hoeppel.
4. Meeting Location: Hyatt Regency Monterey, Monterey, CA.
5. Workgroup Agenda: The Workgroup Agenda is provided at Appendix 1.
6. Participants: The list of Workgroup Participants is provided at Appendix 2.
7. Presentations: Copies of viewgraphs and/or presentation text or outlines are provided at Appendix 3 as provided by the presentors.

1.1 Workgroup Background

Military operations generate significant amounts of hazardous wastes. In fact, Army installations alone annually generate more than one-half million tons of hazardous wastes just in the United States. For example, some of the Army's activities that ultimately produce hazardous wastes are industrial and manufacturing operations to produce, repair and dispose of military equipment and weapons systems, production, testing and disposal of munitions, research and development of new equipment, military training, and medical support for the soldier. Collectively these activities result in a wide array of contaminated sludges, solvents, toxins, acids, bases and heavy metal wastes. Tables 1-1 and 1-2 list typical hazardous waste generating activities and types of wastes, respectively.

The improper disposal activities associated with these wastes in the past has lead to instances of soil, air, and surface/groundwater contamination at numerous DOD installations. The pollution incidents have resulted because many of the generated wastes are/were deposited at authorized or unauthorized landfill sites (on or off base), placed in storage areas awaiting disposal, or occurred as a result of spills, leaks or demilitarization. This situation requires a remedial action program/management/technology equivalent to and in parallel with the Environmental Protection Agency (EPA) Superfund activities.

TABLE 1-1 TYPICAL ARMY HAZARDOUS WASTE GENERATING ACTIVITIES

Process, Operation or Condition
Electroplating
Paint Stripping, Solvents
Paint Stripping, Plastic Beads
Painting
Cleaning/Degreasing
Vehicle Maintenance
Electrical Maintenance
Metalworking
Fueling Operations
Battery Shop Operations
Munitions Demilitarization
Load, Assembly and Pack Operations
Industrial Wastewater Treatment Sludges
Other Treatment-Generated Hazardous Wastes
Other Operations and Processes

TABLE 1-2 HAZARDOUS WASTES BY TYPE

Type Number	Characteristic of Type	Determination	Representative Examples
1	Ignitable	EPA D001 by test	Paint thinner, miscellaneous solvents
2	Corrosive	EPA D002 by test	Acidic and alkaline solutions
3	Reactive	EPA D003 by test	Munitions demil, oxidizers
4	Toxic	EPA D004-017 by EP Toxicity Test	Electroplating waste, sewage, sludges
5	Non-specific	EPA F list	Paint and waste sludges, other solvents
6	Specific	EPA K list	Spent carbon, explosive scrap
7	Acute Hazardous	EPA P list	Pesticides, rodenticides
8	Toxic	EPA U list	Benzene, peroxide formaldehyde
9	Used Oils	DESR Report	Petroleum and other used lubricants
10	Unclassified	--	PCB's, miscellaneous wastes

The DOD has programs to address the compliance requirements for proper disposal, management and operation of hazardous waste facilities. The DOD environmental programs address many aspects of the hazardous waste management problems which include:

1. Identification and evaluation of the past hazardous material storage and disposal practices and sites.

2. Minimization of hazardous waste generation.

3. Monitoring and control of contaminant migration.

4. Decontamination by treatment and detoxification.

A wide range of technologies and strategies exists for managing and treating hazardous wastes. The major functions addressed by existing techniques are:

1. Volume reduction
2. Component separation
3. Detoxification
4. Destruction
5. Storage
6. Material recovery

Since no single process performs every function, several different processes are employed for adequate treatment. Selection of appropriate techniques depends on the type of waste, physical/chemical/biological characteristics, volume, economics of treatment method and government regulations.

One process which is especially promising (based upon theory and actual field experience) is biotechnology. The Office of Technology Assessment defines biotechnology as "Any technique that uses living organisms (or parts thereof) to make or modify products, to improve plants or animals, or to develop microorganisms for specific uses." Biotechnology has been employed in the pharmaceutical and fermentation industries for many years. Application of the principles of biotechnology require a thorough understanding of the metabolic pathways the biota used to transform feedstock to product, and the environmental support conditions which optimize the transformation rate.

If the "feedstock" is a hazardous waste and the products are inert/non-toxic materials, then biotechnology can be of great benefit to society. Whereas biological systems have been employed in waste treatment in the past, these applications have lacked the sophistication require to define and understand the metabolic pathways and environmental conditions needed to optimize the system.

Recently, the success of genetic engineering has driven other researchers to take a biotechnology approach to waste treatment. This has been particularly true in the oil-spill cleanup business. One difficulty lies in determining the effectiveness of the biotechnology approach.

Within the past 15 years, biodegradability studies have been conducted on a wide variety of organic wastes including phenols, chlorinated hydrocarbons and cyanide compounds. The observed biodegradability of compounds previously believed to be resistant to biodegradation suggests that biological treatment techniques are, in fact, viable methods of hazardous waste treatment.

Recent studies of subsurface microbial activity have shown that biological processes are important determinants of the fate and transport of organic contaminants in the subsurface environment. Interest in utilizing natural biological processes to eliminate or treat hazardous wastes has resulted in accelerated research efforts in the field of subsurface microbiology.

Furthermore, recent developments in molecular biology and chemistry made it possible to manipulate and use microorganisms, enzymes, and genetic material in a variety of fields such as medicine, agriculture and pharmacology. This technology can be developed and applied to solve a number of the DOD's environmental problems. These include:

1. The use of biochemical sensors to determine environmental stress in organisms.
2. In-situ or on-situ detoxification/biodecontamination of pollutants and hazardous materials in soils and waste streams.
3. Removal and recovery of metals in waste streams with metallothioneins which are metal binding proteins.

It is claimed that biodegradation is a cost-effective treatment technique which reduces initial capital expenditures, minimizes site disruption in the event of spills and decreases hauling and associated disposal expenses. In addition, the need for new disposal facilities and associated siting problems may be reduced.

The Army consists of several Major Commands (MACOM's) located worldwide, with different responsibilities, that contribute to the diversity of the hazardous wastes generated. Similar activities within these MACOM's generate similar hazardous wastes. However, only three of the MACOM's generate the majority of the hazardous wastes; namely, the U.S. Army Material Command (AMC), the U.S. Army Forces Command (FORSCOM), and the U.S. Army Training and Doctrine Command (TRADOC). In the United States alone, the hazardous waste generators within these MACOM's consist of 108 active Army installations. More specifically, because of AMC's industrial mission to support the soldier in the active Army, the Army Reserve and the National Guard, AMC contributes almost 90 percent of the hazardous wastes generated in the Army.

A recent questionnaire of all Army major commands and installations produced important information on hazardous waste generation, treatment, recycling/reuse/resell, disposal, costs of such and waste minimization actions. For those installations responding, the inquiry revealed on a world-wide basis that the Army generated approximately 200,000 metric tons of waste material, excluding industrial wastewaters and sludges, during the three calendar years 1985 through 1987. The hazardous wastes produced cover a broad spectrum of substances including waste solvents, fuels, pesticide residues, battery acids as well as industrial and wastewater sludges. In particular, the hazardous wastes of concern include (but are not limited to) acids, asbestos, caustics, cyanide, heavy metals, non-volatile and volatile organics, ordnance (TNT, RDX, HMX, PETN), PCB's, pesticides (mainly chlorinated hydrocarbons), toxic inorganics, and others.

Based upon actual military installation site investigations a typical contaminant area may include training areas, sanitary landfills, liquid waste storage areas, an oil dump site, and an unofficial dump site. Based upon field investigations involving the installation of groundwater wells and the collection of numerous soil, sediment, surface water and groundwater samples (which were analyzed for volatile organic compounds, total organic halogens, total organic carbon, arsenic, trace metals, oil and grease, total phosphorous and nitrates) elevated contaminant levels are often found on military bases.

Hazardous wastes were reported in ten waste types listed in Table 1-2. AMC, FORSCOM, TRADOC an CONUS installations reported waste quantities in all ten types. OCONUS installations reported waste generation among seven types. As expected, the most hazardous categories, Types 7 and 8, showed accumulations in the least quantities. Conversely, the general or unclassified category, Type 10, had the largest accumulations for the three year period. Polychlorinated biphenyls (PCBs) accounted for a substantial amount of Type 10 waste, a result of deactivation of PCB contaminated electrical transformers and PCB contaminated soil. PCB contaminated waste will eventually be eliminated as the use of transformer oil is being phased out.

One source estimates the military services are responsible for about 98,000 underground fuel tanks in the U.S. The EPA estimates that as many as 35% of all underground fuel tanks would fail a "tightness test" and may be leaking.

MACOM's are responsible on a geographical and functional basis for the operation of all Army installations, both in the Continental United States (CONUS) and the overseas installations (OCONUS).

The activities generating the most significant quantities of hazardous wastes report to the AMC. To support the Army soldier in the active Army, the Army Reserve, and the National Guard, AMC requisitions, overhauls, and repairs hundreds of thousands of pieces of equipment at its maintenance and repair depots every year. It also produces, tests, stores, distributes, and disposes of virtually all the ammunition for the DOD. These installations are responsible for the manufacture and repair of all Army Weapons systems and equipment maintenance.

TRADOC and FORSCOM have activities which generate similar kinds of waste, such as equipment maintenance and repair. Consequently, the most common hazardous wastes are cleaning and degreasing solvents, contaminated waste oils and fuels, battery acids and paint stripping wastes. The other MACOM's (CONUS) and OCONUS commands also generate similar hazardous wastes but in reduced quantities.

Complicating the issues is the fact that each military installation (Army, Air Force or Navy) is confronted with a unique set of external and internal forces that will shape its approach to management/mitigation of its contamination problem. The technical issues are crosscut by regulation, politics, economics, future force structure, competing interests and the incongruent role (or lack of presence) of the various DOD, Federal, state and local agencies dealing with hazardous wastes issues.

And new problems continue to emerge. Approximately 400,000 new organic compounds are synthesized worldwide each year (DOD, 1988).

1.2 Workgroup Objectives and Organization

The overall Workgroup objective was to examine biotechnology applicability (unique or highly significant) to the military environment (i.e., a technology assessment involving the civilian, industrial, academic and governmental sectors).

This objective was achieved by:

1. Bringing together 38 individuals actively involved in either biotechnology research and development (R&D) or management of the military's environmental programs to exchange information and identify and prioritize the military's biotechnology R&D needs associated with hazardous wastes in an intensive three-day Workgroup.

2. Providing the Workgroup participants with a background paper prepared by Dr. Ed Smith of CERL prior to the meeting.

3. Having each military service describe their hazardous waste problems and ongoing and planned biotechnology R&D programs.

4. Having the EPA and the U.S. Geological Survey (USGS) present their ongoing and planned biotechnology R&D programs and obtaining and distributing to Workgroup participants a report of research needs prepared during the International Conference of Physiochemical and Biological Detoxification of Hazardous Wastes sponsored by the National Science Foundation. This report is included in Appendix 3.

5. Having 11 researchers present their biotechnology projects in some detail, followed by group discussions of their presentations.

6. Forming the following five subgroups for the final day:

    a. Management & Policy
    b. Fuels
    c. Ordnance
    d. Chlorinated Solvents
    e. Inorganics/Heavy Metals

    Each subgroup met separately. The management and policy subgroup addressed planning, funding, coordination, technology transfer and other topics essential to the DOD's biotechnology R&D program. The four other subgroups prepared write-ups for each biotechnology research topic. These write-ups contained a background statement, scope and expected output of the R&D. The subgroups also prioritized the research which their subgroup identified. The assignments of participants to the respective subgroups is listed in Appendix 4.

The Workgroup was conducted in three phases. Phase one included presentations by each of the three military services presenting their hazardous waste problems and discussing, in general terms, biotechnology R&D needs associated with these problems. Phase two included presentations and discussions of ongoing and planned biotechnology R&D by the three services, the EPA, USGS and 11 university and private sector researchers. The final phase was devoted to the development of specific R&D project statements by the subgroups. These statements are included in the final section of this report.

2.0 SUBGROUP RECOMMENDATIONS

2.1 Management and Policy Subgroup

The purpose of this subgroup was to define procedures for coordinating and integrating biotechnology into Navy, Army and Air Force systems to improve these systems and to eliminate or mitigate adverse effects to the environment. This included aspects of both site remediation and hazardous waste producing processes.

The following actions were defined by the subgroup:

1. Develop Tri-Service Research Plan
    a. Define mission needs resulting in technical research needs:
        - Business Plan for next three years.
        - Strategic Plan for next 15 years.

    b. Coordination
        - Define Current Program
            - Develop integrated matrix for technology exchange. Matrix to include vendors, researchers, site data, project data, etc.
            - Define topics for continuing working group meetings in technical areas and assign lead agencies for coordination.
            - Get involvement by other Federal Agencies (i.e., USGS, EPA, DOE).
            - Get involvement from university and commercial/industrial sector.
            - Develop lead agency coordination for technology areas.
            - Plan to be updated each year.
        - Funding
            - Define funding requirements for research needs
            - Define funded project by Army, Navy, Air Force
            - Identify funding gaps
        - Prioritization
            - Develop forecast of Navy, Army and Air Force needs

  - Identify applications first
  - Define research to back-up applications

2. Other items discussed by the management and policy subgroup, which will require policy and management decisions and will be addressed as issues in the Plan are:

   a. Protocol for Biotechnology Application

      - Standards development

        - Peer review
        - Site condition protocol
        - Tech data packages
        - Proposals peer reviewed

      - Technology transfer from research community to user
      - Scale up of research
      - Post application evaluation
      - Standard economic analysis
      - Risk assessment
      - Regulatory and DOD interface

   b. International Issues

      - Technology of NATO countries
      - Problems and requirements
      - International protocols

2.2 Chlorinated Solvents Subgroup

This subgroup identified six research projects. The subgroup prioritized the projects into four priority levels (with a 1 as the highest priority and a 4 as the lowest).

2.2.1 Dechlorination and Oxidative Mechanisms of Microflora; Priority Level 1, a 6.1 Project

Background: The metabolic pathways employed by microbes in the dechlorination and ultimate oxidation of chlorinated solvent substrates are important for the eventual design of efficient systems.

- Different microbes carry the breakdown process to varying endpoints through varying pathways.

- Some intermediate products may be highly toxic.

- Abiotic mechanisms may also be important in some circumstances.

Scope:

- Select a suite (e.g., three to four) of DOD-relevant solvents that are of major significance as contaminants at Defense Environmental Restoration Account (DERA) sites.
- Define the microbial consortia that can be demonstrated to degrade these solvents.
- Determine toxicity thresholds for these microbes in terms of solvent concentrations tolerated.
- Determine the lower concentration limits that can be achieved by use of such microbe populations.
- Analyze degradative pathways of individual microbes identified as important species within the consortia.

Output:

- Determine the species make-up and survivability of synthetic consortia of microbes individually shown to be active solvent degraders.
- Test these populations in reactor systems.
- Ultimately test successful microbial consortia in-situ.

2.2.2 Inventory of Chlorinated Solvent Types Used by the Military; Priority Level 3, a 6.2 Project

Background: Much of the military's current Installation Restoration (IR) effort is devoted to recovering from past practices. Indiscriminate use and disposal of chlorinated solvents have led to contaminated soil and groundwater. To properly determine the feasibility of applying biotechnology to clean-up, the nature, form and allowable impurities of past chlorinated solvents procured for use by the military must be catalogued.

Scope:

- Archival search of Mil Specs in the FSC 6850 series to identify chlorinated solvents (and allowable impurities) procured for military use.
- Comparison of results of Task 1.0 with the history of chlorinated solvents manufacture.
- Identification of solvents unique to specific applications (e.g., methylene chloride for paint stripping).
- Organization and cataloging of existing data on degree of chlorination and associated toxicity.

- Survey of manufacturers and weapons system developers to forecast future developments in the chlorinated solvents (e.g., solvents compatible with radar reflective paint or carbon composite material).

Output:

- List of current and past chlorinated solvents used
    - Arranged by commodity
    - Arranged by using process(es)
- Compendium of data on biotoxicity of chlorinated solvents
- Future (forecasted) solvents which may require treatment by biotechnology methods

2.2.3 Coupled Bioreactor System (Aerobic/Anaerobic); Priority Level 1, a 6.2 Project

Background: Chlorinated solvent contaminated groundwater exists at many DOD installations and is the subject of regulatory action at some of the installations. Technology exists to pump groundwater to the surface and treat with packed column air strippers and/or carbon adsorption; both of which do not destroy the contaminant. Air stripping transfers the contaminant from water to air with no destruction of the contaminant (a practice that is already banned in some states). Carbon adsorption transfers the waste from the water to the carbon which must be regenerated or disposed. A treatment technology is needed to remove the contaminant from the water and/or air stream and destroy or render non-hazardous.

Scope: Establish two tests; one to test the vapor phase treatment capability of biosystems, and one to test the aqueous phase treatment capability of biosystems to include both aerobic and anaerobic systems composed of sequencing, series and fixed film reactors. Tests will be conducted using single contaminants of concern and multiple contaminants based, to the extent possible, on actual site conditions.

Output: Desired output from these tests will be data that defines the effectiveness of biotreatment systems (vapor phase and liquid phase) to treat chlorinated solvent contaminated groundwater, provides sufficient data to design a pilot scale system to prove out concept, and provides sufficient data to conduct a preliminary economic analysis of treatment cost.

2.2.4 Mixed Waste Treatment; Priority Level 2, a 6.1 Project

Background: Almost all contaminated sites involve the presence of multiple contaminants--either multiple organic solvents or organic solvent/heavy metal mixtures. Therefore, the effect of multiple substrates on the biodegradative capability of the microbial population of interest needs to be investigated.

Scope: Using either batch or bioreactor systems, investigate the effects of multiple organics or heavy metals on the biodegradative capability of a microbial population in terms of:

- Inhibiting degradation of the primary substrate of interest by either having a toxic effect on the microbes or by somehow inhibiting their enzymatic pathways.

- Possibly enhancing the biodegradation of the primary substrate of interest.

Output: Information to assist in predicting the feasibility of using biotechnology to remediate a contaminated site containing multiple sustrates (contaminants).

2.2.5 Development and Standardization of Sampling Protocols; Priority Level 3, a 6.2 Project

Background:

- At present there is relatively little standardization in sampling procedures for chlorinated solvents.

- Such standardization is required to overcome problems of sample storage, settling or volatilization that can profoundly affect analytical results and give rise to errors in site evaluation.

Scope:

- Undertake tests to evaluate such factors as sample size and volume.

- Settling and volatility in varying soil and water matrices.

- Allowable storage times and temperatures before sample degradation or change occurs.

Output:

- Disseminate recommended procedures to appropriate organizations for evaluation.

- Collate results and recommendations into final protocols to be used by all services.

- Undertake periodic review (e.g., biannually) of protocols and update as necessary to keep pace with evolving technology and physical/chemical make-up of new solvents.

2.2.6 In-Situ Treatment; Priority Level 4, a 6.2 Project

Background: Chlorinated solvents have been identified as a major problem in the Installation Restoration (IR) program. Application of biotechnology for remediation could be cost-effective. Cultivation and utilization of naturally existing microbes in in-situ treatment approaches can realize maximum benefit.

Scope: Investigate the existing microbes for their biodegradation capability by measuring redox potentials which will determine aerobic and or anaerobic modes. Determine cultivation potential in terms of nutrients and environmental factor requirements. Lab experiments/field pilot demonstration will establish options for barrier/in-situ/on-site/phases treatment.

Output: in-situ/on-site biodegradation system alternative for treatment of soil/groundwater with contaminated chlorinated solvents.

2.3 Fuels Subgroup

This subgroup identified 10 research projects. The subgroup prioritized each project in relative order from 1 to 10 (with 1 as the highest and 10 as the lowest).

2.3.1 Develop Above-Ground Treatment Processes; Priority 3, a 6.2 and 6.3 Project

Background: Above-ground treatment processes are an integral part of both in-situ and land-surface treatment facilities. Improvement of techniques and methods to properly couple subsurface to surface treatment are required.

Scope: In-situ and above-ground treatment methods for fuels rely on increasing the availability of the hydrocarbons to the microbiota, as well as decreasing intracellular toxicity during the degradation processes. Total destruction of hydrocarbons is seldom realized and the major step to toxicity reduction is decreasing concentrations and removing mobile (e.g., solubilized or emulsified) contaminants.

Research is required to develop cost-effective methods to destroy volatile hydrocarbons (VOC's) produced from point source releases (e.g., soil venting during in-situ reclamation). One potential method is biodegradation of VOC's in bioreactors containing a sorptive medium for sorbing the vapor phase. The determination of the best sorptive medium, microbial growth conditions, and bioreactor design that maximizes VOC biodegradation are required. Effective surfactants/emulsifiers that readily mobilize hydrophobic hydrocarbons in water while being non-toxic and non-inhibitive to hydrocarbon degrader growth/metabolism are required. Development of above-ground bioreactors and processes that rapidly and cost-effectively degrade emulsified hydrocarbons is also needed.

Output: Literature reviews of VOC biotreatment and available surfactant/emulsifier use in fuel treatment need to be developed. Laboratory, followed by field pilot studies, need to be performed to evaluate VOC and emulsion removal for cost and effectiveness. Prototype systems will be developed to help phase products to conventional field use.

2.3.2 Saturated Zone Biodegradation of Fuels; Priority 1, a 6.2 and 6.3 Project

Background: Techniques have been developed in a variety of forms for over 10 years within the private sector. The details of the process have undergone major evaluation over the past five years. Most commercial sites have provided only anecdotal data about critical process variables, and very little data from those sites are in the open literature.

Only three "field demonstrations" have been performed which provide published meaningful process data (API-Granger, AFEPA-Kelly AFB, AF-Eglin AFB). Each of these sites suffered from some operational problems which limited the broad utility of these data. Based on these experiences and the collective input of university and consulting personnel, several areas have been identified where improved understanding is required to establish in-situ bioremediation of contaminated aquifers as a reliable predictable technique. Recommended areas of investigation are summarized below.

Scope:

Fundamental Processes

6.2, 6.3 Understand role of adsorption phenomena in controlling the biodegradation of various constituents.

6.2, 6.3 Develop methods to optimize desorption/bioavailability of hydrophobic contaminants (emulsifiers, biosurfactants, surfactants, etc.).

6.1, 6.2 Develop understanding of preferential biodegradation of complex wastes (aliphatics vs aromatics, branched chain affects) and the environmental characteristics which control these differences.

Oxygenation of In-Situ Processes (Saturated)

6.1, 6.2 Develop improved means of predicting and maximizing hydrogen peroxide (H_2O_2) stability in groundwater matrices (soil characteristics, screening protocol, stabilization techniques).

6.1, 6.2 Determine the role of enzymes, especially catalase, in the stability of hydrogen peroxide in the subsurface and develop methods to control these processes.

6.1, 6.2 Evaluate alternate methods of delivering oxygen through the saturated zone (e.g., electrolysis of water, new peroxide formulations, nitrate, nitrogen oxide gases, pure oxygen).

Nutrient Issues

6.2, 6.3 Evaluate C:N:P ratios which optimize process performance.

6.2, 6.3 Improve techniques for evaluating nutrient formulations for compatibility with various soil types.

6.2, 6.3 Develop methods for evaluating and controlling soil buffer capacity and pH.

Environmental Factors

6.1, 6.2 Define the need of co-factors, co-substrates and micro-nutrients for increasing biodegradation rates.

Output: Better understanding of critical site characteristics, literature review, lab studies with multiple soils, etc., field studies performed under the objectives above.

2.3.3 Vadose Zone In-Situ Biodegradation; Priority 2, a 6.2 and 6.3 Project

Background: At many sites most of the fuel contamination is found above the saturated zone sorbed to or otherwise retained by soils. Further research is required to investigate biodegradation mechanisms and engineering of biodegradation enhancement in the vadose zone.

A multi-faceted program is required to address the following:

6.2, 6.3 An assessment of the biotic and abiotic (volatilization) mechanisms associated with conventional venting.

6.2, 6.3 An assessment of soil venting as a mechanism to introduce oxygen and stimulate biodegradation in the vadose zone.

6.2, 6.3 An effort to initiate the process of engineering biodegradation through soil venting. The issues to be addressed include (but are not limited to):

- Moisture augmentation
- Nutrient delivery methods and requirements
- Venting rates to optimize in-situ volatile organic compound (VOC) biodegradation and minimize VOC removal from the soil.

6.1, 6.2 A clear understanding of sensitive parameters affecting biotransformation in the vadose zone.

Output:

- A better understanding of the effects of conventional soil venting on biodegradation.
- An understanding of processes necessary to allow engineering of in-situ biodegradation in the vadose zone.
- Research is to be scaled from bench-scale laboratory studies to field scale pilot studies. Prototype systems will be developed to help phase RDT&E to field demonstration-scale studies.

2.3.4 Anaerobic Processes; Priority 5, a 6.1 and 6.2 Project

Background: Anaerobic processes have been shown to be significant hydrocarbon removal mechanisms (especially for aromatic compounds) in both field and lab studies. However, the environmental conditions which affect rates and the extent of transformation of individual hydrocarbon components are poorly understood. Therefore, such processes have not been considered in the design of soil/groundwater treatment strategies. It is recognized that the pay-off could be very significant if anaerobic processes could be relied upon to clean up spills of fuels and other hydrocarbons.

Scope: Execute detailed and long-term field studies on sites where anaerobic transformation of aromatic and other hydrocarbons have been observed. These field studies should be supported by detailed laboratory studies designed to provide insight into the transformation processes.

Lab studies should be designed to identify the factors which affect adaptation times and transformation rates.

Output: The research outlined above will lead to information useful for:

- Predicting the fate of aromatic hydrocarbons in anaerobic subsurface systems, including residence times and intermediates metabolites.
- Understanding geochemical conditions suitable for anaerobic transformation of aromatics.
- Recommending ways to stimulate/accelerate hydrocarbon biotransformation rates.

2.3.5 Develop In-Situ Methods for Assessing Microbiological Activity; Priority 6, a 6.2 and 6.3 Project

Background: In order to effectively develop biocleanup technologies (stimulation of microbiological activity) either in-situ or above-ground methods are required to obtain real-time data on system performance. Both traditional analytical techniques applied in the field and biosensors can be appropriate to evaluate system performance.

Scope: Development of reliable field systems for monitoring microbiological activity will require identification of appropriate parameters and adaptation of existing technology from soil science, analytical chemistry and biomonitoring. Traditional analytical instruments will be modified to detect and quantify respired (microbially produced) gases and immunoassay techniques will be developed to detect and quantify enzymes involved in biocleanups. New technologies (e.g., immunoassays, fiber optics) require laboratory evaluation, simulated field testing and field evaluations, while traditional systems can be developed faster.

Output:

- Development of an automated monitoring system for microbiologically important gases, with data-interpretation and a User's Guide.
- Development of immunoassay methods to detect and quantify enzymes important in biodegradation of fuel components.

2.3.6 Develop Reliable Standard Methods for Assessing Hydrocarbon Contamination in Soils; Priority 4, a 6.2 and 6.3 Project

Background: Assessment of the degree of biocleanup success for fuel contamination is difficult without appropriate methods to determine the extent of soil contamination. Current methods give results of questionable quality.

Scope: Current and proposed methods for determining total hydrocarbons in soil will be compiled, along with performance data. The most promising methods will be identified and applied to split samples of contaminated soils from diverse DOD facilities.

Output: A detailed, simple analytical protocol will be developed, with precision and accuracy data describing performance.

2.3.7 Develop Better In-Situ Techniques for Determining Mass Balances of Carbon, Nitrogen and Oxygen Compounds; Priority 9, a 6.2 and 6.3 Project

Background: In order to effectively evaluate the performance of biocleanup systems, the utilization of added nutrients, electron acceptors, and contaminants and important metabolic pathways must be known.

Scope: Based on experimental data from monitoring at the site of a biocleanup, methods for development of mass-balance expressions and/or evaluation of the importance of various site-specific conditions at locations of biocleanups will be developed.

Output: A User's Guide on carbon, nitrogen and oxygen mass balances in biocleanups will be prepared.

2.3.8 Development/Improvement of Oil-Water Separation Equipment Using Ultra-/Microfiltration Technology; Priority 7, a 6.2 and 6.3 Project

Background: Heavily contaminated fuel spill sites are often remediated by a "pump and treat" process which injects and/or reinjects pumped water after removing free fuel product. Much fuel remains interstitially and partially emulsified by natural emulsifiers in the soil profile. This remaining product is slow to solubilize or separate from soil particles, which is necessary for bioremediation to proceed rapidly. More product needs to be physically removed to facilitate final cleanup by bioremediation.

Scope: State-of-the-art oil/water separation including ultra- and microfiltration can completely separate fuel even at the colloidal level. Deliberate addition of a biodegradable emulsifier would remove fuel from soil pores, which would then be separated by ultra- or microfiltration.

Output: A complete, light-weight, portable, prototype ultra-/microfiltration system would be developed for evaluation at a site. The protocol would include use of appropriate synthetic or natural emulsifying agents to help extract fuel sorbed to soil particles.

2.3.9 Development/Improvement of Toxicity Determination for Soils; Priority 10, a 6.1 and 6.2 Project

Background: Numerous methods for determination of toxicity exist, and all can provide useful empirical data. However, methods to interpret these data so that they can be used to develop risk-based cleanup criteria are limited.

Scope: An evaluation of existing toxicity methods for fuel components and any correlations (or lack thereof) with risk of groundwater use will be performed. Upon completion, a decision will be made on the feasibility of a second phase, namely, to develop mathematical relationships between toxicity test results and potential risk; this would include required experimental research for verification.

Output: A DOD report, entitled The Use of Toxicity Test Results in Risk Assessment, will be prepared and will include worksheets, flow diagrams and formulae for use in development of risk-based site cleanup action levels.

2.3.10 Development of Early-Warning Groundwater Monitoring Methods to Detect Biogenic Changes in Groundwater Quality; Priority 8, a 6.2 and 6.3 Project

Background: Natural biomineralization of organic contaminants can eliminate contaminants from groundwater, precluding down-gradient contamination. However, detection of effects of these biodegradation processes on groundwater characteristics can provide a sensitive early-warning system for detecting organic leakage from tanks or landfills.

Scope: A system of several physico-chemical sensing probes will be designed and assembled for inorganic water-quality parameters sensitive to biomineralization. The design will allow for input of reference data (e.g., pH, redox potential, temperature, conductance) to eliminate false positive and false negative information. The system will be evaluated for its sensitivity to hydrocarbon degradation processes through field application in a contaminated aquifer.

Output: A DOD report will be prepared on Development of an Early-Warning System for Detection of Organic Groundwater Contamination. It will include design specifications and laboratory and field data from several sites.

2.4 Ordnance Subgroup

This subgroup identified research projects in five categories. The subgroup elected to estimate the payoff and risk (probability of success) of the proposed research using descriptive words (high, moderate and low).

Background

Types of contamination:

Contaminated Sites

- Flora and fauna
- Soil
- Surface water and runoff
- Groundwater
- Structures
- Equipment
- Impacted areas

Operations Generating Wastes

- Production by-products, off-grade
- Spill and area washdown wastes
- Obsolete, unserviceable conventional munitions
- Items to be destroyed by treaty agreement
- Training residues

Consequences of contamination:

- Unsafe water sources
- Possible contamination of food chain
- Restricted use of structures and equipment
- Restricted land use
- Significant cost to treat currently generated wastes to achieve regulatory compliance

Significant portion of installation restoration program costs are dedicated to ordnance related contamination.

R&D Support Required

Evaluation of flora and fauna as required by NEPA--also protection against law suits.

R&D Support Desired

2.4.1 Projects Associated With Contaminated Flora and Fauna

6.1 Complete plant uptake studies in lab, if uptake evaluated/found in field. If found, evaluate consequences including metabolic pathways.

6.2 Develop analytical procedures for flora/fauna measurement. Evaluate vegetative uptake as a cleanup mechanism, migration retardant and run-off retardant.

6.3 Development/prove-out of 6.2 analytical procedures in field. Demonstrate developed run-off prevention/retardant techniques.

Output:

- Protocol/techniques for evaluating impact of ordnance contamination on flora/fauna

- Techniques for minimizing migration and run-off

2.4.2 Projects Associated With Treatment of Soil

6.1 Isolation/characterization of strains.
Applicability of fungi, molds, earthworms, etc.
Impact of critical parameters (pH, temperature, etc.).

6.2 In-situ mechanisms R&D.
Evaluation of alternative nutrients, carbon source.

6.3 Structural alternative evaluations.
Batch vs semi-continuous vs continuous.

Output: More cost-effective process for treatment of contaminated soil

2.4.3 Projects Associated With Contaminated Structures and Equipment

6.1 Evaluate feasibility of masonry and metal decontamination by bacteria, fungi, molds.

6.2 Evaluate bioenhancement of hot gas technology.

Output: More effective structural decontamination technology.

2.4.4 Projects Associated With Production Wastes

6.1 Metabolic pathway of pertinent energetic compounds.
Review past studies to biotreat contaminated ground/surface water, pink water and red water.

6.2 Evaluate pretreatment processes for individual waste stream components that would enhance combined waste biotreatment.

Output: More cost-effective means for treatment of production operation wastes.

2.4.5 Projects Associated With Items to be Destroyed by Treaty Agreements (Rocket motors, etc.) and training residues (propellant bags, etc.)

None, biotechnology approaches not considered feasible for these items.

Probable Pay-Off of Projects

Moderate Risk - High Payoff

Composting could reduce cost of soil treatment by $200 per cubic yard. Total program savings of about $1 billion.

Low Risk - Moderate Payoff

Ability to evaluate effect on flora/fauna could avoid/reduce expensive law suits and adverse publicity.

Low Risk - Moderate Payoff

Minimizing migration and run-off could be reduced by vegetative filtering technologies to produce effluents meeting regulatory standards without further treatment.

High Risk - Moderate Payoff

Use of vegetative uptake as a decontamination technique could further reduce restoration costs.

Moderate Risk - High Payoff

Enhanced structural decontamination technology--use and equipment of hot gas and bio-surface treatment.

High Risk - Low Payoff

Biotreatment technologies for red water, pink water and ordnance contaminated ground/surface water.

High Risk - Low Payoff

Improved pre-treatment processes for combined waste biotreatment plants.

2.5 Inorganics/Heavy Metals Subgroup

This subgroup identified six research projects. Like the chlorinated solvents subgroup this subgroup prioritized the projects into four priority levels (with a 1 as the highest and a 4 as the lowest).

2.5.1 Development of a Relatively Inexpensive System for Reducing the Mobility and Toxicity of Heavy Metals in Soil and Sediments; Priority Level 1

Background:

- Methods for remediating soil and sediment contaminated with heavy metals are expensive and, in some cases, difficult to treat.
- Microbial systems at contaminated sites may provide a method for immobilizing and reducing the toxicity of heavy metals in soil and sediment.
- Laboratory studies have demonstrated that sulfate-reducing bacteria in soil and sediment convert soluble heavy metals in these environments to the metal sulfides (which reduces the mobility and toxicity).
- These studies have also shown that supplementing soil and sediment with nutrient increases the activity of these bacteria and the rate of conversion.
- Since the costs of these supplements are very inexpensive, this process would have application for reducing the mobility and toxicity of heavy metals in soil and sediment.

Scope:

- Before these systems can be used, a need exists for evaluating its potential in the field.
- Identify a field site.
- Evaluate the effectiveness of nutrient addition on the activity of sulfate-reducing bacteria, immobilization of the heavy metals and the potential toxicity of the contaminated site.
- Evaluate the cost-effectiveness of the system.

Output:

- The addition of nutrient to the contaminated site should reduce the toxicity and mobility of heavy metals observed at the treated site.
- The successful demonstration of this system will provide DOD with a relatively inexpensive system for remediating sites contaminated with heavy metals.

2.5.2 Methods for Reducing the Volume of Sludge Contaminated With Heavy Metals is Needed Because the Cost is so Expensive and Method of Storage May Soon be Banned by the EPA; Priority Level 1

Background:

- Sludge is currently disposed of by burying containers with the contaminated sludge. Since the process is expensive and because EPA may ban underground burying, areas exist for developing a new process for treating heavy metal contaminated sludge.
- Microbial systems have been identified that leach heavy metals from sludge. Systems have also been identified that precipitate heavy metals from solution (i.e., converting ionized metals to the elemental form).
- These microbial systems are relatively inexpensive and provide a simple method for reducing the volume of heavy metal contaminated sludge.
- Since these microbial systems have only been tested with small volumes of sludge, a need exists for evaluating these systems with relatively large volumes of contaminated sludge.

Scope:

- Evaluate the microbial systems for their ability to quantitatively leach heavy metal from sludge.
- Evaluate the microbial system for quantitatively for precipitating the heavy metal from solution.

- Evaluate the cost-effectiveness of the microbial systems.
- Evaluate any environmental concerns using this system.

Output:

- Successful demonstration of this microbial system will provide a method for reducing the volume of heavy metal contaminated sludge at costs which are relatively inexpensive.
- This system will also provide a treatment method for sludge before EPA bans underground storage of heavy metal contaminated sludge.

2.5.3 Methods to Preclude Specific Metal-Binding Substances Such as Metallothioneins; Priority Level 2

Background:

- Heavy metals in contaminated waters require removal to meet environmental regulations.
- Additionally, heavy metals desorbed from contaminated solids or sludges and contained in leachates must be removed before it can be disposed.
- Criteria for allowable levels of heavy metals in water is becoming increasingly stringent.
- Metal-binding substances, such as metallothionein, are highly specific and efficient in removing metals from liquids.

Scope:

- Develop method to produce large quantities of metal-binding protein from animals that have demonstrated high binding capacities.
- Use cloning methods based on recombinant DNA procedures.
- Develop methods to use the proteins for metal removal.

Output: Documented procedures for large-scale production of metal-binding proteins.

2.5.4 Field Screening Techniques for the Determination of Heavy Metals in Soil and Groundwater DERA/Superfund Sites; Priority Level 3

Background: Present analytical methodologies for heavy metals are time consuming. It involves samples digestion for several days, dilutions and analysis on AA or ICP. Development of efficient sample preparation schemes coupled with proper selection of instrumentation will allow for same day determinations. Cost-effective analytical methods for field screening of hazardous waste and daily monitoring of cleanup are needed.

Scope:

- Develop and/or evaluate sample preparation techniques for available instrumentation that are well suited for field determinations of heavy metals (6.1 program).
- Demonstrate field sample preparation methods and same day analytical determination procedures for heavy metals at DERA site. (6.2 program).

Output:

- Mobile laboratories equipped with field instruments and sample dissolution apparatus for preparing and quantitatively determining heavy metals in plant, soil and water matrices.
- Documented cost-effective procedures for screening environmental samples for heavy metals.

2.5.5 In-Situ Immobilization/Mobilization of Heavy Metals in Soils, Sediments, Sludges and Aqueous Solution; Priority Level 1

Background: The solubility product of heavy metals in water vary greatly but in general they are low at normal and high pH (about pH 6). The sulfide and oxide forms have the lowest solubilities. A need exists to evaluate potential use of sulfate-reducing bacteria for in-situ formation of sulfide; therefore, precipitate heavy metals from solution. In contrast, formation of metal-organic complexes can increase the solubility of metals, then the solution can be collected, pumped above ground and treated. This will expedite the cleanup process of the soil and perhaps allows for metal recovery and reuse.

Scope:

- For the immobilization technology, a 6.1 research is needed to evaluate the concept in laboratory test tube study. If successful, soil column (a 6.1, also) can then be initiated. If successful, and once the limiting factors are sorted out, a 6.2 research using larger scale prototype systems should be conducted. Eventually, a demo, 6.3 study should be conducted.
- For the mobilization/recovery/treatment, the principals behind the technology is available. This technology, however, needs further column testing using actual soils from a hazardous waste site polluted with heavy metals. This is a 6.1/6.2 research. This study will lead to a full scale demo (6.3 program).

Output: Two technologies for cost-effective cleanup of soil contaminated with heavy metals.

2.5.6 Heavy Metals Speciation in DERA/Superfund Sites; Priority Level 4

Background: Impact, toxicity and degree of bioaccumulation/biomagnification of metals depend not only on the total amounts of each metal but also, and probably more importantly, on the forms of the metal. In addition, the forms of each metal change depending on soil-water or sediment-water environmental factors such as pH, Eh, temperature, dissolved organics and the amounts and types of inorganic anions.

Scope:

- Develop and/or evaluate analytical techniques for determination of metal speciation (6.1 program).
- Develop and/or evaluate field in-situ methods for monitoring the concentration of metal species (6.2 program).
- Develop and/or evaluate mathematical models for prediction of the forms and amounts of metal species in soil solution (6.1 program).
- Demonstrate model prediction and field methods at a DERA site (6.3 program).

Output:

- Availability of field methods such as fiber optics, X-ray florescence to measure and/or to monitor heavy metal concentrations.
- Availability of mathematical models for prediction of the fate, transport and transformations of metal species in soils and groundwater.
- Ability to predict the bioaccumulation/biomagnification of metal species.
- Cost-effective cleanup of hazardous waste sites contaminated with heavy metals.

2.5.7 Field Screening Techniques for Soil and Water Analysis of Volatile and Semi-Volatile Organic Compounds; Priority Level 3

Background: Present analytical protocols for volatile organics are time consuming which prevent timely determination of hazardous levels of volatile and semi-volatile organics in environmental samples. Development of direct methods for field determinations, have the advantages of being cost-effective, provide for early detection, evaluation of existing problems and daily monitoring of cleanup progress.

Scope:

- Develop and/or evaluate sample preparation techniques and field instruments for same day determination of volatiles and semi-volatiles organic compounds (6.1 program).

- Test protocols in the lab and conduct a round robin test (6.2 program).

- Demonstrate field sample preparation methods for soil and groundwater matrices for the analysis of key organic compounds found in fuel and cleaning solvents at DERA sites (6.3 program).

Output:

- Mobile laboratories equipped with field instruments and sample preparation apparatus capable of preparing and qualitatively and quantitatively determining key volatile and semi-volatile organic compounds in soil and water matrices.

- Documented procedures for screening environmental samples for volatile and semi-volatile organic compounds.

3.0 REFERENCES

DOD. 1988. Department of Defense. Hazardous Waste Generation Profile. Draft USA Engineering and Housing Support Center and Army Environmental Office Report; DA Hazardous Waste Minimization Program.

APPENDIX 1

FINAL BIOTECHNOLOGY WORKGROUP AGENDA

BIOTECHNOLOGY WORKGROUP

Agenda

February 20, 1989

6:00 - 8:00 p.m.	Reception at Hyatt Regency Monterey Hotel Hors D'oeuvres and Cash Bar	

Technical Sessions

February 21, 1989	Chairperson Lingua	
8:30 - 8:45 a.m.	Opening Remarks/Introductions	Jain
8:45 - 9:00 a.m.	Administrative Announcements/Workgroup Organization	Reuter
9:00 - 10:30 a.m.	Biotechnology R&D Needs/Hazardous Waste Problems of the Military	
	Service Keynote presentations:	
	- Navy (2 presentors)	Lingua & Wilkins
	- Army	Bartell
	- Air Force	Cornette
10:30 - 10:45 a.m.	BREAK	
10:45 - 11:45 a.m.	Overview of Navy's On-Going and Planned Biotech R&D	Hoeppel and Yamamoto
11:45 - 1:00 p.m.	LUNCH	
1:00 - 2:00 p.m.	Overview of Army's On-Going and Planned Biotech R&D (4 presentors)	Sisk, Mikucki, Lee & Iskander
2:00 - 2:30 p.m.	Overview of Air Force's On-Going and Planned Biotech R&D	Cornette
	USEPA's Ongoing and Planned Biotech R&D (2 presentors)	
2:30 - 3:15 p.m.	- USEPA's Hazardous Waste Engineering Research Laboratory Program	Sferra

continued-

Agenda - continued

3:15 - 3:30 p.m.	BREAK	
	USEPA's Ongoing and Planned Biotech R&D - continued	
3:30 - 4:15 p.m.	- USEPA Robert S. Kerr Laboratory's Program	McNabb
4:15 - 5:00 p.m.	U.S. Geological Survey Ongoing and Planned Biotech R&D	Ragone
5:00 - 5:45 p.m.	Biotechnology Research at UCLA Engineering Research Center for Hazardous Substances Control	Monbouquette
<u>February 22, 1989</u>	Chairperson Chan	
8:30 - 9:15 a.m.	In-situ vs. plant/facility decontamination.	Hinchee
9:15 - 10:00 a.m.	Screening criteria for in-situ bioremediation of contaminated aquifers.	Brubaker
10:00 - 10:15 a.m.	BREAK	
10:15 - 11:00 a.m.	Pilot demonstration project on enhancement of microbial degradation of groundwater and soil contamination from aviation gasoline and jet fuel spills.	Armstrong
11:00 - 11:45 a.m.	Oxygen sources for biotechnological applications.	Brown
11:45 - 1:00 p.m.	LUNCH	
1:00 - 1:45 p.m.	Anaerobic biodegradation of oils and lubricants	Reinhard
1:45 - 2:30 p.m.	Strategy for assessing the ability of native bacteria to biodegrade JP-5	Arnold
2:30 - 3:15 p.m.	Immobilization of lead, cadmium and mercury in soil sludge and aqueous solution.	Revis
3:15 - 3:30 p.m.	BREAK	
3:30 - 4:15 p.m.	Biomonitoring	Kerfoot

continued-

Agenda - continued

4:15 - 5:00 p.m.	Bioreactors	Pickwell
5:00 - 5:45 p.m.	Enhanced in-situ aquifer biorestoration of halogenated alkenes.	Roberts
5:45 - 6:00 p.m.	Air Force Planned Biotechnology R&D	Downey

February 23, 1989

8:30 - 4:00 p.m. (One hour lunch break. Coffee will be available but no scheduled breaks)	Workgroup Discussion Involving All Attendees Discussions will be focused on the following topics/questions and will be discussed in this order:

- What additional information should be gathered/studies performed to better answer the first topic?
- What are apparent "gaps" in on-going R&D work? Within DOD? Within entire research community?
- Suggestions on approaches to prioritizing R&D needs/opportunities.
- Based on information presented to attendees what are recommended biotech R&D projects?

APPENDIX 2

WORKGROUP ATTENDEES LIST

WORKGROUP ATTENDEES LIST

Individuals Making Presentations

Dr. John Armstrong
2480 Gale Road
Ann Arbor, MI 48105

Dr. Susan Landon-Arnold
Northern State College
Biology Department
Aberdeen, SD 57401

Mr. Bob Bartell
USATHAMA
CETHA-TE-D
Aberdeen Proving Ground, MD 21010-5401

Dr. Dick Brown
Groundwater Technology, Inc.
Chadds Ford West
Route 1
Chadds Ford, PA 19317

Dr. Gaylen Brubaker
127 Kingston Drive, Suite 105
Chapel Hill, NC 27514

Dr. James Cornette
Senior Scientist
HQAFESC-RDV
Tyndall AFB, FL 32403

Mr. Douglas Downey
Senior Research Engineer
HQAFESC-RDV
Tyndall AFB, FL 32403

Dr. Rob Hinchee
Battelle Columbus Laboratories
505 King Avenue
Columbus, OH 43201

Dr. Ronald Hoeppel
Naval Civil Engineering Laboratory
Code L71
Port Hueneme, CA 93043

Dr. Alex Iskander
USA CRREL
75 Lyme Road
Hanover, NH 03755-1290

Dr. Ravinder Jain
USA CERL
2902 Newmark Drive
Champaign, IL 61820-1308

Mr. Henry Kerfoot
Lockheed
1050 E. Flamingo Road
Las Vegas, NV 89109

Dr. C. Richard Lee
USAE-WES
P.O. Box 631
Vicksburg, MS 39180

Ms. Mary Lingua
Naval Civil Engineering
Laboratory
Code L70PM
Port Hueneme, CA 93043

Mr. James McNabb
Environmental Protection
Agency
Robert S. Kerr Environmental
Research Laboratory
Ada, OK 74820

Mr. Walter Mikucki
USA CERL
2902 Newmark Drive
Champaign, IL 61820-1308

Dr. H. G. Monbouquette
University of California
at Los Angeles
Chemical Engineering Depart.
5531 Boelter Hall
Los Angeles, CA 90024

continued-

Dr. George Pickwell
Code 521
Naval Ocean Systems Center
San Diego, CA 92152-2760

Mr. Steve Ragone
USGS
Mail Stop 410
National Center
Sunrise Valley Drive
Reston, VA 22092

Dr. Martin Reinhard
Department of Civil Engineering
Stanford University
Stanford, CA 94305-4020

Dr. Nate Revis
Director
Oak Ridge Research Institute
Oak Ridge, TN 37830

Dr. Paul Roberts
Department of Civil Engineering
Stanford University
Stanford, CA 94305-4020

Dr. Patrick Sferra
U.S. Environmental Protection Agency
Risk Reduction Engineering Lab
26 West Martin Luther King Drive
Cincinnati, OH 45268

Mr. Wayne Sisk
USATHAMA
CETHA-TE-D
Aberdeen Proving Ground, MD 21010-5401

Ms. Elizabeth Wilkins
Navy Facilities Engineering Command
Headquarters
Code 183
200 Stovall Street
Alexandria, VA 22332-2300

Dr. Sachio Yamamoto
Commander
Code 52
Naval Ocean Systems Center
San Diego, CA 92152-2760

Other Attendees

Dr. Mick Arthur
Battelle Laboratories
Columbus Division
505 King Avenue
Columbus, OH 43201

Mr. Frank E. Bales
U.S. Army Corps of Engineers
Hazardous & Toxic Waste Treatment
601 E. 12th Street
Kansas City, MO 64106

Mr. Bruce Bauman
American Petroleum Institute
1220 L Street, NW
Washington, DC 20005

Dr. D. B. Chan
Naval Civil Engineering Laboratory
Code L71
Port Hueneme, CA 93043

Mr. Brian J. Hausknecht
Naval Energy and Environmental
Support Activity
Code 112 E
Port Hueneme, CA 93043

Dr. Clemens Meyer
U.S. Army Corp. of Engineers
20 Massachusetts Avenue, NW
DAEN-RDM
Washington, DC 20314-RDM

Dr. Roy Reuter
ICAIR, Life Systems, Inc.
24755 Highpoint Road
Cleveland, OH 44122

Mr. Paul Schatzberg
Annapolis Laboratory, David Taylor
Naval Ship Research and Development
Center
Code 2830
Annapolis, MD 21402

Dr. Ronald Simms
Utah State University
Utah Water Research Laboratory
Civil & Environmental Engineering
Department
Logan, UT 84332-8200

Dr. Ed Smith
USA CERL
2902 Newmark Drive
Champaign, IL 61820-1308

Captain Cathy Vogel
University of Arizona
Civil Engineering Department
Room 206
Tucson, AZ 85721

Dr. Rick Watts
University of Nevada at Reno
Department of Civil Engineering
Reno, NV 89557

APPENDIX 3

PRESENTATION OUTLINES, TEXTS AND VIEWGRAPHS. ARRANGED IN ORDER OF AGENDA PRESENTATION

PRESENTATION BY MS. MARY LINGUA

Lingua

2/14/89

NAVAL CIVIL ENGINEERING LABORATORY

Point Paper
on
Navy Shore Facilities RDT&E Environmental Program

Purpose: The purpose of this paper is to provide definition, information and current status of the Navy's Environmental Protection RDT&E Program. Specific details are provided on hazardous waste minimization efforts.

Naval Civil Engineering Laboratory Mission: The mission of NCEL, as promulgated by NAVFACINST 5450.92A, is to be the principle RDT&E center for shore and fixed surface and subsurface ocean facilities and for Navy and Marine Corps Construction Forces. The Environmental RDT&E Program is designed to maintain Navy compliance with legislation such as RCRA, CERCLA, and international treaties. In doing so the Navy will be allowed to continue to operate without an interruption in mission requirements throughout the world. NCEL's mission in this program is to conduct RDT&E and provide leadership in the coordination of the overall Navy RDT&E efforts relating to the Navy shoreside mission.

Program Organization: The Navy Shoreside Environmental RDT&E Program is organized into 5 major functional categories for purposes of program management, and to facilitate product implementation. These categories are:

**Technology Base Development: This category focuses on the research and development to define new systems and system processes to integrate environmental compliance into Navy system development. Technology base covers a variety of disciplines:

- Biotechnology
- Analytical Procedures
- On Line Diagnostic Sensors

**Pollution Abatement: This category focuses on the testing and evaluating the reliability and life cycle costs of equipment to meet future and impending environmental requirements.

- Air Emissions: Volatile Organic Carbons (VOC, Treatment technologies and Environmentally Acceptable Coatings
- Water and Groundwater: Non Point Source Discharges
- Spill Contingency
- Environmental Effects

**Hazardous Waste Minimization: This category focuses on operationally testing systems and components to eliminate the generation of hazardous waste.

- Industrial Procedures and Supply Policy
- Industrial Processes
- Ordnance Operations and Waste Disposal
- Emptying/Cleaning/Derusting Bilges and Tanks
- Painting/Stripping Operations
- Boiler Lay-up and Cleaning Operations
- Lubricants and Other Fluids Changeouts
- IWTP Reagents Use and Reuse

**<u>Installation Restoration</u>: This category focuses on field intensive testing and validation of cost-competitive and innovative remediation techniques.

-Remediation Technologies
  Biological
  Chemical
  Physical

-Site Restoration And Closure Techniques
-Integrated assessment and Decision tools

**<u>Occupational Safety and Health</u>: This category focuses on the development of guidance and procedures for eliminating Asbestos and Indoor air quality hazards from the Navy workplace.

-Asbestos
-Indoor Air Quality

<u>Installation Restoration Program Goal</u>: The goal of this RDT&E program is to develop processes, equipment, systems, and procedures to eliminate the hazards and liabilities of past Navy waste disposal practices Emphasis is placed on insitu and on site decontamination technologies which will result in returning the contaminated land to military use.

<u>Current Situation</u>: The Navy in FY86-87 conducted an extensive evaluation of past disposal operations at shore installations. This included an evaluation of ongoing remedial investigation, assessment of the state of technology and technological projections that would eliminate long term liability to the Navy in dealing with these sites. Extensive technology assessments were done to identify technologies for insitu and onsite remediation. The following are the results. of this evaluation.

** Navy Installation Restoration is best represented by five generic waste types: 1.Organics contamination of groundwater and soil
2.Combined waste in landfills
3.PCBs and Pesticides contaminating Groundwater and soil
4.Ordnance-related compounds contaminating groundwater and soil
5.Heavy metal contamination of groundwater and soil/sediments

** 28 Technologies were identified for application at Navy Sites. These technologies ranged from Pump and Treat Systems, Chemical Fixation Processes, Oxidation Systems, to Biological Treatment Systems.

**The Strategy that the Navy is currently pursuing to demonstrate these technologies is to develop RDT&E Projects addressing a site cleanup demonstration with the technology in question.

<u>Current status</u>: Currently 10 Installation Restoration RDT&E projects are underway addressing the following technologies:

1. Insitu Biodegadation of Jet Fuel Oil
2. Insitu Biodegradation of Gasoline
3. Chemical Fixation of Heavy Metals
4. Bioreactors for On-Site Treatment
5. Composting/Biodegradation Ordnance-related waste

6. Chemical Treatment of PCB contaminated soil on site
7. UV and Photochemical Oxidation
8. Remote Detection techniques of Hazardous waste sites
9. Underground fuel spill Cleanup techniques
10. Biodegradation Guidelines

__Joint__ __Efforts:__ The Navy, Army, Air Force, as well as research laboratories of EPA and DOE have common goals to develop logical solutions to maintain the natural environment of this planet and eliminate, in the Navy's case, military-caused environmental concerns. As a result of these common goals, expertise and systems developed by other agencies are utilized and cooperatively/jointly evaluated for implementation.

This Point Paper was prepared by the Naval Civil Engineering Laboratory.

WILKINS PAPER NOT AVAILABLE AT THIS TIME

PRESENTATION BY MR. ROBERT P. BARTELL

APPLICABILITY OF BIOTECHNOLOGY TO ARMY ENVIRONMENTAL PROTECTION PROGRAMS

ROBERT P. BARTELL
21 FEBRUARY 89

KEY WORDS

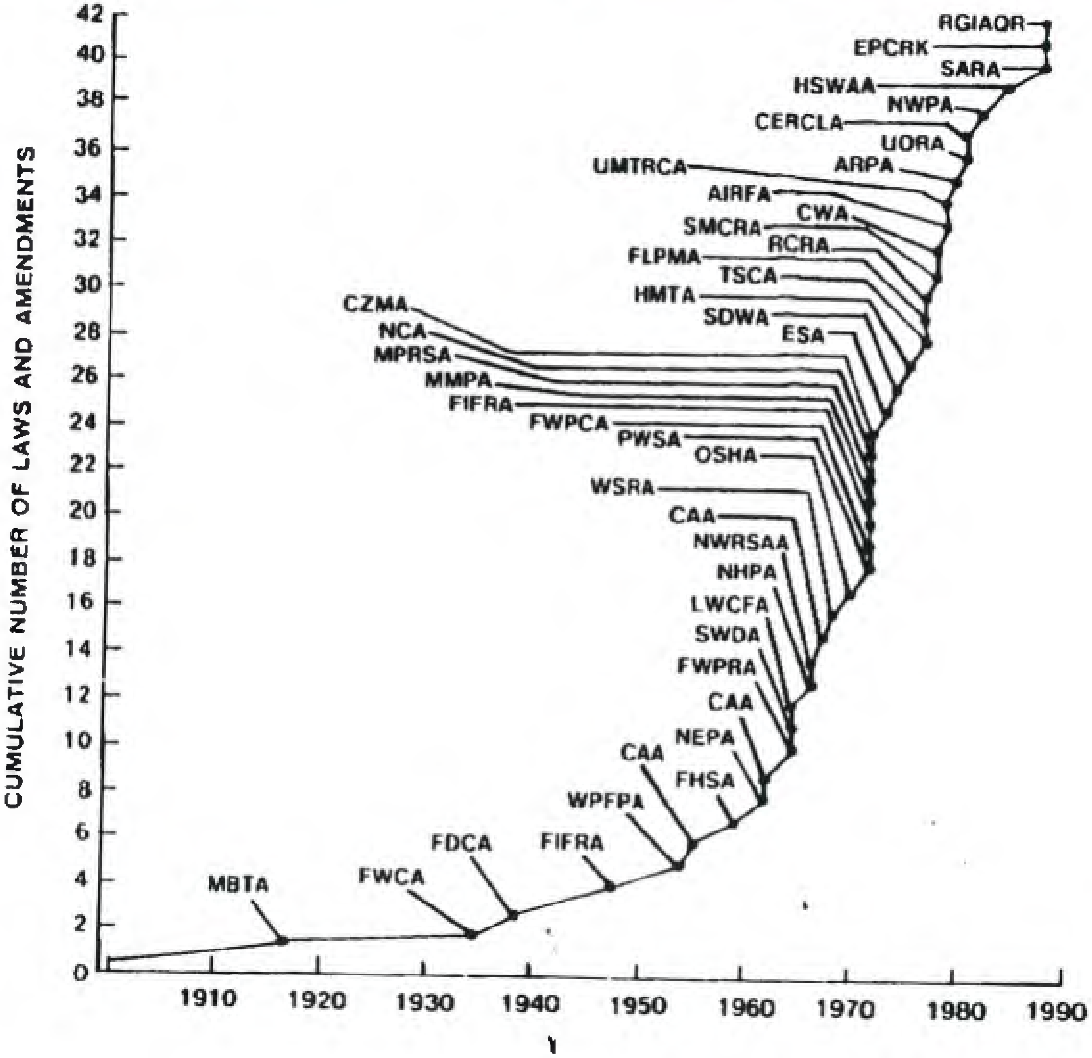
IMPLEMENTATION

COST

NEW TECHNOLOGY DEVELOPMENT
PRIMARY THRUSTS

- DEVELOP NEW TECHNOLOGIES AND IMPROVE EXISTING TECHNOLOGIES TO MAINTAIN ONGOING OPERATIONAL COMPLIANCE WITH ENVIRONMENTAL REGULATIONS
- DEVELOP NEW TECHNOLOGIES NECESSARY FOR ATTAINMENT OF HAZMIN GOALS
- REDUCE ONGOING POLLUTION CONTROL AND INSTALLATION RESTORATION COSTS BY ONGOING PROCESS IMPROVEMENTS AND NEW TECHNOLOGY DEVELOPMENT
- DEVELOP NECESSARY NEW POLLUTION CONTROL TECHNOLOGY TO ASSURE OPERATIONAL READINESS

FEDERAL ENVIRONMENTAL LEGISLATIVE POWER CURVE



REQUIREMENTS IMPOSED BY INCREASED REGULATION

- HIGHER POLLUTANT CONTROL/DESTRUCTION EFFICIENCIES
- MORE ACCURATE/SENSITIVE MONITORING
- HIGHER ADMINISTRATIVE COSTS FOR REQUIRED ASSESSMENTS/PERMITTING/REPORTING

DA HAZMIN PROGRAM

GOAL: BY 1992, REDUCE PRODUCTION OF WASTE BY 50%
(1985 BASE YEAR)

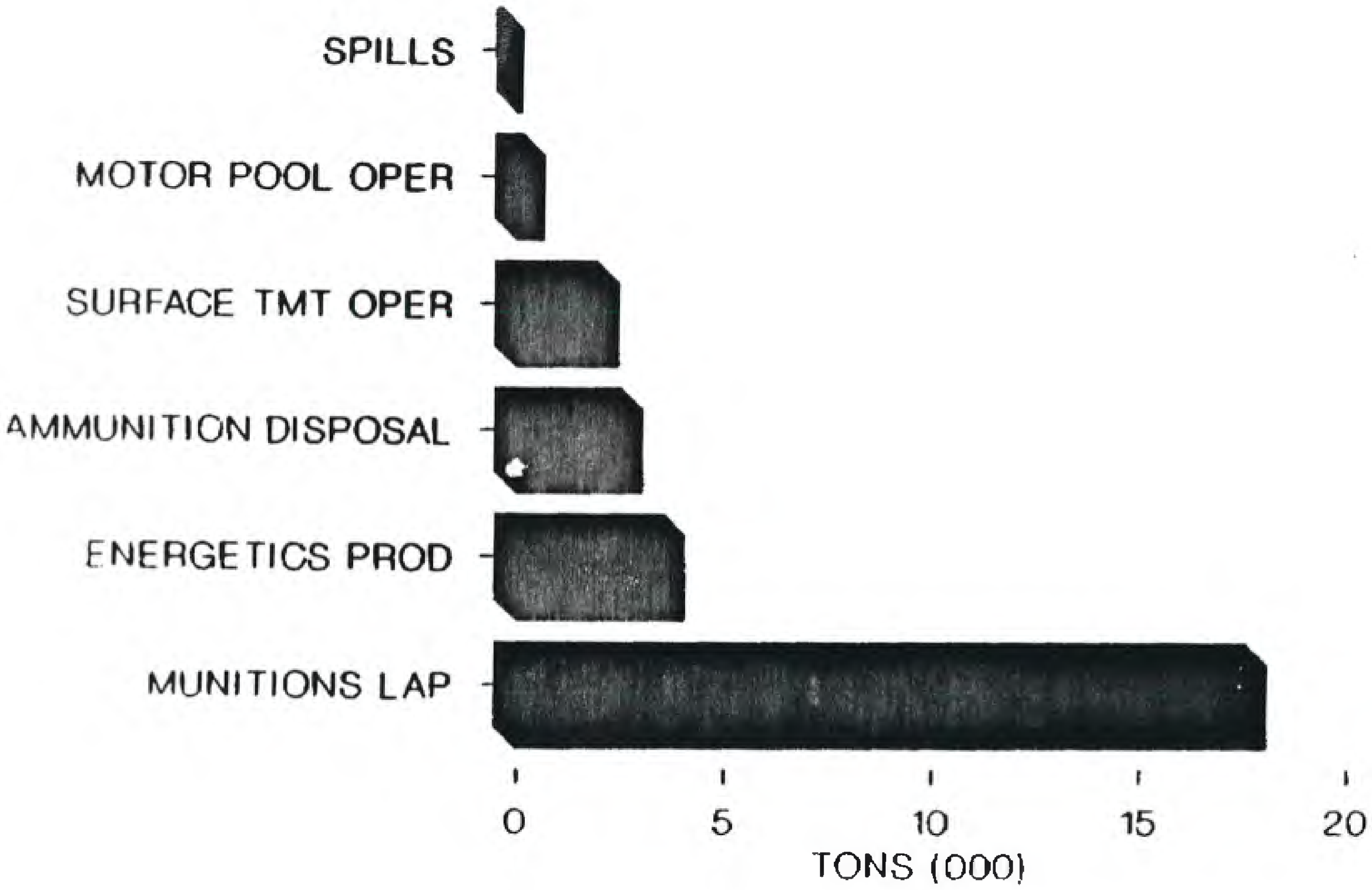
YEAR	85	86	87	88	89	90	91	92	
%REDUCTION GOAL	BASE YEAR	FREE YEAR	12.5	20.0	25.0	30.0	37.5	50.0	
WASTE PRODUCED*	72.5	60.2	64.0						
HAZMIN GOAL			63.5	58.5	54.4	50.8	45.3	36.3	GOAL

* Does not include demil waste, building rubble, or spill clean-up

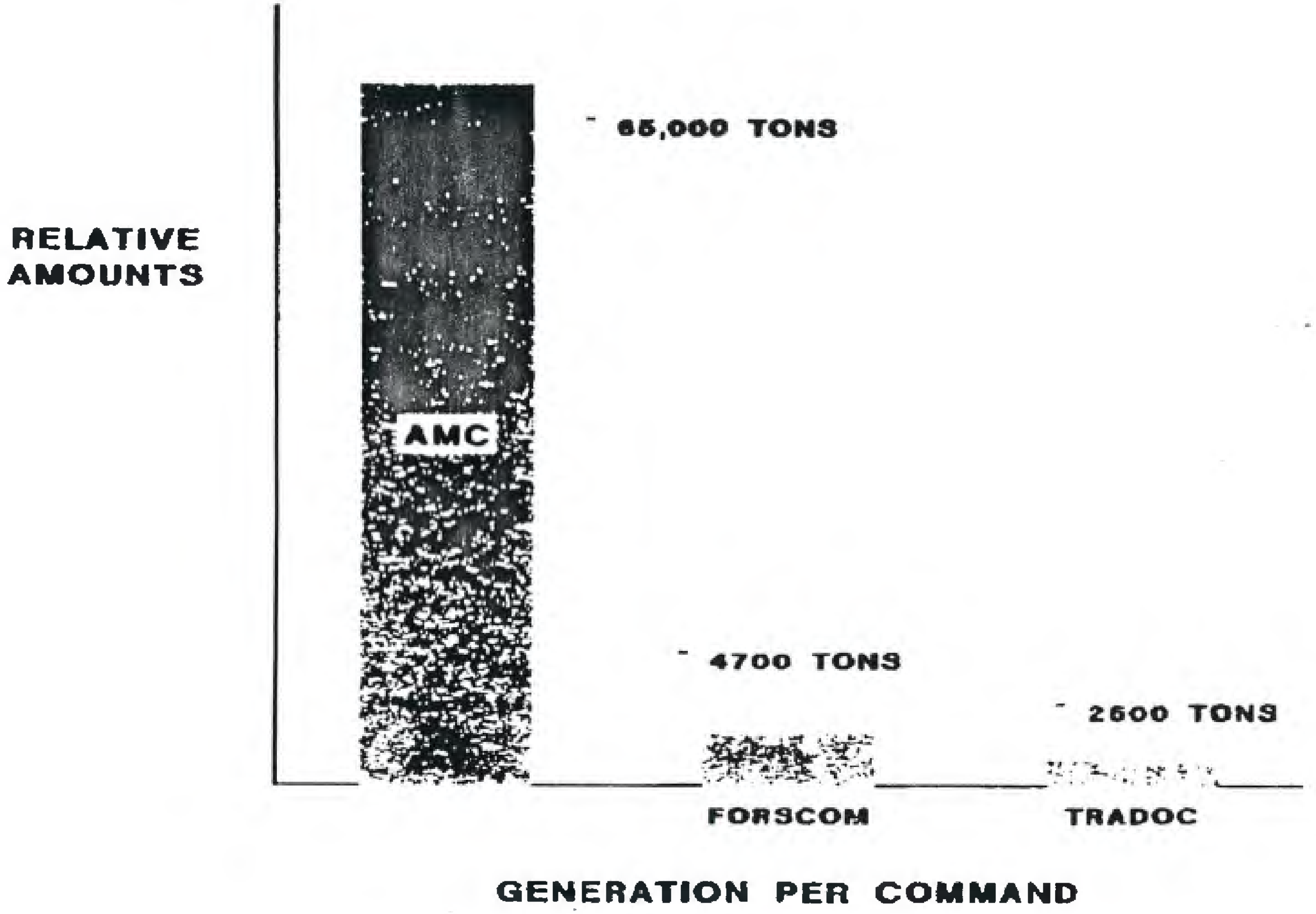
Quantities in 1000 metric tons

HAZARDOUS WASTE GENERATION

GENERATION PER OPERATION IN AMC



HAZARDOUS WASTE GENERATION

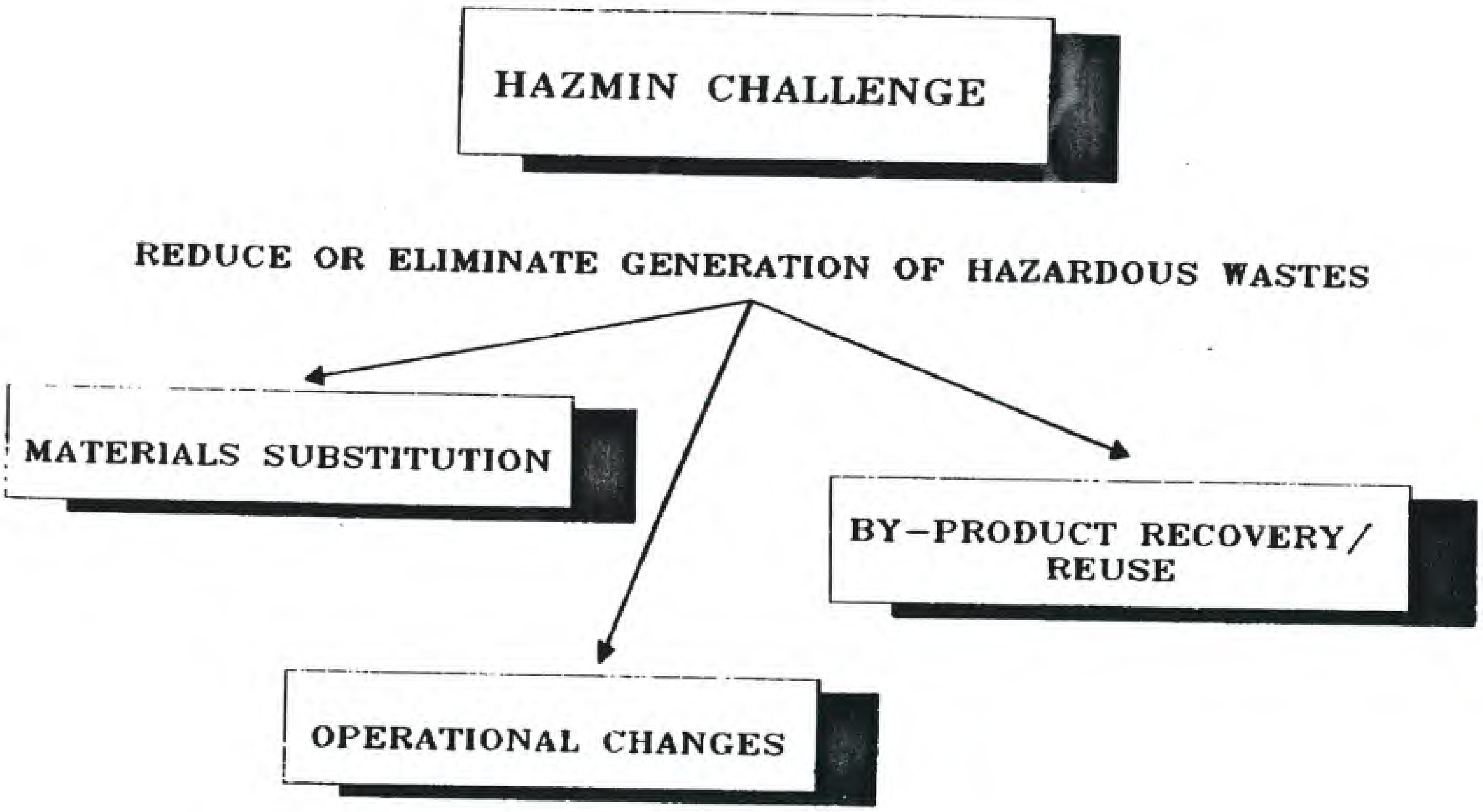


U.S. ARMY
INSTALLATION RESTORATION PROGRAM

- 1391 INSTALLATIONS
- 400+ LAGOONS
- 100,000+ TONS/YEAR OF HAZARDOUS WASTES
- MILITARY UNIQUE COMPOUNDS
- TECHNOLOGY REQUIREMENTS/EXPERTISE
- PAST–CURRENT–FUTURE OPERATIONS
- LONG TERM LIABILITY
- REGULATORY COMPLIANCE--EPA/STATE/LOCAL
- REPORT REQUIREMENTS--CONGRESS/DOD/DA
- COST: MULTI–BILLIONS OF DOLLARS--DECADES OF TIME
- 50 YEAR RECORDS

WASTE DISPOSAL/INSTALLATION RESTORATION TECHNOLOGIES CURRENTLY EMPLOYED

- CONTROLLED INCINERATION
- OPEN BURNING/OPEN DETONATION
- CHEMICAL DEGRADATION/TRANSFORMATION/NEUTRALIZATION
- BIOTREATMENT
- PHYSICAL SEPARATION - CARBON ADSORPTION, AIR STRIPPING
- IMPOUNDMENT
- LANDFILLING
- SALES



HAZMIN CHALLENGE

REDUCE OR ELIMINATE GENERATION OF HAZARDOUS WASTES

MATERIALS SUBSTITUTION

BY-PRODUCT RECOVERY/ REUSE

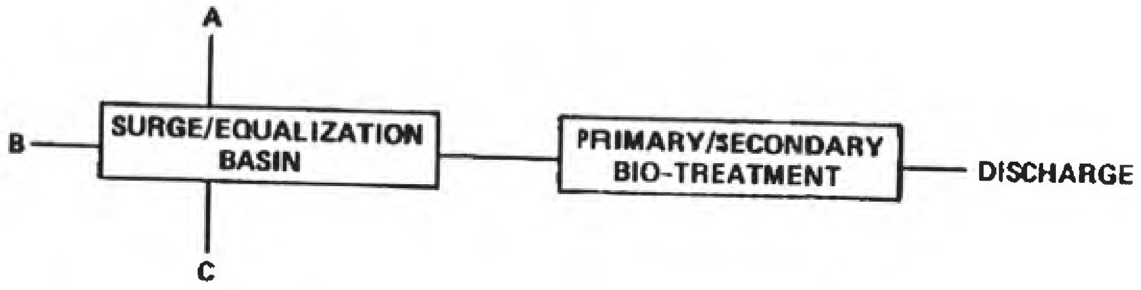
OPERATIONAL CHANGES

RESULTS:
IMPROVED PRODUCTION RATES
IMPROVED PRODUCT QUALITY
DECREASED OPERATING COSTS

CURRENT APPLICATIONS OF BIOTECHNOLOGY

- CONVENTIONAL DOMESTIC WASTEWATER TREATMENT
  - WIDESPREAD
- PRIMARY/SECONDARY TREATMENT OF ENERGETICS PRODUCTION WASTES
  - RADFORD ARMY AMMUNITION PLANT (PROPELLANTS)
  - HOLSTON ARMY AMMUNITION PLANT (RDX/HMX/COMP B)
  - BADGER ARMY AMMUNITION PLANT (BALL POWDER – IN DEVELOPMENT)

COMBINED WASTE TREATMENT



A

B

C

SURGE/EQUALIZATION BASIN

PRIMARY/SECONDARY BIO-TREATMENT

DISCHARGE

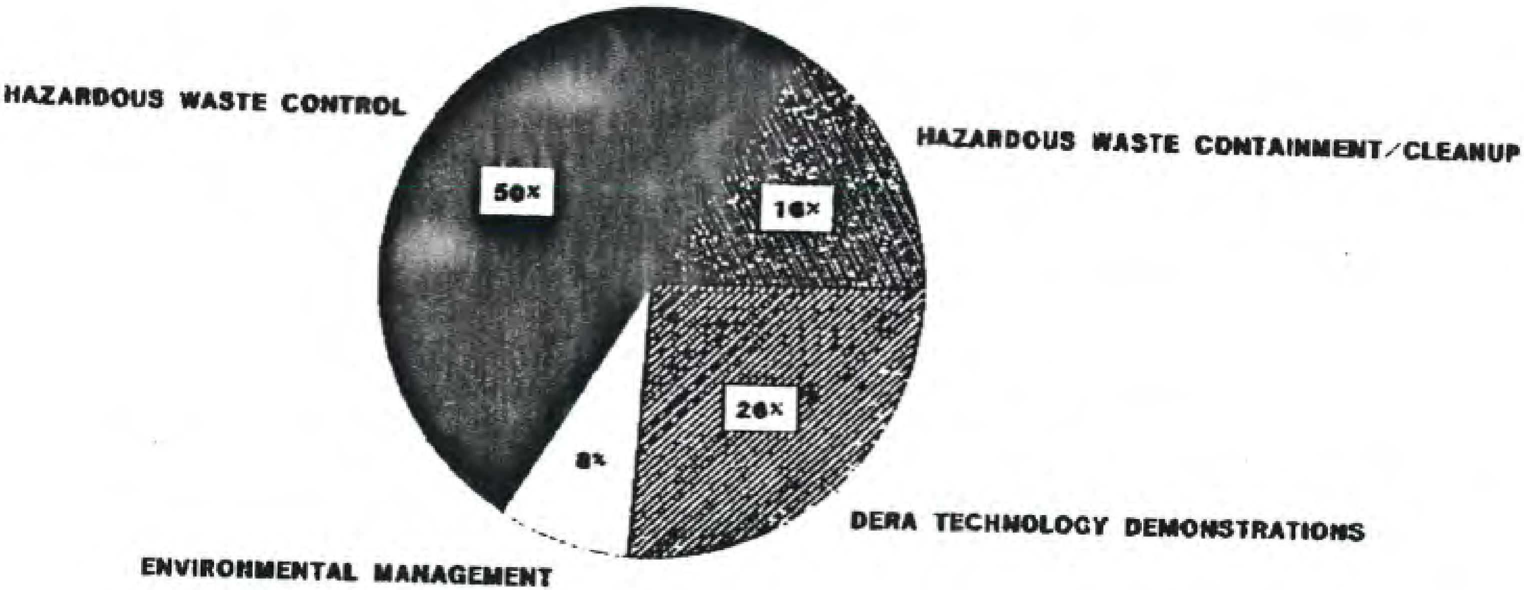
FEATURES:
- HIGH THROUGHPUT (10^5-10^7 GPD)
- PRETREATMENT OF INDIVIDUAL INFLUENTS AS REQUIRED
- LOW UNIT TREATMENT COSTS

PLANNED FUTURE BIOTECHNOLOGY APPLICATIONS

- MORE EXTENSIVE USE FOR ENERGETICS PRODUCTION WASTES
- COMPOSTING OF EXPLOSIVE AND NITROCELLULOSE CONTAMINATED SOIL
- COMPOSTING OF NITROCELLULOSE FINES
- IN SITU TREATMENT OF SOLVENT/POL CONTAMINATED SOIL AND GROUND WATER
- CONTROL OF cuc AIR EMISSIONS FROM SOLVENT CONTAMINATED SOIL/GROUND WATER TREATMENT PROCESSES

FY 1989
ENVIRONMENTAL QUALITY TECHNOLOGY PROGRAM

~\$25m year



NEW INITIATIVES IN TRI-SERVICE COOPERATIVE EFFORTS

- PLASTIC MEDIA BLASTING FOR DEPAINTING OPERATIONS
  - AIR FORCE LEAD
- BIOTREATMENT TECHNOLOGY DEVELOPMENT FOR CONTAMINATED SOIL AND WATER
  - NAVY LEAD
- CONE PENETROMETER DEVELOPMENT FOR COST EFFECTIVE INNOVATIVE FIELD SAMPLING
  - ARMY LEAD

ONGOING AND PLANNED STUDIES AT NAVAL CIVIL ENGINEERING LABORATORY PERTINENT TO ADVANCEMENTS IN BIOTECHNOLOGY

by Ron Hoeppel

Introduction

Research and Development in the Environmental Protection Division (Code L71) at the Naval Civil Engineering Laboratory (NCEL) has emphasized microbial degradation/transformation of organic hazardous wastes, especially those that are most frequently encountered at old landfills, storage areas, and fuel farms at Naval facilities. Of 813 sites at 101 Naval activities identified in June 1987 as requiring RI/FS attention, over a third constituted contamination by fuels, fuel oils and other petroleum products; nearly 10% of the sites are contaminated with organic solvents (mostly chlorinated alkanes and alkenes). More than 5% of Naval sites are contaminated by ordnance wastes.

The Navy is purported to have over 6,000 large underground storage tanks, of which over half are older than 20 years and more than 15% are composed of concrete, which is considered permeable to most organic fluids. Over 75% of the tanks contain refined oils or fuels, such as gasoline grades, diesel grades, and jet fuels (JP-4 and JP-5). The largest underground tanks, exceeding 50,000 gallon capacities, usually contain jet fuels. The number of tanks that are actually leaking is unknown at this time but the above information suggests that underground storage tank leakage is a major problem for the Navy.

Based on limited, mostly demonstration-scale data, on-site microbiological methods for reclaiming contaminated soils, subsoils, surface waters and groundwaters appear to be some where between one-fourth to one-tenth the cost of most competing technologies. In situ methods, using natural indigenous microbiota, should be the most acceptable and least costly treatment technique. However, high variability and poor access to the subsurface environment make in situ bioreclamation more of a science than an art.

Not all hazardous and toxic wastes are known to be biodegraded in an acceptable time period in the natural environment. The reasons for recalcitrance are many. For most petroleum product wastes and many other hydrophobic contaminants, the initial problem may be in producing a larger surface area and more intimate contact between the contaminant molecules and microbial cell membrane. Toxicity through altering metabolic processes, genetic function, or other cytologic systems usually commences once intracellular uptake occurs. If biotechnological advances can produce "superbugs" capable of degrading metabolically recalcitrant compounds in an acceptable time frame through metabolic enhancement, bioaugmentation of contaminated sites with these microorganisms could accentuate removal rates and reduce costs. If recalcitrance is caused mainly by accessibility of contaminant molecules to the microbes, then innovative methods for increasing contaminant surface area per unit volume should increase degradation rates, especially at the higher contaminant concentrations. Production of emulsifiers by "superbugs" is an example of a biotechnological advancement that could feasibly solve this problem.

Regardless, in situ bioaugmentation with genetically altered microorganisms may not be allowed for some time and their use in degrading complex mixtures of organic compounds may never become practical. The thousands of compounds formed during degradation of fuel mixtures are known to involve complex consortia of microorganisms and hundreds of metabolic pathways. Some degradations may also be elicited only under changing environmental conditions (e.g., fluctuating redox potential). Thus in situ and aboveground treatment processes that are engineered for treating wastes through sequential biological or combined biotic and abiotic (physicochemical) processes must also be developed. Development of stable extracellular enzyme systems and studying microbial transport in subsurface environments may also help to bridge the gap between creating bioengineered microbiota and eventually using them in the field.

NCEL Biodegradation Research

Most of the funded research studies concerning biotechnology applications for contaminant removal are tied into actual site cleanups, using funds available under the Defense Environmental Restoration Account. The sites presently being remediated totally or in part by NCEL are listed in Table 1.

The bioremediation technologies being used at each field site are considered to be innovative, thus requiring extensive background feasibility and treatability studies. Details of each site bioremediation and the basic and applied biotechnological research that has or will result during each field site cleanup are given below.

NCS Thurso

This site had about 2200 cu yds of organic soils that were contaminated to a depth of 14 ft with about 1000 gallons of #2 diesel fuel. The chronic leakage contaminated soil under several buildings and migration from the primary spill site contaminated down-gradient surface soils, encompassing an area 100 ft wide, 150 ft long, and about 6 ft deep.

PRESENTATION BY DR. RONALD HOEPPEL

INHERENT PROBLEMS IN IMPLEMENTATION OF NEW TECHNOLOGY

- PERFORMING ACCURATE COST ANALYSES
- USER ACCEPTANCE
- ACQUISITION OF OPERATING/CONSTRUCTION FUNDS

SUMMARY

- A GREATER ROLE OF BIOTECHNOLOGY IN THE DA ENVIRONMENTAL PROTECTION PROGRAM IS FORECASTED
- BIOTECHNOLOGY MUST BE COMPETITIVE WITH OTHER TECHNOLOGIES IN MEETING USER CRITERIA
- BIOTECHNOLOGY DEVELOPMENT EFFORTS MUST BE ACCOMPANIED BY USER INPUT/COORDINATION AND COST ANALYSES AT ALL STAGES

TABLE 1

Biodegradation/Biotransformation Research at NCEL in Support of Field Site Remediations

Navy Activity	Site	Date Initiated	Known Contaminants	Methods of Remediation
NCS THURSO, Scotland	Storage Tank Leak	Mar 85 (Completed Oct 85)	#2 Diesel	*In Situ Sprinkler Irrigation *Trench Leaching *Bioreactor, Bio mass/ Surfactant Additions
PATUXENT RIVER NAS, Maryland	Fuel Farm (Surface/ Subsurface)	Jan 87	JP-5, AVGAS, TCA, TCE	*Free Product Pumping *In Situ Soil Venting & *Bioreclamation
MCAGCC 29-PALMS, Calif.	Small Spills at Field Staging Sites (Surface Soil Spillage)	Aug 88	JP-5, Diesel, Lub Oils, Transmission Fluids	*Bioreactor with VOC Control (Design Stage)
SEAL BEACH NWS, Calif.	Gasoline Station Adjacent to Saltmarsh (Subsurface)	Nov 88	Gasoline #2 Fuel Oil?	*Free Product Pumping *In Situ Soil Venting & *Bioreclamation
SUBASE BANGOR, Wash.	Site D: Ordnance Disposal (Surface Soil)	Nov 88	TNT, RDX, Other Nitro-Organics	*Accelerated Natural Selection of Degraders *Composting

A surface soil treatment system was developed, using a water injection system (5 ft depth) in the primary spill area and surface spray irrigation in the secondary contamination zone. Total flow rate to the system was 10 gpm. Surface runoff from the secondary treatment area was collected in a trench and the water recycled upslope, following treatment in a bioreactor and clarifier; both the primary and secondary treatment areas were treated with recycled water. A proprietary mix of microorganisms (Poly-E bacteria) were added to the bioreactor by the contractor (Polybac Corporation, Allentown, PA) at biomass loadings of 5 pounds the first day, 1 pound on days 2 to 10, and 0.25 pound for the rest of the 105 day treatment period. Ammonium-nitrogen, inorganic phosphate-phosphorus, an emulsifier, and air (oxygen) were added continuously to the clarifier at concentrations of 5, 1, 5, and 3 mg/l, respectively. The average field temperature was 13 degrees C; phosphate buffered the pH between 6 and 7.

This reclamation was successful in removing up to 80% of the fuel that was pumped to the bioreactor during the three month study period. This resulted in converting a merky and oily effluent into a clear and odorless runoff. Vegetation soon developed on the previously denuded soil. Other research projects were not conducted at the site due to insufficient analytical backup and site isolation.

<u>NAS Patuxent River</u>

Sand subsoil, groundwater, and surface water are contaminated by leakage of JP-5 jet fuel and probably aviation gasoline from the main fuel farm. Suspected sources are five 100,000 gallon underground concrete tanks and one 560,000 aboveground steel tank. A spill of several thousand gallons of JP-5 occurred in 1976 and recent leakage from the large steel tank was discovered and corrected in late 1987. Chronic leakage of aviation fuel is thought to be continuing from the fuel farm area. Based on remedial investigations, including installation and monitoring of 34 wells, 222 soil gas analyses, and other geophysical investigations of a 30 acre area, the contamination covers about 15 acres, with the heaviest contamination at depths of 10 to 15 ft. A nearby creek intercepts a confining clay horizon that lies below the contamination. This forces contaminated groundwater and free product to the surface, polluting adjacent surface water bodies. Free product that appears to be weathered JP-5 occurs at the fuel farm proper, free product having gasoline signatures and less weathering is found 1100 ft northwest of the fuel farm, and fresh JP-5 has saturated a couple of acres of subsurface sand immediately west of the above-ground JP-5 tank. Chlorinated solvents are also found in many wells, with concentrations of TCE and 1,1,1 trichloroethane of up to 0.3 and 3 mg/l, respectively. Most of the contamination is present in Ice Age riverine mixed fine sand to gravel strata.

Feasibility and treatability laboratory studies were initiated in Spring 1987, simultaneously with the remedial investigations. These research studies are ongoing. Site remediation is planned to start in late Spring or early Summer 1989. Laboratory research studies are being conducted mainly by universities and research institutes. These include: University of Nevada at Reno (Dr. Rick Watts); Skidaway Institute of University of Georgia (Dr. Dick Lee); University of North Carolina at Chapel Hill (Dr. Fred Pfaender); and Battelle Columbus Labs (Drs. Mickey Arthur and Rob Hinchee). Small business researchers include Dr. Eve Riser (microbiologist, compiling a Users Data Package) and Henry Kerfoot (geophysicist, conducting soil gas analyses and research). Large consulting firms involved in research include International Technology Corporation (Lee Nesbit, Judy Becker, Steve Posten) and Groundwater Technology Inc (Dr. Dick Brown, Mark Hannish).

The University of Nevada biological studies have evaluated:

(a) biotic versus abiotic factors associated with hydrogen peroxide treatment of the subsurface;
(b) stimulation of fuel biodegradations by addition of alternate carbon sources and nitrogen;
(c) soil venting to accentuate biodegradation rates, and
(d) effect of different hydrocarbon concentrations (mainly branched-chain alkane fraction) on biodegradation rates and hydrocarbon degrading populations.

The hydrocarbon dilution experiments, using VOC vials with septa for incubation and sterile clean sand for dilution, showed that degradation rates followed a somewhat geometrical decline with increasing concentrations, as shown in Figure 1. Hydrocarbon concentrations of 500 ppm were degraded in about 75 days. However, when initial total hydrocarbon concentrations exceeded 800 ppm, total degradation was not attained in the 120 day experiment and degradation rates were noticeably reduced. These data may show the effect of drastic decreases in surface area to volume ratio of hydrophobic contaminants in soil matrices when concentrations increase by small increments. At some point the contaminants begin to fill soil pores, which greatly reduces the contaminant surface area and, likewise, microbial activity. The soil venting study, using 2-inch diameter soil columns and high air flow rates, resulted in almost total removal of hydrocarbon contaminants from the sand. The vapor phase has a very high surface area to volume ratio and thus should be readily biodegradable.

The Skidaway Institute research evaluated degradation rates and pathways, using carbon-14 labelled compounds (ring and side chain labelled for aromatics) for contaminants found in fuels (benzene, toluene, naphthalene, methyl-naphthalene,

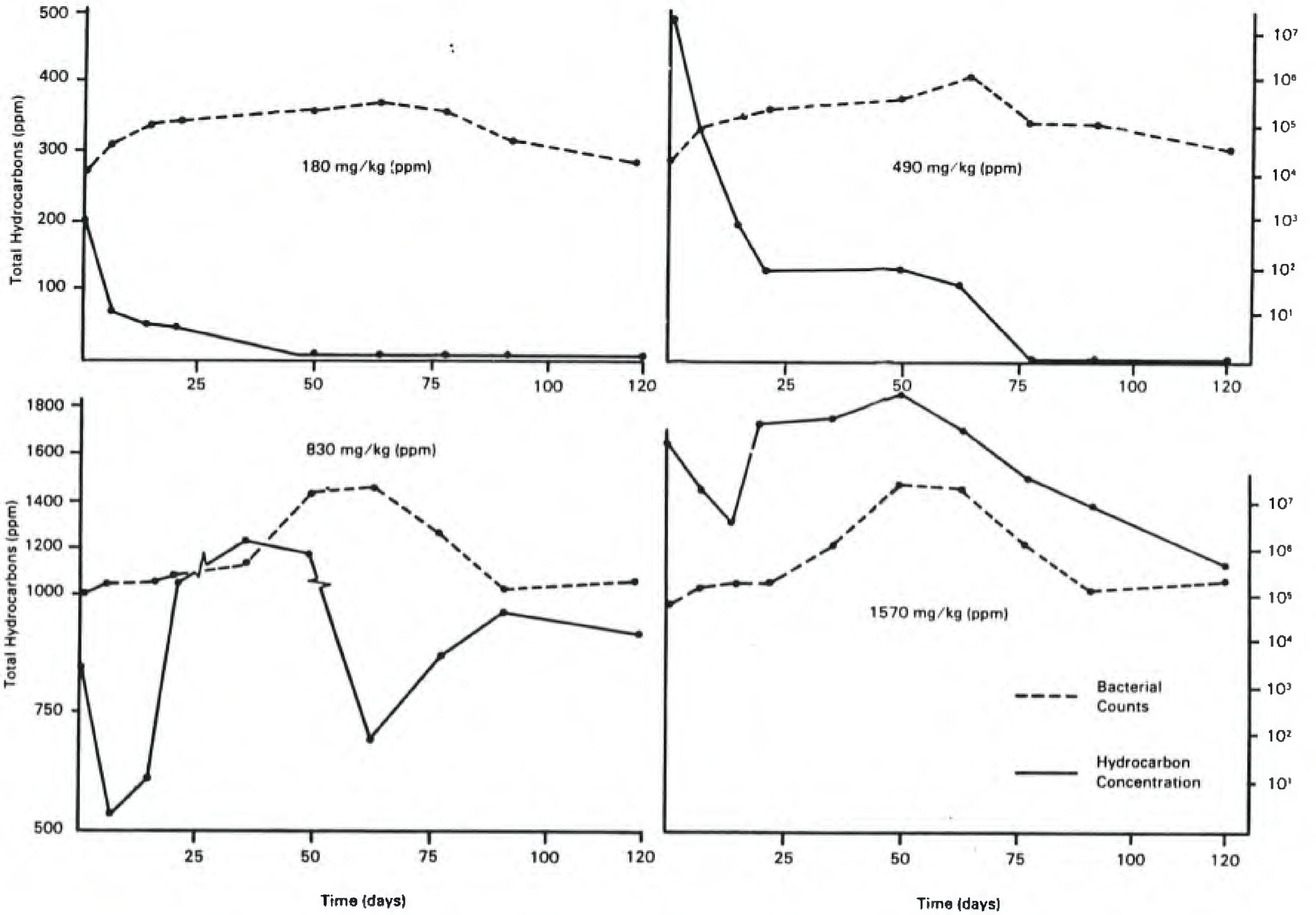


Figure 1. Effects of hydrocarbon and bacteria concentrations in sand on hydrocarbon degradation rates.

phenanthrene, and hexadecane), and compared these data to PAHs not found at the field site (anthracene and fluorene). The aromatic compounds, including phenanthrene, were completely degraded in very short time periods (half-lives of 1 to 6 days) when added to previously contaminated sand or peat from the field site at concentrations of 1 to 2.5 ug/g substrate. PAH compounds not found in fuels were degraded very slowly. In contrast, hexadecane was degraded three times faster in uncontaminated surface sand than in contaminated sand from the fuel farm. Competing substrate utilization may control alkane utilization and enzyme induction appears important for aromatic degradations. Future research will emphasize biodegradation pathways for branched-chain alkanes and cycloalkanes, both groups being important in aged fuel contamination.

Studies at Battelle and Groundwater Technology showed microbial sensitivity of soil hydrocarbon-degrading microflora to hydrogen peroxide concentrations greater than about 100 ppm (even 10 ppm for some groups). Additionally, forced aeration to unsaturated sediment in 1 ft diameter soil columns was as effective as hydrogen peroxide in adding oxygen to the subsurface. Battelle screened 60 commercially available surfactants and found three that were nontoxic to hydrocarbon degrading microflora while mobilizing the contamination at rates superior to acetone extraction. These surfactants will be used in upcoming research aimed at increasing biodegradation rates of high hydrocarbon concentrations. Groundwater Technology, while conducting microcosm studies, noted abrupt increases in hydrocarbon degradation rates in contaminated field sand after 120 days incubation. This increase was associated with a ten fold increase in the aqueous phase hydrocarbon concentration. This suggests that natural surfactants may play an important role in the degradations or that specific consortia of microorganisms are required to degrade soluble metabolites. The relation of these findings to those of the University of Nevada will be evaluated in future studies.

Field research conducted during remedial investigations at the fuel farm included:

(a) comparisons of different soil gas analysis methods;
(b) comparisons of well head space and groundwater analyses with remote soil vapor data; and
(c) evaluations of field analytical procedures (e.g., field GC data) with laboratory analyses.

Most data showed highly variable concentration patterns in the subsurface, indicating the importance of micro-environments and microbial interactions on degradation of complex mixtures of hydrocarbon compounds. Remote soil vapor data proved to be an invaluable semiquantitative tool for rapidly and cost effectively characterizing the subsurface near the Patuxent River fuel farm. Future planned work may include continuous remote monitoring of hydrocarbon vapors and carbon dioxide at the field Pilot Study area to evaluate the correlative value of such data to actual site decontamination.

MCAGCC Twentynine Palms

The Marine Corp Combat Center at Twentynine Palms, which covers an area almost the size of Rhode Island, has accumulated approximately 40,000 cu yds of hydrocarbon-contaminated sand and sandy soil at a 5 acre storage facility. The annual input is about 10,000 to 15,000 cu yds. This contamination consists mainly of diesel and JP-5 fuels but also includes some leaded aviation gasoline (AVGAS), unleaded gasoline, #6 fuel oil (bunker), lubricating oils, transmission fluids, and some degreaser solvents. The contamination comes mainly from large fuel bladder and small storage tank leakage as well as soil from vehicle washdown pads. While most existing contamination is weathered, having set at the storage area for up to three years, new materials are being added regularly and the pollution sources will be continuous for many years to come. The desert climate has promoted volatilization and probably inhibited biodegradation.

A permanent treatment facility will be constructed at the base, with a final design based on Pilot-scale studies. This facility must inhibit the release of VOCs to the atmosphere and is planned to promote biodegradation. To date, contaminated soil samples have been collected from the storage area as well as fresh contaminated sand from each main source area of contamination. Hydrocarbon degrading microbial populations have shown minimal metabolism in the usually dry sandy soils of this desert site. Several designs for the enclosed treatment facility are being developed.

The pilot studies for different selected treatment systems will follow results from ongoing laboratory feasibility and treatability studies. These studies are being conducted at the University of Southern California (Dr. Joe Devinny); AeroVironment, Monrovia, CA (Dr. Kalique Khan); and OH Materials Corp, Findlay, OH (Paul Flathman). Field pilot studies will probably not be conducted until sometime after next year.

NWS Seal Beach

Contamination at Seal Beach is leaded gasoline that leaked from an underground tank at the base gasoline station prior to 1983. Analytical signatures, odor and specific gravity of free product indicate that the site is also contaminated by diesel fuel. Most contamination lies just above the water table, which fluctuates between 7 to 9 ft depth. Since the site is adjacent to a saltmarsh (Seal Beach National Wildlife Refuge), groundwater is influenced by the tides, with maximum fluctuations of 0.5 ft. The subsurface stratigraphy varies from tidal clay at the surface to sand in the zone of contamination. The clay stratum helps to prevent rapid water infiltration into the shallow aquifer from the adjacent wetlands and ocean.

Site remediation is being conducted by the Navy Energy and Environmental Support Activity (NEESA). Their tentative plans call for free product removal from extraction wells, soil vapor extraction, and/or bioreclamation. NCEL will be conducting laboratory feasibility and treatability studies and a small pilot study. The research areas include:

(a) characterization and identification of aerobic and anaerobic gasoline-degrading microorganisms;
(b) nutritional regulation of gasoline hydrocarbon metabolism;
(c) bacterial mobility and survival in the subsurface;
(d) hydrogen peroxide transport and reactivity in the subsurface;
(e) influence of soil physicochemical characteristics on biodegradation kinetics; and
(f) anaerobic biotransformation of fuel hydrocarbons.

Microbial identifications will be aided by use of computerized gel electrophoresis, which separates and categorizes specific microbial proteins following incorporation of radiolabelled sulfur into their structure. Information concerning mobility and survival of microorganisms in the subsurface is important if microorganisms are added to <u>in situ</u> bioreclamations to accentuate contaminant degradations. Tracer microorganisms would be added to laboratory columns of different soil types. Conservative tracer microorganisms, that migrate only by passive diffusion, could include endospores from the anaerobic gut bacterium <u>Clostridium perfringens</u> or spores from <u>Thermoactinomycetes</u> sp.; these propagules are the size of bacteria but the spores germinate only at elevated temperatures and on highly selective media. Growth of select hardy and sensitive bacterial strains that have very unusual metabolic mutations (e.g., resistance to two antibiotics) would measure active movement of bacteria through the subsurface. The aerobic treatability studies, bacterial characterizations, and bacterial mobility studies will be conducted at the Orange County Water District, Fountain Valley, CA (Dr. Harry Ridgway).

Research devoted to understanding and accentuating anaerobic degradations of fuel hydrocarbons is important because movement of free oxygen into contaminated subsurface environments is often the most difficult and labor intensive step in conducting successful <u>in situ</u> bioreclamations. The proposed research will concentrate on increasing the degradation rates of simple aromatic hydrocarbons. Since nitrate can serve as both nitrogen and oxygen sources for nitrate respiring (denitrifying) bacteria, the denitrifier group (which are aerobes in the presence of free oxygen) will be emphasized. However, bacteria that use other reduced compounds as terminal electron acceptors will also be evaluated. Stanford University (Dr. Martin Reinhard) and the Army Corps of Engineers at the Waterways Experiment Station, Vicksburg, MS (Dr. Doug Gunnison) will be doing most of the anaerobic work. The research concerning the Seal Beach site has just been initiated, with no significant results as yet.

Subase Bangor

Highly nitrated compounds such as munitions (TNT, RDX) have not been shown to be biodegradable under controlled laboratory conditions. However, there are many references to complete biodegradation of these materials in natural, poorly controlled systems involving many microorganisms. The white-rot fungus, Phanerochaete chrysosporium, has recently been implicated in munitions degradations but data is minimal. NCEL will attempt to further the present knowledge concerning biodegradation and biotransformation of munitions, using contaminated soils from an ordnance disposal and detonation area (site D) at the submarine base near Silverdale, Washington. The site has variable soils, composed mainly of glacial till and underlying clayey subsoil, both of which are contaminated. Most of the contamination is restricted to the surface soil layers at this site and the major method for mobilization is from surface runoff and soil erosion.

Planned research, which has just been initiated, will address several different treatment options involving biotechnology for the main treatment mode. These include:

(a) analog enrichment, using more easily degraded chemical analogs to induce enzyme production or proliferation of microorganisms elliciting degradation;
(b) anaerobic degradation that might promote use of nitro groups by natural microbial populations as terminal electron acceptors;
(c) coupling of different specialized preadapted microbial populations in serial arrangement to further degrade intermediate metabolites, using perhaps varying soil environmental conditions;
(d) chemical pretreatment to remove one or more nitro groups that seem to inhibit enzyme attack on the molecular ring structures; and
(e) use of radiolabelled munition compounds to assess bacterial and fungal degradation pathways in complex soil matrices.

In each of the above cases a preliminary microbial characterization would be conducted and analytical procedures developed for determining intermediate metabolites. Microbial determinations of the field site are ongoing and are being conducted by ECOVA Corporation, Redmond, WA. Plans are for

Utah State University (Dr. John Bumpus) and ECOVA to conduct fungal and bacterial treatability studies, respectively. USATHAMA, Aberdeen Proving Grounds, MD (Wayne Sisk) will monitor and evaluate results of ongoing field demonstrations at different sites where composting of ordnance wastes is being assessed. The EPA Gulf Breeze (FL) Laboratory (Dr. Hap Pritchard) may provide peer review of these studies.

Suggested Research Topics

Literature reviews, technology assessments and research being conducted by NCEL have shown a number of gaps in present knowledge concerning use of biotechnology for destroying hazardous and toxic wastes. These gaps, problem areas or suggested treatment techniques are discussed below by type of contaminant or treatment method.

Biodegradation of Fuels and Refined Oils

Several Navy bioreclamations will be evaluating multiple treatment schemes involving physical treatment by soil venting combined with conventional biological treatment. We have proposed both aboveground and *in situ* treatments of fuel contaminated soils that combine these two technologies to reduce fuel concentrations in soil at the most rapid and cost effective rates. For example, soil venting/stripping have been shown to be effective for removing gasoline to low final levels in porous media; research indicated the same for jet fuels (JP-4, JP-5). Our research findings have shown that JP-5 biodegrades rapidly when soil concentrations are less than about 500 mg/kg sand. Besides decreasing contaminant concentrations, soil venting/stripping have the potential for moving oxygen at appreciable rates into unsaturated subsurface or excavated contaminated soils. Some research has shown a positive correlation between forced vapor extraction and biodegradation rate but hard data are lacking.

Figure 2 is a schematic of an aboveground bench or pilot-scale treatment system that uses biodegradation for final removal of all contaminant phases: vapor phase, emulsified phase, water phase, and adsorbed phase. This system would be totally contained so that a balanced sheet showing contaminant routes and quantities could be determined. Such a system would give us concise information about the efficiency of vapor extraction/bioreclamation combined treatment.

The described soil treatment system (STS) outlined in Figure 2 would consist of an enclosed vessel or small building impermeable to liquids, with air tightness and a slightly negative atmospheric pressure inside. The bottom (subflooring) of the STS would be equipped with air lines connected to many evenly spaced air diffuser blocks and water lines would be connected to high velocity spray nozzles. Hydrocarbon-contaminated sand from field sites would be

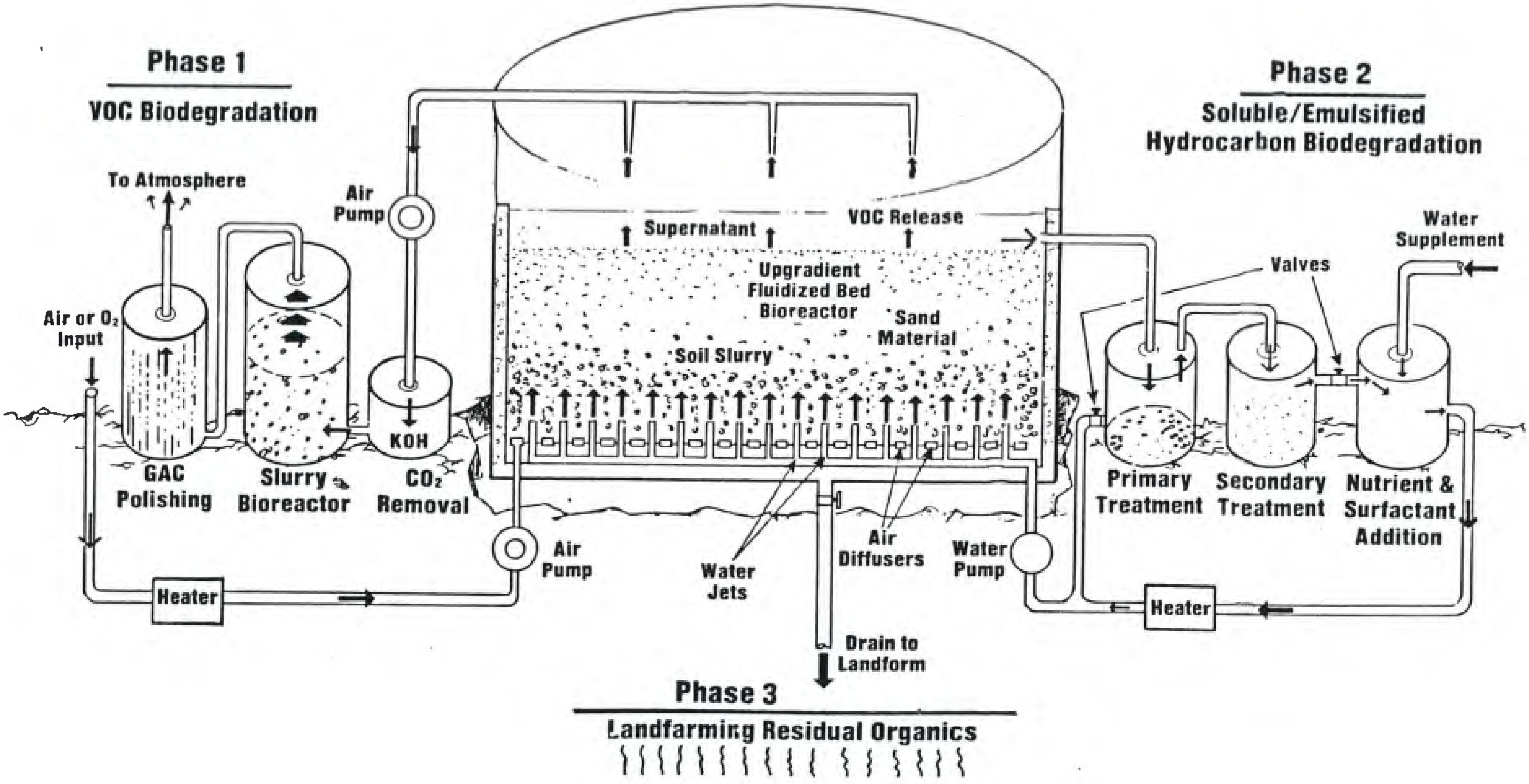


Figure 2. Design for On-Site Controlled-Environment Bioreactor Systems for Treating Soil, Vapor Phase and Aqueous Phase Hydrocarbon Contaminants

placed in the STS. Treatment would occur in three phases, the first two in the enclosed STS. Phase 1 would entail air stripping and soil venting the sandy soil to remove the volatile hydrocarbons (VOCs) at an accelerated rate. The air would continuously percolate through the sand, even during phase 2, and initially the air could be water saturated prior to stripping to promote biodegradation activity. The air would be sucked from the top of the STS and diffused in a small bioreactor containing a sorptive solid such as activated carbon, which would promote biodegradation of the vapor phase. If carbon dioxide inhibits biodegradation, an alkaline trap could be installed before the vapor phase bioreactor. An activated carbon or ion exchange column could be used to trap any remaining hydrocarbons in the air. Phase 2 treatment would be initiated when VOC levels in the air vented from the STS start to decline noticeably. This treatment phase would concentrate on removal of remaining contamination that is soluble or readily emulsified in water containing microbial nutrients and a nontoxic biodegradable surfactant. In large scale, these additives could be automatically injected into the final tank of a small wastewater treatment system, which would elicit primary and secondary treatment. Water would be pumped initially from an external source but most water would be recycled through a closed loop between the STS and the wastewater treatment plant. High pressure injection nozzles beneath the STS should churn the contaminated liquified sand like a fluidized bed biological reactor. Fine particles and oil would be removed from above the sand zone and this effluent would flow by gravity back to the wastewater treatment system. Oily sludge that collects in the primary treatment system (settling tank) could be reinjected through backflushing into the next batch of contaminated dry soil at the beginning of phase 2 treatment. This sludge should contain high numbers of acclimated microorganisms and such injection could speed up acclimation of the new batch of sand. Phase 3 is initiated when hydrocarbon concentrations in STS effluent level off. This phase, in large scale, entails removal of treated and drained soil from the STS and further treatment like a landfarming operation. Water drained from the STS and nutrients would be added periodically to induce degradation of the more recalcitrant hydrocarbon compounds. This phase would require minimal management since the sands should not contain highly mobile fractions that would readily contaminate the atmosphere or groundwater.

Questions to be answered concerning <u>in situ</u> and aboveground treatment of fuel-contaminated soils include:

(a) Can VOCs be rapidly degraded in bioreactors?
(b) Will soil venting/stripping increase biodegradation rates <u>in situ</u> and aboveground when both procedures are used in tandem?
(c) Will biodegradable and nontoxic surfactants aid or hinder biodegradation rates when used <u>in situ</u> or

aboveground?

(d) How do the best oxygenation methods for saturated soils compare to aeration of the vadose zone in terms of contaminant removal rate and cost?

Site Monitoring Problems

Laboratory data obtained by the University of Nevada at Reno and field data obtained at the NAS Patuxent River fuel farm indicate that soils are highly variable (thus promoting highly variable degradation pathways in microenvironments). These data also indicate that we need to further assess our analytical capabilities. EPA-approved analytical procedures developed for petroleum may be totally unacceptable for analyzing highly volatile fuels. Also, how do gas chromatograph and other analytical instruments differ in sensitivity to degradation products (having different water solubilities, volatilities or soil binding energies) and how do changing sensitivities affect final interpretation of data collected routinely during a bioreclamation?

Microbial Transport and Survival

If microorganisms are ever to be considered for injection into the subsurface during *in situ* remediations to enhance degradation rates, we need to know if they will remain viable or be mobile enough to reach the contamination zones within an acceptable time frame. Some research indicates that passive diffusion of bacteria sized particles through soils can be very slow, even through sand. Other data suggest rather rapid transport of viable microorganisms into compact sandstones. Additional studies need to be conducted to determine if injection of special microorganisms will be of value for treating the subsurface.

Anaerobic Degradation Technology

Information and technology concerning degradation pathways, rates and requirements for anaerobes is lacking. Recent data certainly brought anaerobic wastewater treatment to the forefront while, previously, few had confidence in such systems from a practical standpoint. Future research at varying redox potentials may likewise make anaerobic treatment technology practical for *in situ* degradations.

Munitions Degradation Research

Research on microbial metabolism of highly nitrated munition compounds and wastes is far behind that for nitrated pesticides. Findings are mixed and test tube data seem to be different from results obtained with munition-contaminated soils. Although biotransformations of these compounds occur, producing even more toxic or mutagenic products (e.g., where nitro groups are reduced to amino groups), it is not well known if these products are toxic or mutagenic in soils. Microorganisms tend to strongly bind many harmful compounds into soil organic matter. Further research may be needed to evaluate the fate of munitions compounds in different soils.

PRESENTATION BY DR. SACHIO YAMAMOTO

ENVIRONMENTAL BIOTECHNOLOGY

NAVY EXPLORATORY DEVELOPMENT PROGRAM

- Biodecontamination of On-Shore HW (JP-5)
- Bioreactors for HW Degradation (Otto fuel, Organo-tin)
- Analytical Methods Development
  - Fiber Optic Chemical Sensors
  - Spectrometric determination of classes of organic compounds

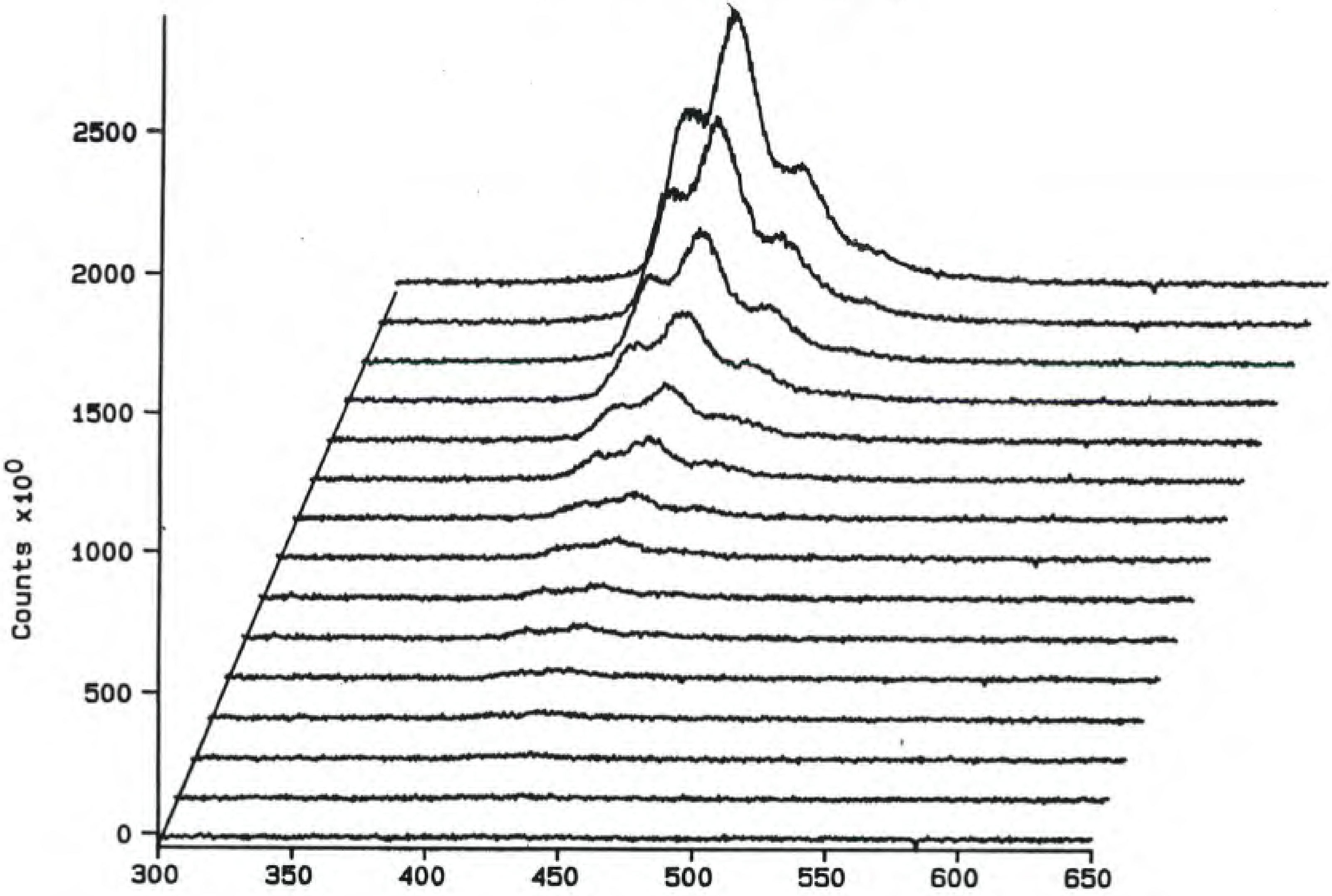
FUNDING:	FY89	FY90	FY91	FY92
	300K	300K	430K	400K

Fiber Optics Chemical Sensors (6.2 & [illegible])

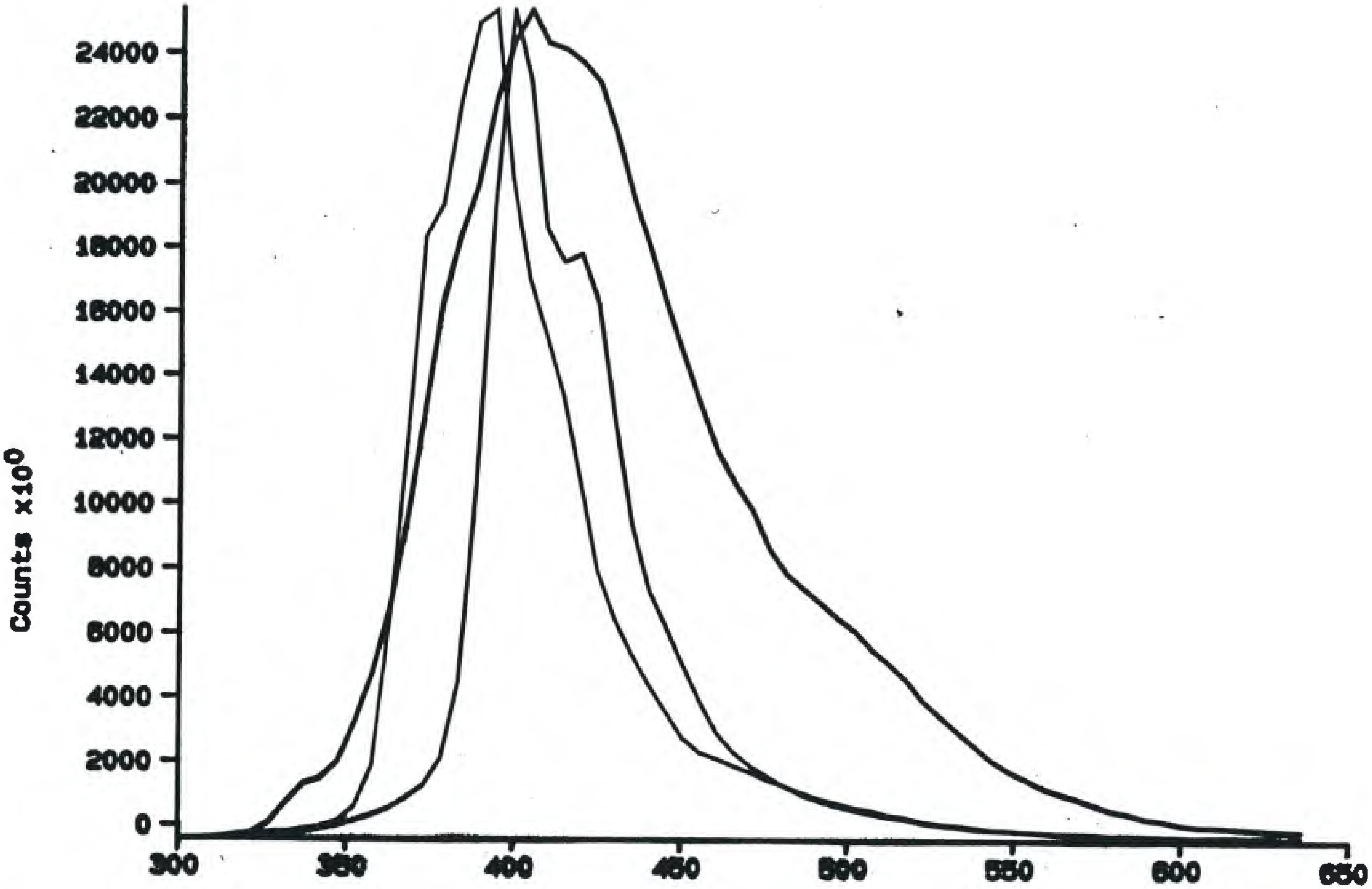
[illegible]

- Develop fiber-optic-based sensors [illegible] fluorescent [illegible] of organic compounds
- Optimize sensor specificity
- Develop "indicators" for nonfluorescent species

Time-Resolved Fluorescent Decay Spectra of Anthracene



Anthracene in EtOH, 2 ns increments, Disk 2, File 19, 4/12/88.



lack - anthracene, Blue - pyrene, Red - chrysene.

Decay-times of PAHs in Seawater

Fluorophore	Emission	Decay-time	Lit
Anthracene	404 nm	4.0 ns	5.0
Chrysene	399 nm	9.7 ns	43
Pyrene	394 nm	128.9 ns	290
Benzo(a)pyrene	425 nm	4.7 ns	15
Fluoranthene	466 nm	32.8 ns	53

ORGANICS ANALYSIS

CONVENTIONAL APPROACH

TAKE FIELD SAMPLE TO LABORATORY
MEASURE INDIVIDUAL PRIORITY POLLUTANTS
MULTIPLE SOLVENT EXTRACTIONS
CHROMATOGRAPHIC SEPARATION AND DETECTION

NEW APPROACH

DETERMINE CHEMICAL CLASSES
SIMPLE GROUP MEASUREMENTS
RAPID FIELD DETERMINATIONS

APPLICATIONS

QUICK RESPONSE
MAP IMPACTED AREA
IDENTIFY SAMPLES FOR DETAILED ANALYSIS
MONITOR CLEAN-UP EFFORTS

ORGANICS ANALYSIS

APPROACH

- Determine classes of organic compounds by spectrometric means
- Develop simple group measurement system using suite of spectrometric methods (UV, IR, Raman)
- Establish Database

GROUP CHARACTERISTICS

Compound	UV	Fluorescence	IR
PCBs	250 nm log ε 2	no	3100– 3000 cm-1
PAHs	250 nm log ε 4–5	yes	3100– 3000 cm-1
NAHs	no	no	3000– 2850 cm-1

PCB = polychlorinated biphenyl
PAH = polyaromatic hydrocarbon
NAH = non–aromatic hydrocarbon

CHEMICAL CLASSES	SOURCE
NON-AROMATIC HYDROCARBONS	
HEXANE	SOLVENT
AROMATIC HYDROCARBONS	
BENZENE	SOLVENT
TOLUENE	
CHLORINATED HYDROCARBONS	
1,1,2-TRICHLOROETHANE	SOLVENT
1,2,4-TRICHLOROBENZENE	
PCBs	TRANSFORMER FLUID
POLYAROMATIC HYDROCARBONS	
BENZOPYRENE	INCOMPLETE COMBUSTION
PHENANTHRENE	

ENVIRONMENTAL BIOTECHNOLOGY
ORGANICS ANALYSIS

MILESTONES

FY88
COLLECT AND MEASURE SPECTRA

FY89
DEVELOP SPECTRUM ANALYSIS PROTOCOL

FY 90
TEST ON BIOREACTOR PROCESS

FY91-92
DEVELOP PROTOTYPE ANALYZER

QUANTIFICATION IN MIXTURES

$$A_n = C_1(\varepsilon_1)_n + C_2(\varepsilon_2)_n$$

$$A_m = C_1(\varepsilon_1)_m + C_2(\varepsilon_2)_m$$

m,n = two different wavelengths
A_n, A_m = absorption at n and m
C_1, C_2 = concentrations of 1 and 2
$(\varepsilon_1)_n, (\varepsilon_2)_n$ = extinction coeff. of C_1 and C_2 at **n**

PRESENTATION BY MR. WAYNE SISK

APPLICABILITY OF BIOTECHNOLOGY TO ARMY ENVIRONMENTAL PROTECTION PROGRAMS

WAYNE E. SISK
21 FEBRUARY 89

PROJECT NO. 1L161102AH68
PHYSICAL/CHEMICAL MECHANISMS

SUMMARY OF PROJECT OBJECTIVE

DETERMINE BASIC CHEMICAL/PHYSICAL/BIOLOGICAL MECHANISMS OF SELECTED POLLUTANT TREATMENT/REDUCTION/CONTAINMENT PROCESSES TO ENHANCE EFFECTIVE FUTURE DEVELOPMENT OF POLLUTION ABATEMENT TECHNOLOGIES ON A TECHNICAL RATHER THAN EMPIRICAL BASIS.

BASIC RESEARCH

CURRENT EFFORTS

- EVALUATION OF NATURAL DEGRADATION OF SELECTED ROCKY MOUNTAIN ARSENAL CONTAMINANTS
- DEFINITION OF FATE OF CHEMICAL AGENTS IN CONTACT WITH MASONRY MATERIALS

PLANNED/UNFUNDED

- ADDITIONAL STUDIES TO DEFINE FATE OF CHEMICAL AGENTS AND EXPLOSIVES IN CONTACT WITH MASONRY MATERIALS
- EVALUATION OF BIODEGRADATION MECHANISMS FOR VOC'S IN AIR EMISSIONS, SOIL AND GROUNDWATER
- EPA JOINT STUDY ON MECHANISMS FOR RECOVERY OF HEAVY METALS FROM SOLID WASTES

POLLUTION ABATEMENT AND ENVIRONMENTAL CONTROL TECHNOLOGY (PAECT)

TO DEVELOP PHYSICAL, CHEMICAL, AND BIOLOGICAL TREATMENT METHODS WHICH WILL ASSURE MEETING DISCHARGE CRITERIA AT ARMY INDUSTRIAL FACILITIES; TO DEVELOP PROCESSES AND ECONOMICS FOR COST EFFECTIVE RECOVERY OF ENERGY AND REUSABLE MATERIALS FROM EXCESS OR OBSOLETE CONVENTIONAL MUNITIONS; AND TO DEVELOP COST-EFFECTIVE TREATMENT METHODS FOR HAZARDOUS MATERIALS IN METAL FINISHING WASTEWATERS AND SLUDGES, PAINT WASTES, AND DEGREASER SOLVENTS

PROJECT TASK AREAS

- MUNITION WASTE
  - CONTROL OF POLLUTANTS FROM MUNITIONS MANUFACTURING/HANDLING OPERATIONS

- TACTICAL VEHICLE MAINTENANCE WASTE TREATMENT
  - CONTROL OF PAINTING/PLATING/DEGREASING WASTES

- HAZMIN
  - RECOVERY/REUSE
  - IN-PROCESS CHANGES
  - MATERIAL SUBSTITUTIONS

PROJECT DO48 POLLUTION ABATEMENT AND ENVIRONMENTAL CONTROL TECHNOLOGY (PAECT)

PRIMARY THRUSTS

- BALL POWDER, NITROCELLULOSE, TNT, AND LIQUID PROPELLANT PRODUCTION WASTE TREATMENT
- RECOVERY/REUSE OF WASTE EXPLOSIVES AND PROPELLANTS
- IMPROVED DISPOSAL TECHNOLOGY FOR SOLID WASTES FROM MAINTENANCE OPERATIONS
- PAINT STRIPPING HAZMIN BY IN-PROCESS CHANGES AND MATERIALS SUBSTITUTION

PE/PROJECT 6.27.20/DO48
FY87 ACCOMPLISHMENTS

- COMPLETED STUDIES TO PROVIDE IMPROVED WASTE TREATMENT DESIGN CRITERIA FOR NEW RDX/HMX PRODUCTION FACILITIES
- COMPLETED LABORATORY SCALE DEVELOPMENT OF TREATMENT TECHNOLOGY FOR BALL POWDER PRODUCTION WASTEWATER
- DEMONSTRATED SAFETY AND TECHNICAL FEASIBILITY ON PILOT SCALE (2MM BTU PER HR) OF USING WASTE EXPLOSIVES AS SUPPLEMENT TO FUEL OIL FOR INSTALLATION HEATING PLANTS
- DEVELOPED TECHNOLOGY FOR RECYCLE OF OFF-GRADE TNT INTO PRODUCTION PROCESS AT RADFORD AAP
- DEVELOPED BASIC DATA FOR IMPLEMENTATION OF PLASTIC MEDIA BLASTING IN MAINTENANCE OPERATIONS AT SACRAMENTO ARMY DEPOT

POLLUTION ABATEMENT AND ENVIRONMENTAL CONTROL TECHNOLOGY (PAECT)

CURRENT ACTIVE PROJECTS

- COMPOSTING OF NC FINES
- PROPELLANT RECOVERY/REUSE (PBMA COORDINATION)
- DISPOSAL OF SPENT ACTIVATED CARBON
- USE OF WASTE EXPLOSIVES AS SUPPLEMENTAL FUEL FOR INSTALLATION HEATING PLANTS
- EQUIPMENT DECONTAMINATION
- STRUCTURAL DECONTAMINATION
- RED WATER ALTERNATIVE TREATMENT TECHNOLOGIES ASSESSMENT
- BALL POWDER PRODUCTION WASTE TREATMENT
- RADFORD AAP - COMBINED WASTE TREATMENT IMPROVEMENT STUDY
- LIQUID PROPELLANT DISPOSAL

USATHAMA HAZMIN TECHNOLOGIES UNDER DEVELOPMENT

- RECOVERY/REUSE
  - REQUALIFICATION/RECYCLE OF OFF-GRADE TNT DURING PRODUCTION
  - RDX AND TNT AS FUEL OIL SUPPLEMENTS FOR INSTALLATION HEATING PLANTS
  - RECOVERY OF PROPELLANTS BY SOLVATION AND SELECTIVE SOLVENT EXTRACTION (BEING TRANSFERRED TO PBMA)
  - RECOVERY OF PROPELLANTS FOR REUSE BY RESOLVATION/SOLVENT EXTRACTION
- PROCESS CHANGE
  - ALUMINUM ION DEPOSITION AS AN ALTERNATIVE TO ELECTROPLATING
  - PLASTIC MEDIA BLASTING FOR PAINT STRIPPING
  - FLUIDIZED BED PAINT STRIPPING FOR SMALL PARTS
- MATERIALS SUBSTITUTION
  - ALTERNATE CHEMICAL PAINT STRIPPER FORMULATIONS

PROJECT AF25 INSTALLATION RESTORATION TECHNOLOGY DEVELOPMENT

PRIMARY THRUSTS

* DECONTAMINATION OF STRUCTURES
  - HOT GAS TECHNOLOGY

* CONTAMINATED SOIL TREATMENT
  - COMPOSTING
  - AQUEOUS THERMAL DEGRADATION
  - TECHNOLOGY FOR HEAVY METAL CONTAMINATED SOILS

* GROUNDWATER TREATMENT
  - IN-SITU TECHNOLOGIES
  - MORE COST-EFFECTIVE VOC AIR EMISSION CONTROLS

PE/PROJECT 6.27.20/AF25
FY88 ACCOMPLISHMENTS/PLANS

ACCOMPLISHMENTS

- COMPLETED INITIAL PILOT TESTING OF STRUCTURAL DECON TECHNOLOGY FOR AGENT CONTAMINATION AT DUGWAY PG
- ESTABLISHED NATIONALLY ACCEPTED SAMPLING PROCEDURES FOR VOC'S IN SOIL
- COMPLETED BENCH SCALE DEVELOPMENT/TESTING OF PROCESSES TO CLEAN UP BASIN F, ROCKY MOUNTAIN ARSENAL

PLANS

JUN 88 – COMPLETED FIELD DEMONSTRATION OF COMPOSTING EXPLOSIVE CONTAMINATED SOIL AT LOUISIANA AAP

JUL 88 – COMPLETE DEVELOPMENT OF REAL TIME ANALYTICAL SYSTEM FOR EXPLOSIVE CONTAMINANTS ON STRUCTURAL SURFACES

SEP 88 – COMPLETE FIELD DEMONSTRATION OF COMPOSTING NITROCELLULOSE CONTAMINATED SOIL AT BADGER AAP

PE/PROJECT 6, 27, 04/AF25
FY87 ACCOMPLISHMENTS

- COMPLETED TECHNICAL IMPLEMENTATION PACKAGE FOR THERMAL STRIPPING TREATMENT OF VOC CONTAMINATED SOIL
- COMPLETED INITIAL TECHNICAL EVALUATION OF IN-SITU BIODEGRADATION PROCESSES FOR GROUNDWATER AND SOILS
- COMPLETED DEVELOPMENT AND PROVE-OUT OF INCINERATION FEED SYSTEM FOR EXPLOSIVE CONTAMINATED SOILS
- COMPLETED INITIAL FULL SCALE PILOT TEST OF STRUCTURAL DECON TECHNOLOGY AT CORNHUSKER AAP
- COMPLETED LABORATORY DEVELOPMENT OF STANDARD ANALYTICAL METHOD FOR ANALYSIS OF EXPLOSIVE CONTAMINATED SOILS

INSTALLATION RESTORATION RESEARCH AND DEVELOPMENT

.PROGRAM GOAL TO REDUCE COST OF CLEANUP AND MEET REGULATORY REQUIREMENTS

.AREAS OF CONCERN

- ARMY UNIQUE COMPOUNDS (EXPLOSIVES/AGENTS/ETC) AND INDUSTRIAL SOLVENTS
- CONTAMINATED SOILS, GROUND AND SURFACE WATERS AND BUILDING/EQUIPMENT

.CURRENT PROGRAM EMPHASIS

- IN-SITU SOIL AND GROUNDWATER TREATMENT FOR VOLATILE ORGANIC COMPOUNDS
- IMPROVED ABOVEGROUND GROUNDWATER TREATMENT TECHNOLOGIES TO REMOVE VOLATILE ORGANIC SOLVENTS, EXPLOSIVES AND HEAVY METALS
- NON-DESTRUCTIVE DECON OF BUILDINGS FOR EXPLOSIVES/AGENTS
- COMPOSTING AND CHEMICAL TREATMENT OF EXPLOSIVES IN SOIL
- THERMAL STRIPPING OF POL COMPOUNDS IN SOIL
- FIELD PORTABLE SAMPLING AND ANALYTICAL INSTRUMENTATION
- QUALITY ASSURANCE AND QUALITY CONTROL PROCEDURES
- DATA MANAGEMENT AND REPORTING

. TECHNOLOGY TRANSFER MECHANISMS

- DOD IR TECHNOLOGY COORDINATING COMMITTEE– TRI SERVICE– CHAIRED BY CMDR USATHAMA
- DA/EPA MOU
- DOD/DOE/EPA COOPERATIVE STUDIES

INSTALLATION RESTORATION TECHNOLOGY DEVELOPMENT

BIOTREATMENT TECHNOLOGY DEVELOPMENT

ONGOING:

- BIOTREATMENT PILOT STUDY OF BALL POWDER PRODUCTION WASTEWATER
- CHEMICAL ANALYSIS AND TOXICITY TESTING OF COMPOSTED EXPLOSIVE CONTAMINATED SOIL

PLANNED:

- VOC BIOTREATMENT STUDY – BASIC RESEARCH
- EXPLOSIVE CONTAMINATED SOIL COMPOSTING FOLLOW–ON
- CHEAPER CARBON SOURCE FOR COMPOSTING

MIKUCKI PAPER NOT AVAILABLE AT THIS TIME

PRESENTATION BY DR. CHARLES RICHARD LEE

Biotechnical Management of Contaminated Soil

Charles R. Lee
USAE-WES
P. O. Box 631
Vicksburg, MS 39180

The Environmental Laboratory at the U. S. Army Engineer Waterways Experiment Station is currently conducting research in the management of contaminated soils to minimize contaminant mobility from sites of concern. Research is evaluating the stabilization of contaminated soil to control surface runoff and migration of contaminants into groundwater and into foodchains associated with installations. Restoration of contaminated soil is evaluated using greenhouse and growth chamber plant tests and rainfall simulator/ soil bed lysimeters to develop relationships of vegetative cover to surface runoff water quality. Plant uptake of contaminants are evaluated to develop management strategies to control migration of contaminants into foodchains. Use of plants that do not take up contaminants eliminate potential foodchain impacts. Use of other plants to take up and clean contaminated soil is also being considered. Use of microorganisms living in the soil to facilitate biological degradation of contaminants is being studied. Use of activated sludge bacteria for degradation of organic contaminants is also being evaluated. These techniques are being evaluated for potential application at installations requiring contaminant remediation.

PRESENTATION BY DR. ALEX ISKANDER

US Army Cold Regions Research and Engineering Laboratory
72 Lyme Road
Hanover, New Hampshire 03755

SUMMARY OF RECENTLY COMPLETED, ONGOING AND PLANNED R&D RELATED TO BIOTECHNOLOGY FOR HAZARDOUS WASTE CLEANUP

Presented by
I. K. Iskandar
Chief, Geochemical Sciences Branch

Presented at the
Biotechnology Workshop
February 20-24, 1989
Monterey, California

INTRODUCTION

The Cold Regions Research and Engineering Laboratory (CRREL) in Hanover, New Hampshire, is the largest cold regions research organization in the free world. The total staff of CRREL numbers 300, including more than 100 research scientists and engineers. The technical staff includes civil, hydraulic, electronic, chemical and mechanical engineers; life scientists, chemists, geographers, geologists, geophysicists, glaciologists, hydrologists, meteorologists, and soil scientists. About 10 percent of CRREL's technical staff members are specialists in waste disposal and treatment, particularly in cold climates. Because of its specialized cold regions mission, CRREL's research facilities are unique. CRREL has 25 coldrooms, six chemistry laboratories, two physics labs, soil physics and soil chemistry labs, a complex for soil studies, a modern computer-controlled Frost Effects research laboratory, a large-scale soil decontaminating freezing facility, several electronics labs with highly specialized equipment, two walk-in growth chambers, a greenhouse, two clean rooms (Class 10,000 rooms with Class 100 work areas), and field prototype test facilities. Also available for research are the following:

a. Modern laboratory and field instrumentation for physical and chemical characterization of toxic and hazardous materials.

b. Remote sensing and geophysical instrumentation.

c. Complete facilities for field sampling and analysis of plant, soil, water and air samples.

d. Complete facilities and expertise for mathematical modeling and data analysis.

e. High-speed mainframe computer, computer workstations, and PCs.

f. Dual-energy gamma-ray device for nondestructive testing.

The following is a brief summary of CRREL's recently completed, ongoing or future R&D related to biotechnology.

RECENTLY COMPLETED R&D RELATED TO BIOTECHNOLOGY

A. Land Treatment Program: During the period from 1973 to 1982, CRREL conducted a major R&D program on "Land Treatment of Municipal Wastewater." The objective was to provide, through research, definitive criteria and procedures to enable the cost-effective and environmentally safe treatment of wastewater by soil. For the first time, the term "land treatment" vs. "disposal" was rationally defined. Three different types of waste applications were identified and studied in detail: slow rate, overland flow and rapid rate systems.

To maximize application rates without affecting ground and surface water quality, it was necessary to understand the physical, chemical and biological processes that took place in each system. Nitrogen, for instance, was found to be the limiting factor for high-rate application in slow rate systems.

Therefore, the biological-chemical processes of the N cycle in soil under the conditions of wastewater land treatment were studied. Stable isotope ^{15}N was used to determine the kinetics of nitrification, denitrification, immobilization, mineralization and plant uptake. Several nitrogen models were developed and evaluated to varying degrees (Iskandar 1981).

In addition to nitrogen, phosphorus and some volatile, semi-volatile or explosive residue (such as TNT) were also studied. The findings from these studies were published in series of more than 240 journal articles, government reports, symposia proceedings, book chapters and a textbook. For a list of publications, see Iskandar and Wright (1983).

B. Fate and Effect of Crude Oil Spilled in Alaska: In 1976, CRREL and EPA jointly conducted field experiments in Alaska where 2000 gallons of oil was intentionally spilled on the tundra. The objectives were to study:

1. The movement of the oil downslope with time.
2. Downward movement of oil in soil.
3. Biological degradation processes.
4. Botanical effects of oil contamination.

It was concluded, that evaporation of volatile components of oil was the most significant natural weathering process during the first two years. C_1 and C_2 components were lost from surface oil in the first 24 hours, with only small concentrations of components (smaller than C_8) present after five months. Somewhat slower evaporation of volatiles from oil was carried deeper into the soil profile.

The indigenous soil microbial populations responded differently to winter and summer oil applications. The responses ranged from inhibition to stimulation, with stimulation of growth and activity appearing to predominate.

Vegetation showed both immediate and long-term damage effects from oil.

Details may be found in CRREL Report 80-29 by Johnson et al. (1980) or in EPA 600/3-80-040, March 1980.

C. Removal of Volatile Organics: The efficiency of soil in removing up to sixteen organic substances from wastewater was studied in large scale prototype systems simulating slow rate and overland flow. In the slow rate system, the concentration of organics in the applied water was about 50 $\mu g/L$ each, up to 70% of the volatile organics were lost to the atmosphere during spraying, In the soil, additional removal occurred by biodegradation-biotransformation. PCB's and diethylphthalate were the most persistent. For more details on this study, see Parker et al. (1984).

In the overland flow experiment (Jenkins et al., 1985), the spiked wastewater was applied at the top of a slope of inclosed test cells and was allowed to run slowly on the grass covered shallow soil. More than 94% of each substance was removed at an application rate of 0.4 cm hr^{-1}. The percent removals declined as application rates were increased. Removal from solution was described by first-order kinetics. A model based on the two film theory was developed using three properties of each substance (the Henry's Constant, the octanol-water partition coefficient and the molecular weight) and two system parameter (average water depth and residence time).

We believe that this technology can be used for cleanup of volatile organic contaminated groundwater.

ON-GOING R&D ON TOXIC AND HAZARDOUS WASTE

A. Methods for Analysis of Explosives-Contaminated Soils: CRREL developed and evaluated a number of analytical methods for identification and quantification of military explosive residues in soils, sediments, waters and plant materials. This research was initiated in 1980 and is funded by US Army Toxic and Hazardous Materials Agency (THAMA). A list of published papers and reports is attached (Appendix A). The contaminants of interest include TNT, RDX, HMX, and tetrazine. Sample collection, handling and extraction, as well as statistical treatment are also being studied.

B. Fiber-Optic Sensors: THAMA is funding a basic research to develop and evaluate fiber-optic sensors for in-situ measurement of TNT in groundwater. CRREL also signed an agreement with a private corporation to evaluate the performance of its sensor for detecting volatile and nonvolatile compounds in soils.

C. Mathematical Models: As a follow-up to the land treatment modeling effort, CRREL continues to improve this technology. Currently, we are focusing on the fate of heavy metals such as Ni, Cr and Cd in soils. Attempts are being made to obtain model parameters from batch studies. The water submodel for the heavy metal model under development is similar to the one developed under the land treatment program. Our near-future interest is to develop a model for RDX and TNT transport and transformation in soils.

D. Composting of Soil Contaminated with TNT: THAMA asked CRREL to evaluate the impact of low temperature on the engineering aspects of composting TNT contaminated soils. This study has just been initiated.

FUTURE PLANS

In the absence of environmental data on toxicity and environmental effects, regulations tend to be conservative, and compliance is often unnecessarily costly. Therefore, developing a better understanding of the release, transformation and transport characteristics of contaminants in soils is a timely and important concern. By the same token, unless we are able to analyze for the contaminant with a certain degree of confidence, the information we obtain or predict will be questionable. CRREL will concentrate on the following four topics:

A. Analytical Methodology: Continue ongoing activities of method development, evaluation and standardization. Develop and/or evaluate field methods for volatile organics. Continue current effort on sample collection, preservation, processing and data analysis.

B. Mathematical Models: Expand on existing models to include groups of organics/inorganics or energetic contaminants. Initial research will concentrate on leaching, sorption and release chemistry according to THAMA's need and field sampling techniques.

C. Evaluation of Chemical Sensor Performance Under Freezing and Frozen Soil Conditions: Currently, we have sensors for temperature and conductivity installed and evaluated. Other sensors for chloride and volatile organics in soil and TNT in groundwater are being acquired and will be tested.

D. Provide Technical Support to THAMA's Installation Restoration Program: Cleanup of toxic and hazardous waste sites in cold regions may be different than in non-cold regions. For example, we learned from the oil spill experiment, that it is more advantages to leave the site as is than disturbing the tundra and permafrost. Therefore, the methods developed for site restoration in non-cold regions should be researched and adopted to the cold areas.

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APPENDIX A

USATHAMA SPONSORED RESEARCH PUBLICATIONS

JOURNAL ARTICLES:

1. Reversed-Phase High Performance Liquid Chromatographic Determination of Nitro-Organics in Munitions Wastewater. T.F. Jenkins, D.C. Leggett, C.L. Grant and C.F. Bauer, Analytical Chemistry, 58, 170-175 (1986).

2. Interlaboratory Evaluation of High Performance Liquid Chromatographic Determination of Nitro-Organics in Munition Plant Wastewaters. C.F. Bauer, C.L. Grant and T.F. Jenkins. Analytical Chemistry, 58, 176-182 (1986).

3. Suitability of Polyvinyl Chloride Pipe for Monitoring Munitions in Groundwater. L.V. Parker and T.F. Jenkins. Ground Water Monitoring, pp. 92-98, 1986 (summer).

4. TNT, RDX, HMX and 2,4-DNT in Wastewater and Groundwater: Liquid Chromatographic Method. J. Assoc. Off. Anal. Chem., 69:366-367 (1986). Standard Method No. 33.B01.

5. Comparison of Extraction Techniques for Munitions Residues in Soil. T.F. Jenkins and C.L. Grant. Accepted by Analytical Chemistry, to be published May 1987.

6. Effects of Analytical Calibration Models on Detection Limit Estimates. K.G. Owens, C.F. Bauer and C.L. Grant in Detection Limits: From Basic Concepts to Pratical Applications, L.A. Currie, Ed., ACS Symposium Series (in press).

CONFERENCE PRESENTATIONS:

1. Munition Compound Analysis Standardization. T.F. Jenkins. DARCOM Environmental Quality Symposium, Ocean City, MD, 2-4 June 1982.

2. Reversed-Phase HPLC Method for Analysis of Munition Wastewaters. T.F. Jenkins et al. Pittsburg Conference, New Orleans, LA 25 February - 1 March 1985.

3. Interlaboratory Evaluation of HPLC Determination of Nitro-Organics in Munition Plant Wastewater. C.F. Bauer, C.L. Grant and T.F. Jenkins. Pittsburgh Conference, New Orleans, LA, 25 February - 1 March 1985.

4. Steps in Developing a Standardized Procedure for Analysis of Nitro-Organics in Munition Wastewater. T.F. Jenkins. U.S. Army Corps of Engineers Chemist Conference, Cincinnati, OH, 8-10 April 1986.

5. TNT, RDX, HMX and 2,4-DNT in Wastewater and Groundwater: Liquid Chromatographic Method. T.F. Jenkins, D.C. Leggett, C.F.

Bauer and C.L. Grant. 100th AOAC Annual International Meeting, Scottsdale, AZ, 14-18 September 1986.

6. Effects of Analytical Calibration Models on Detection Limit Estimates. C.L. Grant and K.G. Owens, 191st American Chemical Society National Meeting, New York, NY, April 1986.

7. Suitability of PVC for Ground Water Monitoring. L.V. Parker. ASTM Meeting on Standards Development for Ground Water Monitoring, Tampa, FL 20-23 January 1987.

8. Liquid Chromatographic Method for Determination of Explosives Residues in Soil. T.F. Jenkins and M.E. Walsh. Fifteenth Corps of Engineers Division Laboratories Conference, San Francisco, CA 9-13 March 1987.

CRREL AND SPECIAL REPORTS:

1. Evaluation of Procedures for Determining Selected Aquifer Parameters. C.J. Daly, CRREL Report 82-41.

2. Reverse Phase HPLC Method for Analysis of TNT, RDX, HMX and 2,4-DNT in Munitions Wastewater. T.F. Jenkins, C.F. Bauer, D.C. Leggett and C.L. Grant, CRREL Report 84-29.

3. Suitability of Polyvinyl Chloride Pipe for Monitoring TNT, RDX, HMX and DNT in Groundwater. L.V. Parker, T.F. Jenkins and B.T. Foley, CRREL Special Report 85-12.

4. TNT, RDX and HMX Explosives in Soils and Sediments: Analysis Techniques and Drying Losses. J.H. Cragin, D.C. Leggett, B.T. Foley and P.W. Schumacher, CRREL Report 85-15.

5. Sample Digestion and Drying Techniques for Optimal Recovery of Mercury From Soils and Sediments. J.H. Cragin and B.T. Foley, CRREL Special Report 85-16.

6. Sorption of Military Explosive Contaminants on Bentonite Drilling Muds. D.C. Leggett, CRREL Report 85-18.

7. Comparison of Extraction Techniques and Solvents for Explosive Residues in Soil. T.F. Jenkins and D.C. Leggett, CRREL Special Report 85-22.

8. Losses of Munitions Residues on Disposable Membrane Filters. T.F. Jenkins, L.K. Knapp and M.E. Walsh, CRREL Special Report (in press).

9. Development of an Analytical Method for Explosive Residues in Soil. T.F. Jenkins and M.E. Walsh, CRREL Report (in press).

10. Comparison of Methanol and Tetraglyme as Extraction Solvents for Determination of Volatile Organics in Soil. T.F. Jenkins and P.W. Schumacher, CRREL Special Report (in press).

11. A Critical Comparison of Moving Average and Cumulative Summation Control Charts for Trace Analysis Data. I. McGee and C.L. Grant, CRREL Special Report (in press).

12. Development of a Fiber Optic Sensor for TNT. Y. Zhang, W.R. Seitz, D. Sundberg and C.L. Grant, CRREL Special Report (in press).

OTHER FINAL REPORTS:

1. Sorption and Release of Metals by Soils. M.C. Amacher, J. Amacher, I.K. Iskandar and H.M. Selim (September 1984).

2. Some Statistical Procedures for Evaluation of Analytical Calibration and Quality Control Data. C.F. Bauer and C.L. Grant (Phase 1 - 1984; Phase 2 - 1985).

3. Development of a Fiber Optic Sensor for In-Situ Measurements of TNT and RDX in Groundwater. E.C. Shane, M.S. Girardi, C.L. Grant and W.R. Seitz (November 1985).

4. Assessment of an Alternative Strategy for Assigning Response Factors for Unexpected Compounds in GC/MS. T.F. Jenkins (May 1986).

5. Estimation of Octanol-Water Partition Coefficients from HPLC Capacity Factors. T.F. Jenkins (July 1986).

PRESENTATION BY DR. JAMES CORNETTE

HQ AIR FORCE ENGINEERING AND SERVICES CENTER

AIR FORCE ENGINEERING AND SERVICES LABORATORY

DR JIM CORNETTE
SENIOR SCIENTIST

BIOTECHNOLOGY

OFFICE OF TECHNOLOGY ASSESSMENT

DEFINITION

"ANY TECHNIQUE THAT USES LIVING ORGANISMS (OR PARTS THEREOF) TO MAKE OR MODIFY PRODUCTS, TO IMPROVE PLANTS OR ANIMALS, OR TO DEVELOP MICROORGANISMS FOR SPECIFIC USES."

AIR FORCE CONTAMINATION PROBLEMS

BIOTECHNOLOGY PROGRAM

PROBLEM DEFINITION

3000 IDENTIFIED IRP SITES
AT LEAST 50% JP-4 SPILLS
CHLORINATED SOLVENTS
ABANDONED FIRE TRAINING PITS
HEAVY METAL SLUDGES

AIR FORCE ENVIRONMENTAL ISSUES
CATEGORIES

- PAST CONTAMINATION
  - IRP
- UNCONTROLLED RELEASE
  - NEW MATERIALS
  - ENVIRONMENTAL DOCUMENTATION
- DEFENSE SYSTEMS
  - MANUFACTURE
  - MAINTENANCE
  - RENOVATION
  - DISPOSITION

HQ AIR FORCE ENGINEERING & SERVICES CENTER
ENGINEERING & SERVICES LABORATORY

BIOTECHNOLOGY PROGRAM DRIVERS

- INSTALLATION RESTORATION
- OPERATIONAL COMPLIANCE/COST REDUCTION
- COST REDUCTION FOR NEW SYSTEMS
  - ADVANCED TECHNOLOGY BOMBER
  - ADVANCED TACTICAL FIGHTER
  - ADVANCED LAUNCH SYSTEM
  - ADVANCED STRATEGIC MISSILE SYSTEM

BIOTECHNOLOGY FOR
ENVIRONMENTAL REMEDIATION/WASTE TREATMENT

CRITICAL ISSUES

- LACK OF FOCUS
- INADEQUATE FUNDING
- TECHNICAL PROBLEMS
  - INHIBITORS
  - TEMPERATURE
  - SALT CONCENTRATION
  - pH

HQ AIR FORCE ENGINEERING & SERVICES CENTER

ENGINEERING & SERVICES LABORATORY

<u>CONFERENCE BIOTECHNOLOGY CHALLENGES</u>

IDENTIFY DOD PROBLEMS

IDENTIFY RESEARCH OPPORTUNITIES

SELL PROGRAM

HQ AIR FORCE ENGINEERING & SERVICES CENTER
ENGINEERING & SERVICES LABORATORY

BIOTECHNOLOGY RESEARCH PROGRAM

OBJECTIVES

DISCOVER BIOCHEMICAL MECHANISMS

DEVELOP MICROBES WITH ENHANCED ABILITY

PREDICT BEHAVIOR

AIR FORCE ENVIRONMENTAL ISSUES
CATEGORIES

- PAST CONTAMINATION
  - IRP
- UNCONTROLLED RELEASE
  - NEW MATERIALS
  - ENVIRONMENTAL DOCUMENTATION
- DEFENSE SYSTEMS
  - MANUFACTURE
  - MAINTENANCE
  - RENOVATION
  - DISPOSITION

HQ AIR FORCE ENGINEERING & SERVICES CENTER
ENGINEERING & SERVICES LABORATORY

BIOTECHNOLOGY PROGRAM DRIVERS

- INSTALLATION RESTORATION
- OPERATIONAL COMPLIANCE/COST REDUCTION
- COST REDUCTION FOR NEW SYSTEMS
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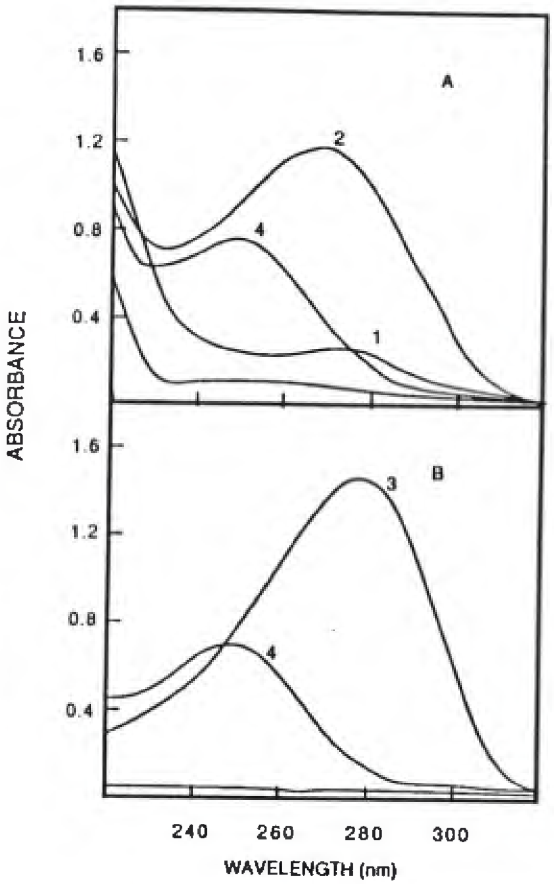
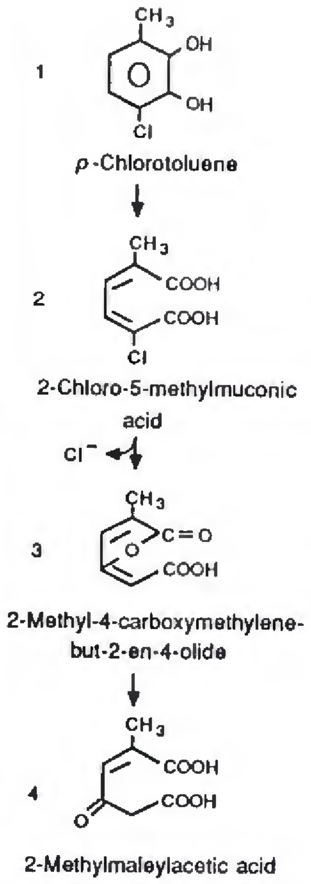
  - ADVANCED TACTICAL FIGHTER
  - ADVANCED LAUNCH SYSTEM
  - ADVANCED STRATEGIC MISSILE SYSTEM

BIODEGRADATION OF HAZARDOUS CHEMICALS

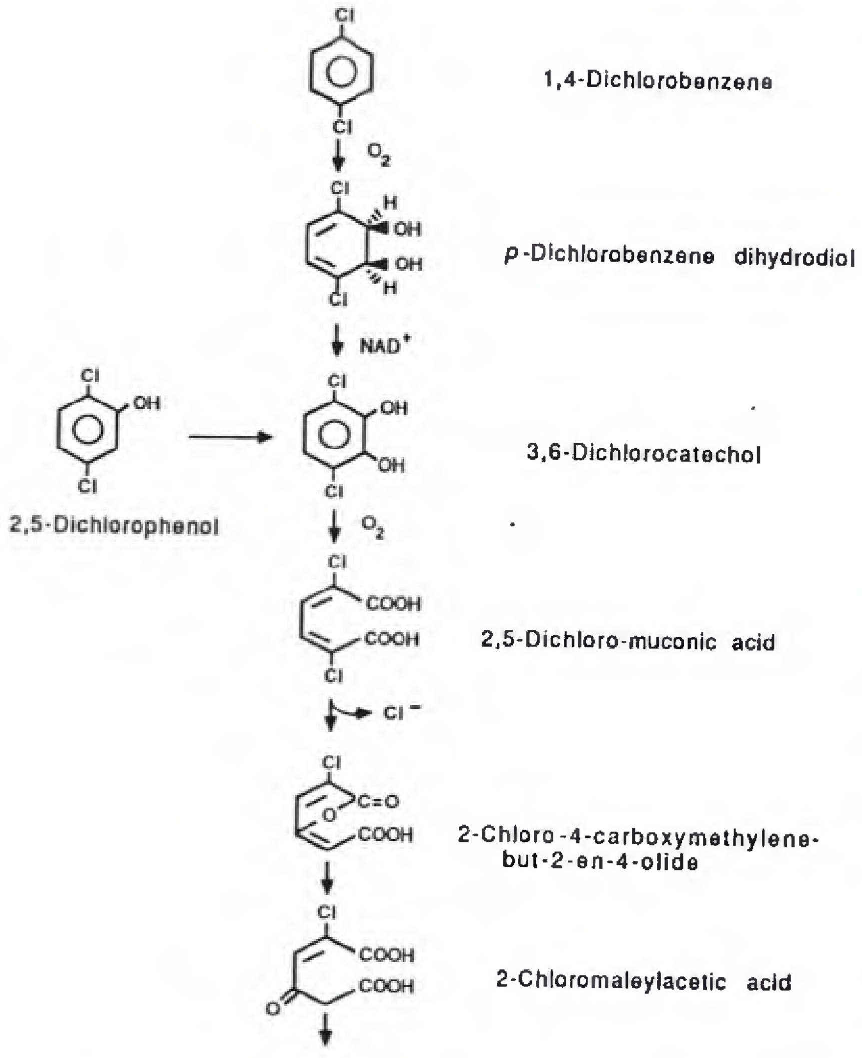




- Substrate range of *p*-dichlorobenzene degrading bacterium extended to include *p*-chlorotoluene
- Pathway involves central metabolite chloromethylcatechol
- Pathway information allows construction of new strains
- Future research on mixtures of pollutants

Metabolism of 3-chloro-6-methylcatechol

BIODEGRADATION KINETICS AND PATHWAYS



Pathway of 1,4-dichlorobenzene degradation

- *Pseudomonas* JS6 degrades wide range of chloroaromatic chemicals
- Pathway involves oxygenase enzymes
- Range extended to include chlorophenols and TCE

Future research

- Enzyme regulation
- Biochemical mechanisms
- Strain construction for specific chemicals
- Designer genes for strain construction

AIR FORCE ENGINEERING SERVICES LABORATORY
Biotechnology
Biodegradation Research Program

Objectives:

The objectives of the Engineering and Services Laboratory biotechnology research program are to: (1) discover the biochemical mechanisms that control biodegradation of Air Force pollutants in groundwater, soil, and aquatic habitats, (2) develop microorganisms with enhanced ability to biodegrade Air Force fuels, chemicals, and hazardous wastes, and (3) to predict the behavior of pollutants in the environment based on knowledge of biodegradation.

Background:

Removal of subsurface contaminants in landfills and waste sites is a major concern for the Air Force. Currently, several billion dollars per year are being spent on cleanup and disposal of hazardous wastes by the Department of Defense. Most of the effort involves pump-and-treat technology or transport of contaminated material to landfills. Biological treatment offers a permanent and less expensive solution to the contamination problem because microorganisms convert toxic organic compounds to harmless products such as carbon dioxide and water. Recent advances in biotechnology allow the development of new biological processes for the degradation of hazardous chemicals. Biodegradation of such wastes can be carried out either in situ or in aboveground treatment systems.

Successful use of microorganisms for degradation of hazardous wastes requires specific organisms able to mineralize the waste components. Microorganisms able to degrade natural chemicals have been studied extensively. In contrast, little is known about microorganisms able to degrade man-made chemicals, such as the halogenated solvents. Some man-made chemicals are not known to be biodegraded, whereas others are only partially degraded or degraded by certain specialized bacteria or fungi. The initial objective of the research described below is to develop microorganisms able to degrade specific chemicals or groups of chemicals. Emphasis will be placed on substituted aromatic compounds. Finding the organisms is only the first step in the development of a treatment process. The biochemical pathways, intermediates, kinetics, and regulation must be understood before the process can be optimized and made predictable. In addition, the organisms themselves should not perturb the natural functions of the ecosystems into which they are introduced.

Present Program Status:

The Engineering Services Laboratory biodegradation research program involves both in-house and contract research with universities, federal agencies, and other research organizations. The in-house program examines basic technical aspects of biodegradation, including strain construction, regulation, biochemical pathways, intermediates, and kinetics. In addition, both formal and informal ties have been established with major universities for purposes of exchanging technical information on biodegradation. Because the details of biodegradation are well understood for some chemical compounds, the program also involves laboratory testing and field demonstration of biodegradation concepts and techniques.

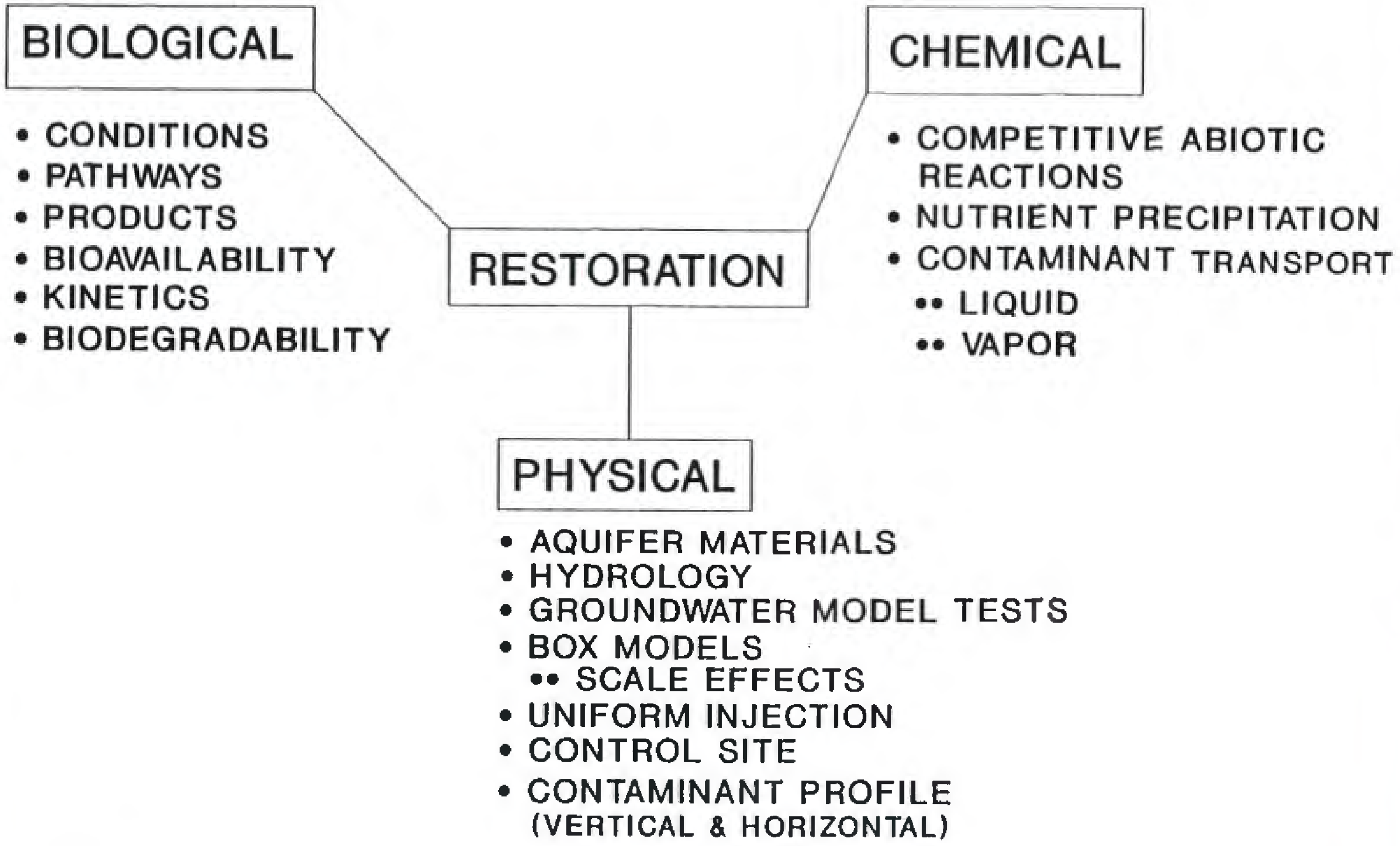
HQ AIR FORCE ENGINEERING & SERVICES CENTER

ENGINEERING & SERVICES LABORATORY

BIOTECHNOLOGY PROGRAM STATUS

- DISCOVERED ORGANISMS
- ELUCIDATED PATHWAYS
- EXTENDED SUBSTRATE RANGE
- FUTURE RESEARCH
  - MIXED WASTES

GROUNDWATER/SOIL RESTORATION
RESEARCH NEEDS



RESTORATION

BIOLOGICAL

- CONDITIONS
- PATHWAYS
- PRODUCTS
- BIOAVAILABILITY
- KINETICS
- BIODEGRADABILITY

CHEMICAL

- COMPETITIVE ABIOTIC REACTIONS
- NUTRIENT PRECIPITATION
- CONTAMINANT TRANSPORT
  - LIQUID
  - VAPOR

PHYSICAL

- AQUIFER MATERIALS
- HYDROLOGY
- GROUNDWATER MODEL TESTS
- BOX MODELS
  - SCALE EFFECTS
- UNIFORM INJECTION
- CONTROL SITE
- CONTAMINANT PROFILE (VERTICAL & HORIZONTAL)

PRESENTATION BY DR. PATRICK SFERRA

Biotechnology Research and Development
Risk Reduction Engineering Laboratory: US EPA

P. R. Sferra, Ph.D.
Hazardous Waste Treatment Branch
Risk Reduction Engineering Laboratory
U. S. Environmental Protection Agency
Cincinnati, Ohio 45268

The EPA's Risk Reduction Engineering Laboratory is conducting a variety of research projects to control hazardous pollutants by biodegradation processes. The ability of some naturally occurring microorganisms to adapt to the presence of xenobiotic chemicals and break them down has been known for many years. On the expectation that intentional use of degradative microorganisms specifically to destroy polluting compounds can be developed as a technology that would be cost-effective and environmentally safe, the EPA has been funding biotreatment research projects. The attempt is made to cover an entire investigative range with a balance of projects that include the search for useful naturally occurring species or strains, the biochemical and genetic characterization of potentially useful organisms, strain improvement through enhanced adaptation and genetic engineering, kinetics and optimizing studies, laboratory-, pilot-, and field-scale testing, the application of engineering techniques to develop the control technology for the ultimate use of biotreatment, demonstrations, the development of testing protocols, biosafety, and technology transfer.

The mission of the Laboratory is to provide an authoritative, defensible engineering basis in support of the policies, programs, and regulations of the EPA with respect to drinking water, wastewater, pesticides, toxic substances, solid and hazardous wastes, and Superfund-related activities.

The Risk Reduction Engineering Laboratory of the U.S. Environmental Protection Agency is the result of a recent reorganization that combined the former Hazardous Waste Engineering Research Laboratory and the Water Engineering Laboratory. Through previous reorganizations these two laboratories evolved from the former Industrial Environmental Engineering Laboratory and the Municipal Engineering Research Laboratory, respectively. The present laboratory, RREL, is located in a seven story concrete building built in 1975 in Cincinnati, across the street from the main campus of the University of Cincinnati.

The Laboratory has Divisions dealing with (1) Drinking Water, (2) Superfund Technology Demonstration, (3) Water and Hazardous Waste Treatment, and (4) Waste Minimization, Destruction, and Disposal Research.

Ten years ago, in 1979, the predecessor lab, the IERL, initiated support for research in degradation by biological processes. Early on, the goals of a program of biological destruction of hazardous wastes were simple and directly related to the mission of the laboratory. These goals were (1) the *in situ* detoxification of hazardous waste sites, and (2) the control of toxic compounds in industrial waste streams.

At that time because of heavy notoriety it would have been expedient to come up with a means of destroying or detoxifying a particular polluting compound the whole world had become aware of, i.e., 2,3,7,8-TCDD. The early support from this Laboratory to do research in biodegradation went to Dr. A. M. Chakrabarty at the University of Illinois Medical Center in Chicago where attempts were made to modify microorganisms so that they would have the ability to degrade dioxin.

Chakrabarty's approach was to place strains of microorganisms from hazardous waste sites in a chemostat, add certain plasmids and nutrients and gradually replace certain chemicals in the hope that the organisms would adapt to the new chemical or chemicals. This slide shows one of the chemostat set-ups in Dr. Chakrabarty's lab. Over a period of time Dr. Chakrabarty's chemostat yielded a pure culture of *Pseudomonas* that was able to get its carbon solely from 2,4,5-T molecules. The strain was named *Pseudomonas cepacia* AC1100 and the method utilizing the chemostat was named "Plasmid Assisted Molecular Breeding". The next step was to attempt to get the strain to adapt to a dioxin. The support for Dr. Chakrabarty continued with a second Cooperative Agreement and he was made a Visiting Scientist to the Laboratory in Cincinnati. The intent was to establish a working research laboratory that did much the same kind of research that was done in Dr. Chakrabarty's laboratory in Chicago with Dr. Chakrabarty overseeing the investigations as a Visiting Scientist for a certain percentage of his time.

This second Cooperative Agreement was titled "Construction and Detection of Genetically Engineered Microorganisms" and by the time it started much chemostat work had been performed in the search for a dioxin destroyer but this work was not successful. New approaches were tried in the new laboratory in RREL in Cincinnati and in time a modified version of strain AC1100 was developed with the capability of degrading 2,4,5-T and 2,4-D. This organism will be used in a combined chemical-biological treatment experiment to clean up stored wastes and contaminated soil at an abandoned herbicide manufacturing facility.

The Laboratory soon was funding other projects. At the University of Cincinnati Dr. John Loper was funded to gene-engineer yeast for use in hazardous waste removal. The intent was to take advantage of particular P-450 gene systems that coded for the capability of detoxicating chlorinated aromatic hydrocarbons like dioxins. Dr. Loper and his group became quite expert in working with the P-450 gene system in yeast but unfortunately this Cooperative Agreement had its funding cut off during a period when the Agency was more interested in projects that were nearer field-testing.

In 1984, the Laboratory awarded funds by means of a Cooperative Agreement to Dr. Steven Aust at Michigan State University to investigate the feasibility of using the white rot fungus in pollution control. The fungus produces a system of non-specific enzymes that operate extracellularly in the breakdown of lignin. If the enzymes were powerful enough to break down lignin then it was theorized that they might have the capacity to break down chlorinated aromatic pollutants. Dr. Aust and Dr. John Bumpus, his coworker, soon published on this capability and white rot fungus, Phanerochaete chrysosporium, became a hot item for pollution control showing the promise according to some people that it could do it all. Dr. Aust's degradation data were based on the ability of the fungus to degrade chemicals in the aqueous solution type of culture of the fungus. The group continued to study the fungus, trying to determine optimum conditions for its use as a degrader, characterizing as much as possible the degradation pathways, and seeking ways to increase enzyme production. Dr. Aust moved to Utah State University where he is heading a biotechnology institute. His funding continued for a time at Utah then suffered the same fate as Dr. Loper's because the work was too much basic research and was not field ready. Drs. Aust and Loper were very productive, both research groups published a number of peer-reviewed papers as a result of funding from RREL.

Other work on white rot fungus is being funded by the Laboratory. One project that studies field conditions to develop the technology is going on at the U.S.D.A. Forest Products Laboratory in Wisconsin. Here, Dr. T. Kent Kirk, is directing the work. Another project is studying the enzymes at the State University of New York in Syracuse under Dr. Kenneth Hammel.

More recently the Risk Reduction Engineering Laboratory entered into a Cooperative Agreement with the University of Texas where Dr. David Gibson directed a biochemical study of PCB degraders and subcontracted to General Electric for genetic studies. General Electric had spent the equivalent of 40 man-years sampling contaminated sites and isolating adapted microorganisms in a search for an effective PCB degrader. The University of Texas-GE collaboration that this Laboratory funded started with the most promising isolate, Pseudomonas putida strain LB400, with Dr. Gibson studying its biochemistry and developing enzyme tests and GE, with the help of Gibson's discoveries, attempting to clone genes coding for enzymes involved in the degradative capacity of the organism. Within the first year of this Cooperative Agreement, three genes coding for action involved in the pathway of degradation of PCB to benzoic acid were cloned. Work has progressed satisfactorily since then. Dr. Gibson has moved to the University of Iowa and recently resumed his work and General Electric is working on genetically-engineered strains with enhanced potential for degrading PCBs. We anticipate funding Dr. Gibson for another Cooperative

Agreement to continue the work with GE collaborating and we also expect to fund GE separately to bench- and field-test their newly-developed strains.

The remainder of this presentation will cover an Agency-wide initiative which came about when the need was recognised for a comprehensive effort to learn about and eventually utilize the biodegradation processes, a description of the Superfund Innovative Technology Evaluation (SITE) Program, and a description of the Biosafety Program.

Biosystems Technology Development Program

The biosystems development program is intended to reduce or eliminate the risk from hazardous wastes that are polluting the environment. The Biosystems Program exists under the rationale that the use of biological processes for pollution control will have the advantages of inexpensive processes, low energy input, degradation activity in situ, and minimum site disruption. For implementation and management purposes the objective of the Program consists of six specific components:

(1) Degradation Processes Characterization,
(2) Biosystems Process Development,
(3) Biosystems Process Engineering,
(4) Fate and Risk Determination,
(5) Control of Adverse Consequences, and
(6) Technology Transfer

Interrelated with these components are the following operational program elements in which projects are grouped:

(1) Bioremediation Technology Development
    (a) Degradation Process Characterization
    (b) Biosystems Process Enhancement
    (c) Biosystems Process Engineering
    (d) Field Demonstration

(2) Risk Protection

(3) Technology and Information Transfer

(4) Protocol and Guideline Development

Research projects in this program are located at, or administered by, five participating EPA laboratories - the Environmental Research Laboratories at Gulf Breeze, Athens, and Ada, the Risk Reduction Engineering Laboratory (RREL) in Cincinnati, and the Health Effects Research Laboratory (HERL) at Research Triangle Park - and the Center for Environmental Research Information (CERI).

The creation of the Agency's Biosystems Technology Development Program is the result of an intense cooperative activity by an interlaboratory committee which is now formally organised as the Scientific Steering Committee for the Biosystems Program. With the experience of past and on-going projects in biodegradation and related subjects, this group determined which activities were vital to guarantee a successful start-up for the Program. Future research goals were formulated and stated and the distribution of first funding from FY88 money and subsequent FY89 money was agreed upon.

FY'88 resources were distributed to the particiapating Laboratories for use in the following projects

I. Bioremediation Technology Development

A. Field Application Projects

1. Alkyl Benzene Degradation Under Nitrate Reducing Cnnditions
2. White Rot Fungus Degradation of PCP in Rotating Biological Contactors

B. Research Projects for Field Application

1. Aerobic Degradation of Trichloroethylene
2. Remediation of PCB-Contaminated Soils with a Recombinant Organism
3. Combined Biological and Chemical Treatment of Dioxins
4. Degradation of Complex Wastes in Soil with White Rot Fungus
5. Anaerobic Dehalogenation of Chlorinated Phenols
6. Aerobic Degradation of PCBs in Sediments

C. Supporting Research Projects

1. Enhancement of Waste Material Bioavailability
2. Physiological Aspects of Enzyme Production from White Rot Fungi

II. Risk Control

1. Application of Existing Genotoxicity Tests to Field and Laboratory Treatments

III. Technology and Information Transfer

1. All Investigators Research Review

IV. Protocol and Guideline Development

1. Aerobic Soil Treatability Protocol

Funds from FY89 were distributed for use in the following projects

I. Liquid Reactors

   A. Keystone Projects

      1. White Rot Fungus Test in Rotating Biological Contactor
      2. Aerobic/Anaerobic Two Sludge Digestor for Leachate

3. TCE Treatment in POTW

   B. Supporting Projects
      1. Gene Enhancement of TCE Degradation
      2. Bench-Scale Treatment of CERCLA Leachates by Carbon-Assisted Fluidized Beds
      3. Single Sludge Treatment POTW

II. Soil/Sediment Treatment

   A. Keystone Projects

      1. White Rot Fungus Cultivation for Soil Treatment
      2. PCP Anaerobic Dechlorination
      3. PCB Aerobic Degradation in Sediments

   B. Supporting Projects

      1. Efficacy of Extracellular Hydrolase and Oxygenase Enzymes from Bacillus spp. for Metabolism of Aromatic Compounds
      2. Biotechnology of PCB Degradation
      3. Effectiveness of Recombinant PCB Degrader
      4. Surfactants/Sorption Effects on Biodegradation
      5. Anaerobic Degradation of Phenolics

III. Combined Treatment

   A. Keystone Projects

      1. Combined Use of KPEG Chemical Treatment and Anaerobic Composting with Municipal Sludge for Treatment of Contaminated Soils

   B. Supporting Projects

      1. Combined Use of KPEG Chemical Treatment and Anaerobic Treatment for Remediating Contaminated Soils
      2. Development of Combined Physical-Chemical and Biological Techniques for Destruction of Hazardous Wastes

IV. Sequential Treatment

- A. Keystone Projects
    1. Anaerobic Degradation of Creosote
    2. Aerobic Degradation of Creosote

- B. Supporting Projects

    1. Degradation of Dioxins
    2. Degradation of Heterocyclics

V. Ground Water Treatment

- A. Keystone Projects
    1. Carbon Tetrachloride Treatment

- B. Supporting Projects
    1. Characterization of Hydrocarbon-degrading Denitrifier

VI. Metabolic Processes Research

1. White Rot Fungus Enzyme Development
2. Degradation Potential of Oxygenase Enzymes
3. Dehalogenation Potential of Sulfate Reducing Organisms

Superfund Innovative Technology Evaluation (SITE)

The SITE Program is the result of a Section added to CERCLA directing the Agency to establish a research and demonstration program for alternative or innovative technology. This section was added by means of the Superfund Amendments and Reauthorization Act of 1986. The development, demonstration, and use of new or innovative technologies are to be accelerated and new, innovative measurement and monitoring technologies are to be demonstrated and evaluated by the SITE Program. The SITE Program in RREL of Cincinnati is administered by the Site Demonstration and Evaluation Branch of the Superfund Technology Demonstration Division.

Two components make up the SITE Program: (1) a Demonstration Program for the demonstration and evaluation of technologies on a field-scale and (2) an Emerging Technologies Program for the testing and evaluation of technologies from bench-scale through pilot-scale. These two components are administered by the Demonstration Section and the Emerging Technology Section, respectively, of the Site Demonstration and Evaluation Branch.

A variety of chemical, physical, and biological technologies make up the methods under investigation in the present Demonstration and Emerging Technologies Programs. Of the 35 projects in these programs 7 are of a biological nature:

SITE Demonstration Program Biological Projects

1. Biotreatment of groundwater. A commercial process called the Aqueous Treatment System (ATS) is based on the use of specific microorganisms as the sole treatment agent for the biodegradation of toxic organic compounds in groundwater. The ATS consists of submerged packed bed bioreactors, containing microorganisms immobilized as a fixed film with the packed bed submerged in the water stream.

2. Submerged aerobic fixed-film reactor. This treatment relies upon an above ground fixed-film reactor operated on a one-pass, continuous-flow basis.

3. Biological degradation. This process involves the slurrying of contaminated soil with water in an open top agitated tank. Special air inlet distributors supply air required for metabolism by aerobic bacteria and to keep the soil in suspension.

4. Liquid/solid contact digestion. This system uses two or three portable tank digesters or lagoons operating in three phases: (a) primary contact or mixing, (b) primary digestion, and (c) polishing.

5. Powdered activated carbon/biological. In this process, powdered activated carbon is added to active biomass in an aeration basin.

SITE Emerging Technologies Program Biological Projects

6. Removal and recovery of metal ions from groundwaters using algae. This process is designed to remove heavy metal ions from aqueous solutions using immobilized algal cells in a silica gel polymer the mode of action being the strong affinity of the cell walls of the algae for the heavy metal ions.

7. Constructed wetlands-based treatment of degraded waters for toxic metal removal. Influent waters containing high metal concentrations flow through aerobic and anaerobic zones of a wetland ecosystem. Metals are removed by filtration, ion exchange, adsorption, absorption accumulation by plants and microbes, and precipitation through geochemical and microbial oxidation and reduction.

Following is another activity the RREL is involved in and is directly related and important to all of the effort being expended to develop the use of biodegradation processes.

Biosafety. Projects in this category will develop engineering-related data required to support the Biotechnology Risk Management Program and the Biosystems for Pollution Control Program. This work will specifically cover

(1) the containment, decontamination, and control of worker exposure to genetically engineered microorganisms in large-scale manufacturing facilities, and

(2) the means to control the unintentional spread of genetically engineered microorganisms deliberately released for use in the environment.

Products will be

(1) reports addressing the following aspects of biologically-based manufacturing facilities

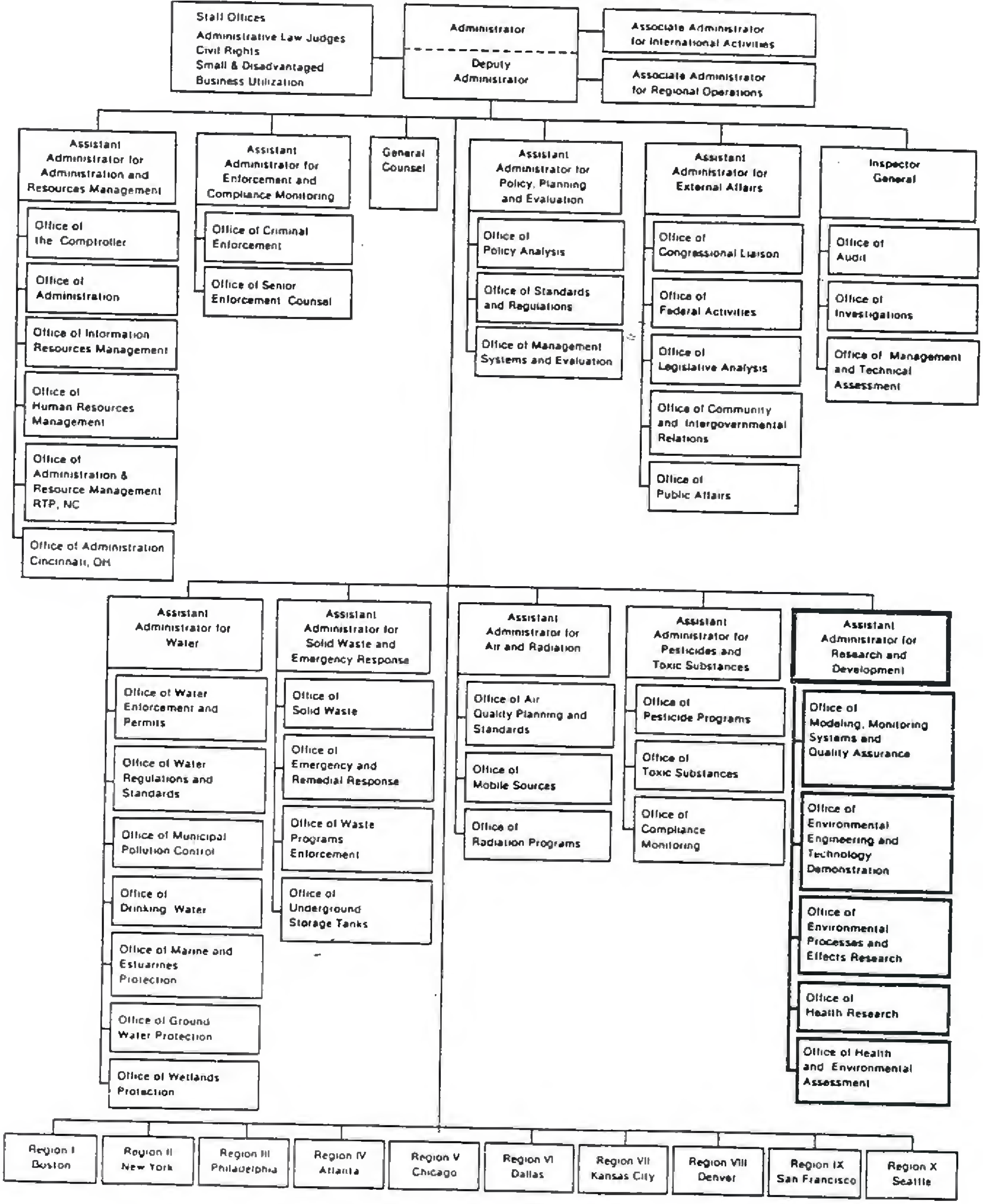
    (a) process analysis/potential for release,
    (b) containment technology,
    (c) decontamination technology,
    (d) potential for worker exposure,
    (e) worker protection technology,
    (f) monitoring needs and strategies, and

(2) reports addressing the following aspects of deliberate environmental release

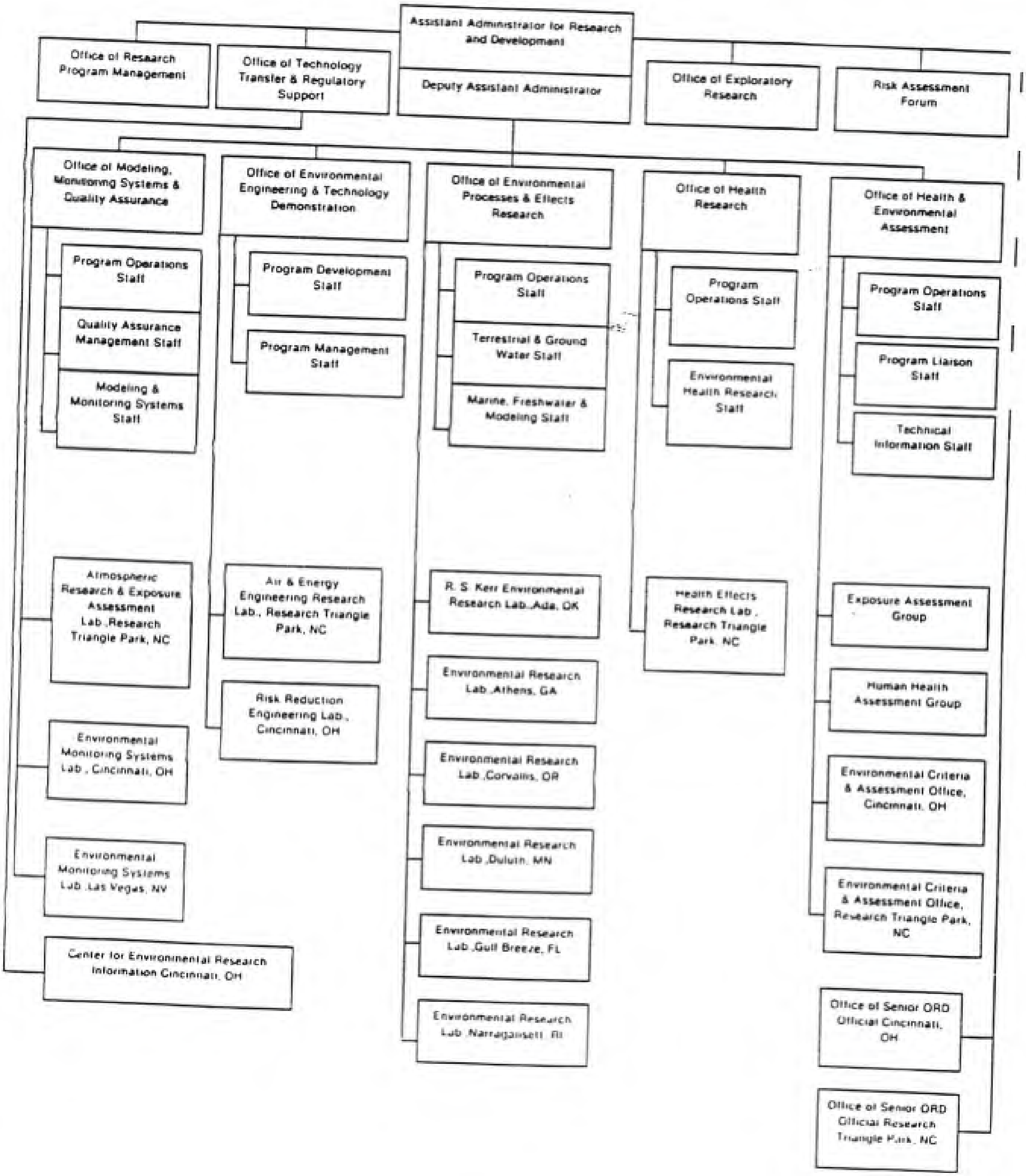
    (a) site profile evaluation procedures,
    (b) site containment alternatives,
    (c) monitoring needs and strategies,
    (d) site decontamination alternatives, and
    (e) evaluation of inoculation techniques.

PRESENTATION BY MR. JAMES McNABB

U. S. Environmental Protection Agency Organization Chart



Office of Research and Development Organization Chart



Robert S. Kerr Environmental Research Laboratory

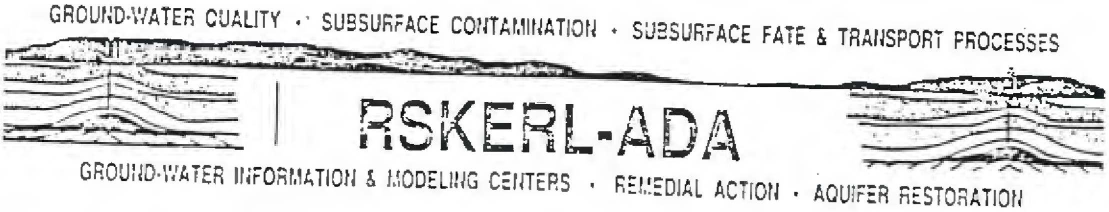
P.O. Box 1198, Ada, Oklahoma 74820
FTS 8-743-2011
(405) 332-8800

The Robert S. Kerr Environmental Research Laboratory (RSKERL) is EPA's center for ground-water research and problems related to subsurface contamination. Current research focuses on providing the necessary fate and transport data bases and support to help the Agency:

- ☐ Establish criteria for waste disposal activities to prevent contamination of ground water or movement of contaminants through the subsurface to points of withdrawal or discharge;
- ☐ Assess the impacts of existing pollution on ground water at points of withdrawal or discharge;
- ☐ Develop remedial actions for protecting and restoring ground-water quality that are neither unnecessarily complex and costly nor restrictive of other land uses; and
- ☐ Regulate the production, use, and disposal of specific chemicals possessing an unacceptably high potential for contaminating ground water when released to the subsurface.

Much of the laboratory's research is process oriented, focusing on the hydrologic, abiotic, and biotic processes governing the fate and transport of contaminants in the subsurface. Simulation models are developed based on soil and subsurface process descriptions for describing and predicting the migration, dissipation, and transformation of pollutants likely to be encountered in soil and subsurface environments under varying conditions of environmental release. The use of natural soil and subsurface systems for the attenuation and degradation of wastes is studied as a treatment option for both point and nonpoint sources of pollution as well as for remedial action in connection with land-based spills or accidents and existing waste disposal sites.

RSKERL is also developing methodologies for aquifer restoration. A major research objective is to demonstrate reliable and effective management of subsurface treatment systems that are applicable to various climatic conditions, soil types, waste characteristics, degrees of pretreatment, and other system variables. Applied research on specific problems related to underground injection control, underground storage tanks, and land treatment of hazardous wastes is also conducted. RSKERL has been instrumental in sponsoring the International Ground-Water Modeling Center at Holcomb Research Institute in Indianapolis, Indiana, and Delft, the Netherlands.



RESEARCH PROGRAM

Robert S. Kerr Environmental Research Laboratory
U.S. Environmental Protection Agency
Ada, Oklahoma

February, 1989

Background

While EPA has no single authority under which it is charged to protect ground water, virtually every major piece of legislation that governs the Agency's mission contains authority for this purpose, including the Clean Water Act (CWA), the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA or Superfund), Superfund Amendments and Reauthorization Act (SARA), the Safe Drinking Water Act (SDWA), the Resource Conservation and Recovery Act (RCRA), the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), and the Toxic Substances Control Act (TSCA), together with their amendments. This broad spectrum of statutory authority within the Agency also contributes to a fragmentation of ground-water issues, priorities, and regulations.

RSKERL's ground-water research reflects diverse priorities among seven Program Office clients (Drinking Water, Ground-Water Protection, Solid Waste, Emergency and Remedial Response, Waste Programs Enforcement, Underground Storage Tanks, Toxic Substances) ten Regions, and a number of crossmedia offices and task forces. The overall research program is guided by a complex relationship involving three research committees (Water, Solid Waste and Superfund, Toxics and Pesticides), and is coordinated with the efforts of several Office of Research and Development (ORD) offices including the Officeof Atmospheric Deposition, Environmental Monitoring, and Quality Assurance; the Office of Environmental Processes and Effects Research; the Office of Environmental Engineering and Technology Demonstration; and the Office of Exploratory Research, and is implemented in association with four other laboratories (Environmental Research Laboratory in Athens, Georgia [ERL-Athens]; Environmental Monitoring Systems Laboratory in Las Vegas, Nevada [EMSL-LV]; Risk Reduction Engineering Laboratory in Cincinnati, Ohio [RREL-Cincinnati]; and Environmental Research Laboratory in Gulf Breeze, Florida [ERL-Gulf Breeze]).

Mission Statement

The Robert S. Kerr Environmental Research Laboratory (RSKERL) is the Agency's center of expertise for investigation of the subsurface environment. The Laboratory is responsible for management of research programs (1) to determine the fate, transport and transformation rates and mechanisms of pollutants in the subsurface environment including both the unsaturated soil profiles and the saturated zones; (2) to define the processes to be used in characterizing the subsurface environment as a receptor of pollutants; (3) to develop techniques for predicting the effects of pollutants on ground water, soil, and indigenous organisms; and (4) to define and demonstrate the applicability and limitations of using natural processes, indigenous to the subsurface environment, for the protection of this resource from municipal, industrial, and agricultural activities entailing the release of pollutants to the soil or deeper regions of the subsurface. The RSKERL assists in the development of Agency research policy and program guidelines and long-range Agency research plans and recommends specific projects and programs, including the resources and schedules required to accomplish them. Upon obtaining the resources the RSKERL staff carries out the work through its own facilities or field stations, or under contract, cooperative agreement, or interagency agreement with other organizations. The RSKERL is also responsible for coordination of technical assistance to Agency components and others as requested within resources allocated for this purpose.

Theme Areas

There are three primary research themes within the current RSKERL research program. The first theme area constitutes the RSKERL core research program and is called Subsurface Fate and Transport Research. The second theme area comprises the RSKERL applied research program and is called Subsurface Assessment and Reclamation Methodologies. The final theme research area is referred to as Technical Assistance and Technology Transfer.

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 Core Research -------> Applied Research -----> Information Transfer
   |                         |                          |
Subsurface Fate  ----> Subsurface Assessment ---> Technical Assistance
and Transport          and Reclamation            and Technology Transfer
Research               Methodologies
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Subsurface Fate and Transport Research (Core Research)

According to the Long-Range Research Agenda for the Period 1988-1992, the Agency has certain mandates that form the primary needs which drive a continuing Agency core research program. This program consists of:

1. human health risk methods development and application,
2. ecological risk methods development and application,
3. total exposure methods development and application, and
4. risk reduction research.

The primary needs driving the RSKERL Research Program are numbers two, three and four of the above list.

For the major category of ecological risk assessment, emphasis will be placed on research activities that contribute to improved prediction of impacts on ecosystem function and structure, on techniques for assessing effects from complex mixtures, and on characterizing uncertainties with risk estimates.

In the area of risk reduction/control technology, emphasis will be placed upon developing transport, transformation, and fate models as well as working with industry to explore alternative treatment technologies such as biodegradation.

The RSKERL Research Program will continue to provide research data and information concerning transport processes, transformation processes and site characterization. These topics are in direct conformance to the legislative mandates which form ORD's research activities.

Transport Processes are physical and chemical interactions that cause contaminants to move through the subsurface at rates different from those of water. Transport processes research at RSKERL is focused on four major areas of concern: (1) sorption processes; (2) facilitated transport phenomena; (3) behavior of complex wastes; and (4) transport processes in heterogeneous anisotropic media. Both inhouse and extramural research activities in these areas have the objectives of defining mechanistically the processes involved and evaluating the implications of these processes. A goal inherent in these efforts is the refinement of models directed at representing the processes affecting mobility and fate of contaminants in ground water.

Much progress has been made in recent years on defining the relationship between soil organic matter and sorption of nonpolar organic molecules. A better understanding of sorption in low-carbon subsurface environments is also beginning to emerge although the capability to accurately forecast pollutant mobility is still lacking. There are other important questions remaining in the sorption area. Of primary interest are environmental factors that control the sorption of polar and ionic organic contaminants and the impact of these contaminants on the mobility of nonpolar organics. There are also questions on sorption and retardation in the real-world complex matrices present at contamination sites and on the practical effects of sorption kinetics on contaminant transport.

Facilitated transport is a generic term encompassing phenomena that enhance contaminant mobility. Evidence of such mobility enhancement has been observed at a number of contaminated sites, but the processes responsible have yet to be clearly identified. Several key research areas being pursued at RSKERL are: (1) dissolved organic carbon enhanced transport; (2) particulate transport; (3) mixed-solvent-enhanced transport; and (4) preferentialpathway water movement. These represent widely disparate research areas, but are linked by their common effects on the movement of contaminants.

The behavior of complex wastes has recently emerged as a focal point of abiotic processes research. Three current concerns in this area are the physical characteristics that regulate the movement of immiscible liquids through the subsurface, movement of other relatively immobile organic contaminants partitioned into the immiscible fluid matrix, and the chemical processes involved in the dissolution and weathering of the fluids.

The latter concern is part of a larger question about the efficacy and economies of pump and treat technologies for remediation of complex-waste-contaminated aquifers. Although the physics of water flow in homogeneous media is reasonably well understood, the physics of water transporting contaminants in heterogeneous media is poorly understood. Lack of understanding of how contaminants move in the subsurface severely restricts our ability to make risk assessments or clean-up ground water.

Current RSKERL research is trying to improve our understanding of the physics of fluid flow-through research to determine how immiscible fluids will move through porous media, the impact of immiscible fluids on the physical properties of the porous media, contaminant transport in fractured rock, and delineation of the physical components of dispersion.

During the next five years core transport research will be focused in three areas: (1) improving laboratory methods for obtaining data needed to mathematically describe the partitioning/sorption between phases in the subsurface; (2) laboratory studies to improve our understanding of facilitated transport processes and the mobility of macro-molecular and colloidal materials; (3) development and testing of contaminant transport models describing multiphase fluid flow based on first principles. The outputs from laboratory process studies will produce information required to develop deterministic system models for risk assessment. The transport models will be reported in the form of computer codes and user's guides.

Site Characterization entails hydrologic and geologic descriptions of a contaminated area to better understand the fate and transport of site specific pollutants. Assumptions of homogeneity and complete mixing are probably inappropriate in most subsurface situations. But the impact of the variable distribution of important subsurface parameters on the processes that control pollutant transport and fate has not been investigated in a systematic fashion. The mathematics needed to describe this impact must be further developed and evaluated. RSKERL is pursuing studies in these areas, including application of geostatistical techniques to describe subsurface systems.

During the next five years core research in site characterization will be focused in two areas: (1) developing new field methods for obtaining valid in-tact samples of subsurface media and new field methods for measuring physical and chemical properties of the geohydrologic system; (2) development of numerical methods to characterize the variability of a site. Both geostatistical and stochastic methods will be considered. The descriptions of the geophysical methods will be output as technical notes and journal manuscripts. The geostatistical and stochastic studies will be output in the form of computer software and user's guides.

Transformation Processes are chemically and biologically mediated transformations of contaminants in the subsurface into other compounds. The Laboratory's research efforts on transformation processes are designed to acquire the insight and information necessary to predict the behavior of pollutants in the subsurface environment. Transformations can destroy organic pollutants and immobilize toxic metals, or they can produce new substances that are more hazardous and more mobile than the original contaminants.

Transformation processes also influence the survival and transport of viruses and other infectious agents in the subsurface. An understanding of these fundamental biological processes is needed to develop control and remediation technologies for ground-water quality protection. RSKERL core program supports work at the level of process discovery (what can occur), and process definition (when a process can be expected, and at what rate and extent).

Transformation processes are the ultimate risk reduction mechanism. Most of the effort will be in the form of laboratory studies attempting to define the rate and extent of transformation of xenobiotics as a function of the controlling environmental parameters. Simultaneously, the microbiological community controlling these transformations will be characterized biochemically. The potential for abiotic transformation is still at the discovery level.

Summary of Core Research: Predictive research provides the basis for assessing the risk of ground-water contamination upon the subsurface environment and for understanding subsurface processes that eventually may lead to cleanup methodologies. Sorption, biotransformation, transport, mixed solvent interactions, oxidation reduction, hydrolysis, dechlorination, dispersion, fractured flow, and immiscible flow will be investigated for organic chemicals that could pose significant risk. Research will continue on virus survival and transport, and metals mobility. Contaminant-transport models will be adapted and modified to include the improved process descriptions. Field evaluations will determine the degree of confidence that can be expected from predictive models in various hydrogeologic environments.

Assessing the risks associated with various methods of waste disposal is a critical aspect of the Agency's RCRA program, but is an area of major scientific uncertainty. Developing the scientific and technical information needed to establish the quantity and types of wastes that escape into the environment through different disposal methods, the effects they produce for both human health and the environment and the methods for assessing their risks will remain a significant area for research activity for some time. Moreover, given that most existing information is based on the properties of individual chemicals, rather than the complex mixtures of chemicals typically found in wastes streams, the state-of-knowledge in this area will require several years to develop.

The information developed to support this research area will be used by EPA in permitting and enforcement decision making, regulatory policy making, and implementing the land-banning program. Products will provide more applicable, less expensive, and more accurate information and risk assessment methodologies. Increased development and validation of promising field screening techniques (e.g., cone penetrometer) will have the potential to provide Superfund pollutant characterization.

Subsurface Assessment and Reclamation Methodologies (Applied Research)

The Resource Conservation and Recovery Act (RCRA) authorizes a regulatory program to identify wastes which pose a substantial hazard to human health or the environment, and develop waste management standards which protect human health and the environment. Research support for this program provides the scientific and engineering basis for characterizing wastes, determining the hazards they pose and formulating controls. In addition, Section 311 of the Clean Water Act authorizes research to support prevention and control of hazardous material releases.

The Office of Emergency and Remedial Response (OERR) requires scientific research and technical support from the Office of Research and Development (ORD) to investigate and mitigate health and environmental problems at the priority sites listed under authority of the Comprehensive Environmental Response, Compensation and Liability Act (CERCLA), as amended by the Superfund Amendments and Reauthorization Act of 1986 (SARA). ORD's research program provides a core of scientific and technical information to support the implementation requirements of CERCLA and the enforcement actions undertaken to obtain cleanup and recovery of costs. It concentrates on assessing health and environmental risks posed by Superfund sites and on evaluating equipment and techniques for discovering, assessing, preventing, controlling, removing and ultimately disposing of hazardous substances released into the environment. RSKERL research and support activities consist of programs to develop and evaluate validity of methods for detection and evaluation of adverse environmental effects, to evaluate alternative control and removal technologies, and to develop effective monitoring systems.

Superfund develops technologies and information needed for assessing and managing uncontrolled sites. The success of removal and remedial actions frequently depends on an accurate appraisal of the nature and severity of the problem. Research activities supporting this objective provide the techniques and procedures for on-scene coordinators' needed to quickly and effectively assess the degree of hazard and the cleanup requirements at specific uncontrolled waste sites.

These legislative requirements drive the ORD Research Program which further determines the direction of the RSKERL Research Program. RSKERL continues to provide information concerning (1) subsurface exposure assessment, (2) subsurface reclamation, (3) underground injection control, and (4) wellhead protection.

Subsurface Exposure Assessment includes the estimation of the temporal and spatial distribution of contaminants in the environment. Exposure assessment is a requisite initial step in any risk analysis. In this theme area, studies are performed evaluating exposure assessment methodologies that have been developed primarily in the form of mathematical models. Outputs include: data bases utilized in making exposure assessments, expert systems for more appropriate application of exposure assessment models, and demonstrations/evaluations of the application of site specific and generic mathematical models.

There are presently over 600 documented mathematical models describing movement of fluids in the subsurface ranging from simple analytical solutions to highly complex numerical models. RSKERL has an extensive program to develop, evaluate and improve mathematical models for use by Federal and State agencies in predicting the transport and fate of contaminants in the subsurface. The principal avenue of model information transfer is the International Ground Water Modeling Center (IGWMC) at Holcomb Research Institute, Indianapolis, Indiana. The Center maintains annotated data bases of mathematical models used to simulate fluid movement and contaminant transport, offers hands-on training courses and conducts research to develop benchmark methods for the intercomparison and validation of existing models.

Subsurface Reclamation involves restoring the contaminated subsurface to a predetermined level of cleanliness. All research characterizing the nature of a release, as well as the site itself, is requisite for selection and implementation of site remediation technology. In addition, factors such as cost, resources at risk, and ground-water value, must be weighed in selection of a remediation technology. It is obvious that no universal formula can exist prescribing successful site remediation; each site provides its own unique set of problems determined by site specific characteristics. It is, therefore, imperative that sufficient site characterization information be available to allow informed selection of a remediation technology. A remediation technology successful at one site may be totally inappropriate at another site. Remediation technology effective in the early stages of remediation may not be effective at later stages. Hence, integration of several remediation technologies may , in some cases, be the most efficient option.

Implementation of a site remediation technology entails involvement in reducing contaminant concentration and mitigate release effects through contaminant containment, removal, or transformation. RSKERL research programs have investigated chemical techniques using cationic surfactants for contaminant migration control, or containment. Research on contaminant sorption-desorption kinetics has uncovered limitations of pump and treat technology for contaminant removal. CERCLA lists various approved methods for remedying releases and additionally mandates the development of cost-effective remediation alternatives to off-site treatment or disposal. RSKERL has committed substantial resources to laboratory and field-scale development of remediation alternatives, such as enhanced biological contaminant transformation methods.

Successful implementation of remediation technology is dependent on continuing and initiating the following research:

1) Microbial transformation of contaminants and the effects that transformation enhancement amendments (methane, oxygen, hydrogen peroxide, nitrate) have on aquifer chemistry and physics.

2) Staged aerobic/anaerobic treatment technology. Different classes of contaminants are more easily degraded in oxydizing or reducing environments.

3) Removal of residual saturation of an immiscible phase. The residual saturation will be very difficult to remove by "soil washing" techniques and will constitute a long lived source term for contaminant input to ground water.

4) Chemical techniques for contaminant migration control.

5) Development of decision support systems such as expert systems for making decisions on site remediation.

The Underground Injection Control Program is concerned with the safety of underground injection as a hazardous waste disposal method. Both the Safe Drinking Water Act of 1974 and the Hazardous and Solid Waste Amendments of 1984 address the question of protection of ground-water quality as a result of injection of waste into the subsurface by means of deep wells. EPA regulations in support of this legislation have been based on assuring that the use of injection wells for the disposal of waste will not endanger human health or the environment.

Research in support of the underground injection control program is investigating three areas: the mechanical integrity of the injection well itself; the injection zone and confining bed; and the interaction of injected waste with the formation or formation fluid within the injection zone.

RSKERL has research projects in each of these areas through the extramural research program. In addition, RSKERL has constructed three research wells near Ada for the purpose of assessing methods for determining the mechanical integrity of injection wells. The research wells, which are 1215, 1530, and 1550 feet deep, are designed to evaluate the integrity of cement behind both steel and fiberglass casing and to evalute a variety of methods for determining leaks in tubing, casing or packers and fluid movement behind the casing. These research wells provide EPA with a unique research facility unmatched in government, academia, or industry.

This research will be extremely important over the next few years due to the regulatory requirements of the Hazardous and Solid Waste Amendments of 1984. EPA is required to reconsider the safety of underground injection as a hazardous waste disposal method and to ban such injection should there be migration out of the injection zone. EPA has a number of research activities under way to aid the Office of Drinking Water in making these determinations, including determining the fluid movement from wells, describing the interaction of injected fluids with the geological strata, and characterizing saline formations in the Texas Gulf Coast as receptors of hazardous wastes. Research will determine the mechanical integrity of injection wells, the location of abandoned wells and the practices associated with nonhazardous injection.

The Wellhead Protection Program focuses on developing methods and information needed to develop strategies for wellhead protection areas. Current research is focused on improving the methods currently being used to delineate wellhead protection areas and evaluation of the the ground-water flow, and fate and transport models available for wellhead protection and delineation. In addition, ongoing research will provide analysis of the impact on wellhead protection areas from multiple sources of contamination and evaluate the effectiveness of control methods both technical and institutional on the prevention of ground-water contamination in a wellhead protection area.

Summary of Applied Research: Research will continue at RSKERL in characterization of contaminant dispersion in ground water using data from recent field experiments to develop a realistic approach to modeling dispersive transport in exposure assessments. In addition, work will continue into the development of criteria for the selection, development and modification of mathematical models that can be applied to exposure assessments.

Aquifer cleanup research will provide cost-effective methods for cleanup of contaminated soils and ground water. Alternatives are needed to current approaches such as withdrawal and treatment or containment. Promising laboratory methods for enhancing subsurface biotransformation will be field tested, the safety of using genetically-engineered organisms for biodegradation will be determined, and the application of these methods to leaks from underground storage tanks will be evaluated.

Underground injection control research will be extremely important over the next few years due to the regulatory requirements of the Hazardous and Solid Waste Amendments of 1984. EPA is required to reconsider the safety of underground injection as a hazardous waste disposal method and to ban such injection should there be migration out of the injection zone. EPA has a number of research activities underway to aid the Office of Drinking Water in making these determinations, including determining the fluid movement from wells, describing the interaction of injected fluids with the geological strata, and characterizing saline formations in the Texas Gulf Coast as receptors of hazardous wastes. Research is ongoing to determine the mechanical integrity of injection wells, the location of abandoned wells and the practices associated with nonhazardous injection.

RSKERL research will be used to improve and evaluate the ground-water flow, and fate and transport models available for wellhead protection and delineation. Methods and information will be developed on critical wellhead protection factors such as radius of influence around a well or well field, depth of drawdown of water table by well or well field, and the time and rate of travel of various contaminants in various hydrogeological settings. In addition, the research will provide analysis of the impact on wellhead protection areas from sources of contamination and evaluate the effectiveness of control methods both technical and institutional on the prevention of ground-water contamination in a wellhead protection area.

Technical Assistance and Technology Transfer (Information Transfer)

To be effective, research results must target and be disseminated to operational personnel, program managers, and decisionmakers in a timely manner. Field personnel in EPA Regions, states, and local government agencies must deal with an extremely broad and complex range of data and information, and deserve close support from scientists and engineers in ORD laboratories. While there is no technical assistance line item specific to ground water, ground-water issues are becoming a major focus of technical requests from client offices. Therefore, the RSKERL has made significant advances in disseminating its research products and making available its research scientists for requested technical assistance from EPA Regions, state and local authorities and Program Office personnel.

The Superfund Subsurface Remediaton Technical Support Program at RSKERL receives its statutory authority from the Superfund Amendments and Reauthorization Act of 1986 (SARA) which directs the EPA, as part of the overall Superfund site clean-up program to conduct a program of research, evaluation and demonstration of alternative or innovative technologies for response actions that will achieve more permanent solutions. As part of that effort the Office of Solid Waste and Emergency Response and the Office of Research and Development have established a Subsurface Remediation Support Program (SRSP) at RSKERL that provides decision-makers with a source of easily accessible, up-to-date subsurface fate and transport information with the associated expert assistance requried to effectively use this information. Components of SRSP include:

- Subsurface Remediation Technical Support Team--composed of ten scientists and engineers to provide a readily available source of technical assistance;
- Subsurface Remediation Information Clearinghouse--designed to provide the user community highly specialized, fate, transport, and remediation information;
- National Center for Ground Water Research--consortium of Oklahoma, Oklahoma State and Rice Universities charged with developing and conducting a long-range exploratory research program to help anticipate and solve the Nation's emerging ground-water problems;
- International Center for Ground Water Modeling--Holcomb Research Center Indianapolis, clearinghouse for ground-water modeling software, providing research, short courses, seminars and educational activities;
- RSKERL Extramural Research Program--expertise of subsurface processes and systems from more than 30 universities and research insitutions.

UPDATE ON BIORESTORATION RESEARCH AT RSKERL

2/89 John Wilson

Removal of vinyl chloride in an aquifer — Stanford at Moffett Field

In this field season, the Moffett Field demonstration site is examining the co-oxidation of vinyl chloride during the aerobic metabolism of methane. Removals are dramatic, more than 98% in a single pass.

Removal of trichloroethylene from well water — USAF/Battelle

In the spring of 1989, Battelle will install a bioreactor to treat TCE contaminated water from a monitoring well at Tinker AFB in Oklahoma City.

Removal of trichloroethylene from air — U. of Oklahoma/in-house

A laboratory bioreactor has successfully treated TCE vapors in air. The reactor can reduce the concentration of TCE an order of magnitude in 15 minutes.

Removal of trichloroethylene from purge wells — For Emergency Response Team, in-hous

The laboratory bioreactor is being scaled up, to build a mobile unit that can remove TCE from water produced during pump-and-treat activities. The unit will contain an air stripper to treat the water. The gas from the air-stripper will be regenerated in the bioreactor, then recycled through the air-stripper. This will advoid TCE release to the atmosphere. The unit will be field tested in the summer of 1989.

Removal of a fuel spill using hydrogen peroxide — U.S. Coast Guard/in-house

A 50 foot interval of aquifer contaminated with aviation gasoline has been remediated by circulating water amended with mineral nutrients and hydrogen peroxide. Based on the total amount of fuel in the aquifer, each liter of ground water in the contaminated interval would require 90,000 mg of oxygen for complete bioreclaimation. The demonstration only required 3,000 mg oxygen per liter of ground water. This corresponds closely to the theoretical oxygen demand of the BTX fraction of the fuel spill. Apparently, biological activity selectively removed the compounds of regulatory concern.

Remediation of an oily waste using nitrate — U.S. Coast Guard/in-house/U.S. Air Force

Nitrate will be used to remediate a jet fuel spill. Nitrate does not cause some of the plugging problems often seen with hydrogen peroxide. The demonstration will start in the winter of 1988/89.

in-house

Microcosms are being used to define the kinetics and stoichiometry of denitrification of the alkylbenzenes, and other components of fuels.

Removal of carbon tetrachloride from an aquifer — Biosystems/Stanford

In the spring of 1989, the Moffett Field facility will be used to study co-oxidation of carbon tetrachloride supported by denitrification of certain alcohols.

Natural anaerobic bio-transformation of TCE in aquifers

USCG/ in-house

Cores will be acquired from an aquifer that transforms a plume of TCE to dichloroethylene and vinyl chloride. The cores will be used to construct microcosms. The kinetics of TCE biotransformation in the microcosms will be compared to the actual rate of transformation in the field.

Natural removal of atrazine in water-table aquifers

NSI/ in-house

Aseptic cores will be acquired from water table aquifers to assess the capacity of microorganisms that occur naturally in aquifers to degrade atrazine. Cores will be obtained from areas that have received atrazine and control areas that have not. The first set of cores will be obtained in 0/89.

Anaerobic and aerobic biotransformation of PCBs

NSI/ in-house

Prospects for the transformation of PCBs will be examined in mixed oily waste. The possibility of co-oxidation of PCBs during aerobic metabolism of high-molecular-weight hydrocarbons will be examined. Reductive dechlorination of PCBs during anaerobic metabolism of butyrate will also be examined. The wastes have been acquired from Superfund sites and are undergoing an initial chemical screening.

Environmental biochemistry of TCE co-oxidation

NCGWR at OSU

The mono-oxygenases of a variety of microorganisms are being compared at the genetic and biochemical level. The monooxygenases are being screened to identify the most apporpriate enzyme for the construction of a strain capable of degrading TCE as a sole carbon and energy source.

Kinetics of denitrification in aquifers

Oregon State University/ in-house

The kinetics of denitrification of simple organics such as ethanol are being studied in a large laboratory physical model. The object is to develop in-situ techniques for nitrate removal. The effect of substrate concentration on aquifer plugging is being systematically explored.

Biotransformation of BTX by sulfate reducing organisms

in-house

Mixed cultures capable of removing alkylbenzenes under sulfate-reducing conditions have been isolated from a drip-gas site. Work is in progress to identify the biochemical pathways, and to characterize the organisms involved.

Survival of genetically-engineered organisms in hazardous waste sites

in-house

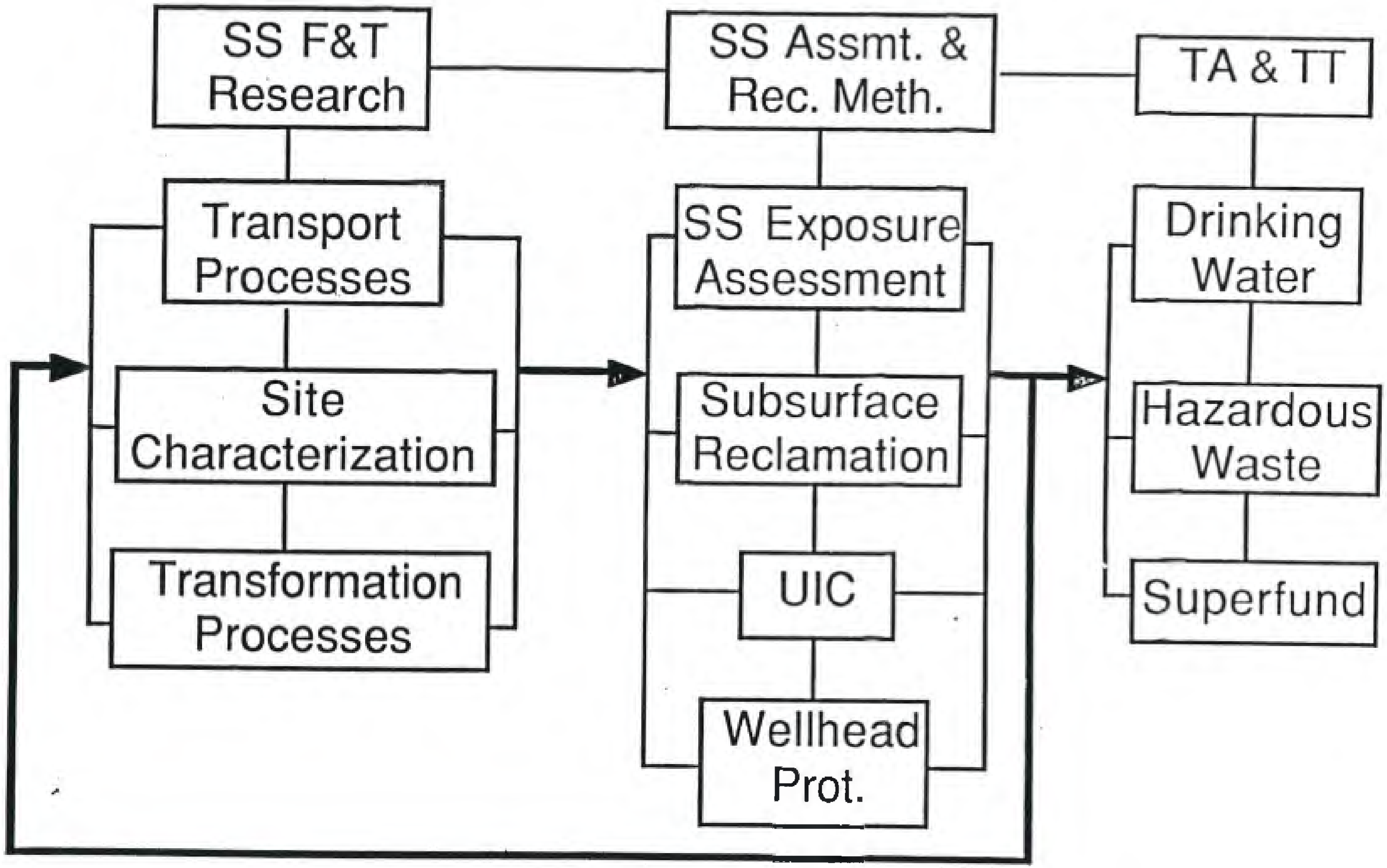
A microbe capable of denitrifying toluene will be labelled using genetic techniques, then the propagation and survival of the organism will be studied in aquifer material contaminated with JP-4 jet fuel. The plasmids necessary to construct the strain have been obtained.

AGENCY MANDATES

- Source Control
- Damage Assessment
- Remedial Action

RSKERL MAJOR RESEARCH THEMES

- Subsurface Fate and Transport Research
- Subsurface Assessment and Reclamation Methodologies
- Technical Assistance and Technology Transfer


SS F&T Research
SS Assmt. & Rec. Meth.
TA & TT
Transport Processes
Site Characterization
Transformation Processes
SS Exposure Assessment
Subsurface Reclamation
UIC
Wellhead Prot.
Drinking Water
Hazardous Waste
Superfund

BASIC CORE RESEARCH

- Subsurface Fate and Transport Research
    - Transport Processes
    - Site Characterization
    - Transformation Processes

APPLIED RESEARCH
(Program Office Needs)

- Subsurface Assessment and Reclamation Methodologies
    - Subsurface Exposure Assessment
    - Subsurface Reclamation
    - Underground Injection Control
    - Wellhead Protection

INFORMATION TRANSFER

- Technology Assistance and Technology Transfer
    - Drinking Water
    - Hazardous Waste
    - Superfund

RSKERL MAJOR RESEARCH THEMES

- Subsurface Fate and Transport Research
- **Subsurface Assessment and Reclamation Methodologies**
- Technical Assistance and Technology Transfer

In Situ Biodegradation Methodologies for Restoration of Contaminated Aquifers

Project Objective: The basic objectives of this portion of the project are to optimize the proposed method for the enhanced in situ biodegradation of halogenated aliphatic compounds and to determine the necessary processes to treat a real contamination incident.

In Situ Biorestoration of a Gasoline Spill

Project Objective: The objective of this project is to develop and evaluate engineering design criteria for the use of hydrogen peroxide to restore aquifers contaminated by gasoline spills.

TCE Aerobic Degradation

Project Objective: To provide information required for the intelligent design of ground water reclamation methodologies based on the biodegradation of trichloroethylene and related compounds by microorganisms that aerobically metabolize gaseous aliphatic hydrocarbons.

A Soil-Bioreactor to Treat Vapors Produced by In Situ Restoration of Hazardous Waste Sites

Project Objective: To conduct field demonstrations of the use of soil bioreactors for treating hydrocarbon vapor streams produced by in situ air stripping at sites of subsurface contamination by petroleum hydrocarbons.

In Situ Biorestoration of a Gasoline Spill Utilizing Nitrate - Respiring Bacteria

Project Objective: The objectives of this project are to: (1) build a model describing the degradation of BTX by nitrate-respiring bacteria in aquifers contaminated with oily materials; (2) use the model to design a Biosystem demonstration for in situ clean up of oily wastes; (3) carry out the Biosystem demonstration; and (4) evaluate the model by comparing the actual progress of the clean up to the projections of the model.

CTORS CONTROLLING THE RATE AND EXTENT OF EMEDIATION AT FIELD SCALE

- Rate of supply of essential nutrients, usually the electron acceptor
- Spatial variability in flow velocity
- Seclusion of the waste from the microorganisms

INTERPRETATION OF TREATABILITY STUDIES FOR SUBSURFACE REMEDIATION

A good treatability study determines whether bioremediation is **possible**, and whether there are any **biological barriers** to attaining the goal for clean-up. It can also provide an estimate on the rate of remediation that can be attained if the organisms are not limited by the rate of supply of some essential nutrient.

[illegible] 51

ch 4.5

;TS ASSOCIATED WITH ƷURFACE REMEDIATION

ΓERIZATION

Wells, Soil Gas Survey, Coring and Core Analysis, Geological Section, Aquifer Tests, Tracer Tests

REMEDIAL DESIGN

Treatability Tests, Mathematical Modeling

SYSTEM DESIGN

Permits, Negotiating trade-offs between cost and time required

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4-6

MORE COSTS ASSOCIATED WITH JBSURFACE REMEDIATION

1 INSTALLATION

ls, infiltration galleries, pumps, pipelines, tanks, control devices, treatment systems

MATERIALS AND OPERATING EXPENSES

Water, electron acceptor, fertilizer, inoculant, maintenance, power, sewer charges

MONITORING

Monitoring wells and pumps, cores and their analysis

SITE SECURITY AND OPERATIONAL OVERSIGHT

EVALUATION
OF
TREATMENT

5-11-35
[illegible]

THE OPERATION SYSTEM AS WELL PERFORMANCE

- Delivery of mineral nutrients
- Delivery of electron acceptor
- Position in the water table
- Effectiveness of containment

HOW OFTEN SHOULD MONITORING WELL BE SAM

The frequency of sampling should be related to th
for significant changes to occur along the most co
path.

IMPORTANT CONSIDERATIONS

- Time required for water to move from injection wells to the monitoring wells
- Seasonal variations in water-table elevation or hydraulic gradient.
- Changes in the concentration of electron acceptor.
- Cost of monitoring compared to day-to-day cost of operation.

PROBLEMS WITH WELLS AS MONITORING TOOLS

Treatment can occur in the well itself. The water in the well may not be representative of the water in the aquifer.

A conventional monitoring well produces **a composited water sample.** Water from the most contaminated flow path is diluted by water from many other flow paths that are less contaminated.

A water sample from a well tells nothing about the amount of hazardous material that is **absorbed** to aquifer solids or is **trapped** as an oily phase.

PRESENTATION BY MR. STEVE RAGONE

Ragona

WATER FACT SHEET
U.S. GEOLOGICAL SURVEY, DEPARTMENT OF THE INTERIOR

TOXIC WASTE-- GROUND-WATER CONTAMINATION

BACKGROUND

Safe cleanup and management of hazardous wastes is one of the most critical environmental problems that will confront the United States for the next several decades. Of the 35 million tons of hazardous waste disposed of in 1979, the U.S. Environmental Protection Agency (EPA) estimates that 90 percent was handled improperly. Improper disposal of hazardous waste imposes high costs on the public and affects human health and safety. The cost to clean up and contain contaminated ground water can be as high as several million dollars per site. Although most of our Nation's ground water remains uncontaminated, in local industrial and agricultural areas some ground water has been severely affected. In recent years, hundreds of wells supplying drinking water to millions of people have been condemned because of contamination.

The variety of toxic substances requiring disposal is increasing and the quantity of waste is growing 3 to 5 percent annually. Safe management of these wastes merits as much attention as cleanup of wastes that already contaminate or threaten the environment. Present technology and regulations are not adequate to protect the public from all hazardous-waste contamination in a cost-effective manner. Major technical questions are yet to be answered regarding the behavior of specific wastes under different hydrogeologic conditions and on the safety, suitability, and economics of waste disposal and site-restoration methods.

THE U.S. GEOLOGICAL SURVEY TOXIC WASTE—GROUND-WATER CONTAMINATION PROGRAM

Waste disposal and contamination of water are inseparable issues. Water flowing on or beneath the land surface dissolves and transports wastes. The natural water circulating in its geologic framework (soils and rock) both spreads and, in some situations, cleanses contaminated water. The U.S. Geological Survey (USGS), since its inception in 1879, has been concerned with the development of earth-science information related to water contamination. Its many studies in hydrology, the study of the movement of water and its chemical constituents through surface- and ground-water systems, and in geology, the study of the earth and the processes acting on it, provide the scientific information necessary to properly manage hazardous waste.

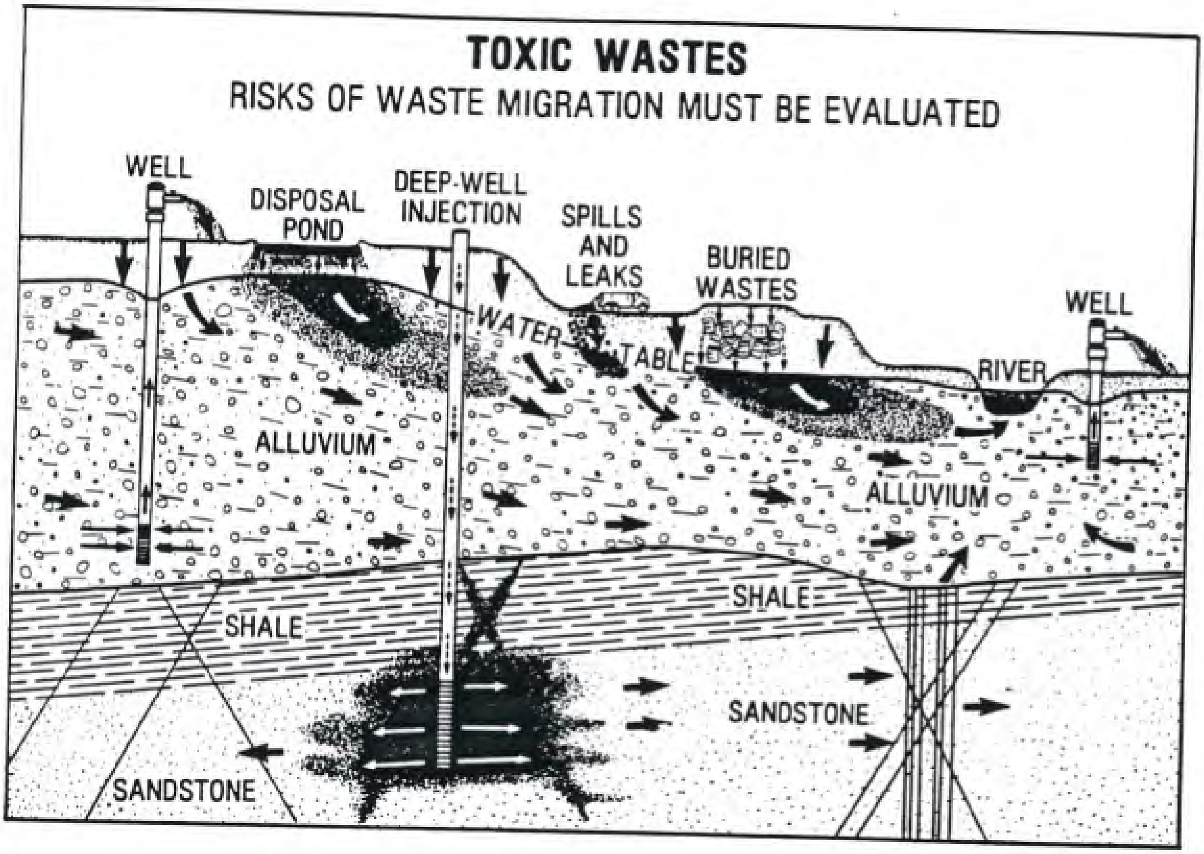
In fiscal year 1982, the USGS began an interdisciplinary program—the Hazardous Waste Hydrology Program—to provide the Nation with earth-science information that can be used to improve waste-management practices and to help solve problems of toxic-waste management and ground-water contamination. The Hazardous-Waste Hydrology Program, which includes the Toxic-Waste—Ground-Water Contamination Program and programs concerned with the disposal of low-level and high-level nuclear wastes, builds upon information about the impact of hazardous wastes on water quality derived from past and present related USGS programs, such as Radioactive Waste Disposal—Nuclear Hydrology, Regional Aquifer-Systems Analysis (RASA) Program, Federal-State Cooperative Program, Subsurface Waste-Storage Program, and studies of glacial deposits in the Eastern United States.

Purpose:

The purpose of the Toxic Waste—Ground Water Contamination Program is to provide other Federal agencies (such as the EPA), State and local governments, and industry with earth-sciences information that they need in order to improve waste disposal-practices and regulations and to solve existing and prevent future problems related to management of toxic-wastes.

Activities:

- Bring together USGS scientists representing different disciplines, such as hydrology, geology, geochemistry, and microbiology, to study the behavior and fate of contaminants in the subsurface environment.



- Develop technical guidelines for application of earth-science principles to the disposal of toxic wastes and the management of contamination problems.
- Assess the general state and trends of ground-water quality across the Nation.
- Improve monitoring, sampling, and "exploration" procedures that define and analyze the potential for or extent of ground-water contamination.
- Develop predictive models and assess their reliability and value for determining the potential for or extent of ground-water contamination from disposal sites.
- Provide information in readily understandable language and usable formats to Federal, State, and local agencies and to industry.

Information on technical reports, overview reports, and hydrologic data related to USGS Toxic Waste—Ground-Water Contamination Program can be obtained from:

Hydrologic Information Unit
U.S. Geological Survey
419 National Center
Reston, Virginia 22092

November 1983

PRESENTATION BY DR. H. G. MONBOUQUETTE

BIOTECHNOLOGY RESEARCH AT THE UCLA ERC FOR HAZARDOUS SUBSTANCE CONTROL

Presented by H.G. Monbouquette, Assistant Professor
Chemical Engineering Department
5531 Boelter Hall
University of California, Los Angeles
Los Angeles, CA 90024-1592
213-825-8946

Introduction

The Engineering Research Center Program was initiated by the National Science Foundation (NSF) in 1985 to utilize American universities on the front line of research and education directed at the needs of technology-intensive US industries. With their interdisciplinary emphasis and strong systems orientation, these centers have been conceived to play a vital role in our nation's competitiveness in world markets.

Although there are currently 18 Centers operating across the country, ours at UCLA is the only one with an environmental mission. In addition, NSF sponsorship gives our program a longer term and broader perspective than those found in EPA-sponsored and industrial-consortium type centers. Our Center is also inherently much more involved in developing educational and fundamental research programs designed to attract "the best and the brightest" students to the field of environmental engineering.

To solve our environmental problems we must encourage a positive, complementary relationship between government and industry. Our Center contributes to this process by offering a neutral forum where key technical professionals in government gain exposure to the needs and concerns of industry. These same government people will ultimately influence their non-technical regulatory colleagues to adopt an approach to environmental problems that is both fair to industry and yet protects society.

By providing seed funding, NSF has taken an important step in creating an incentive for cross-disciplinary, problem-focused, collaborative research and training within our educational system. By seeking industrial participation in the oversight and support of NSF Centers, it is providing the additional incentive for testing the relevance of Center-sponsored research to real industrial and societal needs.

Our primary goals are to conduct ground-breaking research on advanced technologies and risk assessment; to transfer effectively new technologies to industry, government and academia; to promote and conduct quality education in the environmental area; and to foster industry and government collaboration in our endeavors. An interdisciplinary approach is taken toward the achievement of our Center goals. Representatives from five departments in the School of Engineering and Applied Science, from the School of Public Health, from the Department of Chemistry and Biochemistry, and from the Graduate School of Library and Information Science participate in research, education and training.

Center activities are administered by a Directorate composed of our Director, Executive Director, and Thrust Leaders in each of four research areas: Waste Minimization, Treatment and Disposal, Risk and Systems, and Remediation (see Fig. 1, "ERC Organization"). Industry and Government representatives contribute valuable input to our program through the Policy Advisory Group and the Technical Advisory Committees (Fig. 2) and by collaborating in research, education and technology transfer at the Center. Research in biotechnology at our ERC for Hazardous Substance Control cuts across three of our Research Thrust areas, waste minimization, treatment and disposal, and remediation. Ongoing projects include ground-breaking, exploratory research into new methods for the biodegradation of hydrocarbons and halogenated hydrocarbons and the selective recovery of toxic heavy metal species from dilute aqueous solution.

Microbial Degradation: Detoxification by Bacterial P-450 Monooxygenase--V. Vilker, Chemical Engineering Department

Biotreatment relies on microorganisms that have the ability to degrade specific contaminants to harmless byproducts. Halocarbon contaminants, which are a widespread problem affecting subsurface water quality, have been generally regarded as biorefractory because few microorganisms possess the enzymes to degrade part-per-million or lower concentrations of halocarbons.

Most microbial treatment processes for elimination of organic chemicals like trichloroethene (TCE) or dibromochloropropane (DBCP), regardless of whether they occur naturally in a soil remediation or if they are "engineered" into the design of a wastewater treatment device, require microorganisms that use the substrate to be removed as a primary carbon or energy source. In our research, we are looking at microorganisms and/or the enzyme systems which they generate that can be engineered to carry out detoxification reactions on organics for which the concentration levels are so low that they would not satisfy food or energy needs.

Our current studies are on the generation and use of microbial P-450 monooxygenase enzyme systems in specially-cultured *P. putida* bacteria for the detoxification of these hazardous organic compounds. The conversion of BTM (Cl_3CBr) to chloroform (see Fig. 3) indicates a reductive biodehalogenation mechanism in the aerobic *P. putida* cultures. Also, we have been investigating the activity of resting cultures of *P. putida* PpG-786 for degrading trace levels of the substrate 1,2-dibromo-3-chloropropane (DBCP) as a function of the intracellular concentration of the enzyme cytochrome P-450_{cam} monooxygenase. The enzyme is generated only when camphor is used as sole carbon source during culturing of the bacteria. Spectrophotometric measurements of intracellular P-450 monooxygenase concentration correlates with the ability of *P. putida* cells to carry out biodehalogenation, although other intracellular electron transfer reactions are also significant to determining the overall rate. Comparing reported octanol/water partition coefficients and measured degradation kinetics for BTM and DBCP suggest that the overall biodehalogenation by resting bacteria cells is under intrinsic enzyme kinetic control, and not by rate of halocarbon permeation through the cell membrane.

	$K_{o/w}$	$t_{1/2}$, minutes
BTM	1000	90
DBCP	100	15

The stability of both the intracellular enzyme concentration and dehalogenation activity were shown to decline when the resting cultures were aged up to 48 hours at room temperature. The activity and enzyme were retained when the cultures were aged to 48 hours at refrigeration temperatures. This data is especially exciting because it offers first evidence that there may be a way to stabilize monooxygenase activity for long enough time to use the organisms in field remediation situations. Hopefully, this stabilization will be achievable at temperatures somewhat higher than 4°C. Dehalogenation activity did not correlate as well with cell viability as with enzyme concentration. DBCP toxicity toward the bacteria was present over the entire substrate concentration range that was investigated, and at about one millimolar initial DBCP concentration, dehalogenation activity was almost completely lost (see Fig. 4).

During the next twelve months, the focus of the research will be on two projects. In the first we will continue our efforts to understand better how the culturing conditions of *P. putida* during camphor metabolism, and to obtain high productivity of cytochrome P-450 enzyme that is also active for biodehalogenation. The advantage of a continuous culture system is that the effect of individual parameters can be analyzed, and it provides a constant source of cells in an exponential phase of growth. The studies will be conducted in a chemostat, which can be operated in a batch or continuous mode, and which consists of a 2 liter stirred vessel and peripheral equipment to control temperature, pH, agitation and dissolved oxygen.

In the second project, we intend to study the activity of this bacteria/enzyme combination for degrading trace levels of phenanthrene, a polynuclear aromatic hydrocarbon. Microsomal enzymes, particularly microsomal P-450, and fungal enzyme systems have been shown to degrade phenanthrene and other PAH's via a monooxygenase enzyme catalyzed pathway. Studies on bacterial enzyme systems to date have focused, however, on the dioxygenase enzyme catalyzed degradation pathways of organisms induced by or grown on the compound of interest or one which is closely related. Degradation of trace amounts of contaminants is not possible via these dioxygenase systems. In this project, we will assess the feasibility for PAH degradation via the extensively studied bacterial P-450_{cam} monooxygenase enzyme study of *P. putida* PpG-786.

Microbial Degradation: Halocarbon Degradation by Methane-Oxidizing Bacteria--R. Mah, School of Public Health

Introduction: Recalcitrant halogenated and nonhalogenated organic compounds are difficult to eliminate from the environment and are becoming increasingly problematic as pollutants. Trichloroethylene (TCE) and other chlorinated, organic chemicals are widely used as solvents and as feedstock in the plastics manufacturing industry. Recalcitrant, nonchlorinated organic compounds are components of fuels, oils, and lubricants. In aquifers near spill and disposal sites, chlorinated and nonchlorinated compounds are detected at concentrations up to many thousand ppb. They are persistent in the environment and may be transported rapidly in groundwater. Chlorinated organic compounds may be biologically transformed by various consortia of anaerobic bacteria; however, some of the resulting transformation products, *e.g.*, vinyl chloride, are more toxic to man than the original materials. Microbiological evidence indicates a role for aerobic, methane-oxidizing bacteria in the formation of enzymes which may play a role in the co-metabolism of the chemically analogous compounds. One of the goals of our project is to determine if soil samples obtained from contaminated groundwater aquifers may be enriched for aerobic, methane-oxidizing bacteria, if these organisms can aerobically degrade/transform recalcitrant compounds, such as TCE and others, and to study the biotransformation to find ways to enhance *in situ* treatment.

We have isolated in pure culture the methane-oxidizing bacteria from enrichment cultures inoculated with sediment from a contaminated groundwater aquifer and sediment from an uncontaminated freshwater stream. Both organisms are cocco-bacillus shaped, Gram negative, catalase negative, strictly aerobic microorganisms that can use methane or methanol as sole sources of carbon and energy. The ability of the pure cultures to transform TCE was tested and the time-course of TCE transformation was determined; TCE was transformed by the pure cultures as well as the mixed, enrichment cultures. After growth on methanol the ability to metabolize methane and TCE was inhibited. The isolate form the contaminated aquifer could not use chlorinated, short chain carbon compounds, sugars, yeast extract, or Trypticase peptone as sole sources of growth and energy; sludge extract was used. We have determined the temperature, 25°C, and pH, 6.4, for optimum growth of the isolate.

We are conducting further studies to assess the ability of the pure cultures, enrichment cultures, and other samples for the ability to transform chlorinated and other organic compounds.

Methane-oxidizing enrichment cultures: Soil and water from a depth of 9 to 10 meters were collected from a TCE-contaminated groundwater aquifer beneath the Rockwell Corporation, Canoga Park, CA and a sediment sample was collected from a freshwater stream on the UCLA campus. Samples of the aquifer soil were used to inoculate enrichment cultures containing an aquifer-water based medium, the stream sediment was inoculated into synthetic mineral medium; both were given methane gas as the energy substrate. Active methane-oxidizing enrichment cultures were obtained from samples of both sites. When transferred to the same media, the cultures grew more quickly than the first incubation. This finding showed that there are *in situ* methane-oxidizing bacteria in the sediments and that their numbers may be increased by enrichment with methane gas.

The aquifer water medium was replaced with the synthetic medium and the culture grew, even after many transfers, just as well in the synthetic mineral medium, showing that no growth factors in the aquifer water were required. For all further work the synthetic, mineral medium was used.

Experiments to determine if the methane-oxidizing enrichment cultures could transform TCE were initiated by inoculating cultures into medium with added TCE. Methane-dependent TCE-transformation occurred and twice the quantity of TCE was transformed when methane was present compared to control cultures without methane. Figure 5 shows the results of an experiment in which the transformation of TCE was followed over time in the Rockwell enrichment cultures with different conditions. TCE was measured in headspace-gas samples with a gas-chromatograph equipped with an electron capture detector: squares, culture fed 5% methane; diamonds, culture with methanol; triangle, autoclaved control with methane; cross, culture with no substrate.

TCE has been found in waste-site leachates at concentrations of thousands of μg L^{-1}. We found that TCE was not toxic to the cultures at concentrations up to 1000 μg L^{-1}. Figure 6 shows the results of an experiment to determine if the methane-oxidizing enrichment cultures, grown with inoculum from sediment samples collected from the TCE-contaminated aquifer beneath Rockwell International, Canoga Park, California, are inhibited by high concentrations of TCE. The optical density of the cultures is on the y-axis and time, in days, on the x-axis. Conclusions are that the culture was not inhibited by high TCE concentrations and we considered these data when designing experiments to study transformation of TCE.

Pure cultures: To further our studies on the TCE transforming ability of our cultures, we wished to isolate the most numerous methane-oxidizing organisms present in the cultures. To isolate the organisms, we prepared solid plate-medium by adding purified Gelrite, a bacterially derived gelling agent similar to agar, to the synthetic mineral medium. We streaked samples of the liquid enrichment cultures on the plates and incubated them in closed vessels with a methane/air atmosphere. Repeated attempts at isolation on plates gave no methane consuming colonies, only organisms that were metabolizing the impurities in the Gelrite. We postulated that insufficient methane and/or air was entering the plates under the lids and so we devised a different system for isolation of the organisms on solid medium. To improve gas-transfer to the cultures molten solid-medium was solidified on the sides of 65 ml serum vials, serial ten-fold dilutions of liquid cultures were inoculated onto the solid surface by streaking through the opening, the vials were sealed with butyl rubber stoppers, and methane was added. Within several days methane consumption began and colonies were visible. Colonies were picked and streaked on the same medium several times to ensure strain purity and finally transferred to sterile liquid medium.

Both organisms are cocco-bacillus shaped, Gram negative, catalase negative, strictly aerobic microorganisms that can use methane or methanol as their sole sources of carbon and energy. Transmission electron micrographs of whole cells of the isolates show bundles of parallel, stacked membranes within the cells. Clusters of such membranes are typical of methylotrophic bacteria and the characteristic parallel/stacked arrangement shows that our isolates are "Type One" methylotrophs.

The optimum temperature for growth of the isolates is 25°C and the optimum pH is 6.4. The time-course of TCE transformation and methane depletion by the pure cultures has been measured. TCE transformation begins when methane is limiting at the end of the growth phase. TCE was degraded, up to 1500 $\mu g\ L^{-1}$, within four days during the growth of the culture compared to uninoculated controls (Figure 7). This concentration and rate is greater than that reported by other studies of methane-dependent TCE transformation. In Figure 8, it is shown that the Rockwell methanotroph can degrade TCE to 200 $\mu g\ L^{-1}$ within six days (diamonds represent methane consumption).

Nutritutional characteristics and energy substrates of the pure culture were determined. The addition of yeast extract of Trypicase peptone (pancreatic digest of casein) did not enhance the growth rate with methane and they were not used as growth substrates; however, clarified anaerobic digestor sludge supported growth. The chlorinated compounds chloroform, trichloroacetic acid, carbon tetrachloride, ethylene dichloride, 1,3 dibromopropane, 1,1,1 trichloroethane, and the carbohydrates glucose, sucrose lactose, galactose, mannose, xylose, maltose, and fructose were not used as substrates for carbon and energy.

Whole cell deoxyribonucleic acid of the isolates has been purified; the mole percent guanine plus cytosine of the organisms was 62%. This value will be used as a taxonomic characteristic for classification of the microorganisms.

Examination of other classes of compounds: The techniques that we have used to study the transformation of a chlorinated organic compound are classic methods of environmental microbiology. We have employed the basic concepts of enrichment and habitat simulating medium to show that there are naturally occurring microbes in the local environment of a contaminated aquifer site capable of transforming an unnaturally occurring, xenobiotic compound. With these same, and related, techniques we will examine the degradation of other compounds that are recalcitrant and environmentally problematical. We are consulting with other members of the bioremediation group to determine what chemicals or classes of compounds would be appropriate and fitting with the overall goals of the Center.

Optimization of growth conditions: We will continue to study the Rockwell isolate and begin studying the pure culture from UCLA with respect to optimization of TCE transformation and growth. With these data the benefits of altering the physical conditions of aquifer water during on-site, *in situ*, treatment to enhance contaminant transformation can be determined.

Co-metabolic studies: Methane-oxidizing bacteria have been shown to transform TCE, which is regarded as a co-metabolic substrate of the enzyme methane-monooxygenase. The observation that TCE transformation occurs after methane has been depleted strongly supports the theory that TCE is attacked as a co-metabolite by the methane oxidizing enzymes of the cultures. If TCE is a co-metabolic substrate of methane-monooxygenase, then methane would be a direct competitor with TCE at the enzyme's active site. By comparing the TCE transformation rate of methane limited cells with that of cells which are actively metabolizing methane, we can infer competitive relationships. These data may suggest methane feeding regimens at *in situ* treatment sites which could increase TCE transformation with decreased methane requirements. We will perform the same studies with other chlorinated or nonchlorinated, recalcitrant organic molecules.

Degradation and Biotransformation of Xylenols, Cresols, and Polyaromatic Hydrocarbons in Acclimated Activated Sludge: Use of Enrichment Reactors to Enhance this Process--M. Stenstrom, Civil Engineering Department

The activated sludge process is widely used to treat organic wastes in both publicly owned and industrial wastewater treatment plants. The process is being used more and more to treat organic hazardous wastes. Various modifications are being developed to optimize the process for removal of specific compounds. The use of an enrichment reactor, fed specific compounds is one such approach.

Structural analogs of target compounds, intermediates in the degradation pathways of the target compounds, and compounds causing gratuitous induction of enzymes utilized for the degradation of the target compounds are used in the enrichment reactors. The enrichment reactor is separate from the main activated sludge process and can be operated under different conditions and geometries, *e.g.*, batch sequencing reactors, to provide optimal growth conditions (see Fig. 9).

In this study two classes of target compounds are being investigated: menthylated phenols such as xylenols, cresols and trimethylphenols, and PAH's (polyaromatic hydrocarbons) such as napthalene, phenanthrene and anthracene. 2,4, xylenol was used as a structural analogue for the first class of compounds. For the second class of compounds 2-amino benzoic acid and salycilate were used because they are degradation intermediates and/or caused gratuitous induction of enzymes. Figure 10 shows time curves for degradation of napthalene in batch reactors containing activated sludge conditioned on napthalene and glucose/nutrient broth, aminobenzoic acid and succinate, and salicylate. The ordinate on this plot is napthalene concentration normalized to its initial concentration and the average biomass concentration, MLVSS. Results to date show that enrichment reactors can be used to provide improved treatment efficiency by accelerating removal rates for the target compounds. Both classes of compounds showed enhanced removal. Future work will be focused on expanding these findings.

Basic computer modeling and simulation of the volatilization or stripping of organics from activated sludge, using oxygen as the tracer is also included.

Biomimetic Systems for Metal Waste Minimization--H.G. Monbouquette, Chemical Engineering Department

The processing of hazardous metal-laden wastes presents a unique challenge. Unlike toxic organics, dissolved heavy metal species cannot be converted through degradative means to non-toxic substances. There are only two alternatives: isolate the contaminated waste from the biosphere or recover the metal for return to a process. Given the high strategic and economic value of many heavy metals, the latter approach, where possible, is often the most practical.

Highly toxic metal-laden waste is generated by a number of industries including: oil refining, energy, mining, nuclear, microelectronics, aerospace, and electroplating. Since electroplaters use many of the same toxic heavy metals in plating processes as appear in their aqueous waste streams, this industry is a logical target for a waste minimization effort. The major metal-laden waste streams originate from the rinsing of plated substrates between process steps and from spent acid cleaning and plating baths. The rising cost of disposal fees, more stringent regulations imposed by the EPA, and the value of recoverable metals have encouraged electroplaters and other metal waste generators to consider processes and plant designs for "zero metal discharge".

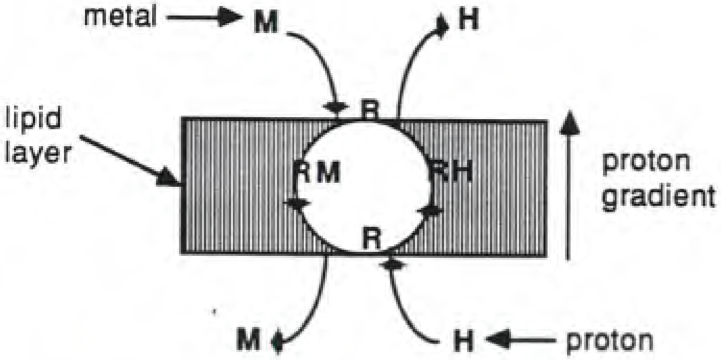
The installation of more efficient continuous plating plants could do much to reduce waste generation. However, implementation of this strategy will require the development of new sensing and control systems to govern the plating process. Currently, electroplating is carried out primarily as a batch process with few controls, *e.g.*, time and current. There is a critical need for sensors capable of monitoring plating bath, rinse and effluent stream composition.

In order to achieve the goal of zero metal discharge, technologies must be developed to ensure that virtually the only metal outlet from the plant is the final plated product. The currently available methods of metal recovery, evaporation, electrodialysis, reverse osmosis, and electrolytic cell processes, all are deficient for one or more of the following reasons: lack of selectivity, inability to reduce metal-ion concentration to EPA standards, instability, and/or high operating cost. There is a growing body of evidence, however, indicating that biologically-derived or biomimetic metal-recovery systems may exhibit the required selectivity (separation factors $>10^3$), metal-sorbing capability (binding constants up to 10^{50}), and low operating cost.

Highly efficient mechanisms such as the inducible synthesis of specific metal-complexing agents (*e.g.*, metallothioneins and siderophores) and membrane-based metal efflux systems have evolved in a wide variety of organisms to control intracellular metal concentrations. DeVoe and Holbein have covalently attached siderophores and synthetic siderophore-like ligands to solid supports such as porous glass to create powerful, highly specific sorbents with the demonstrated capability to reduce heavy metal concentrations in aqueous streams from hundreds of ppm to less than 0.01 ppm. Such biomimetic systems should be explored further, both for the design of metal recovery systems and for the development of specific metal-ion sensors.

We propose the development of membrane-based biomimetic separation systems that utilize highly selective carriers and a proton gradient to "pump" a particular metal species from a dilute waste stream to a metal-rich solution for eventual recycle to a process. By coupling multivalent metal ion transport to an opposing flux of protons, metal ions can be concentrated 10,000-fold or more. The use of ether lipids from archaebacteria in ultrathin (5 nm) biomimetic membrane systems is being pursued in our laboratory. The lipid membranes of some of these extremely thermophilic bacteria that thrive at temperatures of up to 105 °C and pHs <2.0 or >9.0 have been found to resist decomposition in boiling detergent solutions.

Similar "supported liquid membrane" systems consisting of an organic solvent with a dissolved lipophilic metal carrier trapped in the pores of a hydrophobic sheet have been constructed and pilot tested for the the recovery of heavy metals. The best of these systems takes full advantage of the Donnan Distribution Law. For example, by coupling divalent metal ion transport to the opposing flux of protons down a 10-fold concentration gradient, 100-fold metal ion concentration factors may be achieved.



Donnan Dialysis Membranes utilize charged carriers that must bind cations to achieve neutrality and to enable their diffusion in the organic solvent filled pores of the membrane. If the initial concentration of protons on side I of the membrane is much greater than the concentration of a multivalent metal cation on side II, the flux of protons down its concentration gradient can be used to "pump" the metal cation up its concentration gradient. The process continues until Donnan equilibrium conditions are reached,

$$\frac{[H^+]_I}{[H^+]_{II}} = \left\{\frac{[Me^{+z}]_I}{[Me^{+z}]_{II}}\right\}^{1/z}$$

where Me is any metal ion of valence, +z.

A major drawback to currently available Donnan Dialysis Membranes is their instability due to leakage of the solvent and carrier from the membrane pores. In some but not all cases, continuous membrane regeneration schemes have been worked out. In place of these liquid membranes, the use of ether lipids from archaebacteria in ultrathin biomimetic membrane systems is proposed. Due to their extreme thinness (5 nm) much improved fluxes of metal species would be expected.

A planar geometry system has the advantage that the metal ions may be transferred from a continuously flowing waste stream on one side of the membrane to a metal-rich stream on the other. However, due to the extreme thinness of our proposed system these membranes may be quite unstable mechanically, and we will probably have to explore the deposition of lipid layers on mechanical supports such as macroporous polypropylene sheets or carbon fiber mats. Similar systems have been created using normal phospholipids and synthetic surfactants but have not been well characterized. In contrast, vesicles must be harvested in an ultrafilter before the metal can be recovered by lowering the pH of the external solution and reversing the metal pumping action, yet these structures are much more stable than the planar membranes.

The creation of affinity membrane systems for metals could be translated into the development of a new generation of very high sensitivity metal ion sensing electrodes for use in process monitoring and control. Lipids can be induced to self-form a defect-free coating on electrodes, and specific metal binding species can be incorporated into this layer, thereby creating a probe for a particular metal ion. Preliminary work with experimental biomimetic membrane systems based on biological sensing mechanisms indicates that detection limits of at least 10^{-8} molar are possible, which is well into the ppb range for heavy metals.

An Expert System to Help Control an Activated Sludge Treatment Plant--D. Okrent, Mechanical Engineering Department; M. Stenstrom, J.B. Neethling, Civil Engineering Department

Of all the biological treatment processes, the activated sludge treatment plants are best suited to handling industrial waste with organic constituents. These plants are widely used and there is a considerable amount of data experience, and open-literature publication on the subject. Theoretical models have been developed to simulate steady state and dynamic conditions, which are useful for design and economic optimization under normal operation. However, severe transients, such as sludge bulking, do occur; they are not completely understood, and pose a major challenge to the operator to keep the plant on line and still meet effluent permit requirements.

This project will develop an expert system to help the operator recover from conditions that would otherwise lead to a situation severe enough to cause shutdown. In part the expert system will depend on current theories as to the cause and nature of upset conditions. In part, the system will use the results of computer analysis of data from an existing plant. Chevron Research has expressed an interest in working with us on the project and steps have been taken to make available data from the Chevron El Segundo activated sludge plant.

Much of the artificial intelligence methodology developed in connection with the expert system to aid control of an incinerator will be relevant to this project. Professor Stenstrom is familiar with the El Segundo Plant, having worked on its operation and design with Chevron engineers for over five years.

It is anticipated that much of what is developed will be generic and applicable to large numbers of similar plants.

ERC ORGANIZATION

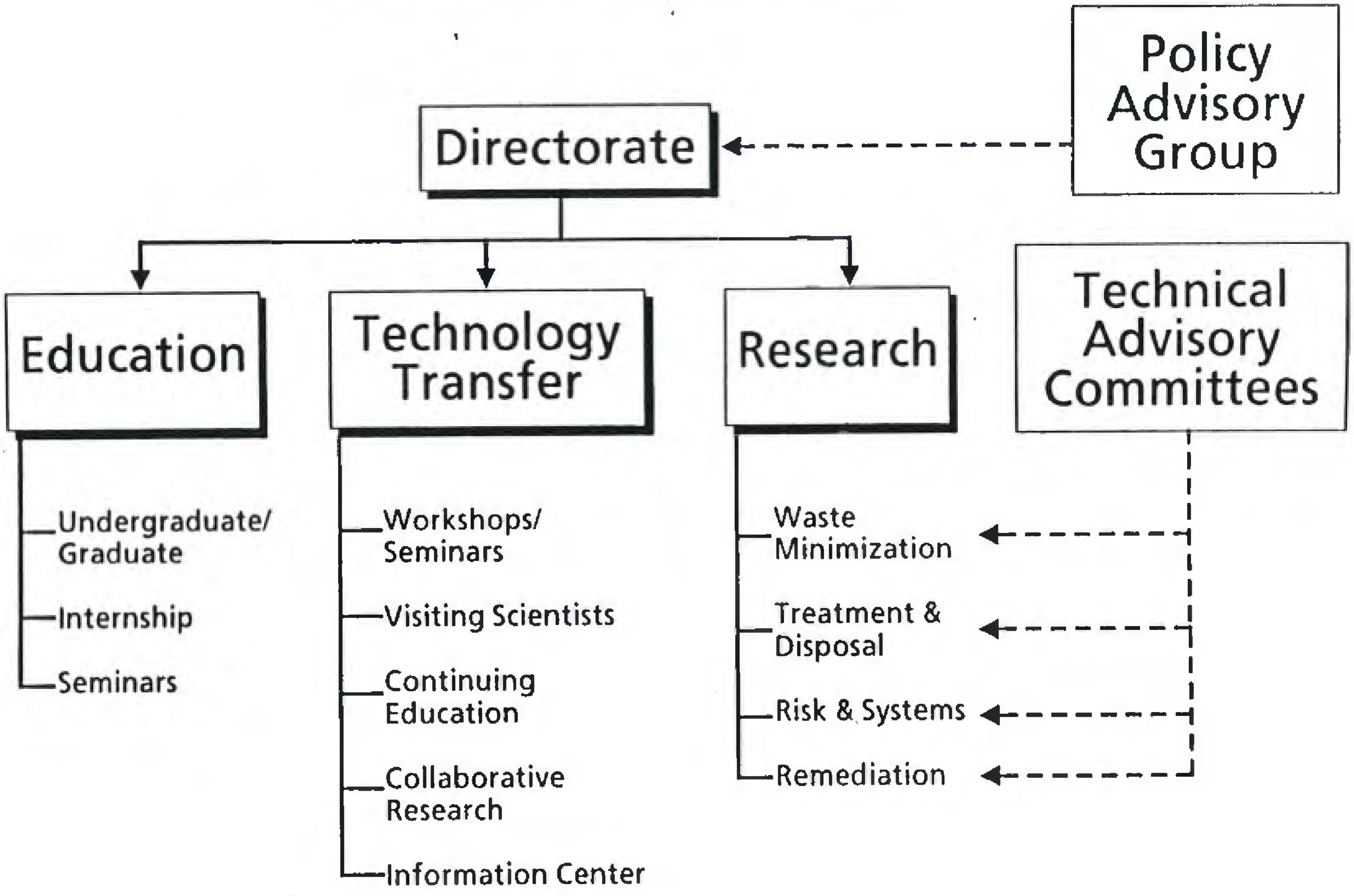


Figure 1

BROMIDE ION RELEASE

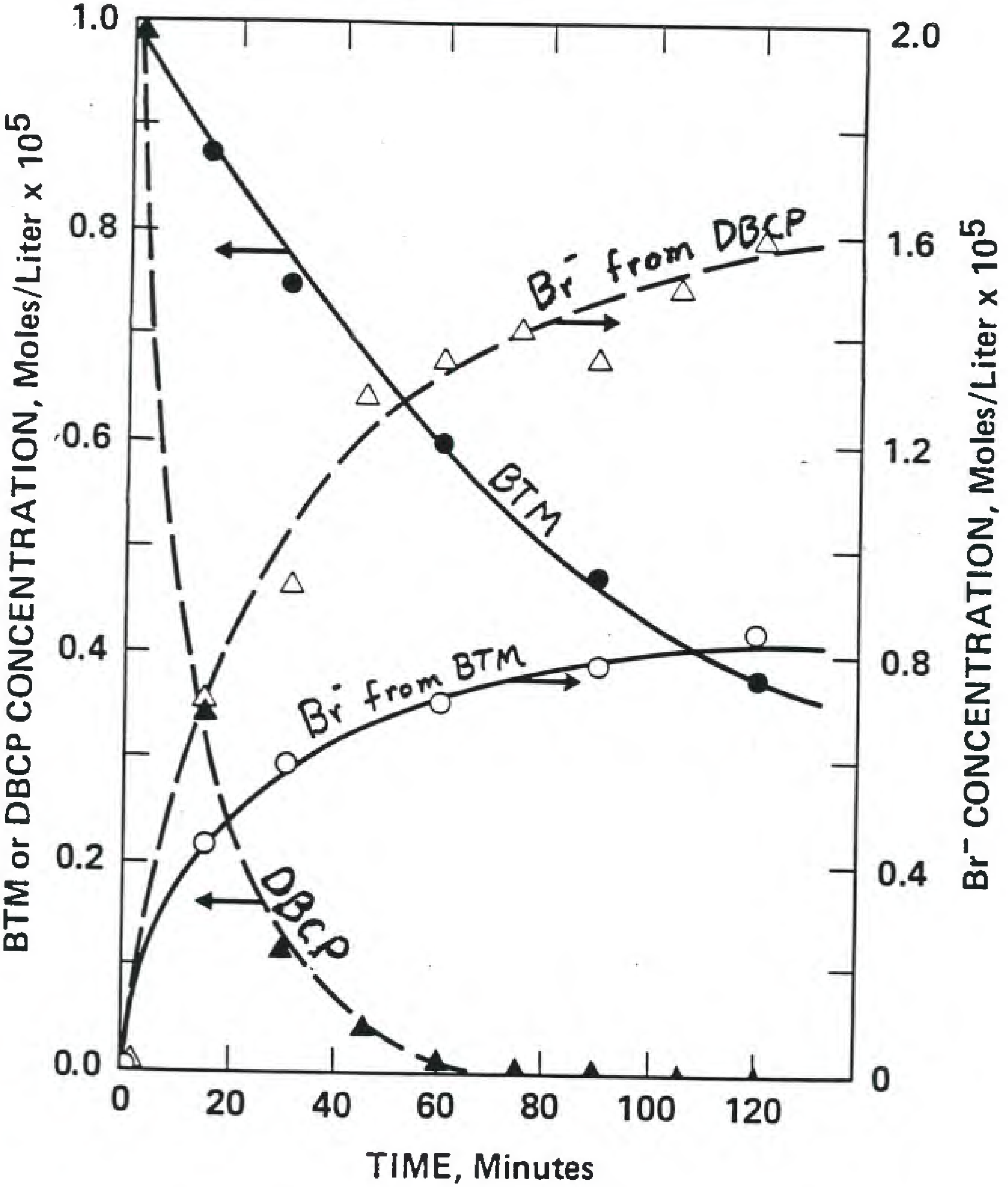


Fig. 3

ENGINEERING RESEARCH CENTER for HAZARDOUS SUBSTANCES CONTROL

TECHNICAL ADVISORY COMMITTEES

Remediation	Treatment & Disposal	Waste Minimization	Systems & Risk
June Andersen IBM	Geoffrey DeZellar SC MWD	Pat Avery ARCO Refinery	Daniel Askenaizer SC MWD
Lisa Anderson SC MWD	Edward Glod Chem Waste Mgmt.	Brad Benton General Dynamics	Joan Berkowitz Risk Science Intl.
Richard Cartwright Chem Waste Mgmt.	Michael Gordbn Portland General Cement	Gail Burke Xerox Corp.	Jerry Blancato US EPA
Joseph Chu General Motors	Richard Grzelak Combustion Engineering	Ian Croll IBM	**Edward Faeder-Chair** Lockheed Aeronautical
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Beverly McFarland-Chair Chevron Research Co.	Jim Ouimette Chevron Research	**Gregory Hollod-Chair** Conoco	Louis Levy CA DHS
Ishwar Murarka EPRI	David Pershing Univ. of Utah	Ravi Jain USA - CERL	Nestor Ortiz Sandia National Lab
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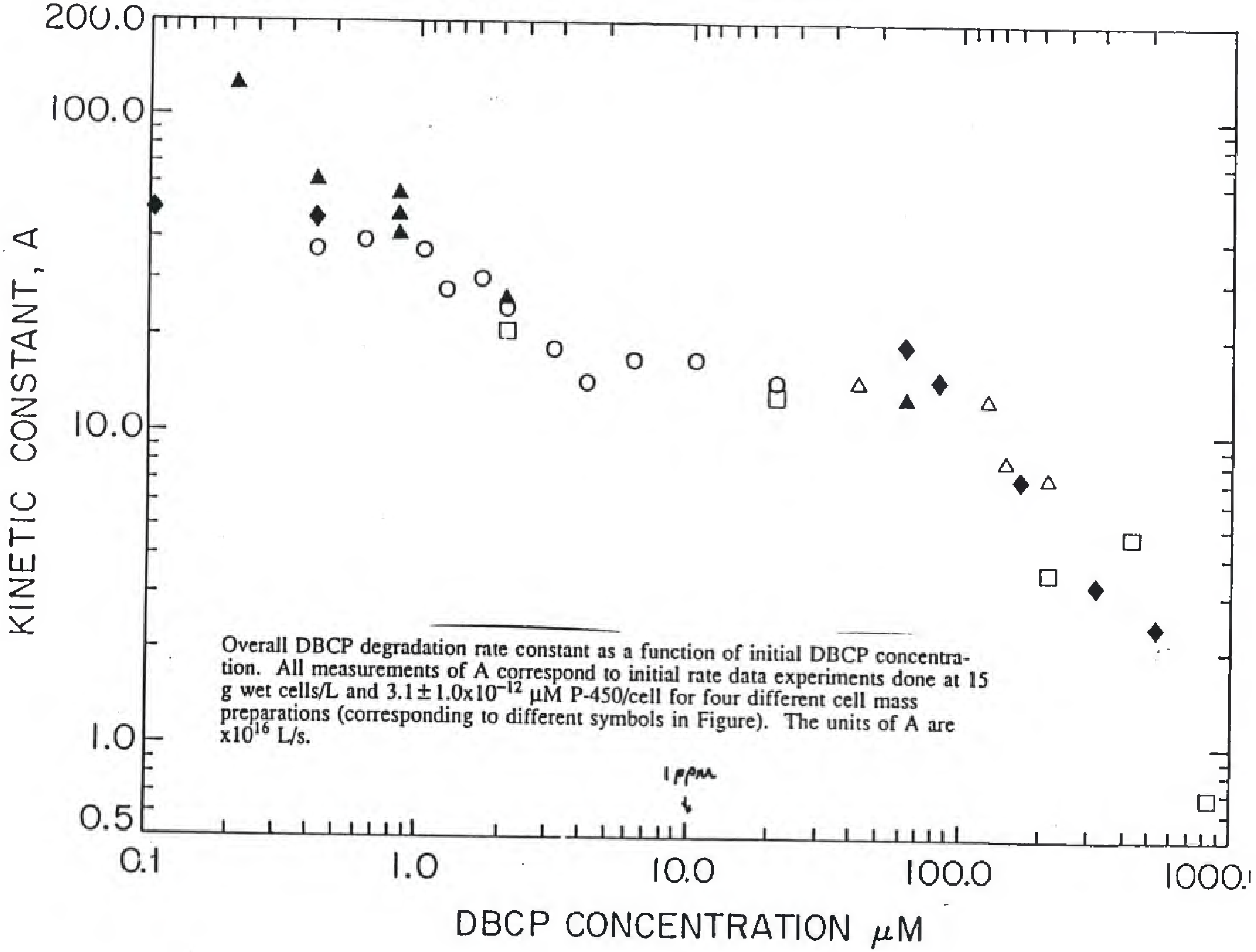
	Loo Tjay Yap The BOC Group	Alex Sapre Hughes Aircraft	Paul Slovic Decision Research
			Detlof Von Winterfeldt USC CRC
			Ron Wysga EPRI

Fig. 2



Overall DBCP degradation rate constant as a function of initial DBCP concentration. All measurements of A correspond to initial rate data experiments done at 15 g wet cells/L and $3.1 \pm 1.0 \times 10^{-12}$ μM P-450/cell for four different cell mass preparations (corresponding to different symbols in Figure). The units of A are $\times 10^{16}$ L/s.

Fig 4

FIGURE
5

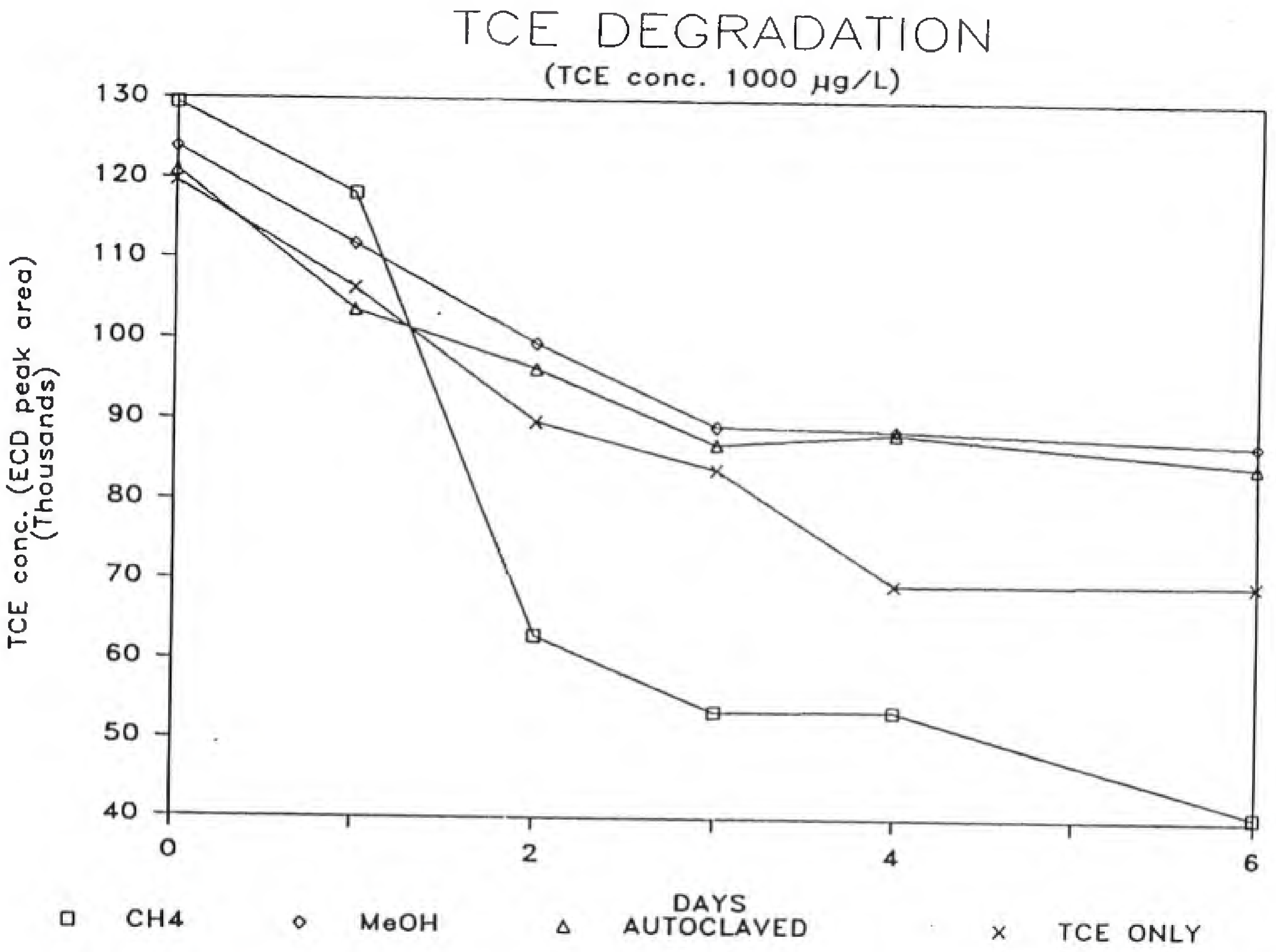

TCE DEGRADATION
(TCE conc. 1000 μg/L)
TCE conc. (ECD peak area)
(Thousands)
130
120
110
100
90
80
70
60
50
40
0
2
4
6
DAYS
□ CH4
◇ MeOH
△ AUTOCLAVED
× TCE ONLY

FIGURE 6

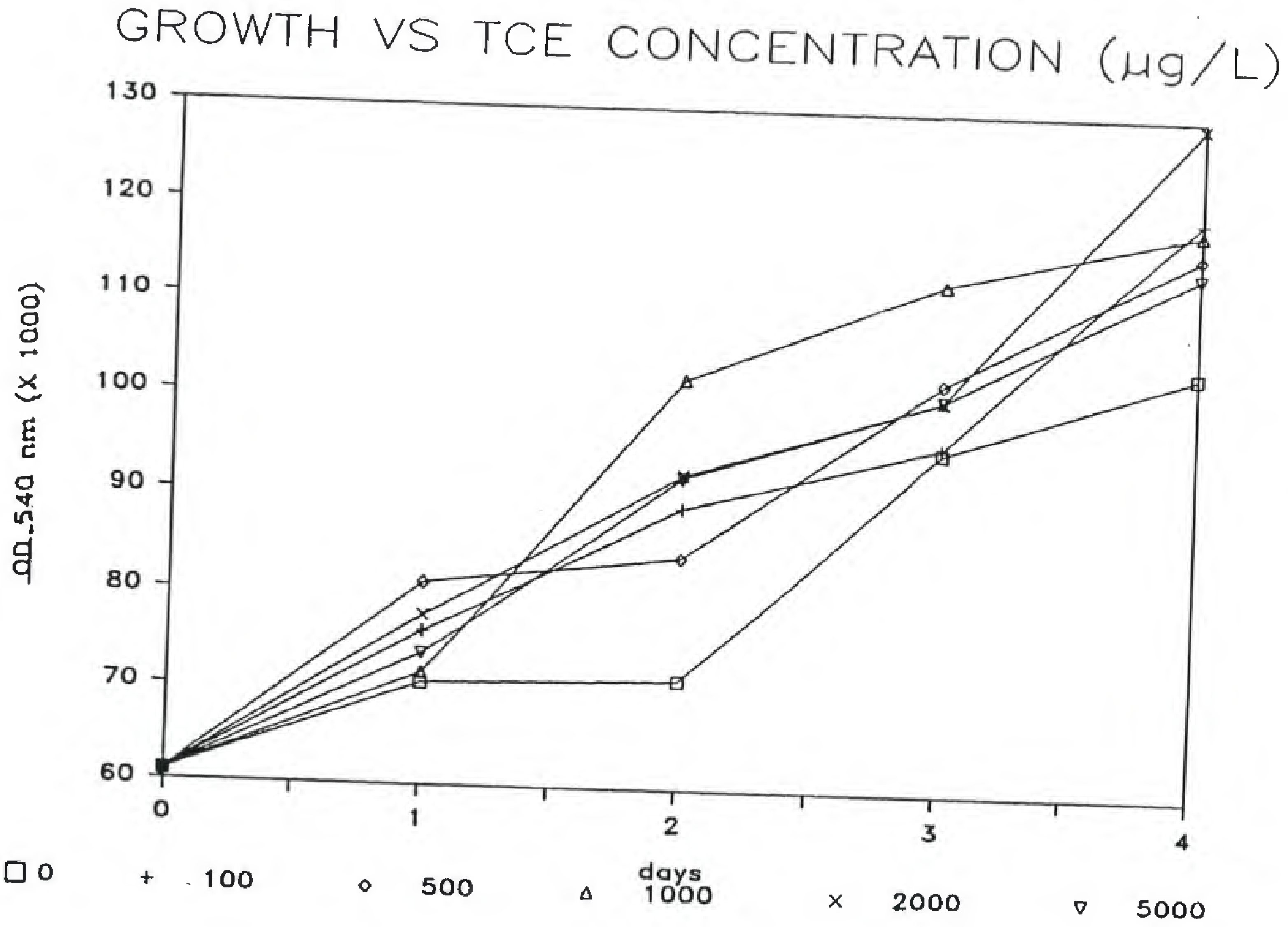
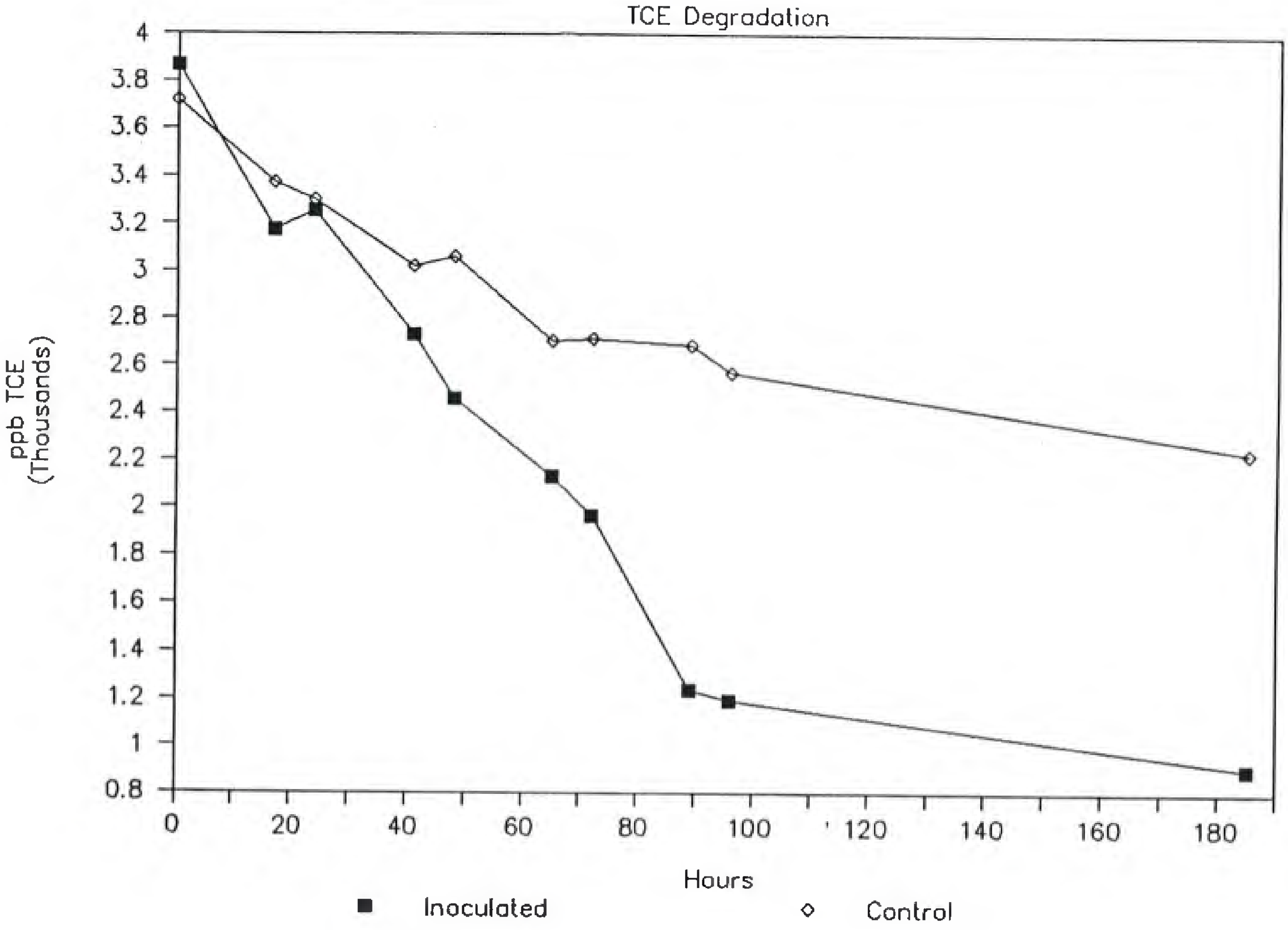

GROWTH VS TCE CONCENTRATION (μg/L)
OD 540 nm (X 1000)
130
120
110
100
90
80
70
60
0
1
2
3
4
days
□ 0
+ 100
◇ 500
△ 1000
× 2000
▽ 5000

Figure 7



TCE Degradation

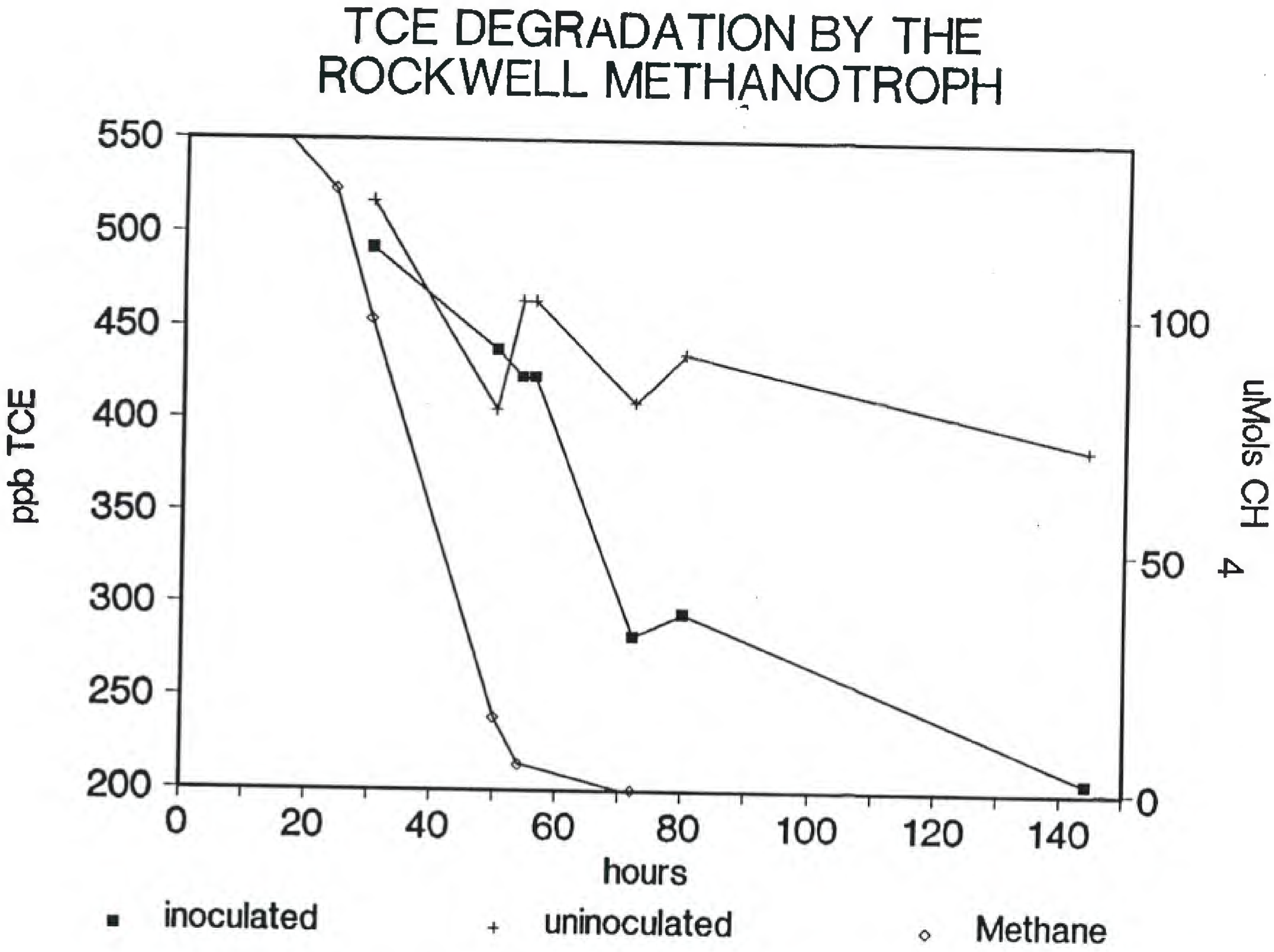

TCE DEGRADATION BY THE ROCKWELL METHANOTROPH
ppb TCE
550
500
450
400
350
300
250
200
0
20
40
60
80
100
120
140
hours
uMols CH 4
100
50
0
■ inoculated
+ uninoculated
◇ Methane

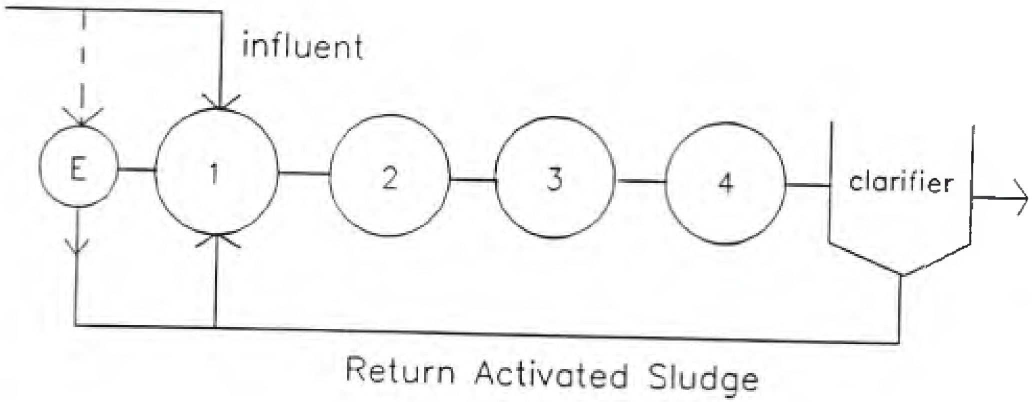
Fig. 8

Fig. 9

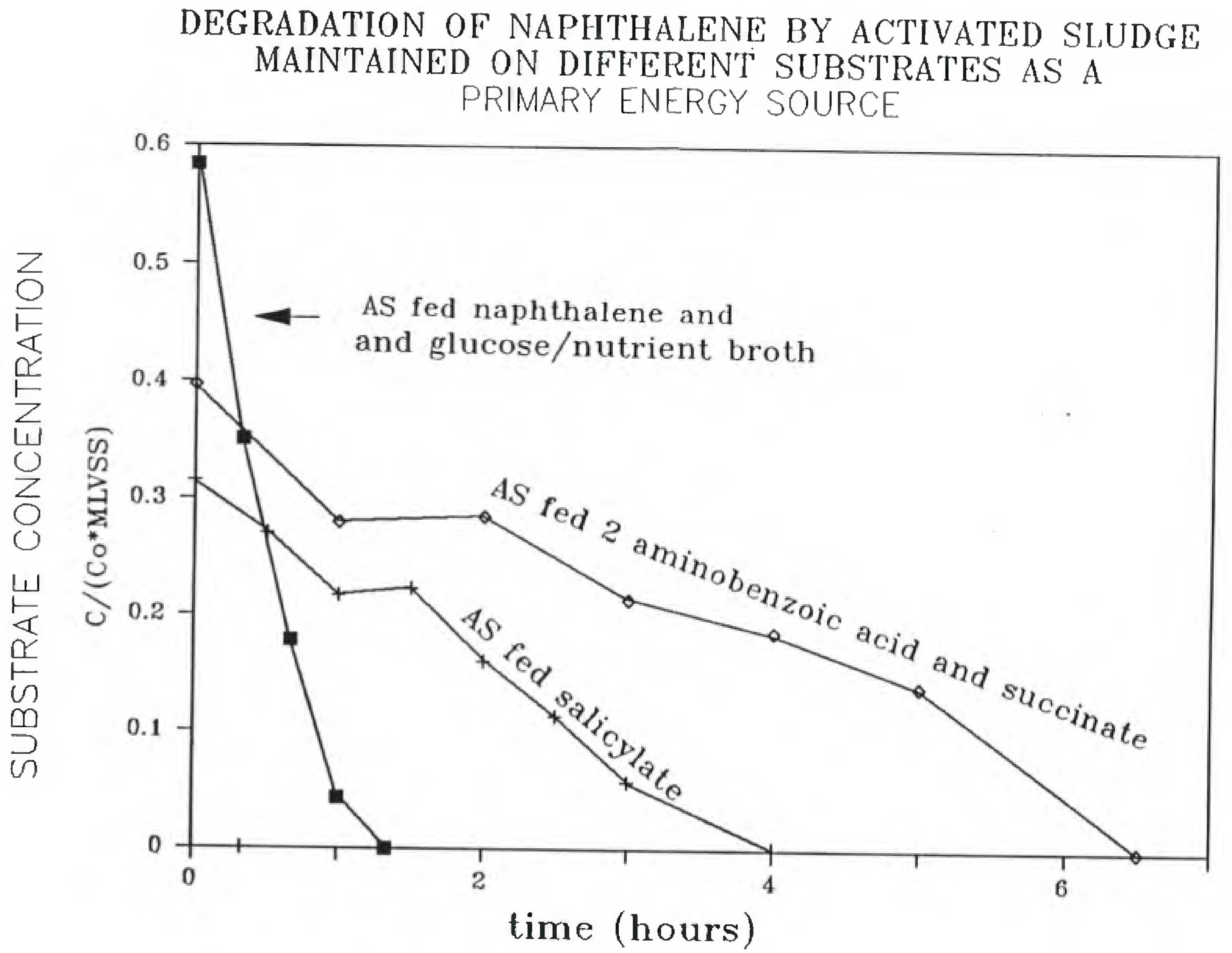
Purpose of substrate:

Serve as a carbon source for the NAP+ cells.

Maintain the genetic integrity of NAP+ cells.



Activated sludge process with enrichment reactor (E)



DEGRADATION OF NAPHTHALENE BY ACTIVATED SLUDGE MAINTAINED ON DIFFERENT SUBSTRATES AS A PRIMARY ENERGY SOURCE

Fig. 10

PRESENTATION BY DR. ROB HINCHEE

NCEL/USACERL

Biotechnology Workgroup

Presentation by

Robert E. Hinchee

February 22, 1989

Monterey, California

Eglin AFB Enhanced Bioreclamation Milestones

April 1984	Spill occured
1984–1986	Preliminary characterization Free product recovery
1985	Hurricane
November 1986	Site characterization
January/ February 1987	Enhanced bioreclamation construction
March 1987	Preliminary start-up
June 1987	Full-scale start-up
August 1988	End of field effort
September 1988	Final sampling

Eglin AFB Initial Site Conditions

Parameter	Soil (mg/kg)	Groundwater (mg/l)
TOC	—	130
TPH	2000	—
BTX	110	6
Bacteria	2×10^5	15×10^5
HC Degraders	$.9 \times 10^5$	1.6×10^5

16,000 ± lbs of residual hydrocarbon

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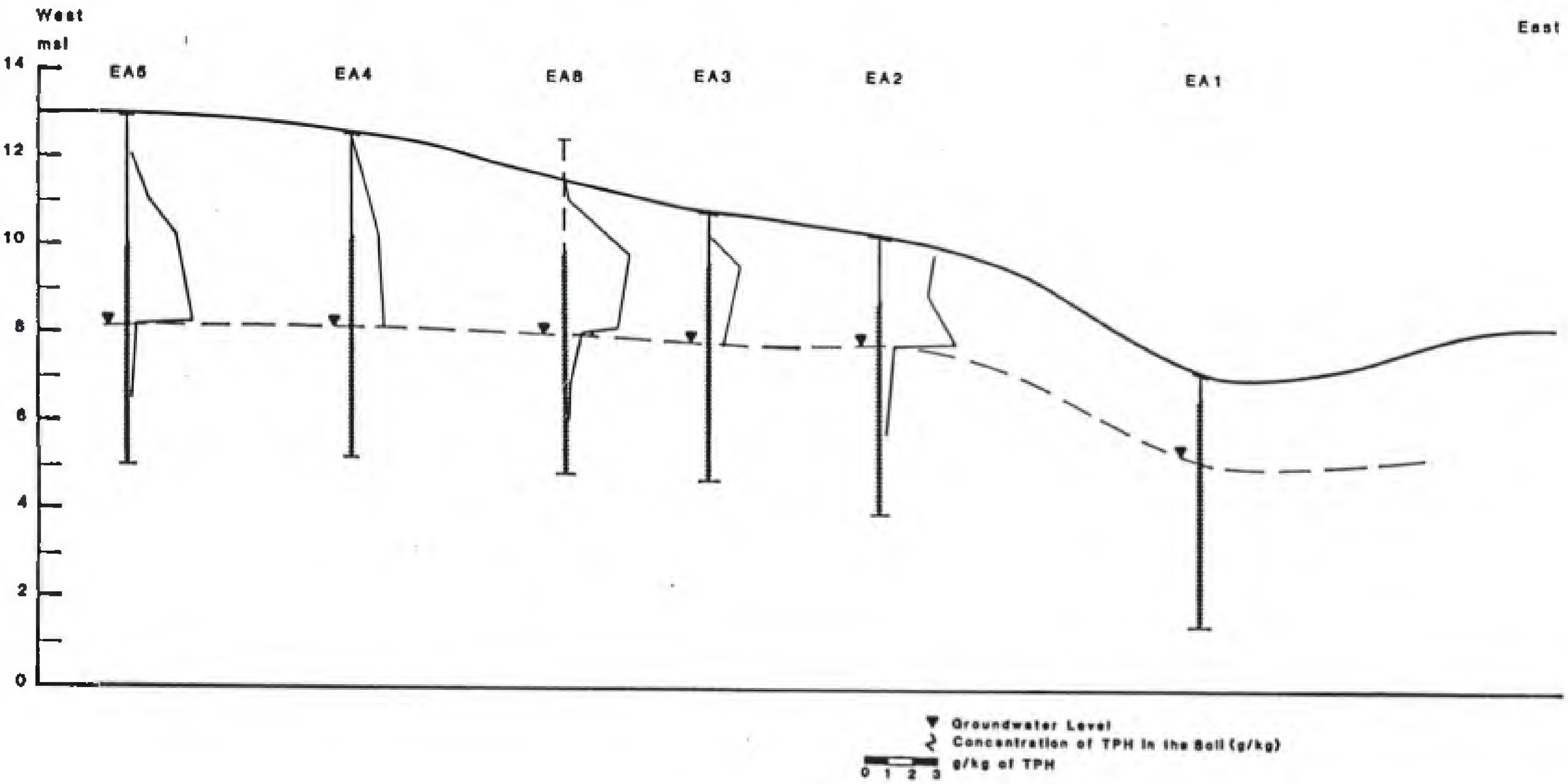


Figure 15. Surface and ground-water levels, well profiles, and distribution with depth of Total Petroleum Hydrocarbons (TPH) in the POL area, Eglin AFB, Florida, November 1986.

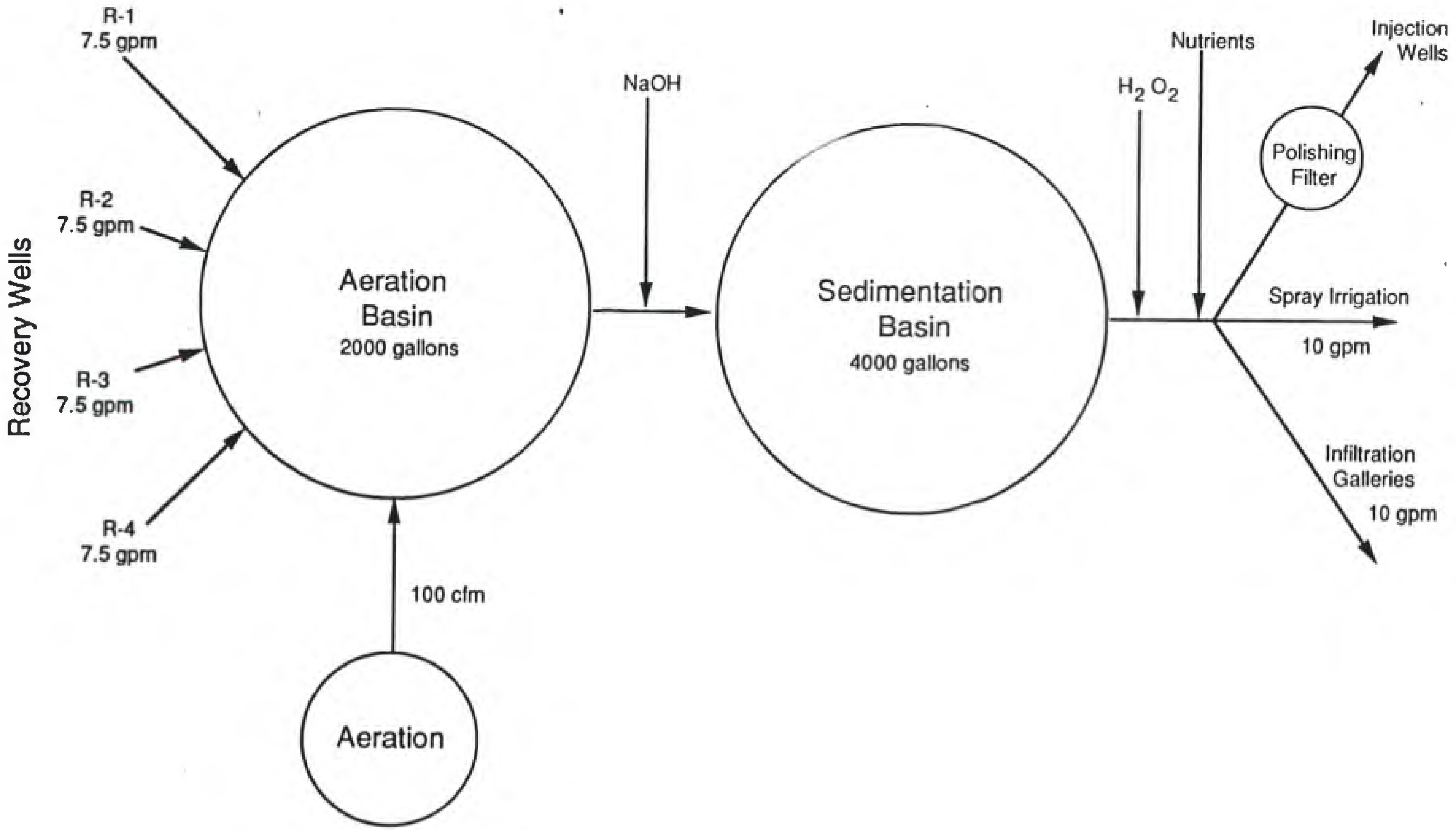


Figure 5. Design schematic for Eglin AFB Enhanced Bioreclamation Demonstration.

Nutrients—Restore 375R

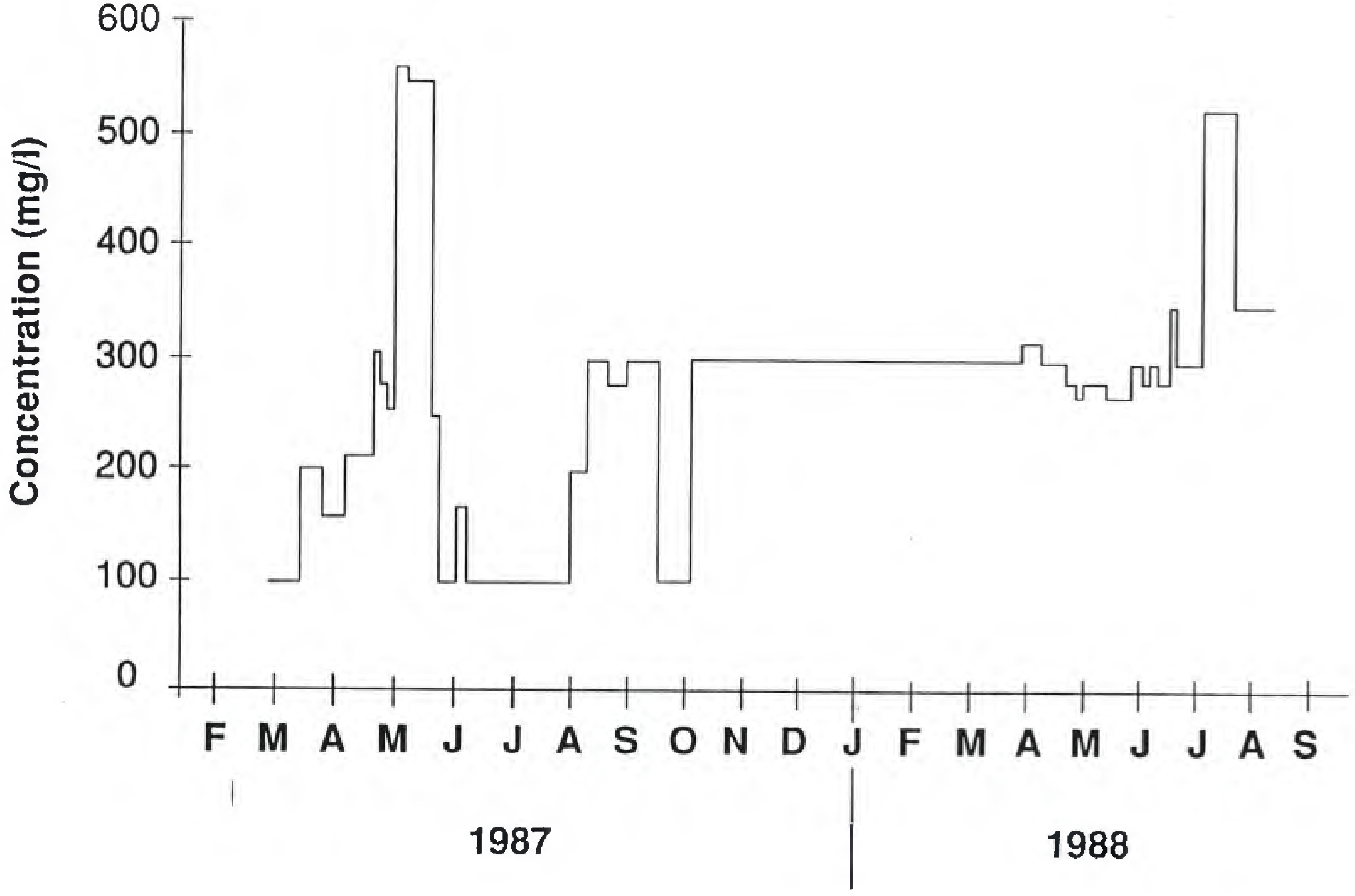
50%	Ammonium Chloride
20%	Disodium Phosphate
17.5%	Sodium Tripolyphosphate
12.5%	Monosodium Phosphate

Volume Pumped

Infiltration Galleries	8,900,000 gallons
Spray Irrigation	9,900,000 gallons
Injection Wells	1,100,000 gallons
Untreated Spray	1,500,000 gallons
Total	21,400,000 gallons

7800 lbs of Nutrient

10,000 gallons of 35% H_2O_2


Concentration (mg/l)
600
500
400
300
200
100
0
F
M
A
M
J
J
A
S
O
N
D
J
F
M
A
M
J
J
A
S
1987
1988

Offgas Composition

	GL2	GL6	GL9
Oxygen	82%	83%	36%
Nitrogen	16%	16%	59%
Carbon Dioxide	1.5%	0.8%	5.4%

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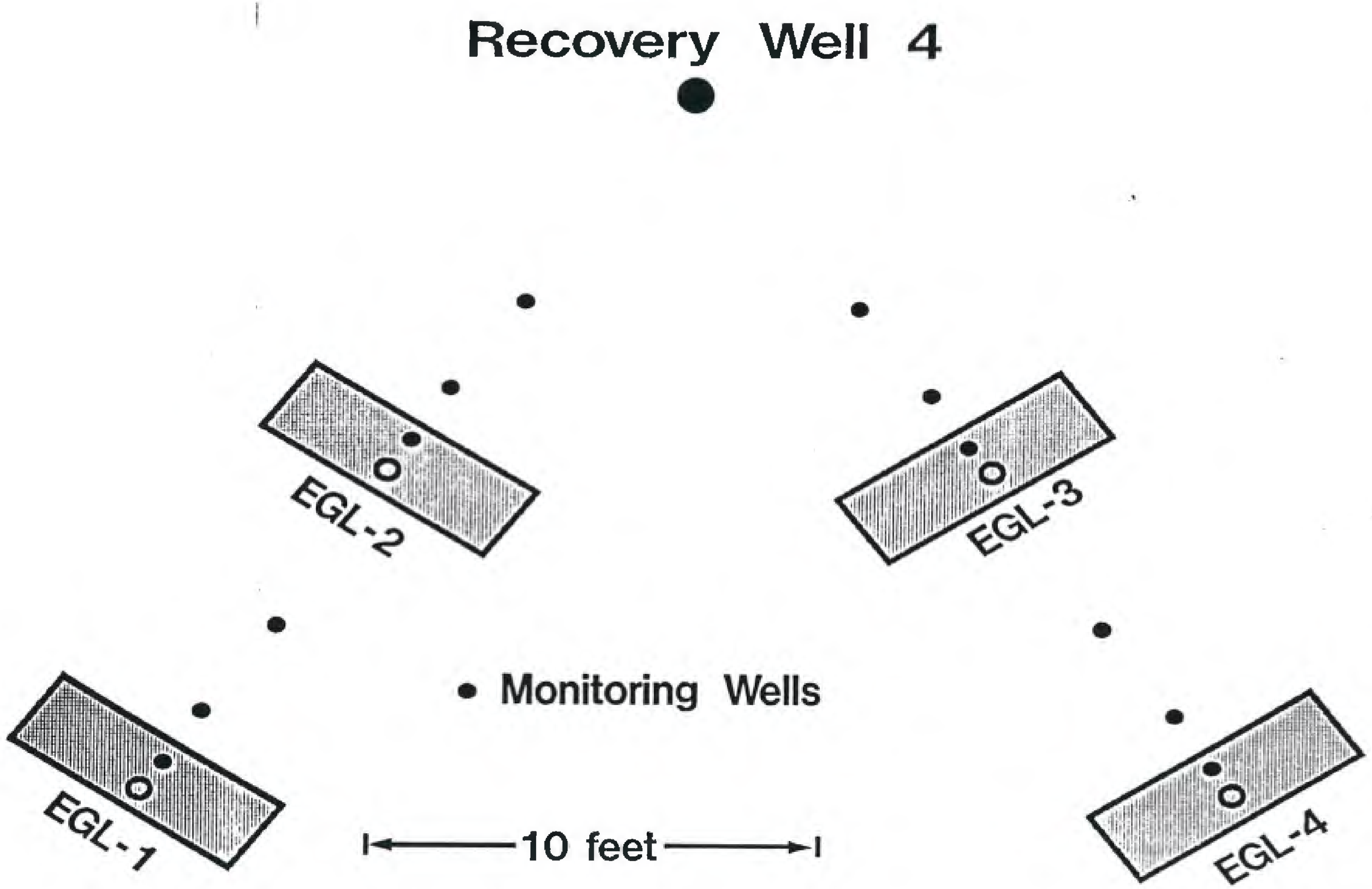
192-8631(863) — Dunn, Sept. 30, 1988


Recovery Well 4
EGL-2
EGL-3
• Monitoring Wells
EGL-1
10 feet
EGL-4

4 TREATMENTS

EGL - 1	NO TREATMENT	CLEAN WATER
EGL - 2	PHOSPHATE PRETREATMENT	CLEAN WATER
EGL - 3	PHOSPHATE PRETREATMENT	SYSTEM WATER
EGL - 4	NO PRETREATMENT	SYSTEM WATER

	CLEAN WATER	SYSTEM WATER
TOC	< 1 mg/l	20± mg/l
TOTAL IRON	< 0.03 /mg/l	3-10 mg/l

SOILS

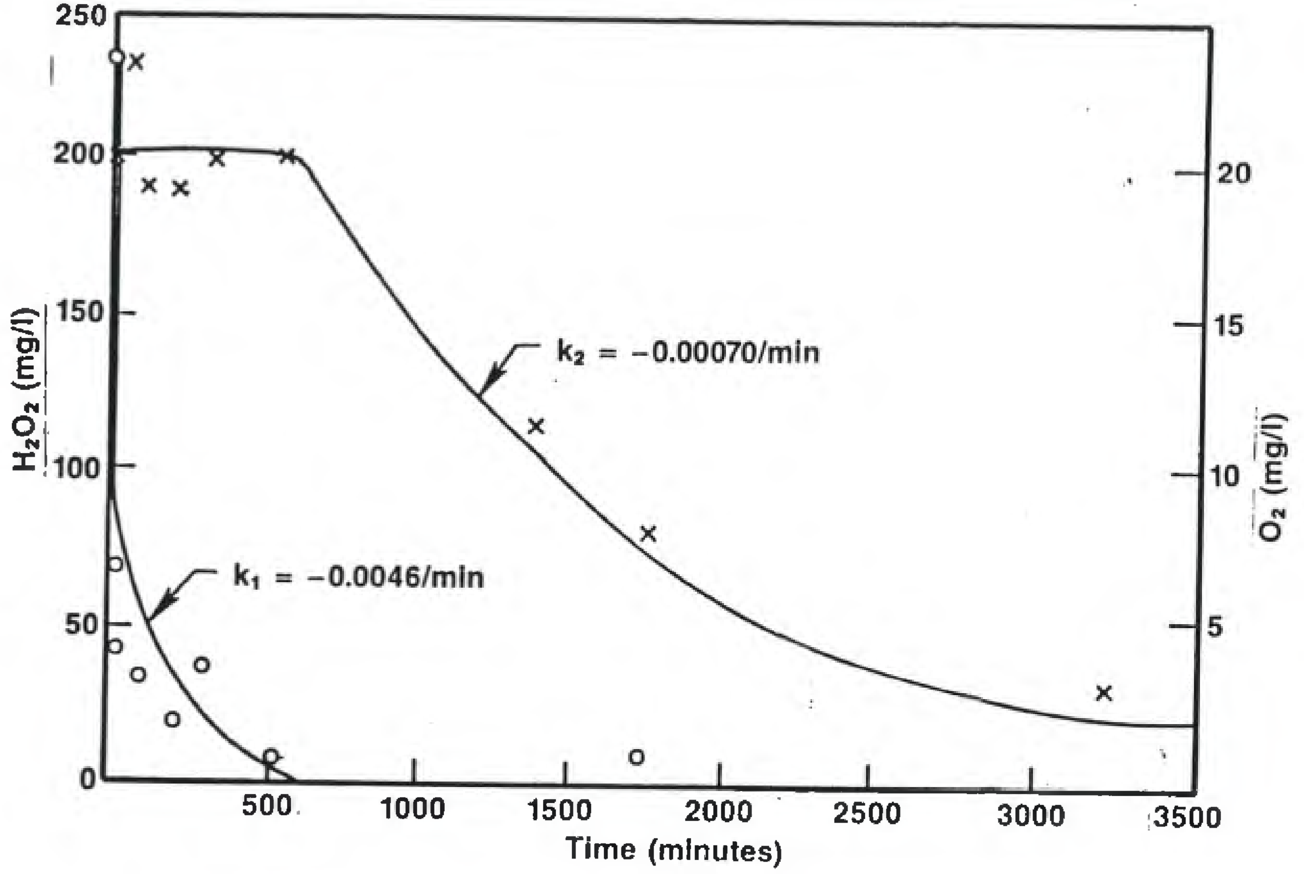
50 lb	DISODIUM PHOSPHATE
45 lb	SODIUM TRIPOLYPHOSPHATE
31 lb	MONOSODIUM PHOSPHATE

IN 10,000 ± gal

OVER 7 DAYS

EGL - 1 JUNE 22, 1988

	DISTANCE	APPROXIMATE TRAVEL TIME	H_2O_2	O_2
EA 27	0	0	4500	81(?)
EA 28	1’	1 hr	200	34
EA 29	4’	8 hr	ND	6


250
200
150
100
50
0
H_2O_2 (mg/l)
20
15
10
5
O_2 (mg/l)
500
1000
1500
2000
2500
3000
3500
Time (minutes)
k_2 = −0.00070/min
k_1 = −0.0046/min

$$\frac{dO_2\uparrow}{dt} = \tfrac{1}{2}K_1\ H_2O_2 \ - \ K_2O_2$$

and

$$\frac{dO_2B}{dt} = K_2O_2$$

Assuming

$K_1 = -0.0046$ /min

$K_2 = -0.00070$ /min

$H_2O_2 = 300$ mg/l

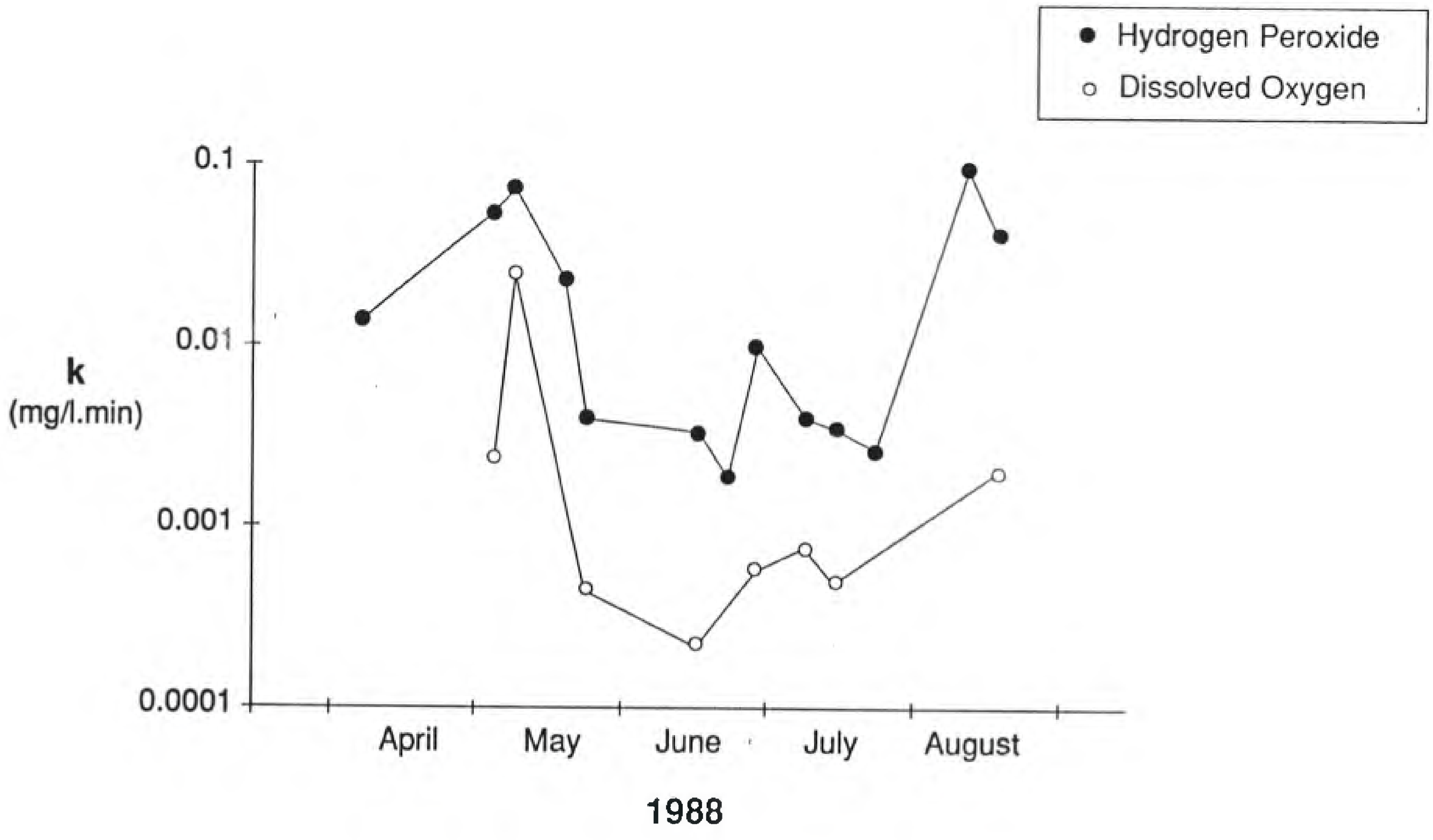
$O_2 = 40$ mg/l

$$\frac{d0_2\uparrow}{dt} = \quad 1/2\ (-0.0046)\ (300) - (-.00070)\ (40)$$

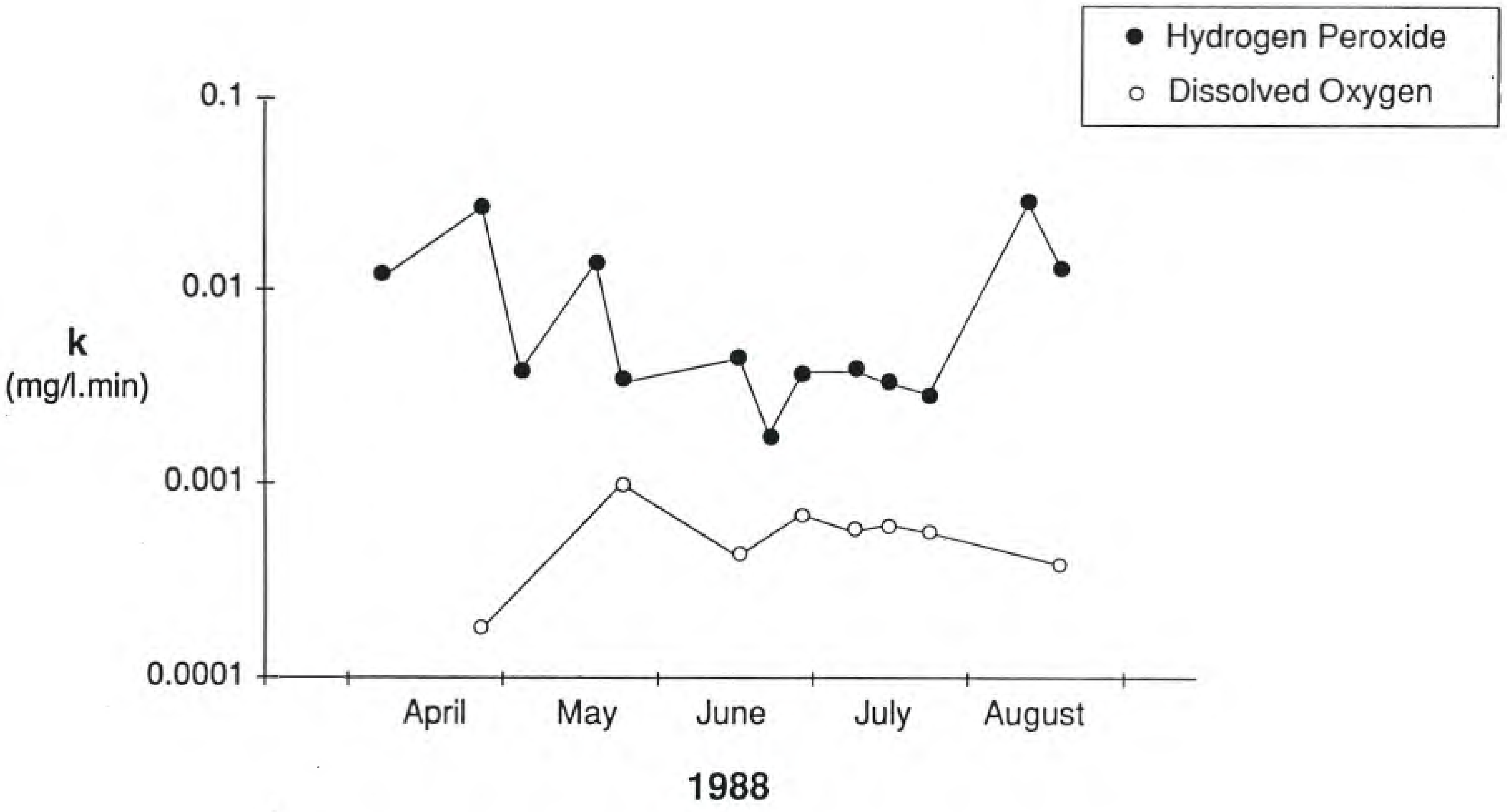
$$\frac{d0_2\uparrow}{dt} = \quad 0.66\ \text{mg/l}\cdot\text{min} \quad \text{or } 96\%$$

$$\frac{d0_2B}{dt} = \quad - \quad (.00070)\ (40)$$

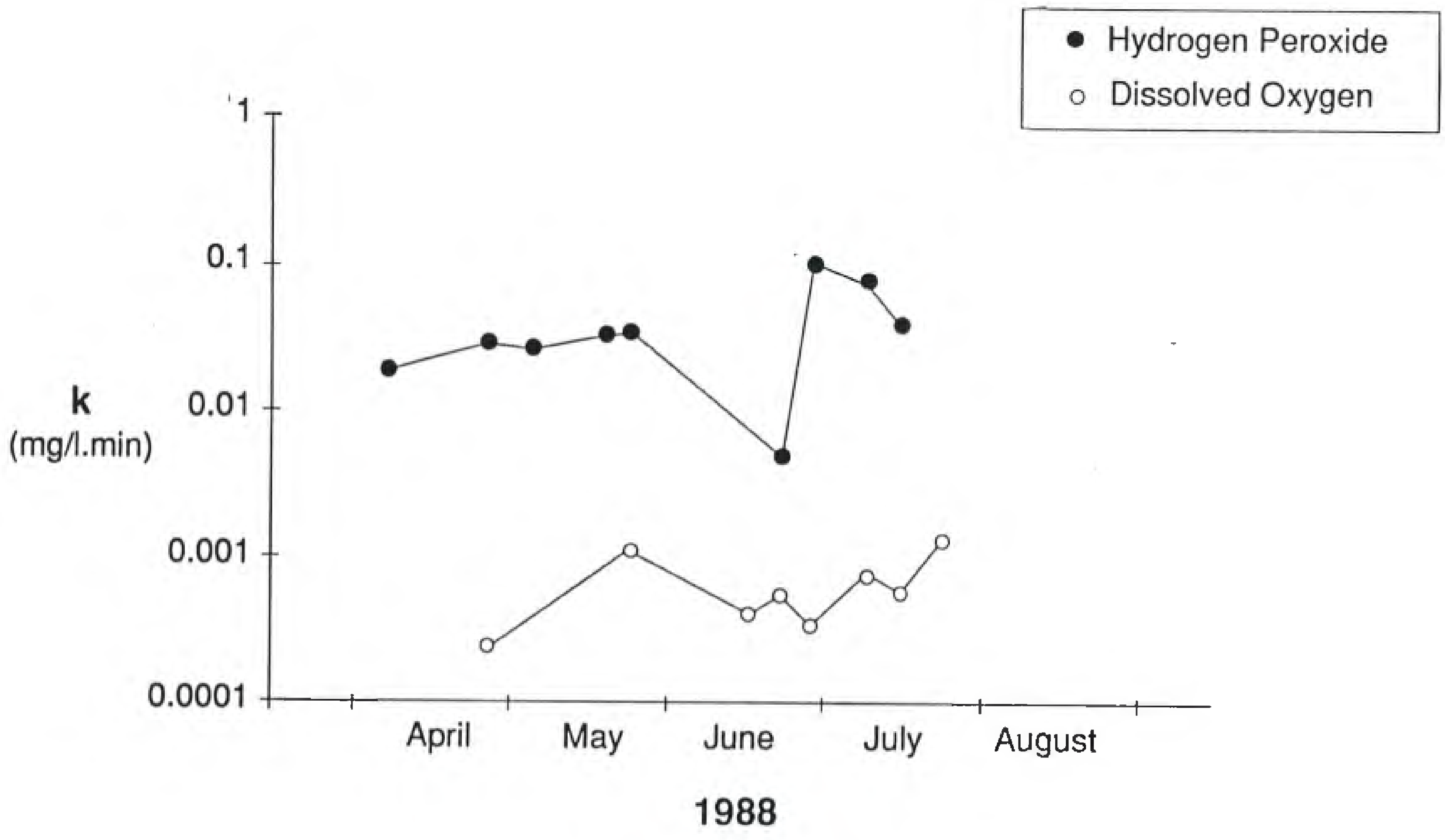
$$\frac{d0_2B}{dt} = \quad - \quad 0.028\ \text{mg/l}\cdot\text{min of } 4\%$$



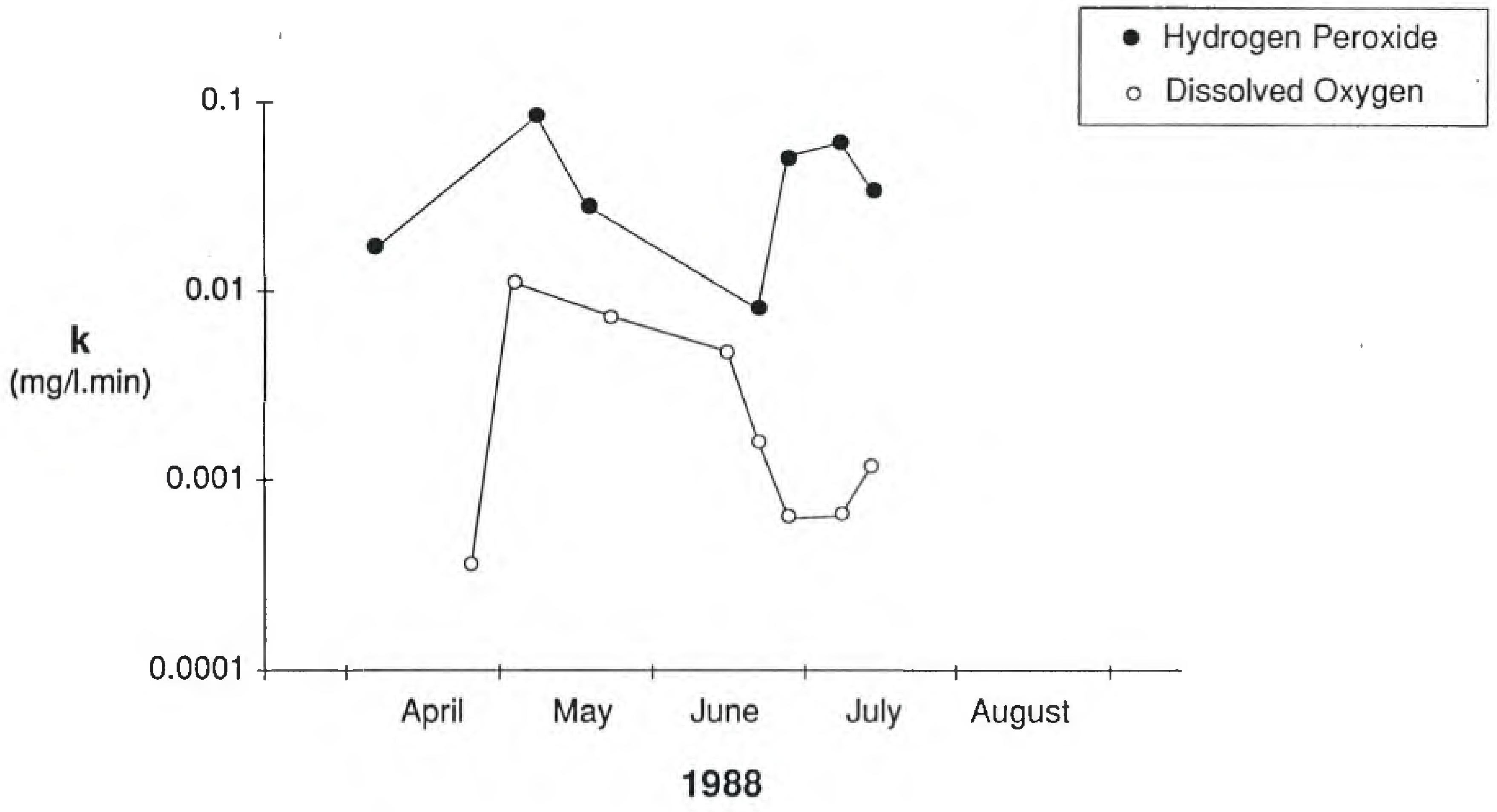
First Order Decay Constants for Hydrogen Peroxide and Oxygen in EGL-1, Well EA-27



First Order Decay Constants for Hydrogen Peroxide and Oxygen in EGL-2, Well EA-30



First Order Decay Constants for Hydrogen Peroxide and Oxygen in EGL-3, Well EA-35



First Order Decay Constants for Hydrogen Peroxide and Oxygen in EGL-4, Well EA-38

Spray Irrigation—Concentrations in mg/l

	Feed	Spray	0 ft	0.5 ft	1.0 ft	2.0 ft
H_2O_2	270	270	270	ND	ND	ND
DO	19	16	17	5.2	5.3	3.3

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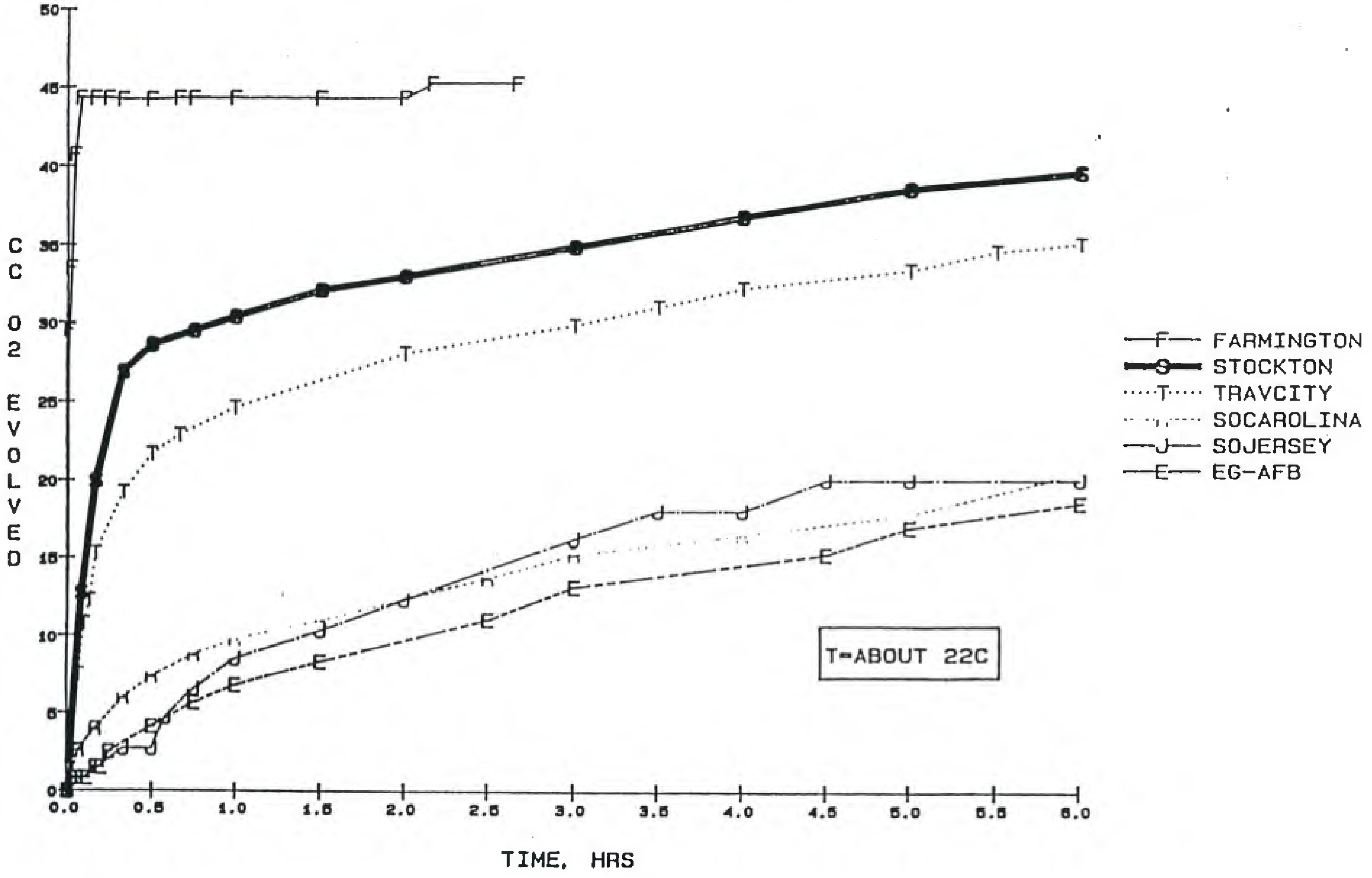
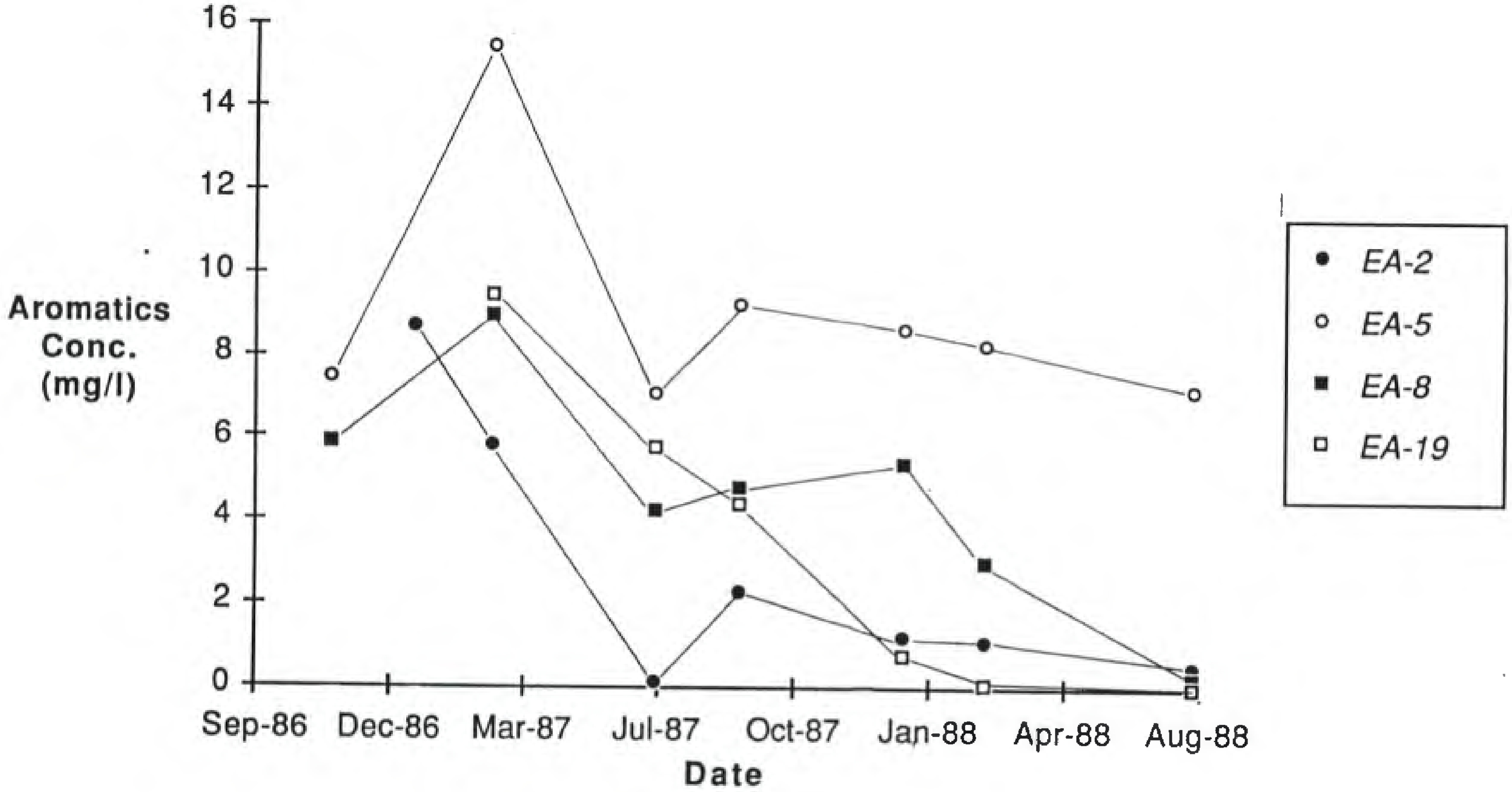
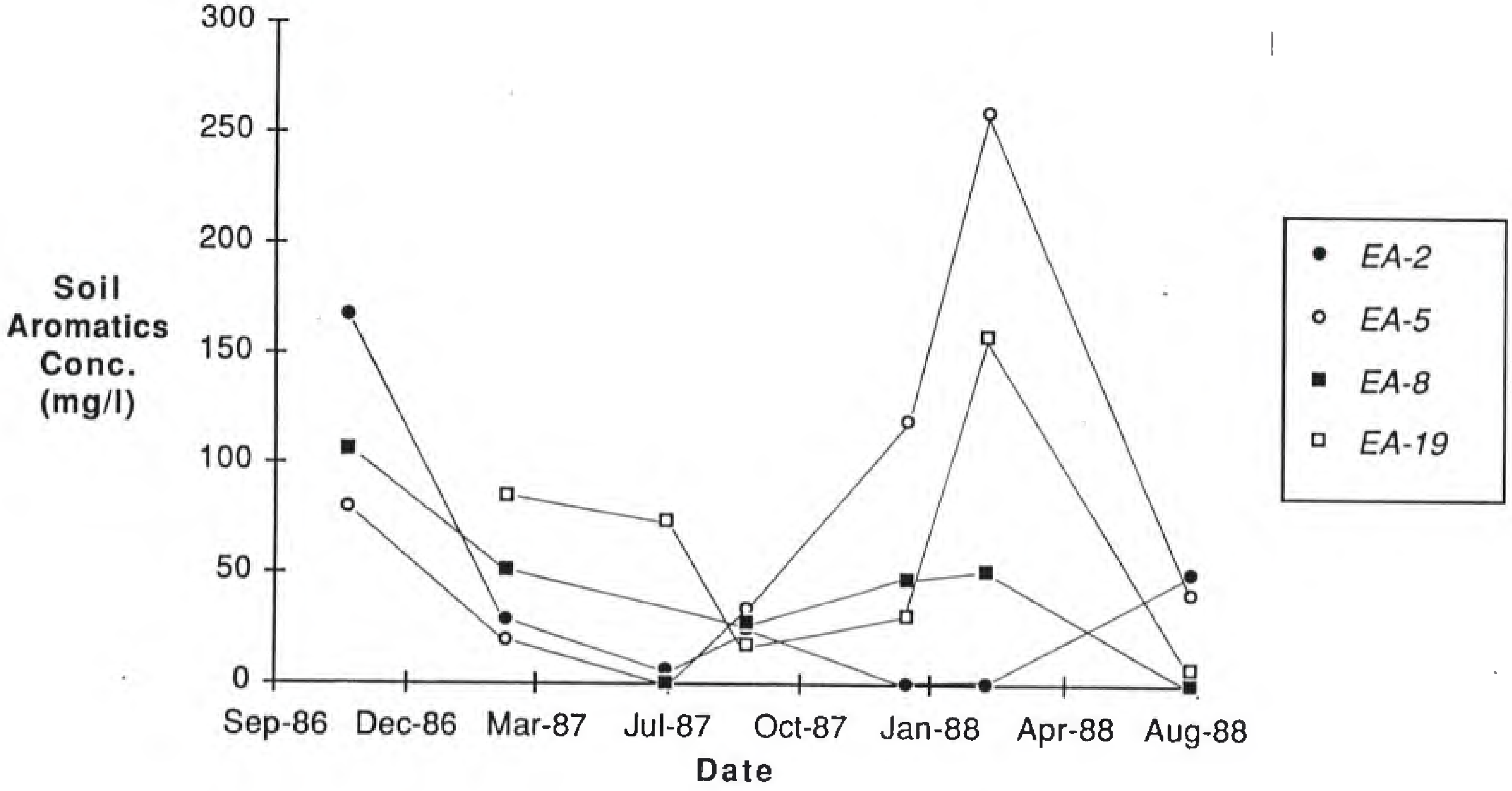
FIGURE 7

DECOMPOSITION OF 0.1% H202 BY SIX SOILS

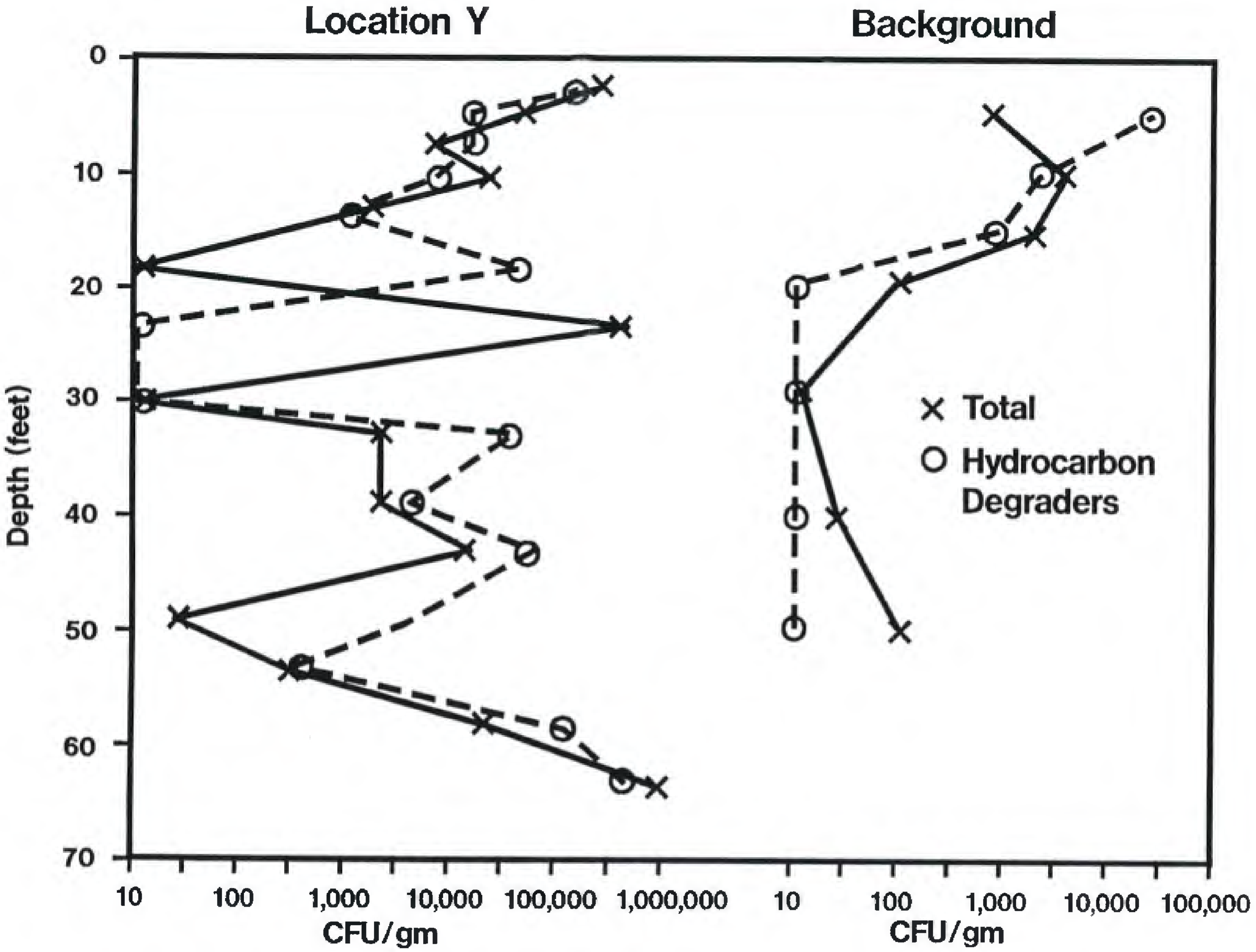



Aromatics Conc. (mg/l)
16
14
12
10
8
6
4
2
0
Sep-86
Dec-86
Mar-87
Jul-87
Oct-87
Jan-88
Apr-88
Aug-88
Date
● EA-2
○ EA-5
■ EA-8
□ EA-19


Soil Aromatics Conc. (mg/l)
300
250
200
150
100
50
0
Sep-86
Dec-86
Mar-87
Jul-87
Oct-87
Jan-88
Apr-88
Aug-88
Date
● EA-2
○ EA-5
■ EA-8
□ EA-19

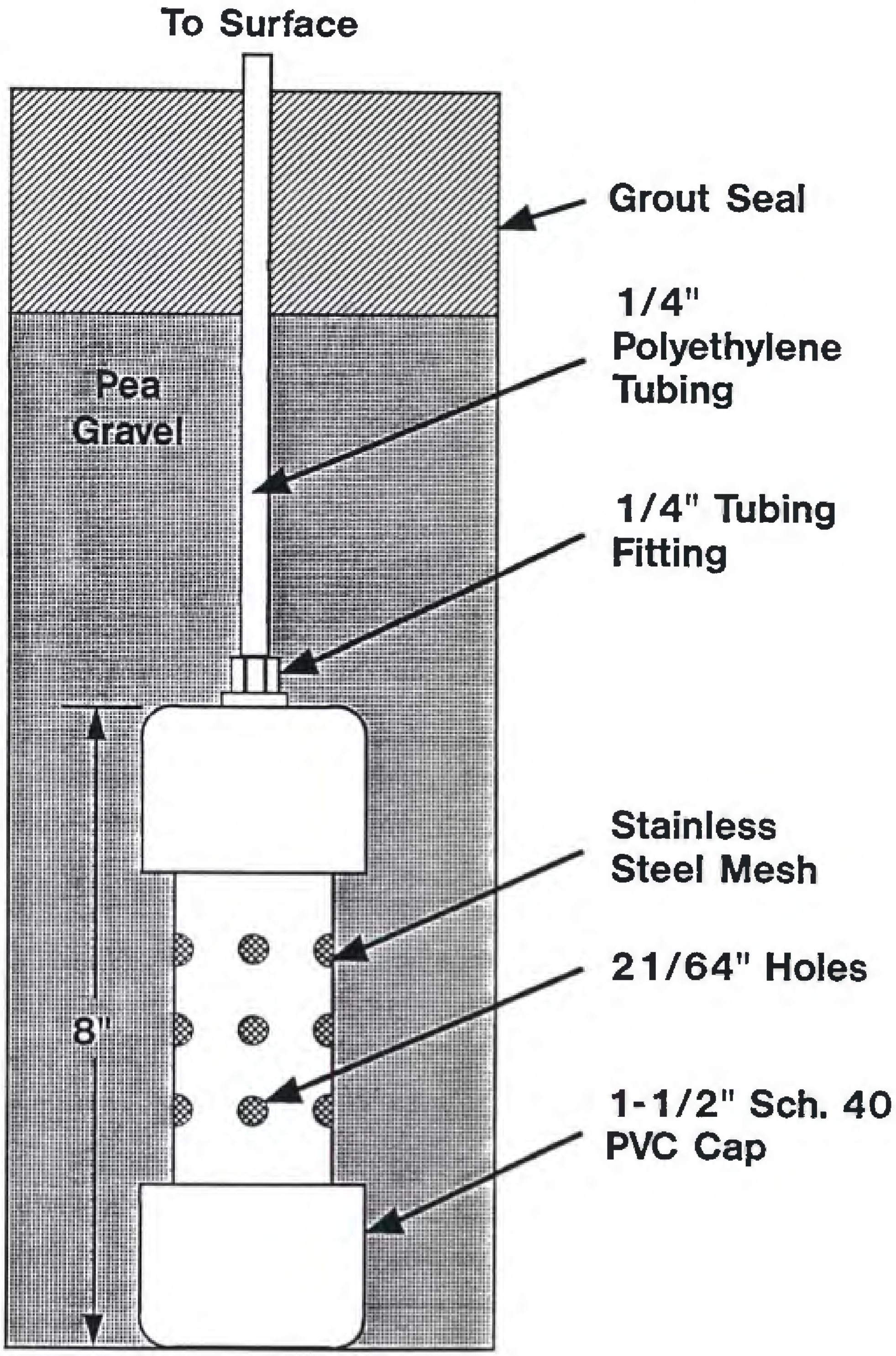
Hydrocarbons Removed

Biodegradation	1400 lbs
Volatilization	3600 lbs

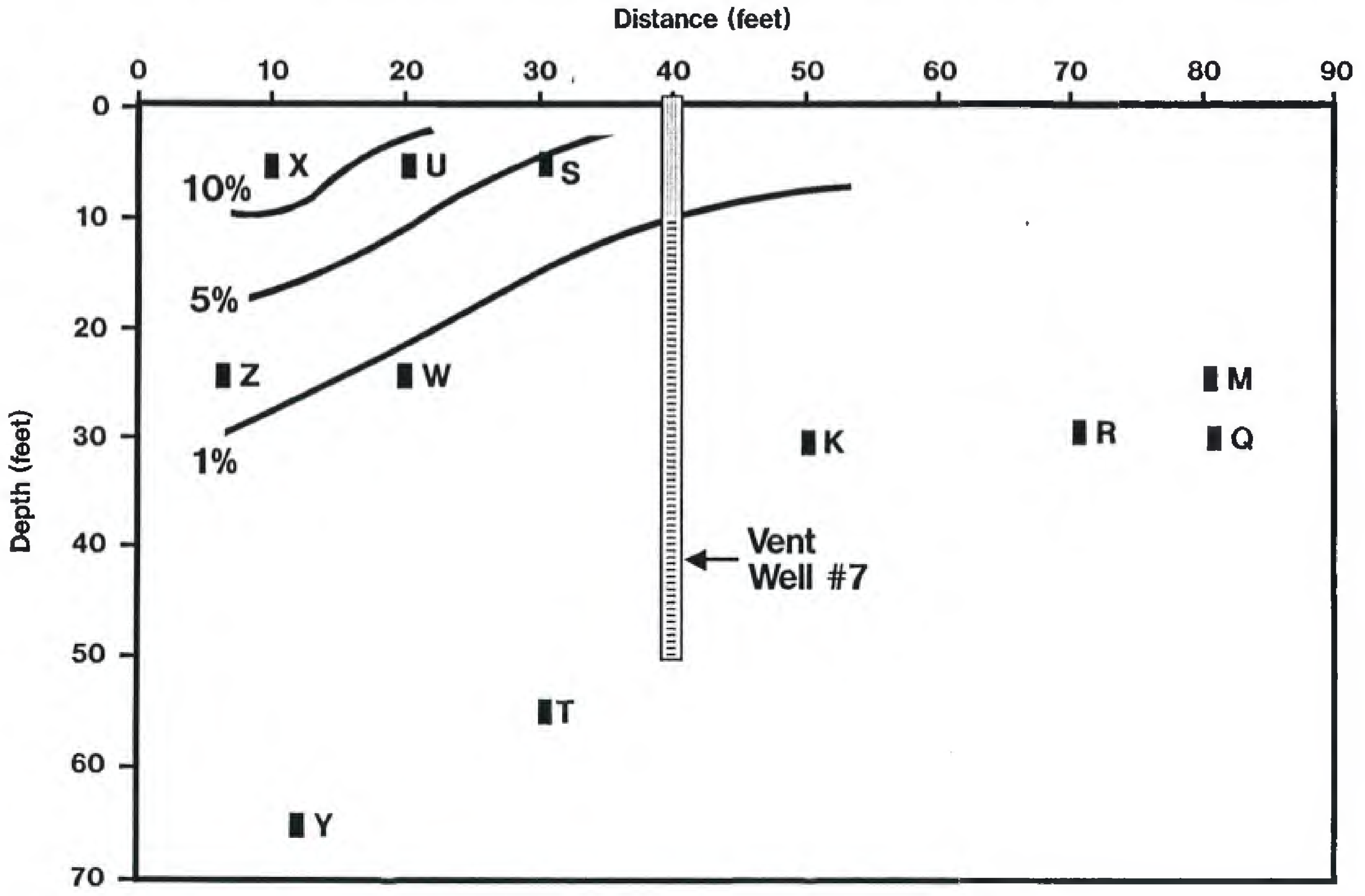


KA\Hncline2-6

Monitoring Point Construction

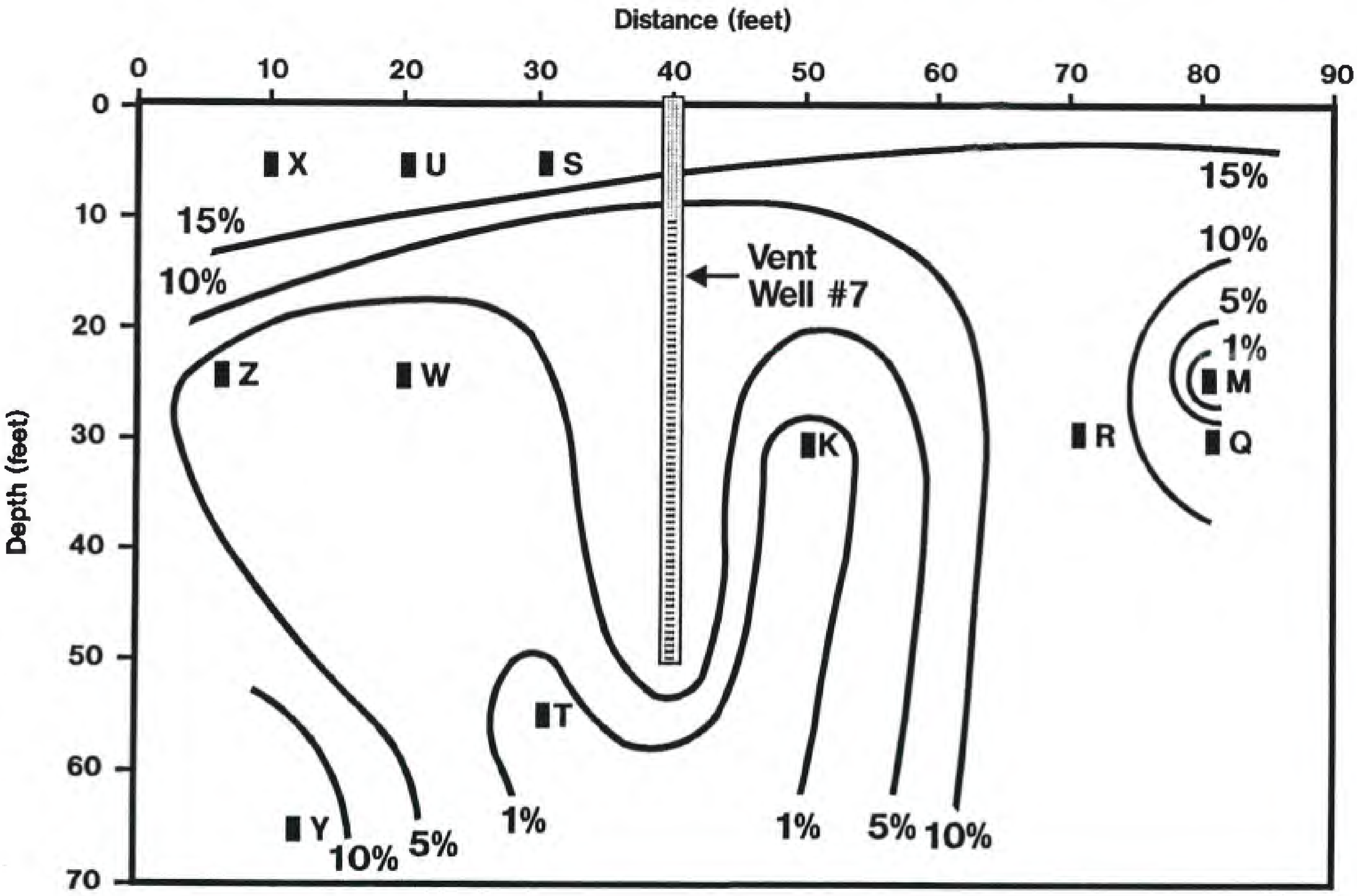


Oxygen Concentration in Vadose Zone Before Venting



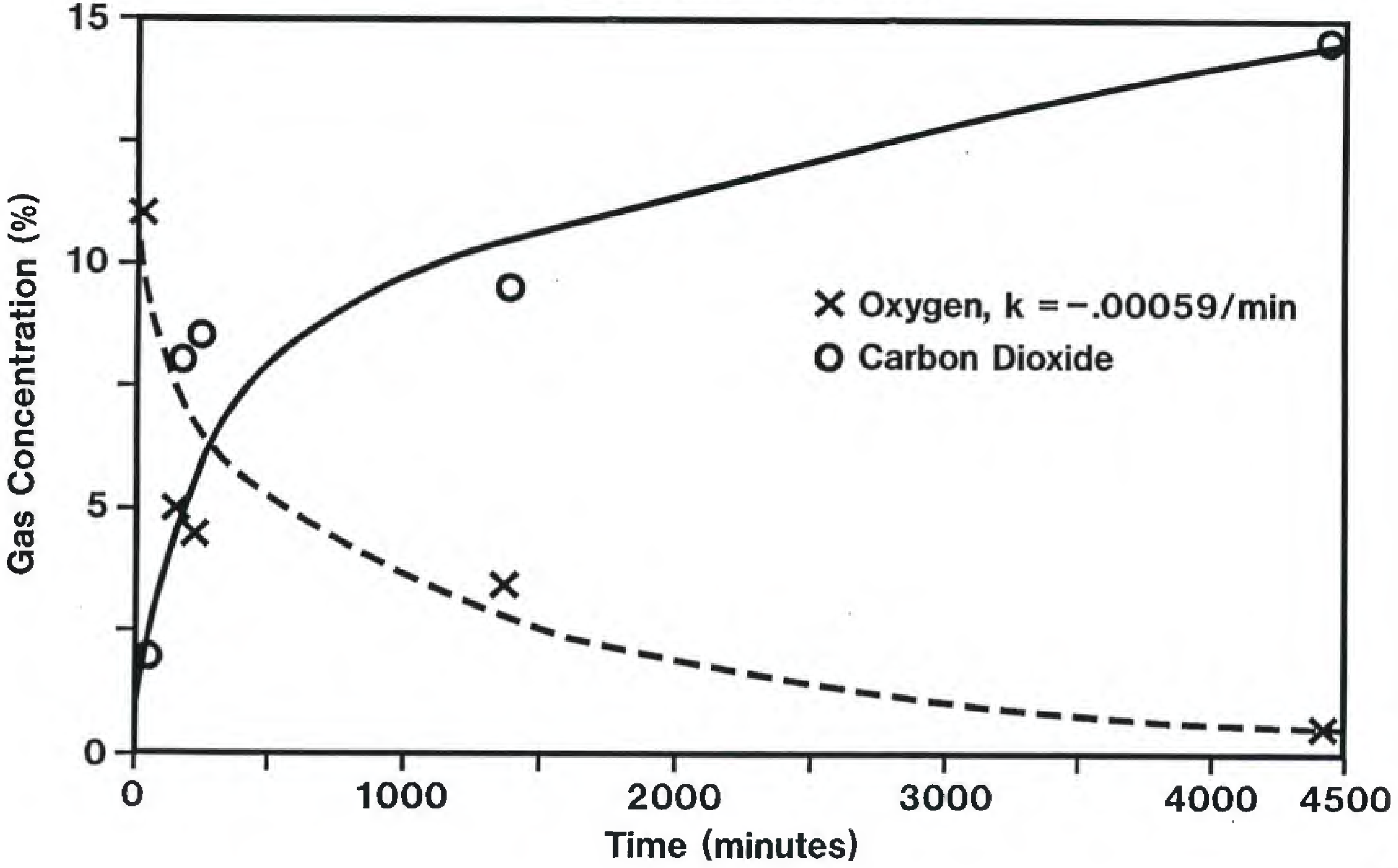
KA\Hinchee2-5

Oxygen Concentration in Vadose Zone After Venting



KA\Hinchee2-4

Monitoring Point Y In-Situ Respiration Test
December 19, 1988



KA/Hinchee2-2

Hill AFB Soil Venting

Effluent 1.8% CO_2
1.0% Hydrocarbon

in 100 cfm of Air

Removal
~ 250 lb/day Volatilization
~ 85 lb/day Biodegradation

PRESENTATION BY DR. GAYLEN BRUBAKER

Screening Criteria for In situ Bioreclamation of Contaminated Aquifers

by

Gaylen R. Brubaker, Ph.D.
Remediation Technologies, Inc.
127 Kingston Place, Suite 105
Chapel Hill, North Carolina 27514

INTRODUCTION

An increasing number of Superfund and other complex industrial sites are moving from the site investigation, or Remedial Investigation (RI) phase, into the Feasibility Study (FS) phase of remediation. In the process, many environmental scientists and engineers will be required to perform site specific evaluations for bioremediation techniques without the benefit of first-hand experience in the design or implementation of those methods. This paper is designed to provide guidance for those faced with this challenge who are considering the in situ bioreclamation of contaminated aquifers.

Several papers in recent years have provided general descriptions of the technology (4,6,14), case histories (3,7,9) and descriptions of laboratory treatability studies (6). This paper provides a simple scoring mechanism (Table 1) to help the environmental engineer evaluate the feasibility of using in situ bioreclamation using the information which is typically gathered during a remedial investigation. It is important to acknowledge that a one-page set of questions cannot replace the insights of an experienced engineer or the results of a detailed feasibility/treatability study; but hopefully it will help define the critical issues for a particular site.

PROCESS DESCRIPTION

In situ bioreclamation involves the introduction of microbial nutrients (typically ammonia and orthophosphate) and an electron acceptor (typically oxygen, delivered as a solution of hydrogen peroxide) into the saturated zone of a contaminated aquifer. Since these materials are all readily soluble in water, the process delivers the nutrients to the contaminants by transporting solutions of the materials through the areas of contamination, converting the region into a "bioactive zone". Groundwater is also withdrawn from the formation to maintain hydraulic containment and to increase the hydraulic head and thus the flow rate of nutrients through the formation. The captured water is typically treated to remove contaminants and then a major portion of the water is amended with nutrients and reinjected (6). This process is much more aggressive than simple groundwater treatment processes which rely on solubilization and transport of the contaminant from the soil/groundwater matrix. In situ processes, when compared to excavation options, also have inherent limitations which make definition of remedial objectives and choice of remedial processes closely related. In situ bioreclamation is a valuable technique for removing a large portion of soil and groundwater contamination and protecting groundwater receptors. However, if remediation objectives require complete destruction of small concentrations of organics within isolated pockets of the site, the probability of success will be very dependent on the homogeniety of the formation.

The design and implementation of an in situ bioreclamation process is controlled by several interrelated characteristics of the site. These include the biodegradability and physical nature of the contaminant, the compatibility of microbial nutrients and electron acceptors with site soils, the hydrogeological setting and the general physical limitations of the site surface. These issues will be summarized by introducing a feasibility scoring system which provides a semiquantitative assessment of the ease with which in situ bioreclamation can be implemented at a given site. It must be remembered, however, that sites which are difficult to treat with this process are often also difficult to treat with other in situ or groundwater treatment processes. This score presents only a comparison of the ease of treating a particular site using in situ bioreclamation relative to other sites, not relative to other processes at the same site. Since metals are not usually mobile in subsurface environments and there is very little experience related to their treatment using in situ microbial processes, this discussion will e limited to a discussion of organic contaminants.

CONTAMINANT CHARACTERISTICS

Although there is no doubt that the microbial environment effects the biodegradability of a particular chemical in a particular location, it is also clear that certain chemical structures are inherently more accessible to microbial degradation than others (10). The aqueous solubility, molecular size, and types of chemical bonds within a particular chemical structure will effect the ability of a bacteria to metabolize the molecule and obtain energy from the process. Several literature reviews have been published which summarize the general "biodegradability" of the types of compounds which are most frequently of concern (1,2,12,13). In addition, at least one reference also provides a guide to the concentrations at which various contaminants may also be toxic to organisms (13). Collectively this literature can be used to provide the reader with a measure of the biodegradability of a particular compound. A brief summary of general trends of aerobic degradability is provided below and reflected in Section 1A of Table 1. For the purposes of this paper, a material is considered easily degradable if the genetic and enzymatic equipment required for the degradation of a compound is widely distributed in nature and if bacteria can obtain sufficient energy from the compound to use the material as a sole carbon source. Although newer innovative techniques may lead to ways of treating the more recalcitrant materials, these processes are likely to be more complex than those currently being used on gasoline contaminants.

> Simple hydrocarbons and light petroleum distillates such as gasoline, kerosene, diesel, jet fuel and light mineral oils are generally degradable. Their rate of degradation decreases with increasing molecular weight and decreasing solubility. Increased branching and cyclic structures also slow the degradation process.
>
> Aromatic hydrocarbons with up to two rings (including benzene, toluene, xylene, ethylbenzene and naphthalene) are readily degradable. The rate of degradation of larger polyaromatic hydrocarbons decreases as size increaes and solubility decreases (a 3 ring PAH contains up to 14 carbons).
>
> Alcohols, amines, esters, mercaptans, carboxylic acids, and nitriles are also usually degradable, but these compounds also tend to be toxic to unacclimated bacteria at high concentrations. Nitro substitution and ether linkages usually make degradation more difficult.
>
> Chlorinated hydrocarbons (both straight chain and aromatic) become increasingly difficult to degrade as the degree of chlorine substitution increases. As a result polychlorinated biphenyls (PCBs) and other polychlorinated hydrocarbons (chloroform, carbon tetrachloride, tetrachloroethylene, trichloroethylene and dichloroethylenes) are not readily biodegraded aerobically, and are toxic at ppm levels.
>
> Pesticides are another very complex set of organic contaminants. In general those which are found at hazardous waste sites (DDT, Lindane, Aldrin, Chlordane etc.) are not readily biodegraded.

The degradation of viscous organics materials like number 6 fuel oil, creosote and refinery wastes are often controlled by their physical condition in the soil/water matrix. If they are present as small droplets of oil occluded within the pores of a soil, there will be very little exposed surface area for degradation and the process will be inhibited.

Although it is difficult to quantify the importance of contaminant distribution to project feasibility, project success will clearly require movement of nutrient-enriched water through those areas of the site which contain the highest concentrations of contamination. Sites which contain a few point sources of contamination, whether a lagoon or a leaking tank, can generally be treated fairly reliably with an in situ treatment method. However, at sites which contain multiple and undefined sources of contamination treatment methods become much more difficult to design and operate in a predictable fashion. The probability of successful remediation is definitely influenced by ones understanding of the sources and transport mechanisms for the contaminants.

HYDROGEOLOGY

In order to transport nutrients through the areas of contamination, it will be necessary to have a detailed understanding of the hydrogeology of a site. The rate limiting step for in situ processes is typically the ability to move nutrient-enriched water through the areas of highest contamination. Since both the soils and the contaminant distribution at a site are typically non-homogeneous, the initial determination of feasibility and cost will usually be limited to an estimate of total volume of degradable material in the area of concern and the anticipated groundwater injection and recovery rates.

Permeability and aquifer thickness are both important aquifer characteristics which effect the rate of groundwater movement through the treatment area. This relates to both the cross-sectional area of flow and to the amount of drawdown which can be created in a well. The drawdown, in turn, influences the hydraulic head that can be created and the size of the area of influence of a well, which in turn effects the number of wells that would be required to capture a contaminated plume. The interrelationship of these characteristics is illustrated in Table 2, which shows average pumping rates from recovery wells within aquifers of various permeabilities and thicknesses (5).

Another characteristic which is important but often overlooked is the depth to the water table. At sites with very shallow water tables, injection of water into a well or trench can easily lead to flooding. Shallow injection points limit the amount of hydraulic head which can be applied to the injection system and limits the area of influence of injection wells. Collectively these parameters effect the rate of travel of injected water into a contaminated area, which in turn effects the required well spacings, capital costs and treatment times. Although a detailed discussion of these issues is beyond the scope of this paper, it should be noted that injection wells and recovery wells are ideally located at a distance that allows injected water (and nutrients) to reach areas of contamination less than six weeks from the time of injection (depending on the compatibility of soils and nutrients). Even in a fine sand aquifer (permeability 10^{-3} cm/sec) with three feet of injection head and seven feet of drawdown this is only about 36 feet. The effect of these variables on project feasibility are reflected in Section 2 of Table 1.

SOIL AND GROUNDWATER CHEMISTRY

Microbial degradation processes are controlled by the presence of appropriate bacteria, the suitability of the environment for microbial degradation and the general chemical structure of the contaminant of interest. Unfortunately very little information about the first two areas is typically obtained during initial site investigations. However, some information can often be obtained by examining the general groundwater parameters.

A review of available ammonia, chloride, phosphate, dissolved oxygen, conductivity and pH data can provide an overview of the microbial environment. Groundwater pH is perhaps the most basic characteristic. Most soil bacteria prefer pH in the range of 6.5 - 8, but are likely to adapt to ranges slightly beyond this range. As with most groundwater characteristics, consistence is almost as important as "optimum" levels.

Elevated concentrations of ammonia and chloride, or sulfide can be an indication of septic or sanitary contamination or a highly anaerobic zone. This could indicate a large oxygen demand from other readily degradable materials which would significantly impact project cost and timing. There are several other site chemistry issues which do not effect microbial health, but may effect process feasibility by effecting nutrient transport.

Although simple in concept, the process of transporting an electron acceptor (typically hydrogen peroxide) through soils can present challenging problems at some sites. In "ideal sites" hydrogen peroxide can provide a very effective means of delivering 150 to 250 ppm of dissolved oxygen in injected water. It is infinitely soluble in water and decomposes in the presence of soil such that 300 ppm of hydrogen peroxide produces about 150 ppm of dissolved oxygen. The main design concern in using peroxide is controlling the rate of this catalytic decomposition. (Hydrogen peroxide can also be used directly by some bacteria and consumed in the oxidation of some chemicals, but these reactions are of limited concern.) In those soils which contain high concentrations of natural organics, iron, nickel, and copper for example, the catalytic decomposition of peroxide can be so rapid that the groundwater quickly becomes supersaturated with oxygen. This can create gas blockage of the formation, loss of oxygen and poor general transport of DO to remote area of the site. Although methods exist for conditioning soils to minimize these effects, this phenomenon can limit the concentration at which the peroxide can be used from 500 ppm, a common concentrations in clean sands, to as little as 100 ppm.

An alternative electron acceptor which may be usable is nitrate. Recent studies have demonstrated that PNAs and other organic compounds can be degraded by denitrifying bacteria. Although this technique still has a limited history in commercial practice, it has a significant potential at sites where peroxide stability is poor and where the hydrogeology will allow for good containment of introduced nitrate.

Nitrogen and phosphorus are usually required in small amounts to support increased bacterial growth, while trace minerals are readily available in soil. These requirements are usually satisfied through the periodic addition of nutrient formulations containing a blend of ammonia and phosphate. However, ammonia and phosphate transport can be difficult at some sites, as these materials can be retarded and consumed while passing through soils. Ammonia can be lost through ion exchange with other cations in clays and soils and phosphate can interact with cations, especially calcium and magnesium. Although these processes do not make the nutrients unavailable, they do limit the distance through the formation where transport is feasible, or effect the formulation of nutrients that are most effective. In addition, the general introduction of these nutrients, with associated ions, might lead to dispersion or swelling of clay particles.

For the purposes of a feasibility study, the interactions of phosphate with calcium and magnesium (both of which form insoluble precipitates with orthophosphate) are probably the only transport issue which can be assessed; both inhibit phosphate transport at high concentrations. Heavy metals, such as cadmium, chromium, arsenic and mercury, are toxic to bacteria in certain forms and concentrations. The presence of these materials, especially if it extends beyond a localized area, should be highlighted as a area of concern when evaluating any microbial-based process.

COST AND TIMING

Although it is not the objective of this paper to present a detailed discussion of the cost of performing an in situ bioreclamation project, a few basic principles deserve to be highlighted.

- For easily degraded contaminants, the kinetics of microbial processes are irrelevant. The rate at which sufficient oxygen can be transported through the contaminated zone should be the basis of projections.

- The amount of oxygen required for a microbial process is approximately three pounds of oxygen per pound of biodegradable organic present within the treatment area. This is best estimated from general indicator measurements such as Total Organic Carbon or Total Petroleum Hydrocarbon, rather than from GC-MS tabulations which only identify selective compounds.

- The rate of oxygen transport can be estimated from the rate at which groundwater can be injected into a site (assume injection rate is 70% of withdraw rate) and the concentration of oxygen or hydrogen peroxide that can be effectively carried in the injected water.

CONCLUSION

Although it is not possible to anticipate all of the results of a full treatability/feasibility study from the limited information available in typical site assessment studies, it is often possible to identify the critical issues for the design and execution of an in situ bioremediation process. A literature review of contaminant degradability and toxicity, an analysis of aquifer depth, permeability and the depth to groundwater, and an estimate of total oxygen demand can provide the basis for an initial assessment. Review of general groundwater and soil data will suggest other areas of concern related to toxicity and nutrient transport. Collectively these data can provide sufficient guidance to evaluate the feasibility of this process relative to others under consideration and to define a suitable feasibility/design study.

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Table 1. Screening Criteria for In Situ Bioreclamation

1. CONTAMINANT CHARACTERISTICS	SCORE
A. Structure	
Simple Hydrocarbon C1 to C15	0
C12 - C20	-1
>C20	-2
Alcohols, phenols, amines	0
Acids, esters, amides	0
Ethers, monochlorinated, nitro	-1
Multichlorinated	-2
Pesticides	-2
B. Sources	
Well defined point sources	+1
Undefined multiple sources	-1
2. HYDROGEOLOGY	
A. Aquifer Permeability (cm/sec)	
$>10^{-3}$	0
10^{-3} to 10^{-4}	-1
10^{-4} to 10^{-5}	-2
B. Aquifer Thickness (feet)	
20 or greater	+1
10	0
5	-1
<2	-2
C. Depth to Aquifer (feet)	
20	+1
10	0
5	-1
<2	-2
D. Homogeneity	
Uniform well defined geology	+1
Heterogeneous poorly defined	-1
3. SOIL AND GROUNDWATER CHEMISTRY	
A. Groundwater pH	
>10	-2
8 - 10	-1
6.5 - 8	0
4.5 - 6.5	-1
<4.5	-2
B. Groundwater Chemistry	
High Fe, S, Ca, Mg, Cu, Ni	-0.5
High NH_4 and Cl	-0.5
Heavy metals (As, Cd, Hg)	-0.5

Interpreting the Total Score

0 or Greater	Site Appears Suitable
-1 to -2	Possible Areas of Concern
-2 to -4	Areas of Significant Concern
Less than -4	Success in Unlikely

Table 2. Approximate Pumping Rates (GPM) from Various Aquifers

Aquifer Material	Permeability (cm/sec)	Saturated Thickness		
		10 Ft	20 Ft	30 Ft
Fine Sand	10^{-3}	10	38	81
Silty Sand	10^{-4}	3	5	10
Silt, Loess	10^{-6}	0.04	0.14	0.28

These values assume a constant pumping rate where $r_w = 0.5$ feet and $S = 0.2$ (Reference 5).

PRESENTATION BY DR. JOHN ARMSTRONG

THE TRAVERSE GROUP, INC.
January 12, 1989

NATIONAL DEMONSTRATION SITE: IN-SITU BIOREMEDIATION OF CONTAMINATED AQUIFER USEPA/TRAVERSE GROUP, INC./U.S. COAST GUARD

John M. Armstrong

I. BACKGROUND

Remediation of contaminated soil and ground water by in situ biodegradation is a much-discussed technique, and has been carried out or studied at several sites with several approaches. The technique of using microorganisms to metabolize organic contaminants is no longer a new idea: In fact, for decades this same process has been used in both municipal and industrial waste water treatment plants, as well as in millions of domestic septic tank/drain field systems.

The purpose of the joint cooperative project for which Traverse Group is the engineering contractor is to provide a well documented and evaluated site where enhanced biodegradation of soils and ground water is accomplished in situ, under realistic, controlled conditions. This project, started in 1987, actually consists of two full-scale field projects at a real contamination site (U.S. Coast Guard air base). One project involves aerobic metabolism of gasoline hydrocarbons in the aquifer. The other, second project involves the anaerobic degradation of jet fuel in soil and ground water. Both projects provide contaminant treatment that spans a wide range of typical contamination problems in the petroleum and chemical industries. The projects are funded by the federal government and will span a three-year period ending in June, 1990. The following sections briefly describe the projects.

II. OVERVIEW

The biodegradation project at the U.S. Coast Guard Air Station in East Bay Township in Northern Michigan is a pilot scale demonstration of in situ enhanced biodegradation for remediation of an aviation gas spill. The Traverse Group, Inc. was chosen as the engineering contractor for the project, which is a jointly funded research effort between the U.S. Coast Guard and the U.S. Environmental Protection Agency (EPA). The alkylbenzenes, benzene, toluene, ethyl-benzene, and xylenes (BTEX) are the object of the regulatory concern, and the bioremediation will be finished when their concentration is brought to a level specified by the Michigan Department of Natural Resources (MDNR).

Extensive soil coring was performed at the site to define the vertical and lateral extent of the "trapped" or retained zone of aviation gas contamination. A special coring tool for obtaining wet aquifer soil samples intact was fabricated for USEPA by Traverse Group, Inc. engineers. This tool is highly successful. (The tool and its associated field techniques have been formally adopted by USEPA and is now the required method for obtaining biological soil samples for determining biodegradability of various contaminants under the USEPA hazardous waste program.) Based on this information, a 50 by 130-foot study area was chosen in the most contaminated portion of the plume. The bulk of the contamination was found to be confined to a narrow interval between 15 and 17 feet below land surface. This interval corresponds closely with the seasonal high and low water table at the site (the "smear zone").

The aim of the first field project is to accelerate natural bacterial action by supplying needed nutrients and oxygen in the form of hydrogen peroxide. In order to impact the interval of highest contamination, two rows of injection wells at different depths were placed at the upgradient edge of the study area (Figure 1). The deep row of wells is injected with clean water from a distant (off-plume) supply well to form a local mound, or rise, in the water table. The wells in the shallow row are placed so that nutrients and peroxide are injected onto this mound and are in direct contact with the zone of highest contamination. This is a method worked out over several months by Traverse Group and USEPA scientists.

Monitoring of the study area is accomplished using a series of 50 mini-well tubing sample points and nine conventional wells. The tubing for each point is routed to a central location to allow indoor sampling. The conventional wells are used primarily for water table elevation measurements. The hydraulic distribution and control system was designed and constructed on a modular basis by Traverse Group engineers and is housed in a small control shed on the site.

III. OPERATION - Biodeg I (the Aerobic Project

In March of 1988, the injection of oxygen was started to acclimate the system. Injection of nutrients (ammonium chloride, disodium phosphate and potassium phosphate) was begun in May, 1988 to increase biological activity. In June of 1988, oxygen injection was stopped and replaced with hydrogen peroxide injection. The injection concentration began at 50 mg/L (Figure 2).

IV. RESULTS

The interval between the injection wells and the monitoring wells was considered remediated when detectable oxygen broke through and alkylbenzenes (BTEX) disappeared (compare Figures 3 and 4). The interval between the injection field and the monitoring well located 31 feet downgradient was remediated after 220 days (Julian Date 8281), and the interval to the monitoring well at 50 feet downgradient was remediated after 270 days (Julian Date 8331).

Shortly after the breakthrough of oxygen in monitoring well BD-31-2, the area near the monitoring well was cored and the wet soil samples analyzed for alkylbenzenes (BTEX) and total fuel hydrocarbons. Results were compared to earlier cores to determine whether any trapped contaminants remained in the aquifer. The aliphatic (non-aromatic) hydrocarbons remained at their initial concentration, but the alkylbenzenes (BTEX) were below the analytical detection limit (see Table 1).

V. THE SECOND PROJECT: Biodeg II (Anaerobic Demonstration)

In October of 1988 a second project was assigned to Traverse Group, Inc. This project will demonstrate the use of a nitrate based metabolism as opposed to the use of oxygen. The project has been designed and is currently under construction by Traverse Group, Inc. It will start up March, 1989.

VI. FUTURE PLANS

The EPA considers the aerobic field project to be a success at this point. Engineering studies are now under way by Traverse Group, Inc. to determine the cost feasibility of converting the pilot scale project to a full scale remediation. The aim is to not only remediate this site, but also to develop enhanced biodegradation as a practical and cost-feasible treatment technology for similar hydrocarbon contamination sites. Traverse Group also has constructed two large model beds in which full scale tests of soil farming, vapor phase biodegradation and other related bio-restoration concepts are being developed.

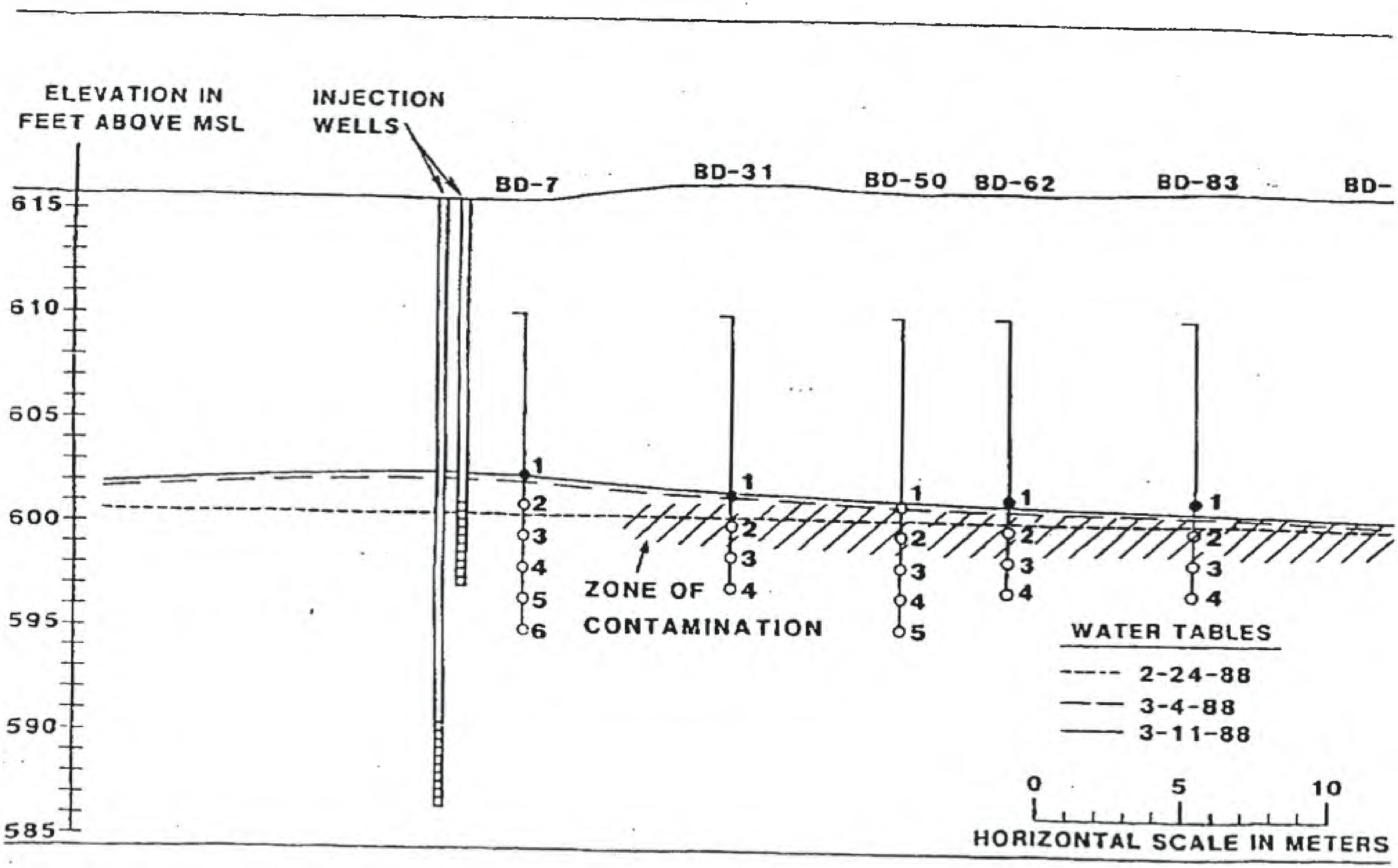

Figure 1. Cross section of a demonstration project for bioremediation of the aviation gasoline spill at Traverse City, Michigan.

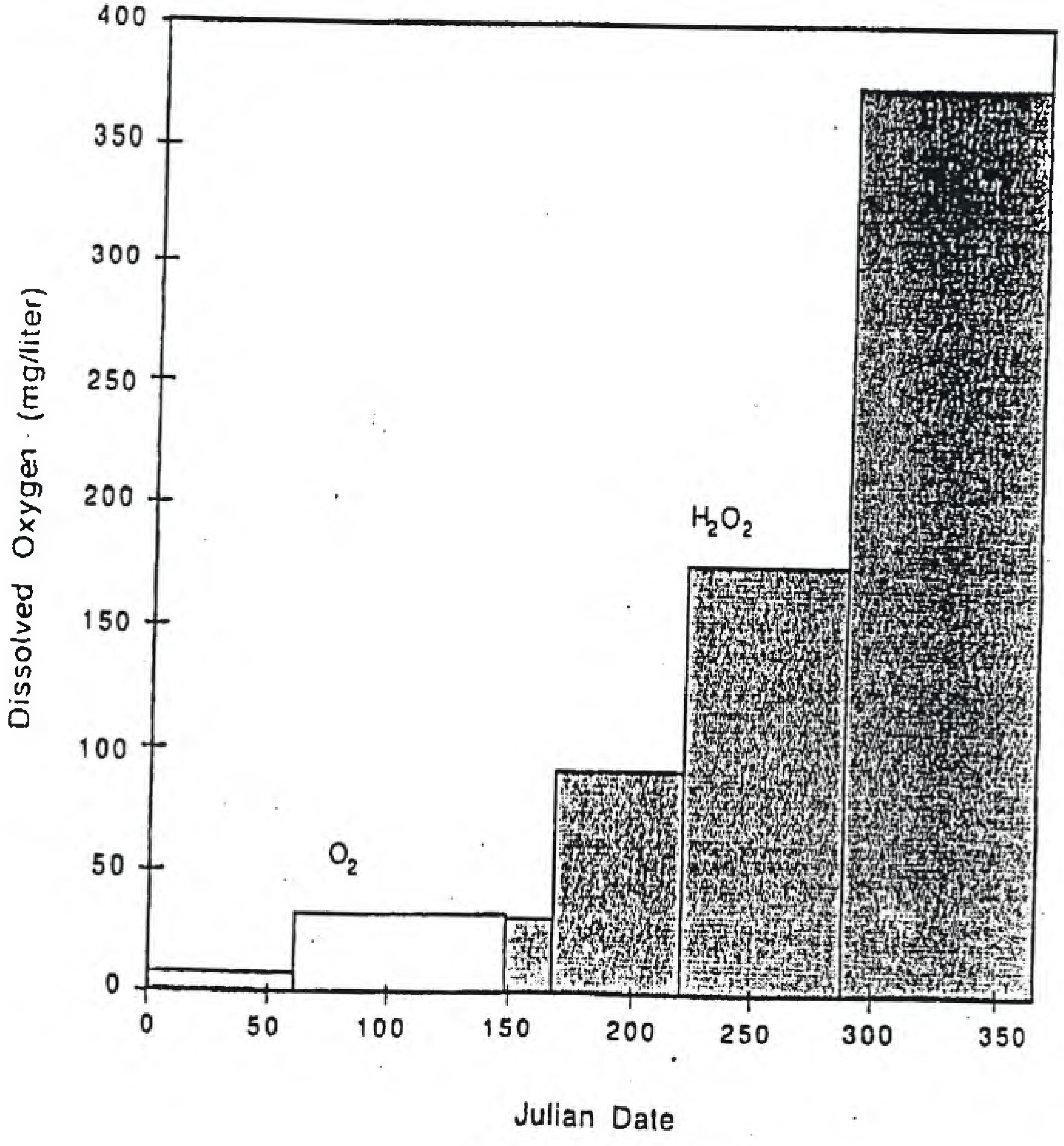

Figure 2. Schedule of application of oxygen or hydrogen peroxide in the first year of the demonstration project (1988)

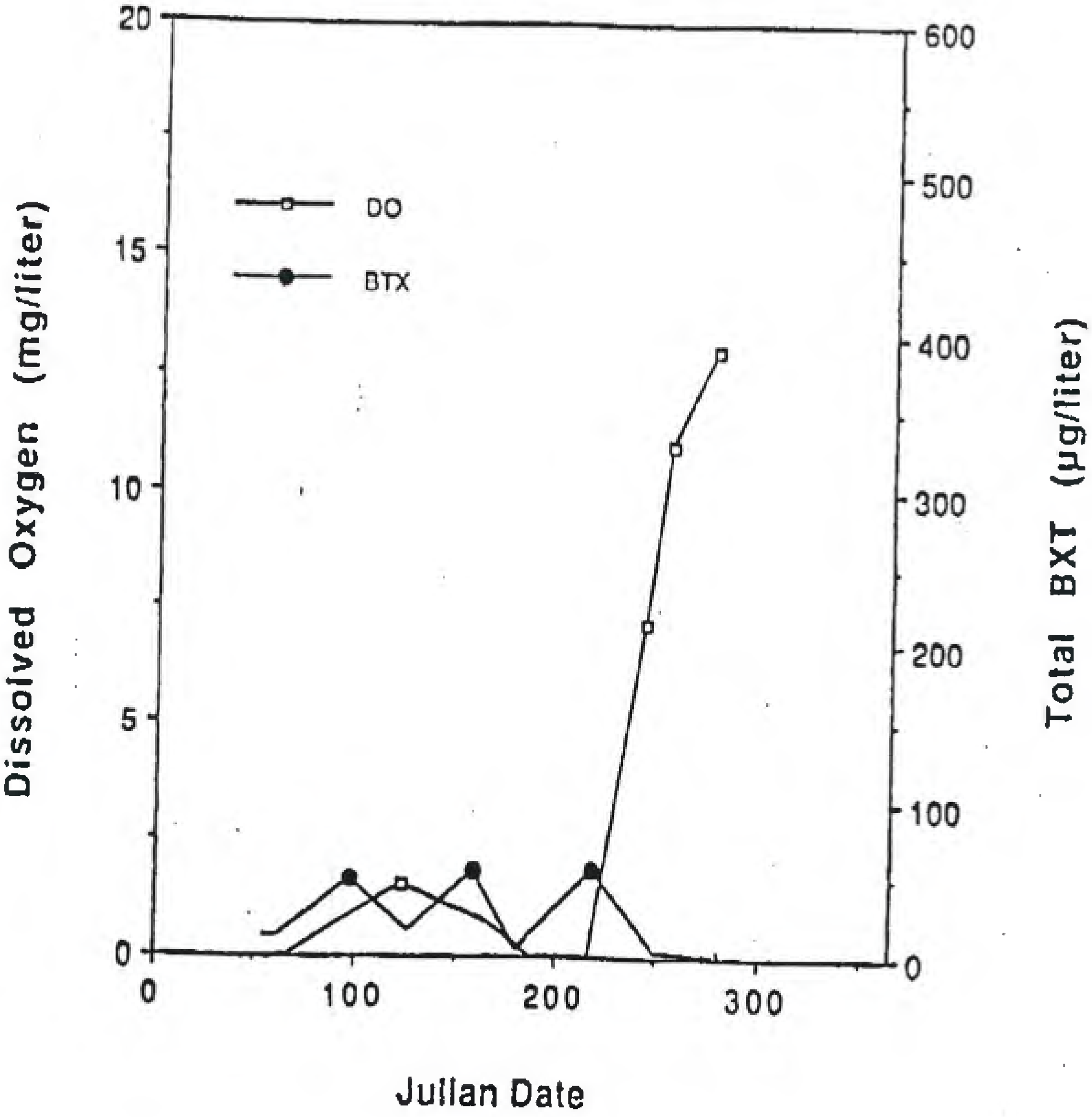

Figure 3. Breakthrough of oxygen and depletion of alkylbenzenes (BTX) in a miniature monitoring well (DD 31-2) 32 feet from the injection wells.

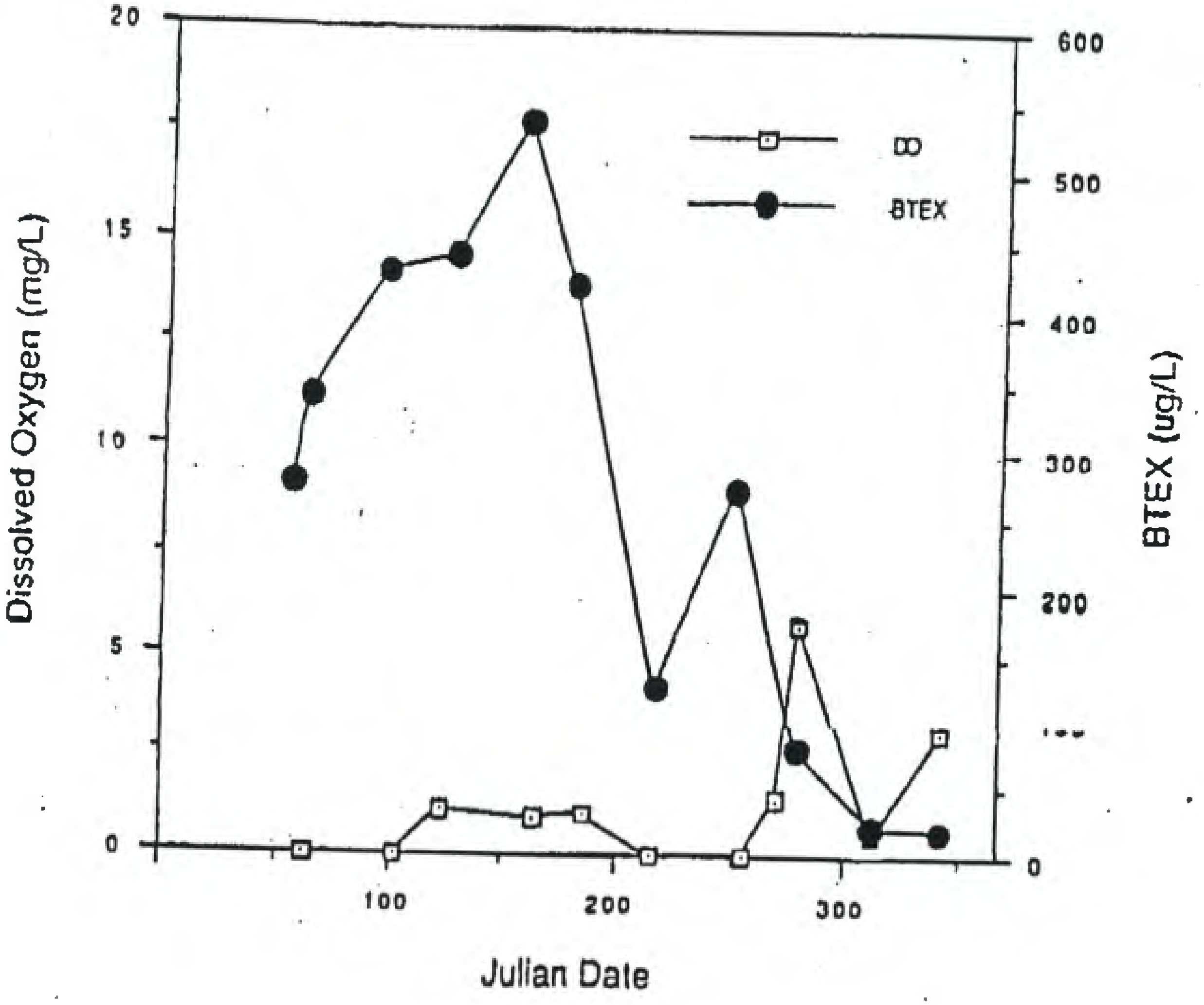

Figure 4. Breakthrough of oxygen and depletion of alkylbenzenes (BTX) in a miniature monitoring well (BD 50B-2) 50 feet from the injection wells.

Table 1. Changes in concentrations of alkylbenzenes and total fuel hydrocarbon in core material during bioremediation of an aquifer contaminated with aviation gasoline.

Date Core Number	Oil and Grease	Fuel Hydro-Carbon	Benzene	Toluene	Ethyl-benzene	Xylenes
	mg/kg wet sample					
Background conditions in an unweathered part of the spill area.						
June, 1988						
50R6		12,150	1.0	107	57	218
50R7		5,220	1.0	170	24	100
Preliminary sampling used to design the bioremediation project near monitoring well 0D-31-2, August 1987.						
50A3	4,310	5,590	0.6	235	33	121
50H4	4,130	6,500	0.3	544	12	48
50D18	1,130	2,500*	0.7	112	11	39
Sampled after four months of perfusion with mineral nutrients and oxygen, June, 1988.						
50T3		3,330*	1.4	1	7.3	23
50H3		4,800*	1.5	1	13	41
Sampled after eight months of perfusion with mineral nutrients and oxygen, October, 1988.						
50AE4		8,400	<0.3	<0.3	<0.3	<0.3
50AE5		2,370*	<0.3	<0.3	<0.3	<0.3

*these cores included some uncontaminated material.

PRESENTATION BY DR. RICHARD BROWN

OXYGEN SOURCES FOR BIOTECHNOLOGICAL APPLICATIONS

Richard A Brown, Groundwater Technology, Inc.

It is well recognized that microorganisms play prominent roles in the transformation and degradation of organic chemicals in virtually every major habitat except the atmosphere. Microbial communities in nature exhibit a truly impressive biochemical versatility in the number and kinds of synthetic organic compounds that they are able to metabolize (1,2).

Virtually the only natural transformation of polluting chemicals that can result in complete mineralization occurs via microbial metabolism. However, there are limits to the metabolic versatility of microorganisms. Many xenobiotic substrates are transformed so slowly that they cause some degradation of environmental quality. This resistance to biodegradation, though, is not a feature that is strictly associated with exotic chemical compounds. Decomposition is a function of the a) structure of the particular contaminant, b) the existing environmental conditions, and c) the physiology of the requisite microorganisms (3,4). Of these, the environmental limitations are the easiest to rectify.

In order to grow, microorganisms need a suitable physical and chemical environment. Microorganisms, like all other forms of life, are primarily composed of C, H, O, N, P, S, although a variety of other elements are also found in trace amounts. These substances are required to varying degrees in order for microorganisms to proliferate; these materials must already be present or be supplied in the proper form and ratios to the requisite microorganisms. Extremes or temperature, pH, salinity, and contaminant concentrations can also markedly influence the rates of microbial growth and substrate utilization. The nature of the limiting environmental factor(s) will often help dictate the strategy in applying biotechnology to hazardous waste treatment.

In most cases the organic pollutants themselves are able to supply the carbon and energy required to support heterotrophic microbial growth. However, the introduction of carbonaceous materials to soils and groundwater aquifers can cause an imbalance in the natural biodegradation processes, limiting the microbial transformation of the organic pollutant. For example, when labile carbon is introduced to an aerobic aquifer, the microorganisms consume oxygen along with the carbon substrate. An anaerobic aquifer can be expected whenever the rate of aerobic respiration exceeds the rate of oxygen input to the site. To sustain aerobic microbial growth, oxygen, therefore, must be supplied to the subsurface microorganisms.

The importance of oxygen supply to in-situ biodegradation was well documented recently in a study of a wood treating site in Conroe, Texas (5). A downgradient portion of the contaminant plume was characterized by low levels of organic pollutants and dissolved oxygen, while inorganic contaminants (i.e., chloride), which were associated with the organic wastes, remained at elevated concentrations. The authors suggested that oxygen was consumed during the aerobic metabolism of the organic contaminants by the indigenous micro-organisms. Hydrocarbons persisted in areas of the plume where oxygen levels were insufficient to support aerobic biological activity.

Artificially increasing the oxygenation of subsurface environments will dramatically increase the growth of heterotrophic bacteria. In a study of petroleum hydrocarbon degradation, sand columns were used to determine the effect of oxygen supply on bacterial growth and degradation of gasoline.

Several columns were prepared under identical conditions using 50 mL of wet sand sieved to 40-60 mesh. Fifty milliliters of gasoline were added to each column and allowed to drain through. An average of 4.3 mL of gas was retained. The columns were then washed with 2 liters of nutrients made up in groundwater. Different levels of oxygen were supplied to the columns by using air, oxygen or hydrogen peroxide dissolved in groundwater. The columns were treated for two weeks. At the completion of the experiments the columns were drained, and analyzed for gasoline content, total organic carbon (TOC), total bacteria and gasoline utilizing bacteria.

Bacterial counts in the interior of the column showed a very strong dependence on the oxygen level:

DEPENDENCE OF BACTERIAL GROWTH ON AVAILABLE OXYGEN

Bacteria, Colony Forming Units (CFU) / Gram Dry Soil

Available Oxygen, ppm (Ave.)	Heterotrophic Bacteria $(x\ 10^6)$	Gasoline Utilizing Bacteria $(x\ 10^6)$
8	.05	.0001
40	5.5	.7
112	75	27
200	207	31
Correlation w D.O.	.979	.933
Ratio of counts @ 200 ppm D.O.: 8 ppm D.O.	4×10^3	3×10^5

As can be seen from the data, the bacterial counts increased dramatically with increasing available oxygen. Gasoline utilizing bacterial are even more sensitive to oxygen levels than are general heterotrophic Bacteria.

The biodegradation of gasoline in the columns was also affected by the oxygenation:

DEPENDENCE OF GASOLINE DEGRADATION ON OXYGEN LEVEL

Available Oxygen	Gasoline Biodegraded		Gasoline Flushed Out		Total Gasoline Removed	
ppm (Ave.)	grams	%[1]	grams	%[1]	grams	%[1]
8	.388	12.9	.71	23.6	1.098	36.6
40	.508	16.9	.77	25.6	1.278	42.6
112	.773	25.8	.59	19.6	1.363	45.4
200	1.272	42.4	.49	16.3	1.762	58.7
Correlation w D.O.		.994		-.93		.974
Ratio gas degraded @ 200 ppm D.O.: 8 ppm D.O.		3.27		.69		1.60

[1] Based on average of 3.0 g originally present.

Several things should be noted from this data. First, the more oxygen that was supplied, the more gasoline that was biodegraded. Second, the rate of biodegradation under highly oxygenated conditions was greater than the rate of physical removal/dissolution.

These sand column studies demonstrate that bacterial growth and metabolism are very dependent on oxygenation. As a result, an important part of the biological treatment of hazardous waste is oxygen supply.

There are basically two methods of oxygen supply - physical and chemical. Physical supply involves forcing air and/or pure oxygen into the contaminated matrix. Chemical oxygen supply involves the addition of substances which can be converted to oxygen, such as hydrogen peroxide (6); or substances which can act as terminal election acceptors directly such as nitrate (7,8). All of these methods have been used in treating contaminated soils and aquifers.

The choice of an oxygenation method depends on several factors. Basically, one wants to achieve maximum efficiency in oxygenation. Too little oxygen supply relative to the amount of contamination results in much longer remediation times. Too much oxygen relative to the amount of contamination being treated can result in elevated remedial costs. The principal is to balance oxygen supply with oxygen demand. The factors that must be considered in achieving this demand are:

- oxygen mass transfer, pounds per unit time, supplied by each method
- contaminant load and location
- ease of transport/utilization

First, considering oxygen mass transfer, it is easy to calculate the amount of oxygen supplied by the different methods. The more oxygen supplied per unit time, the greater the potential level of bioreclamation.

Air sparging, one of the simpler techniques, provides oxygen by diffusing air/oxygen into a well bore. This is accomplished by using a porous stone, scintured metal or fitted glass diffuser. The water in the well bore is saturated with oxygen and diffuses out into the formation. The amount of oxygen supplied is a function, therefore, of the rate of water flow by the well bore. This, in turn, is a function of the hydraulic conductivity, the gradient and the surface area of the formation affected by the well bore. The following matrix calculates the pounds of oxygen per day an air sparger provides per well for different hydraulic conductivities and gradients. The table assumes a 30 foot saturated thickness and that the lateral influence of the well is 3 ft.

POUNDS PER DAY OXYGEN SUPPLIED BY SPARGING, SINGLE WELL

Hydraulic Conductivity gals / Day / ft^2	Hydraulic Gradient ft/ft (high) .1 (air)	(high) .1 (oxygen)	(medium) .01 (air)	(medium) .01 (oxygen)	(low) .001 (air)	(low) .001 (oxygen)
10^4 (gravel)	6	30	.6	3.	.06	.3
10^2 (medium sand)	.06	.3	$6x10^{-3}$	$3x10^{-2}$	$6x10^{-4}$	$3x10^{-3}$
10^{-1} (silt)	$6x10^{-5}$	$3x10^{-4}$	$6x10^{-6}$	$3x10^{-5}$	$6x10^{-7}$	$3x10^{-6}$

As can be seen air sparging is a limited source of oxygen. Sparging pure oxygen instead of air will increase the pounds per day by a factor of five so that the maximum on the matrix would be 30 lbs. oxygen per day instead of 5.

A second system is to pump air/oxygen saturated water into a contaminated aquifer. The pounds per day of oxygen supplied is a function of injection rate:

POUNDS PER DAY OXYGEN SUPPLIED BY AERATED/OXYGENATED WATER INJECTION SINGLE WELL

Injection Rate, gpm	aerated water 10 ppm D.O.	oxygenated water 50 ppm D.O.
1	.12	.60
10	1.2	6.0
100	12.0	60.0

Air venting systems are an efficient means of supplying oxygen through unsaturated contaminated soils. This technique is used in treating vadose zone contamination or in treating excavated soil piles. Air can be added by either injection or by withdrawal. In vadose zone treatment, the common method is vacuum withdrawal. This method has the added advantage of physically removing volatile contaminants in addition to supplying oxygen. The amount of oxygen supplied is a simple function of the air flow rates. The following table uses a 20% oxygen content for air to calculate air supply:

POUNDS PER DAY OXYGEN SUPPLIED BY VENT SYSTEM (UNSATURATED SOILS)
SINGLE WELL

air flow rate SCFM	oxygen supply lbs / Day
1	23.3
5	116.6
10	233.3
20	466.6
50	1,166.4
100	2,332.8

Finally, there are two chemical carrier systems - Hydrogen peroxide and nitrate. While both of these materials are highly soluble, their common use rate is about 1000 ppm (.1%). The number of oxygen equivalents supplied is dependent on the chemistry involved. Hydrogen peroxide is converted through decomposition to oxygen:

$$H_2O_2 \text{ -------- } H_2O + 1/2\ O_2$$

Each part of hydrogen peroxide supplies .47 parts of oxygen. Nitrate is, on the other hand, directly utilized as a terminal election acceptor. Its oxygen equivalents can be calculated by comparing the amount of nitrate required to oxidize a substrate versus the amount of oxygen. Take, for example, the oxidation of Methanol:

Oxygen: $CH_3OH + 3/2\ O_2$ -------- $CO_2 + 2H_2O$

Nitrate: $NO_3^- + 1.08CH_3OH + H^+$ ---- $.065C_5H_7NO_2 + .47N_2 + .76CO_2 + 2.44H_2O$

Based on these above equations, one part of nitrate is equivalent to .84 parts of oxygen.

The oxygen equivalents supplied by these two chemical carriers is a simple function of injection rate.

POUNDS PER DAY OXYGEN EQUIVALENTS SUPPLIED BY CHEMICAL CARRIERS, SINGLE WELL @ 1000 ppm

Injection rate gpm	H_2O_2 (.47 equiv O_2/part H_2O_2)	NO_3^{---} (.84 equiv O_2/part NO_3)
1.0	5.6	10.0
5.0	28.0	50.0
10.0	56.0	100.0
20.0	112.0	200.0
50.0	280.0	500.0

The second factor in considering an oxygen source is the contaminant load and location. Contaminant location is important in that vent systems require unsaturated environments and will, therefore, be excluded in treating contaminants below the water table. Contaminant load, on the other hand, impacts all means of oxygen supply, in that it determines oxygen demand. What drives contaminant load is the phase distribution.

Petroleum hydrocarbons exist in the subsurface as three condensed phases: mobile free product (phase separated), residually saturated soil (adsorbed phase), and contaminated ground water (dissolved phase). The distribution of hydrocarbons into these different phases, while a result of dynamic transport, is ultimately a function of their physical and chemical properties, and the hydrogeological and geochemical characteristics of the formation. One must examine the phase distribution by two means: the areal extent of contamination or the volume of the subsurface impacted by a phase and the severity of contamination or the amount of the contaminant within a phase, measured as either total weight or concentration. The following table gives the phase distribution for a gasoline spill in sand and gravel:

PHASE DISTRIBUTION OF GASOLINE IN SAND AND GRAVEL

	Extent of Contamination		Mass Distribution		
Phase	Volume, cu. yd.	% of Total	lb.	Conc. ppm	% of Total
Free phase[1]	780	5.3	126,800[1]	---	90.9
Adsorbed (soil)	2,670	18.3	11,500	2,000	8.2
Dissolved (water)	11,120	76.3	390	15	0.3

[1] Actual value recovered from site

There are several generalizations that can be made from the above data concerning the distribution of petroleum hydrocarbons between the different phases. First, groundwater flow is the primary long term mechanism for spread of the contamination once the free product layer has achieved flow equilibrium. Thus, the areal extent of groundwater contamination is typically greater than that for other phases. However, the amount of material in the groundwater is small compared to that retained in the soil matrix, less than 5%. The residually saturated soil, if untreated, is a continuing source of groundwater contamination.

In looking at the contaminant load, the presence of and the distribution between the different phases is an important factor. The following table gives the pounds per cubic yard of aquifer for dissolved and adsorbed phase contamination. The calculation assumes a porosity of 30% and a dry soil bulk density of 2700lb/yd^3. The soil levels are generally two orders of magnitude higher than dissolved levels.

COMPARISON OF CONTAMINANT LOADING
DISSOLVED AND ADSORBED PHASE
/ yd^3 OF AQUIFER MATERIAL

Dissolved Phase @	#/yd^3	Adsorbed Phase Phase @	#/yd^3
1 ppm	$5x10^{-4}$	100 ppm	.27
10 ppm	$5x10^{-3}$	1,000 ppm	2.7
100 ppm	$5x10^{-2}$	10,000 ppm	27.0

From this data it is obvious that contaminated soil drives the contaminant load. The more the volume of contaminated soil and the higher the level of contamination, the greater the contaminant load. One cubic yard of soil contaminated at only 100 ppm contains as much contaminant as 5.4 yd^3 of contaminated aquifer material (dissolved phase).

The third factor in considering an oxygen source is the ease of transport and utilization. This involves the means of application, the maintenance of system, and the rate/degree of utilization.

An air sparger system uses a small compressor able to deliver ~1CFM per well. The sparger itself is either a porous stone, a scintured metal diffuser, or a fritted glass diffuser. Power consumption is minimal. The transport of the aerated water is limited by the rate of groundwater flow. The most significant operating cost is an air sparger system is maintenance of the compressor and of the diffuser and well screen. Biofouling or inorganic fouling of the diffuser and well screen can be significant and well therefore require a high degree of maintenance. Bacterial utilization of the dissolved oxygen is very high.

Injection of aerated/oxygenated water is a relatively simple system. The simplest approach is to use an air stripper to aerate the water. Often in treating a contaminated aquifer, groundwater is recovered and air-stripped to achieve hydraulic control of the contaminant plume. Reinjection of the stripped groundwater, can therefore, be accomplished for relatively low cost. The main cost of operation is controlling fouling of injection system. Transport of the oxygenated water is dependent on the geology (hydraulic conductivity). Bacterial utilization of the injected dissolved oxygen is very good.

Venting systems, while limited to unsaturated soils, are very efficient means of oxygen supply. The primary capital cost is the vacuum pump(s) needed to drive the system. Maintenance of the pumps is fairly simple and power consumption is minimal. The efficiency of the vent system is enhanced by volatile removal. The largest potential cost with a vent system is treatment of the vapor discharge. This can be accomplished by using disposable carbon, regenerable carbon or catalytic oxidation. Regenerable carbon and catalytic oxidation are capital systems.

A hydrogen peroxide system is generally a low capital, easy to maintain system. It does entail a fairly high O&M cost due to the chemical cost of the hydrogen peroxide. The cost of hydrogen peroxide is dependent on the volume used. Small quantities cost more per pound than do large quantities. On a per pound of oxygen basis, the cost will range from $1.50 to $2.50. The biggest cost factor involved with hydrogen peroxide is how quickly it decomposes. There are two mechanisms of decomposition - Biological and metal catalysis. Ideally, one would like minimal metal catalyzed decomposition. In some soils, however, that contain high levels of iron or manganese, metal catalyzed decomposition can be severe. In such cases the solubility of oxygen is rapidly exceeded and the water phase degassed, loosing available oxygen and drastically reducing the efficiency of the system.

Finally, nitrate systems are a potential electron acceptor alternative. Operationally, these systems have not been proven.

Capital costs for a nitrate system would be fairly low consisting, as with peroxide, of a supply tank and metering pump. Chemical costs for nitrate are $.60 - .70 / lb oxygen equivalent. The issue with nitrate, however, is not the cost or ease of addition, but instead the biochemistry of utilization and the regulatory issues. In a recent test of nitrate utilization, it was found that even with an extremely labile substrate such as sucrose, there was a significant lag phase in the utilization of the nitrate when oxygen was also available at low levels. It would appear that nitrate utilization requires low oxygen conditions. If the biochemistry of nitrate is complicated, the regulatory issues become significant. Nitrate levels in ground water are regulated at 10 ppm. If nitrate is not rapidly utilized, injection would have to be tightly controlled and may be precluded.

To put the above costs and analyses into perspective, one can compare the operation of the different systems for a sample gasoline problem. The site characteristics are as follows:

Area of contamination

soil - 100 x 50 c 5ft (3ft above; 2ft below water)
groundwater 250 x 70 x 5ft

Contaminant concentrations

soil - 1200 ppm
groundwater - 18 ppm

Aquifer characteristics

hydraulic conductivity	5×10^2 bal/day/ft^2
hydraulic gradient	.08 ft/ft
pumping rate/well	35 gpm
saturated thickness	25 ft.
DTW	15 ft.

Contaminant distribution

soil	2500	lb.(1500 lb above/1000 lb below
GW	30.0	lb.
Total	2530	lb.

The configuration of the systems would vary. The basic layouts would be as follows:

Air Sparging: Sparging would be through 15 wells spaced through-out the plume. There would be no recovery wells.
Water Inspection: The system would consist of 2 recovery wells pumped at 35 gpm each. Each well would have a 25 ft. cross-gradient capture radius. The 70 gpm water would be air stripped through a 2 ft. x 18 ft. air stripper and reinjected through two upgradient galleries.
Vent System: Venting would be through 4 wells placed on 40 ft. centers. Each well would be pumped at 40-45 CFM. The system would require a single 2 H.P. high vacuum blower. Vapor discharge would be treated through a catalytic oxidizer.
Peroxide System: Hydraulic control is maintained by 2 down gradient recovery wells each operated at 35 gpm. Of the 70 gpm recovered, 35 gpm would be reinjected through a gallery and 2 upgradient injection wells. Peroxide would be added at 1000 ppm. Excess water would be air stripped and sewered.
Nitrate System: Because of the concern with off site migration of nitrate, there would be 4 downgradient recovery wells. Two of the wells, on the leading edge of the plume, would be operated at 35 gpm each. This water would be amended with nitrate (1000 ppm) and reinjected through 4 upgradient wells and an injection gallery. The two additional recovery wells would be placed 50 ft. downgradient of the plume to create a barrier to migration of nitrate. They would be operated at 30-40- gpm.

Using this data, the capital and operating costs for each system can be calculated. The following table gives a comparison of the different systems.

Cost/Performance Comparison for Various Oxygen Systems
High Degree of Contamination

System	Costs			Performance				
	Capital	Operation	Maintenance	Lbs/Day Oxygen	% Site Treated	Utilization Efficiency %	Time of Treatment	$/Lb oxygen Used
Air Sparging	$35,000	$800/month	$1200/month	6	41	70	858 days	$25.80
Water Injection	$77,000	$1200/month	$1000/month	8	75	50	1580 days	$28.62
Venting System	$88,500	$1500/month	$1000/month	4000	60	5	132 days	$ 3.82
Peroxide System	$60,000	$10,000/month	$1500/month	190	100	15	330 days	$18.60
Nitrate System	$120,000	$6500/month	$1000/month	211	100	12.5	335 days	$22.06

As can be seen, there is a wide variance in both cost effectiveness and in treatment effectiveness. In terms of cost performance, the order is:

Venting > > peroxide > nitrate > air sparger > water injection

In order of treatment effectiveness the order is:

Peroxide = nitrate > water injection > venting > air sparging

While venting is a very cost effective method it is limited to treating the vadose zone. Consequently, it's treatment effectiveness is limited.

This above analysis is given for a situation with extensive contamination. If the degree of contaminants is changed so that the soil contamination is minimal, the analyses would change. Assuming that there is no soil contamination above the water table and that the soil levels are <100 ppm, the performance of the different systems would be as follows, all other factors remaining constant:

COST/PERFORMANCE, LOW DEGREE OF CONTAMINATION

System	lb$/day O_2	Time of Treatment days	$ lb oxygen used
Air sparging	6	180	43.55
Water Injection	8	330	89.55
Venting	Not Applicable		
Peroxide	190	180	38.39
Nitrate	211	240	47.39

When the degree of contamination is less, simpler systems such as air sparging become more cost effective. When the contamination is only the dissolved phase, an air sparger system is the best choice. The following table summarizes the best choices for different contamination situations.

Prioritization of Systems for Different Contaminant Situations
(1=Best)
Types and location of Contamination

System	Dissolved Only	Dissolved and Soil Below Water	Soil Above and Below Water Table and Dissolved	Soil Above Water Table
Air Sparger	1	2	3	
Water Injection	3	3	2	3
Vent System				1
Peroxide	2	1	1	2
Nitrate	(Not Recommended at present)			

The choice of an oxygen supply is dependent on the contaminant load, the mass transfer and the ease of transport/utilization. Depending on what the degree of contamination is, different systems will be most effective.

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PRESENTATION BY DR. MARTIN REINHARD

INVESTIGATION OF THE TRANSPORT AND FATE OF GASOLINE HYDROCARBON POLLUTANTS IN GROUNDWATER

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<u>Prepared by</u>:

Harry F. Ridgway
Donald W. Phipps
Jana Safarik

Orange County Water District
10500 Ellis Avenue
Fountain Valley, California 92728-8300

AND

Franziska Haag
Martin Reinhard
Harold A. Ball
Perry L. McCarty

Water Quality Research Laboratory
Department of Civil Engineering
Stanford University
Stanford, California 97305

1.0 ABSTRACT

"INVESTIGATION OF THE TRANSPORT AND FATE OF GASOLINE HYDROCARBON POLLUTANTS IN GROUNDWATER"

A 24-month study was conducted to delineate chemical and microbiological factors controlling the transport and fate of gasoline hydrocarbon pollutants in groundwater. Groundwater and aquifer solids for analysis were obtained from a shallow, semi-perched zone consisting primarily of silty/sandy alluvial deposits which had been contaminated with approximately 20,000-30,000 liters of regular, unleaded gasoline from a leaking underground storage facility. The extent of the contamination plume had been described in a previous U.S. Geological Survey investigation of the site (Schroeder, 1985), was located adjacent to an intertidal marsh and wildlife refuge on the U.S. Naval Weapons Station property in Seal Beach, California.

The results of gas chromatographic analyses of specific gasoline hydrocarbons in groundwater samples from the site confirmed the extent of gasoline migration and also revealed an enrichment of naphthalene and p/m-xylene near the periphery of the contamination plume. The latter observation suggests either greater transport velocity and/or reduced biodegradability for the enriched components.

Approximately 300 gasoline-degrading bacteria recovered from the Seal Beach study site were sorted into 111 distinct subpopulations (nutritional groups) based upon their aerobic growth pat-

terns on a battery of 15 specific hydrocarbons which represented the major chemical groups in unleaded gasoline. Each of the 15 test hydrocarbons was found to support the growth of at least one bacterial isolate from the site, suggesting that all of the primary constituents of gasoline could be biodegraded under appropriate conditions. The marked heterogeniety of the gasoline-degrading microbial community at the Seal Beach site was further demonstrated by an independent technique involving computer-programmed analysis of whole-cell protein banding patterns as detected by high-resolution, sodium-dodecylsulfate polyacrylamide-gel electrophoresis. Using this technique, 287 isolates capable of gasoline degradation were sorted into 59 distinct bacterial subpopulations. Bacterial isolates capable of biodegrading the linear, branched, and cyclic alkanes comprised a divergent subpopulation which exhibited protein banding patterns generally dissimilar from those isolates which could metabolize only the aromatic compounds.

The results of microbial enumeration studies indicated that the number of aerobic, gasoline-degrading bacteria detected in groundwater samples typically varied from about one colony-forming-unit (CFU) per milliliter to several hundred CFUs per milliliter, which was consistently several orders of magnitude lower than the total heterotrophic plate count. The number of gasoline-degrading bacteria throughout the site tended to vary inversely as the concentration of hydrocarbon (toluene), probably as a result of specific hydrocarbon-induced injury and/or toxicity. Consistent with this hypothesis was a gradual temporal decline in the number of gasoline-degrading bacteria in the most contaminated well near the center of the plume. Prolonged exposure of one gasoline-degrading microbe from this well to gasoline vapors was associated with physiological injury and accelerated cell death, as well as a measurable increase in the frequency of spontaneous segregation of mutant strains which had irreversibly lost their ability to grow on gasoline as a sole carbon source. The results of mineral deprivation studies suggested that metabolism of one or more specific gasoline hydrocarbons was evidently required for the full microbicidal activity of gasoline to be manifested, suggesting the formation of a toxic metabolite.

Hydrocarbon mineralization activity by the native microbial flora in groundwater samples was determined by (1) quantifying the amount of radioactive carbon dioxide released metabolically from ^{14}C-carbon-labeled hexadecane, (2) computer-programmed nondispersive infrared analysis of unlabled carbon dioxide released from gasoline or individual gasoline hydrocarbons, or (3) direct quantification of a specific hydrocarbon substrate by gas chromatographic analysis.

Despite the relatively low numbers of gasoline-degrading bacteria in most aquifer samples, mineralization of gasoline hydrocarbons by native microbial consortia, as well as by individual bacterial isolates, proceeded rapidly under oxygenated conditions in the presence of appropriate trace mineral supplementation. Biotransformation and/or complete mineralization of selected aromatic gasoline constituents (e.g., toluene and p-xylene) was also demonstrated under anaerobic (denitrifying or methanogenic) conditions. The rate of hydrocarbon mineralization was a function of trace nutrient availability and concentration, soil type and condition, as well as the type and initial concentration of hydrocarbon-utilizing bacteria. The rate of gasoline biodegradation in aerated, fluidized-bed soil microcosms could be significantly accelerated by increasing the size of the initial cell inoculum or by the introduction of hydrogen peroxide. It is concluded that in-situ mineralization of gasoline hydrocarbons can occur rapidly under aerobic conditions, or more slowly under denitrifying or methanogenic conditions using appropriate nutrient supplementation.

PRESENTATION BY DR. SUSAN LANDON-ARNOLD

STRATEGY FOR ASSESSING THE ABILITY OF NATIVE BACTERIA TO BIODEGRADE JP-5.

S. Landon-Arnold, Ph.D.
Northern State University

The contamination of ground water is an ever increasing problem. Approximately 95% of the U.S. freshwater supply is contained in subsurface environments (20). Society's increasing reliance on ground water for domestic agricultural and industrial usages has made quality ground water one of the primary environmental concerns of the 1980's (27).

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 (27). Petroleum fuels are complex mixtures of aliphatic and aromatic hydrocarbons. The major components of refined petroleum fuels are paraffins, olefins, naphthenes, aromatics and alcohols (19). 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. (27). 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 (6).

Underground storage tanks (USTs) are major sources of subsurface contamination (27). It has been estimated that as many as 100,000 buried gasoline tanks in the United States were leaking as of 1985 (7), and as many as 350,000 USTs will leak by the end of this decade (26). It is important, then, to clean up this contamination. Clean-up of subsurface contamination can be extremely difficult due to the inaccessibility 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 (31). Bioremediation as a possible means of decontamination, is often dependent on the biota naturally selected for by each contaminant at a spill site which may be very specific. In situ bioreclamation uses indigenous, subsurface microbial communities to metabolize organic contaminants into carbon dioxide and water. 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 (27). It would be advantageous to have a workable strategy for assessing the ability of naturally enriched, native bacteria to degrade contaminating hydrocarbons such as JP-5.

Subsurface ground waters act as reservoirs of ground water (20). Soil will function as a trickling filter to purify surface water as it trickles down to ground water reservoirs (18). The relative number of studies of subsurface organisms are few when compared to studies dealing with surface soil organisms. The function of surface soil microorganisms and biodegradation is widely documented (3). In subsurface soil, however, evidence is just now being presented on the role of microorganisms and environmental pollutants (13, 15).

The presence of diverse and abundant subsurface microbial populations has been established (17, 30). 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, 27). Ghiorse & Balkwill, (18) characterized indigenous microorganisms of ground water-bearing subsurface environments with respect to their identity, abundance, and metabolic activities. However, traditional cultural techniques are not optimal because organisms selected for by conventional lab techniques may not represent relative proportions of indigenous microorganisms. It has been documented that traditional cultural techniques detect only 1-10% of organisms in soil (2). It is known that heterotrophic microorganisms can metabolize most petroleum hydrocarbons to carbon dioxide, water, and other harmless compounds (24). Hydrocarbon utilizing bacteria have been found in oil- and gas-bearing deposits (13). Ghiorse & Balkwill, (18) presented a nutritionally rich growth medium that was relatively successful in increasing the percent recovery of organisms from the soil. This optimization of recovery is integral in a strategy for isolating native bacteria

Biorestoration efforts may be based on aerobic or anaerobic mechanisms of biodegradation. During aerobic biodegradation, the carbon source is broken down by a series of enzyme-mediated reactions in which oxygen serves as the final electron acceptor. When oxygen is not available, simple organic compounds or inorganic anions like nitrate, sulfate or carbonate can serve as alternate electron acceptors. In the latter cases, the biodegradation is linked to denitrification, sulfate-reduction and methanogenesis, respectively (25). Aerobic biodegradation of petroleum hydrocarbons is much more rapid than anaerobic biodegradation, and thus is preferred for bioremediation (27). Regardless of the electron acceptor and strategy employed in the restoration effort, the electron acceptors should be consumed at a greater rate in treated areas than in unimpacted areas receiving only a control treatment.

STRATEGY PLAN

Since biodegradation is more effective, complete and cost effective, how then can appropriate organisms be found and enhanced. In a strategy of assessing the ability of indigenous bacteria to degrade a contaminant (Figure 1), such as JP-5, the first objective is to identify microorganisms capable of this degradation . The first step is laboratory, in vitro analysis. This is accomplished by acquiring a fresh sample of JP-5 contaminated soil and determining the indigenous population and their ability to utilize the contaminating substrate. Since indigenous organisms have been exposed to the contaminant, the process of "adaptation" or "acclimation" may have occurred. This response is a uniquely biological one and refers to the accelerated utilization of a substrate by microorganisms due to a change in the levels of requisite enzymes of the numbers of degrading microorganisms. While other reasons are also possible for adaptation phenomena, these two are likely to be the most frequently encountered mechanisms (25).

Enumeration of organisms: It is advantageous to get a handle on the number of organisms/gram of contaminated soil. It is known that microbial numbers will change during storage of the soil sample (17). Therefor, it is most effective to use direct observation immediately upon collection or the Aufwuchs method (10) in which a slide is suspended in the material with time, then stained and examined. With respect to direct observations: epifluorescence (28) using acridine orange has proven very effective. In this technique, the organisms fluoresce green indicating the presence of double stranded DNA (11). As previously stated, enumeration of viable organisms via the isolation of colony forming units (cfu) will not give accurate assessment of the relative numbers of organisms in the soil. However, it may allow the isolation of individual, degrading populations and thus may prove worthwhile. It is possible that indirect counts may be more sensitive, such as the measure of muramic acid, a specific bacterial cell wall component (18).

Growth and component utilization: How do you know the organisms are utilizing the components. Once the organisms are isolated from the soil, and the soil has been determined to be colonized, then alterations to the environment can be examined. This should be done'in vitro, as in a chemostat and then with soil columns. The chemostat is a means of continuous culture that corresponds much better than batch (12). A fresh supply of nutrients is added while medium is simultaneously withdrawn from the culture; thus culture volume is constant (16). The continuous culture techniques are important in the study of mixed cultures. The continuous flow system allows the mixed culture to reach a steady state condition or else stay within a narrow range of fluctuation with regard to growth rate and cell age due to wash out (9). The chemostat offers the advantage of acquiring high population densities at low substrate concentration. This then, would mimic the natural environment (29). The chemostat has been especially useful in enrichment studies. When the population of a desirable organism is low, then some enrichment is necessary. Enrichment usually means increasing the population size by altering the environment (21). A diagram of the chemostat physical set up is given in Figure 2. The chemostat is inoculate with pure and mixed identified cultures using the contaminant, JP-5, as the sole carbon source. Analysis of the effluent, is by GC, HPLC, and/or UV for the determination of biodegradation and bioconversion. Then, characterize the degree of contaminant removal and amount of conversion products produced per strain or mixture of microorganisms. Use of the chemostat will give data regarding toxicity concentration, and optimal nutrient amendments for a broth culture. This would allow one to maximize microbial biodegradation by manipulating environmental parameters, such as dissolved oxygen, nutrients, pH and temperature. A major weakness in most claims of biorestoration success is the lack of controls for abiotic mechanisms that might account for the removal process. The increase in the rate of substrate decay in experimental microcosms over sterile abiotic control flasks (i.e. controls) can be attributed to biodegradation, and thus account for abiotic mechanisms of removal.

By nutrient amendments, one refers to the adjustment of oxygen, nitrogen and phosphorous. Usually, the contaminant is the only carbon source used, although cometabolism is not uncommon. In a mixed population and in an uncontrolled environmental matrix such as <u>in situ</u> soil, the addition of an additional, potentially more easily metabolized carbon source may not prove to be helpful. Optimum growth can be, as a result of amendment addition, determined by:

1. Rate of biomass increase and the rate of substrate disappearance, as determined spectrophotometrically and/or by gas chromatography (GC). Soil extraction of contaminant from the sample using conventional extraction and column cleanup procedures as recommended by the EPA (25).
2. GC, HPLC, UV and/or MS analysis of the soil to determine the JP-5 contaminant concentrations. Observation of removal of the substrate is the best way to assess microbial activity.

<u>In Situ Assay</u>: This information can then be used to set up soil columns. Our soil columns are 1m long, 4 cm diameter glass columns, that use an upflow of influent with effluent being discharged from the top of the column. In using a soil column , one tests for adsorbance on soil types, permeability of formation, chemical aspects i.e., precipitation or complexation of critical nutrients, adherence of organisms and adsorbance of waste, partitioning coefficients, adsorbtion and adherence of the influent and retention time needed in addition to fine tuning of nutritional amendments that have been determined via the chemostat experimentation. Again, the depletion of the contaminant, JP-5, is

monitored. This data can then be applied in situ at the field test site. Here enhancement of indigenous organisms, and thus optimizing naturally selected hydrocarbon degraders, can occur.

Metabolite Toxicity: As with all bioconversions, metabolites are produced. It is important that laboratory screening procedures for toxicity of metabolites are conducted. The production of metabolites which are more toxic than the parent compound during biodegradation is a known phenomenon (25). The need to evaluate the level of hazard of compounds has been met by technological methods of 1toxicity testing. Toxicity measurements indicate the level of hazard by determining how quickly a given organism dies in the presence of a given compound at a certain concentration. The speed of death and the numbers of organisms which die indicate the hazard of that compound. Several recent and on-going studies by the EPA and others have attempted to evaluate and rank toxicity indicator organisms in order of sensitivity. Two studies of hazardous waste site materials obtained the following results:

Test organisms listed in order of decreasing sensitivity

1. Alga (Selenastrum capricornutum)
2. Daphnia magna
3. Microtox (photoluminescent bacterium)

However, in certain types of wastes, the ranking of organisms may be reversed (25). Our laboratory used MICROTOX (Microbics, Inc.) toxicity assays (22, 23).

The EPA and others have recommended that at least two toxicity indicators be used in environmental toxicity testing (14, 25,). Severasl laws, such as the Comprehensive Environmental Response, Compensation and Liability Act (CERCLA), Clean Water Act, Resource Conservation and Recovery Act, and Toxic Substances Control Act, require the safe handling and disposal of hazardous wastes. For biodegradation to be considered a safe disposal method, the products of biodegradation must also be examined for toxicity. Toxicity testing should be performed as a part of a site evaluation, laboratory simulations and field treatment studies.

Field Test: The last step in this strategy is the performance of field tests. A baseline level of specific degrader populations and activity measurements should be collected within the impacted area before the biorestoration effort has started and identical data should be collected from outside the polluted area to serve as a background. Also, an impacted control plot should also be established that does not receive the bioremediation treatment to act as a baseline comparison. By carefully monitoring these areas for substrate depletion with time, the effectiveness of the applied biorestoration effort can be evaluated. Thus three test plots are recommended:

1. One contaminated with JP-5 and treated using the data gathered in laboratory tests.

2. One contaminated with JP-5 and not treated.
3. One not contaminated with JP-5 and yet treated.

The depletion of the contaminant is the ultimate interest in any biorestoration effort and is the parameter which should be routinely measured. Thus, by measuring the rate of contaminant depletion in representative samples, the efficiency of the biorestoration effort can be evaluated relative to both baseline and background controls. For instance, before a treatment process is implemented, the rate of substrate decay in laboratory assays may be nil. However, as a result of field treatments, the laboratory determined rates may be accelerated with time relative to appropriate background and baseline controls. It is important to emphasize at this point the need to have performed representative simulations of the environment under examination with appropriate laboratory controls to account for abiotic losses of the substrate (i.e. sorption, volatilization, etc.).

When the desired treatment level is reached (i.e. the contaminant is reduced to predetermined acceptable levels or background) the restoration effort can be discontinued. Such a consideration early in any bioremediation effort helps focus on the extent of the contamination problem, the efficiency of the removal mechanism and the endpoint of the cleanup procedure.

WHAT WE HAVE DONE

In our laboratory, microorganisms indigenous to the 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. (27). Microorganisms capable of growing on fuel hydrocarbons were found in three soil samples examined. It was anticipated that these soils had potential for promoting the growth of the indigenous microflora. However, our data show that the isolated organisms were not able to utilize all the petroleum hydrocarbons tested as determined by biomass increase. However, experimentation is ongoing, using more sensitive assays for the determination of component removal. Also, toxicity analysis was underway, using the Microtox apparatus and individual hycrocarbon components, and also effluents from preliminary soil column work (23).

SUMMARY:

It is suggested that a workable strategy for assessing the ability of native bacteria to biodegrade subsoil and ground water contaminants, such as JP-5 would be as give in Figure 1.

The potential use for such a strategy is the assessment of potential petroleum hydrocarbon degraders and the ability to amplify in situ degradation capabilities. This can be accomplished by enhance growth via the regulation of factors such as oxygen, nitrogen, and phosphorus. This strategy can be applied to many ground water or soil contaminants and may be a viable strategy for setting up successful bioremediation attempts. An added attraction of this strategy is the potential for the isolation of organisms suitable for pan site bioaugmentation.

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Figure 1:

STRATEGY FOR ASSESSING THE ABILITY OF NATIVE BACTERIA TO BIODEGRADE JP-5.

APPROACH:

-Lab Bacteria Population Studies

-Soil/Chemostat Laboratory Tests

-Column Tests

-Field Tests

-Technology evaluation

ISSUES:

What organisms are naturally found in contaminated areas?

Which naturally occurring, site-specific microorganisms can biodegrade specific hazardous wastes?

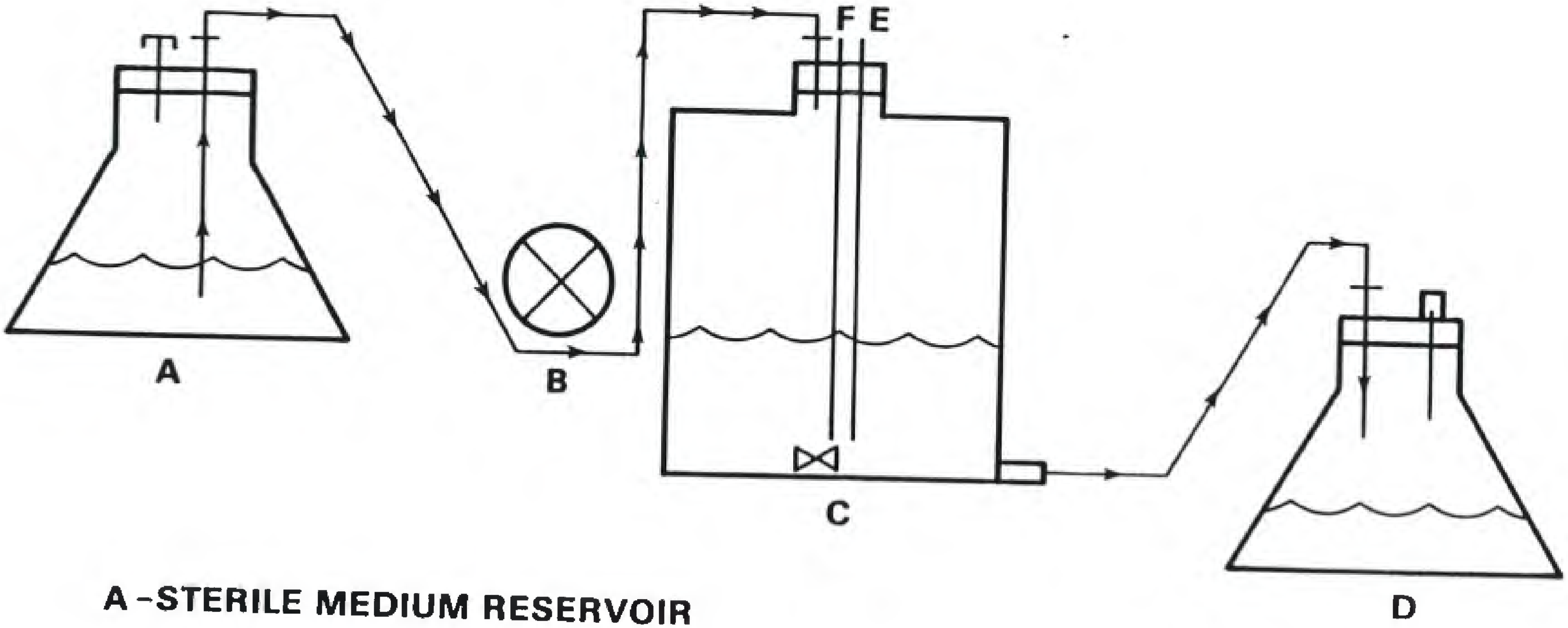
How will environmental parameters affect biodegradation?

What effect will different environmental augmentation have on biodegradation/bioremediation?

How will laboratory testing, such as soil column experimentation, translate to in-situ applications?

How do specific contaminant components affect bioaugmentation?

CHEMOSTAT



A – STERILE MEDIUM RESERVOIR
B – PERISTALTIC PUMP
C – REACTION VESSEL
D – WASTE
E – SAMPLE PORT
F – AERATION

Figure 1 Chemostat.

PRESENTATION BY DR. NATE REVIS

IMMOBILIZATION OF LEAD, CADMIUM, AND MERCURY IN SOIL, SEDIMENT, SLUDGE AND AQUEOUS SOLUTION

Oak Ridge Research Institute
Oak Ridge, Tennessee 37830

N. Revis, T. Osborne, and G. Holdsworth

SUMMARY:

Sulfate-reducing bacteria oxidize sulfur compounds and release hydrogen sulfide during their metabolism. Because many metal sulfides are not soluble in water (Table 1), it could be expected that sulfate reduction in metal-contaminated liquid media will lead to the formation of insoluble metal sulfides (Table 1). The precipitation of heavy metals from wastewaters by sulfide generation has been proposed in the past, and studies of its feasibility have met with varying degrees of success. The addition of sulfide ion to wastewater has been shown to decrease the concentration of dissolved mercury, although sulfide generation by bacteria apparently results in greater metal precipitation per mole of sulfide added to the reaction.

Table 1
Solubility Products of Selected Metals Sulfides in Water

	μg metal/Liter
CdS	0.00000087
Bi_2S_3	0.0000000019
CoS_2	0.000016
Cu_2S	0.0000000000043
CuS	0.000000000000088
FeS	0.00019
PbS	0.0000043
MnS	0.00000044
Hg_2S	0.0000000000073
HgS	0.0000000000000011
NiS	0.00011
Ag_2S	0.0000000084
SnS	0.000048
ZnS	0.0000024

We have been working to develop systems for the removal of heavy metals from wastewater using the microbial production of sulfide by a sulfate-reducing bacteria (Figure 1). If the ions of metals whose sulfides are insoluble in water are present in solution with actively growing sulfate-reducing bacteria, those metals will be precipitated as sulfides and can be removed from the medium by centrifugation or filtration.

Figure 1

The Relation of Sulfate-Reducing Bacteria to Metal Sulfide

Sulfate-Reducing Bacteria + Sulfate + Nutrients

|

Hydrogen Sulfide
+
Ionic Metals
(soluble in water)

|

Metal Sulfide
(insoluble in water)

We have carried out a series of experiments to characterize the precipitation of heavy metals by sulfate-reducing bacteria. Growth media for these bacteria typically contains lactate and sulfate, along with ferrous ion, which reacts with the sulfide generated to form a black precipitate in the medium. This precipitate is thus an indicator of sulfate reduction. Many other metal sulfides are insoluble, notably those of mercury, cadmium, and lead. Therefore, it seems probable that those metals should be removed from solution by the growth of sulfate-reducing bacteria. To test this hypothesis, we added radioactive $HgCl_2$ to a growth medium in which a culture of sulfate-reducing bacteria was then inoculated. After various periods of growth, the medium was filtered and the amount of soluble mercury remaining was determined. Soluble mercury levels were reduced from 10 ppm to 0.05 ppb. Table 2 shows that several other heavy metals commonly found in industrial wastes can also be removed by this process. We have also treated samples of wastewater from the U.S.D.O.E. Y-12 Plant in this way and reduced the level of soluble mercury below the limits of detection as measured by atomic absorption spectroscopy (Table 3).

Table 2
The Removal of Metal Ions from Solution by Sulfate-Reducing Bacteria

Metal	Concentration Initial	Concentration Final	Percent Remaining
	mg metal/liter		%
Cadmium	0.5	0.02	4.0
	500	5.0	1.0
Lead	10,000	620	6.2
Mercury	5.0	0.06	1.2
	100	0.5	0.5
Nickel	10,000	2400	2.4
Zinc	1000	100	10.0

Table 3
Wastewater Samples from Y-12 Plant

Source	Mercury Concentration	
	Untreated	Treated
	ug mercury/liter	
Storm drain	9.6	<0.1
Coal pile run-off	18-20	<0.1
Settling pond	3-8	<0.1

The sulfides are formed at the cell surface or in the near vicinity of the cell, but are not tightly bound to the cell. Accordingly, they do not form deposits that poison the cell or physically block the flow of metabolites into or out of the cell. In fact, the local precipitation of metals seems to be an important factor in rendering these organisms relatively resistant to the toxic effects of heavy metals. We have observed the growth of sulfate-reducing bacteria in media containing 500 ppm Hg^{++} or 50 ppm Cd^{++}, with concomitant removal of the majority of the heavy metals from solution. We believe that these organisms can be utilized in large-scale waste treatment systems to reduce the concentrations of a variety of heavy metal pollutants to acceptable levels.

This microbial system has application to soil, sediment, and sludge. Sulfate-reducing bacteria indigenous to soil, sediment and/or sludge can immobilize a wide range of heavy metals as insoluble metal sulfides (Table 1). The natural production of biogenic hydrogen sulfide is enhanced by the addition of calcium sulfate (gypsum) and a carbon source; they are spread on soil, sediment and/or sludge. The added sulfate and carbon stimulate metal sulfide formation and prevent the transformation of some metals to toxic organic (methylated) forms by sulfate-reducing bacteria.

Most heavy metal ions bind tightly to particulates in soil, and subsequent migration through the soil column is minimal (USEPA, 1983). Spreading of sewage sludges on the land surface distributes mercury and other metals such as antimony, arsenic, cadmium, chromium, lead, mercury, selenium, and zinc in soil. These metals are considered to pose little hazard to plant life. However, plants may bioaccumulate these metals and increase the risk of human exposure through the food chain. Furthermore, bound metals are not necessarily chemically stable and can undergo changes in chemical speciation and type of particulate carrier, and be subject to biological transformation and biomagnification. Biological agents, particularly bacteria and fungi, may either degrade organic matrices to which metals are bound or convert inorganic metal ions to volatile organic forms. These latter conversions are of special interest because they represent a pathway for bound, and apparently stable, metals to be mobilized and enter the food chain and aqueous and atmospheric environments.

Because of their low solubilities, metal sulfides may form readily at normal temperatures and pressures upon the interaction of sulfide and metal ions. As indicated in Table 1, a number of potentially toxic elements (e.g., Cd, Hg, Cu, Pb, Ni, Ag, Sn, and Zn) form relatively insoluble sulfides. These metal sulfides may be formed either abiogenically or biogenically, depending on the source of the reactive sulfide. Sulfate-reducing bacteria require an anaerobic environment, a source of sulfate, and any of several simple organic carbon compounds to produce sulfide in sufficient quantities to react with free or bound metal ions. High concentrations of toxic metals in soils and sediments do not appear to have a significantly toxic effect on these organisms, due to the high degree of metal adsorption to particulates such as clays and organic matter as well as the rapid formation of metal sulfides in their vicinity.

In soil organic matter may interact with elements in solution, chelating them and preventing the formation of metal sulfides. However with time these metals are released from the organic matter and metal can interact with sulfide to form the metal sulfide. Thus metals in soil can either react with organic matter or hydrogen sulfide which will reduce the soil mobility of the metal.

The deliberate enhancement of sulfate reduction activity in soils and sediments to immobilize heavy metals has been suggested previously. In laboratory and field studies, we have observed a reduction in leachability of a variety of toxic metals from soil and sediment (Table 4). As shown the percent leached using the TLCP procedure of EPA for several metals was less than 3% of the total metal concentration present. This observation has been confirmed in field studies. Furthermore, in laboratory studies the formed metals sulfide were stabile over a period of 12 months (i.e., the amount leached on a monthly basis over the 12 month period was similar to the ounts shown in Table 4).

Table 4
Reduction in acetic acid Leachability of Lead, Cadmium, Mercury, Chromium, and Silver from Soil

Metal Complex Added	Final Concentration of Metal in Soil	Additions to Soil	
		Water	Water, Calcium Sulfate Plus a Carbon Source
	ppm	percent of total leached	
Mercuric chloride	600	51	1.5
Lead chloride	300	66	3.6
Cadmium chloride	600	70	2.9
Silver nitrate	300	49	1.3
Chromium hexavalent	600	80	21.6

A 200-g sample of soil was mixed and divided into 100-g aliquots. One 100-g aliquot was heat-sterilized and cooled before addition of the metals shown above. To both 100-g aliquots were added individually, an aqueous solution of the above metal complexes to the final concentration indicated. After the addition of the metal complex, samples were mixed with water (i.e., the heat-sterilized samples) or water plus calcium sulfate plus a carbon source. This slurry was incubated at room temperature for 4 days and extracted with acetic acid using the EPA EP toxicity extraction method. After extraction, the above metals were determined in the leachate using atomic absorption with a graphite furnace. The results are the mean of four extractions and analyses.

These results indicate the potential of immobilizing the metals in soil and sediment. In addition these metals in the sulfide form would be less toxic to mammals because of their low solubility in water. Thus the amplification of sulfate-reducing bacteria in soil should reduce the mobility and potential toxicity of those metals shown in Table 4. It application to sites with heavy metal contamination is apparent.

PRESENTATION BY MR. HENRY KERFOOT

BIOMONITORING TECHNOLOGY: <u>IN SITU</u> MONITORING OF BIOOXIDATION OF HYDROCARBON FUELS

H. B. KERFOOT

BIOMONITORING: A HISTORICAL PERSPECTIVE

EARLY BIOMONITORING:

- -CANARYS TO DETECT METHANE IN MINES
- -MANY MEDICAL APPLICATIONS

EARLY ENVIRONMENTAL APPLICATIONS

- -BIOASSAYS TO EVALUATE WASTE TOXICITY
- -CULTURING AND IDENTIFYING/ENUMERATING SPECIFIC MICROORGANISMS IN WATER
- -CULTURING WATER TO DETECT FECAL COLIFORM RESPIRATION
- -ASSESSING BIOLOGICAL OXYGEN DEMAND

BIOMONITORING: A HISTORICAL PERSPECTIVE

PAST APPLICATIONS OF BIOMONITORING TO HAZARDOUS SUBSTANCES

- U. S. ARMY/ U. S. COAST GUARD ENZYME TICKET TO DETECT CHOLINESTERASE-INHIBITING SUBSTANCES IN WATER
  - non-specific
  - several interferences

EMERGING BIOMONITORING TECHNIQUES FOR HAZARDOUS SUBSTANCES

- RESPIRATION MEASUREMENT
  - Testing ground-water/soil samples for adapted microorganisms
  - *In situ* techniques
- IMMUNOASSAY METHODS
  - *Extreme* specificity
  - Numerous applications
  - Most suitable for high molecular-weight compounds
  - Most suitable for dedicated specific monitoring

IMMUNOASSAY TECHNIQUES

BASED ON THE FORMATION OF AN ANALYTE-ANTIBODY COMPLEX

- ANTIGEN MUST BE LARGE, SO SMALL (VOLATILE) COMPOUNDS MUST OFTEN BE DERIVATIZED TO FORM A LARGER MOLECULE

- SEVERAL APPROACHES TO DETECTING AND MEASURING THE FORMATION OF THE ANTIGEN-ANTIBODY COMPLEX

APPROACHES TO DETECTION OF ANALYTE-ANTIBODY COMPLEXES

- LABORATORY METHODS
    - radiolabelled antigens
    - others
- POTENTIAL FIELD METHODS
    - antigen-coated test tubes
    - antigen-coated fiber optics
    - antigen-coated piezoelectric crystals

NOTE: DUE TO THEIR HIGHLY SPECIFIC NATURE, IMMUNOASSAY TECHNIQUES WILL COMPLEMENT, BUT NOT REPLACE, BROAD SPECTRUM ANALYTICAL METHODS SUCH AS GC/MS. MOST LIKELY THESE METHODS WILL BE USEFUL FOR DEDICATED LONG-TERM MONITORING.

IMMUNOASSAY TECHNIQUES

DEMONSTRATED IMMUNOASSAY ENVIRONMENTAL MONITORING APPLICATIONS

Pentachlorophenol

Benzene, toluene, xylene

Dioxins

ENVIRONMENTAL MONITORING APPLICATIONS IN DEVELOPMENT

-INDICATOR COMPOUNDS

-MONITORING CLEANUPS

Compounds For Which Immunoassay Methods are Under Development

Nitroaromatic compounds

Chlorinated benzenes

Phenol

Benzene

Toluene

Ethylbenzene

Expressed proteins from genetically engineered microorganisms

IN SITU MONITORING OF BIOGENIC SOIL-GAS CARBON DIOXIDE

SOIL-GAS SAMPLING AND ANALYSIS -

A RAPID, COST-EFFECTIVE TECHNIQUE

- Mapping contaminant plumes
- Leak detection

SOIL-GAS TECHNOLOGY DEPENDS UPON:

Efficient soil-gas transport of the target compound from the contamination to the sampling location

Conservative (non-degraded) behavior of target compounds in the subsurface

SOIL-GAS CO2 CONCENTRATIONS: DETECTION OF DISSOLVED HYDROCARBON CONTAMINATION

SOIL-GAS SURVEY PROBLEMS: SUBSURFACE BIODEGRADATION OF HYDROCARBONS

PROBLEM: Subsurface biodegradation of hydrocarbons can result in non-detectable soil-gas hydrocarbon concentrations above significant hydrocarbon contaminations.

PROCESS	REACTION
Aerobic Respiration	$2C_6H_6 + 15O_2 = 12CO_2 + 6H_2O$
Denitrification	$6H^+ + 6NO_3^- + C_6H_6 = 6CO_2 + 3N_2 + 6H_2O$
Mn(IV) Reduction	$30H^+ + 15MnO_2 + C_6H_6 = 6CO_2 + 15Mn^{++} + 18\ H_2O$
Fe(III) Reduction	$60H^+ + Fe(OH)_3 + C_6H_6 = 6CO_2 + 30Fe^{++} + 78H_2O$
Sulfate Reduction	$7.5SO_4^{--} + 5H^+ + C_6H_6 = 6CO_2 + 3H_2S + 2H_2O$
Methane Fermentation	$3.5CO_2 + 5H_2O + C_6H_6 = 6CO_2 + 3.5CH_4$
Nitrogen Fixation	$10H^+ + 5N_2 + C_6H_6 + 12H_2O = 6CO_2 + 10NH_4^+$

Adapted from: Champ, et al, 1978

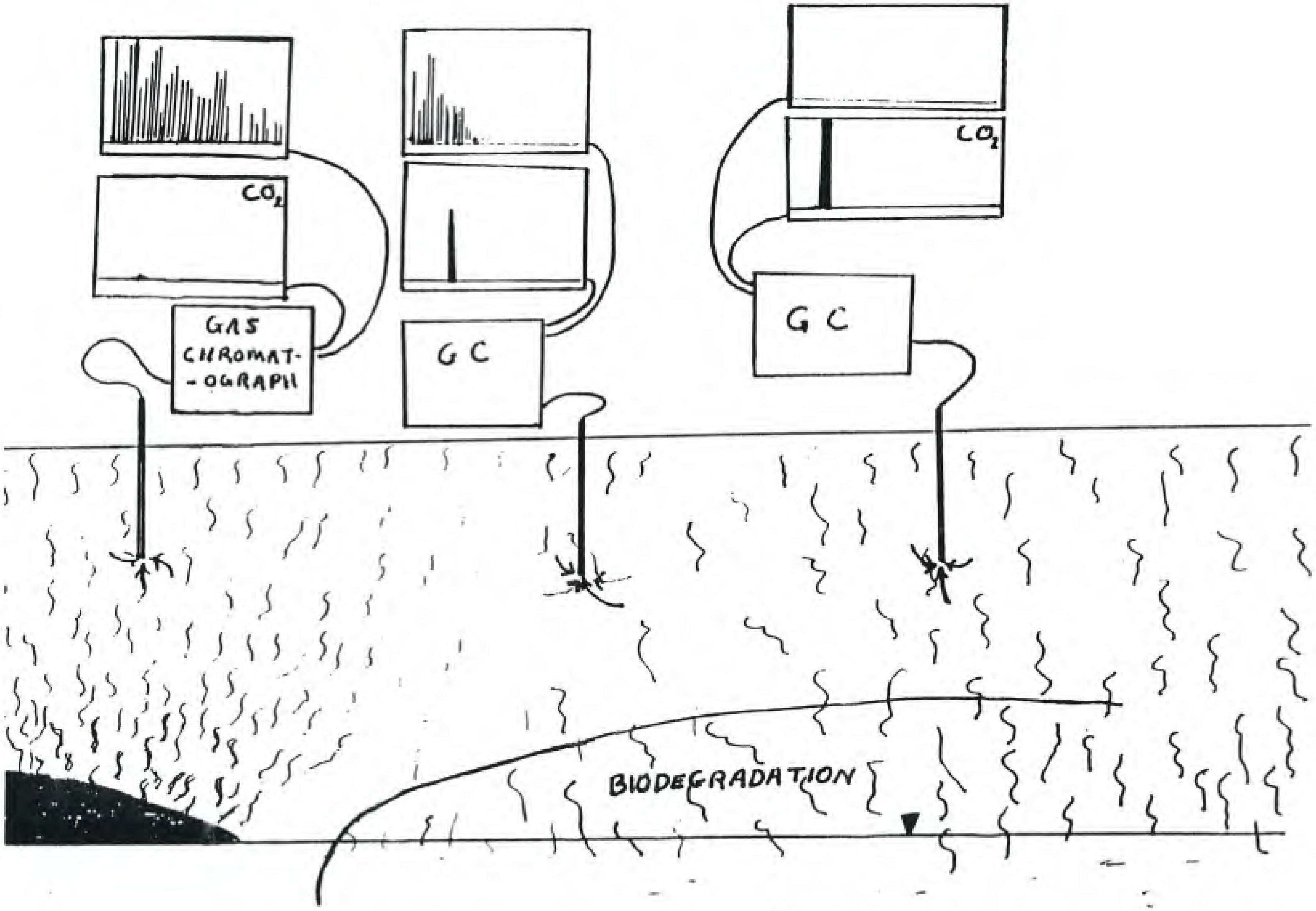
SOLUTION: DETECT THE PRODUCTS OF BIOOXIDATION OF THE HYDROCARBONS

SOIL GAS CONCENTRATIONS ABOVE HYDROCARBON-CONTAMINATED GROUND WATER

Depth to Ground Water (m)	pH	Organic Carbon (mg/L)	Inorganic Carbon (mg/L)	Soil-Gas CO2 (%)
5.5	7.10	2.52	40.8	0.09
2.4	6.95	13.02	76.0	0.45
2.2	7.08	12.27	73.2	0.32
2.8	7.02	14.79	76.8	0.36*
2.8	7.02	14.79	76.8	0.35*
2.7	6.98	12.54	73.6	0.41
2.9	7.20	9.20	57.9	0.10

Source: Ground Water Monitoring Review, Spring, 1988, pp. 67-71.

SOIL-GAS CO2 CONCENTRATIONS: DIFFERENTATION BETWEEN PURE PRODUCT AND DISSOLVED FUEL CONTAMINATION


CO$_2$
GAS CHROMAT-
-OGRAPH
G C
CO$_2$
G C
BIODEGRADATION

POSSIBLE REASONS FOR A POOR CORRELATION BETWEEN CO2 AND TOTAL HYDROCARBON CONCENTRATIONS IN SOIL GASES

-POCKETS OF PURE PRODUCT

-TEMPERATURE EFFECTS

-SPATIAL VARIABILITY IN SOIL-GAS CO2 FLUXES

-NOT A CLOSED SYSTEM

-NOT AN ORGANIC-CARBON LIMITED SYSTEM

POSSIBLE FUTURE WORK

-Evaluate the effects of spatial variability on soil-gas CO2 concentrations

-Evaluate the temporal variability of soil-gas CO2 concentrations and the factors that cause it

-Evaluate the effects of hydrogeologic factors on soil-gas CO2 concentrations near hydrocarbon contamination

-Evaluate the use of soil-gas analysis for CO2, O2, N2O, NO and other compounds for use in process control in biocleanups

Measurement of Carbon Dioxide in Soil Gases for Indication of Subsurface Hydrocarbon Contamination

by H.B. Kerfoot, C.L. Mayer, P.B. Durgin and J.J. D'Lugosz

Abstract

A preliminary field evaluation of a new application of soil-gas measurement for delineation of subsurface organic contamination is described. The method measures carbon dioxide concentrations in soil gases and is based on the hypothesis that carbon dioxide concentrations from subsurface oxidation of organic compounds will be porportional to the extent of organic contamination. A correlation coefficient (r) of 0.81 (n=6) was observed between ground water dissolved organic carbon ground water concentrations and carbon dioxide concentrations in the overlying soil gases at one site. Soil-gas carbon dioxide concentrations measured ranged from 0.09 percent to 0.45 percent.

Introduction

Sampling and analysis of soil gases can be a valuable technique for delineation of the extent of contamination by volatile organic compounds (VOCs). Typically, soil gases are sampled and the VOC concentration in them is determined by traditional gas chromatographic methods or by detectors. This technology has shown success both in field evaluations (Marrin and Thompson 1984, Kerfoot and Barrows 1987, Kerfoot and Mayer 1987) and in actual preliminary site characterization efforts (Evans and Thompson 1987, Marrin and Thompson 1987). The techniques of soil-gas surveying depend on the partitioning of the target compound(s) from soil or ground water into the interstitial soil gases and on the gas-phase diffusion of these compounds through the vadose zone. For these techniques to be successful, the VOCs must be persistent and not undergo oxidation in the subsurface (Evans and Thompson 1987, Marrin and Kerfoot in press). Failures of the technology to detect petroleum hydrocarbons do occur. Compound-specific factors of either low volatility of the hydrocarbons or subsurface oxidation of the hydrocarbons have been cited as causes of [illegible] failures (Evans and Thompson 1987). In this brief article a new approach to soil-gas surveying not subject to these limitations is described. The technique reported here takes advantage of the fact that subsurface oxidation of organic carbon can provide increased carbon dioxide concentrations in soil gas and ground water (Haas et al. 1983, Krauskopf 1967). Soil-gas carbon dioxide concentrations of 0.1 to 5 percent by volume are common (Fernandez and Kosian 1987, Matthess 1982). Above ground water that is highly contaminated with organic compounds, carbon dioxide concentrations of up to 26 percent have been measured (Bishop et al. 1966). These elevated soil-gas concentrations are attributed to subsurface oxidation of organic compounds (Bishop et al. 1966, Haas et al. 1983, Matthess 1982). If this is the case, both the soil-gas carbon dioxide concentrations and the ground water inorganic carbon concentrations should correlate with the dissolved carbon content of contaminated ground water. This article describes measurements made to test that hypothesis.

Oxidation of hydrocarbons, such as benzene, can utilize molecular oxygen or aquifer materials (e.g., pyrolusite) as oxygen sources (Champ et al. 1979):

$$2\,C_6H_6 + 15O_2 \rightarrow 12CO_2 + 6H_2O \qquad (1)$$

$$12H_2O + C_6H_6 + 15MnO_2 \rightarrow 6CO_2 + 30\,OH^- + 15Mn^{++} \qquad (2)$$

Based on the stoichiometry of Equations 1 and 2, approximately 132 milligrams of carbon dioxide would be produced from each 39 milligrams of benzene. At standard temperature and pressure, this corresponds to approximately 6.6 liters of 0.3 percent by volume carbon dioxide in the gas phase. Similarly, 36 milligrams of organic carbon could potentially produce 132 milligrams of carbon dioxide. The presence of high concentrations of free carbon dioxide in aquifers with high dissolved organic carbon concentrations has been observed (Edmunds 1973). The rate of surface oxidation of organic ground water contaminants is quite variable, depending on their molecular structures, physical properties and site-specific factors (Matthess 1982).

Experimental Section

Site

Field measurements were performed at a site of known ground water contamination that has been used previously for studies of soil-gas survey techniques (Kerfoot and Barrows 1987, Kerfoot and Mayer 1987). Ground water monitoring wells at 64-meter (200 foot) intervals along a line perpendicular to the northward flow of ground water transect a plume of benzene and chlorobenzene contamination. Table 1 lists the benzene and chlorobenzene concentrations in wells at the site. Figure 1 shows the hydrogeology at the site and the well locations. Ground water occurs at 2 to 4.4 meters below the ground surface in calcified unconsolidated alluvium. The soil type is a Caliza very gravelly sandy loam with a clay content of 2 to 8 percent that decreases with depth (Soil Conservation Service 1974). The soil permeability is 5 to 15cm per hour at depths of 0 to 40cm and 15 to 50cm per hour at depths of 40 to 150cm. The site has very sparse desert vegetation, and the soil has little or no organic matter. The average annual precipitation is 10 to 15cm.

Apparatus

Measurements of pH were performed in the field with a PHI-21 temperature-compensated portable Beckman pH meter fitted with an Orion-Ross electrode. Organic and inorganic analyses were performed with a Dohrman carbon analyzer. Ground water samples were pumped with a bladder pump into standard 40mL glass septum-capped vials (Pierce Chemical Co.). Soil-gas samples were taken with a manual air-sampling pump (National Draeger Inc.) through a stainless steel soil-gas sampling probe used in an earlier study (Kerfoot and Barrows 1987). Detector tubes (Draeger) with a detection range of 0.01 to 0.3 percent by volume and a precision of 10 to 15 percent relative standard deviation for a 1000 cm^3 sample (Draeger 1976) were used for soil-gas carbon dioxide (CO_2) measurements. A similar measurement approach has been used for soil-gas CO_2 in a study of soil-gas CO_2 concentration in forest soils (Fernandez and Kosian 1987).

Procedure

Ground water samples were collected from five monitoring wells in the area of the benzene and chlorobenzene contamination (well numbers 641, 643, 645, 647, 649) and from one well outside that area (well number 631). Ground water samples were pumped directly into 40mL vials and stored at approximately 4 C until analysis. Ground water samples were analyzed for total inorganic carbon and total organic carbon using an EPA procedure (Kerfoot et al. in press). Gas chromatography/mass spectrometry was performed according to EPA Method 624 (U.S. EPA 1982). Table 1 shows the benzene and chlorobenzene concentrations at the site. Calculations of carbon dioxide equilibrium concentrations due to carbonate species were performed with equilibrium constants for an ionic strength of 0.7 molal. Standard equations were used to calculate CO_2 partial pressures with $pK_H = 1.54$, $pK_{a1} = 5.86$, $pK_{a2} = 8.95$, $pK_w = 13.20$ (Butler 1982).

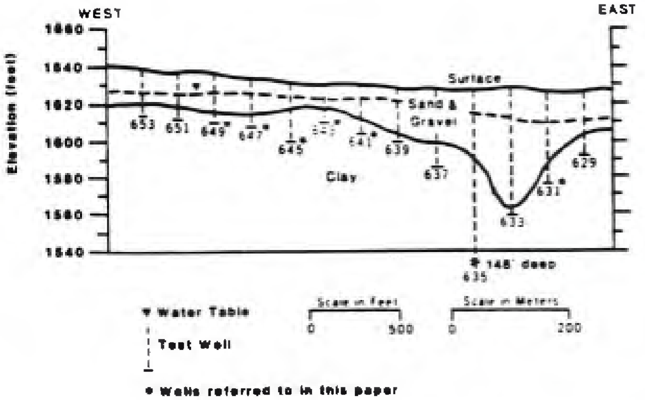


Figure 1. Subsurface hydrogeology and well locations at the study site.

TABLE 1
Benzene and Chlorobenzene Concentrations[a]

Well No.	Benzene ($\mu g/L$)	Chlorobenzene ($\mu g/L$)
631	n.d.[b]	n.d.[b]
641	2550	4520
643	3400	2400
645	3820	5060
647	3500	3600
649	1300	2400

[a]Source: Kerfoot and Barrows 1987
[b]Below detection limit of 10μg per liter

Samples for pH measurement were pumped into beakers and analyzed immediately after collection. For each sample, the pH electrode was allowed to equilibrate, and the value recorded was that obtained when two successive timed readings of freshly pumped sample were identical. Values of pH listed are temperature compensated for the ground water temperatures, which ranged from 22.2 C to 23.7 C.

Soil-gas carbon dioxide measurements were taken at locations 6.4 meters north of the wells. Sampling probes with a void volume of 15 cm^3 were hammered into the ground to a depth of 1.6 meters and the manual air sampling pump was attached. One hundred (100) cm^3 of gas was drawn through the sampling probe to purge it (Kerfoot 1987), a rubber nipple and detector tube attached, and 1000 cm^3 (at location numbers 631, 649) or 800 cm^3 (at location numbers 641, 643, 645, 647) of soil gas was drawn through the detector tube. The use of the smaller volumes was due to higher than anticipated CO_2 concentrations. Carbon dioxide concentrations were obtained by comparing the length of color change in the tube to a scale on the tube. The combined purge and sample volumes (900 to 1100 cm^3) correspond to the volume of soil gas present in a sphere of radius approximately 13cm or less for 10 percent air-filled porosity of the soil. This number provides a rough estimate of the magnitude of perturbations of the soil gases caused by sampling. Adjacent to one well (645), samples were taken through two probes 1 meter apart to obtain an estimate of the precision of the sampling/analysis system, including effects of local geologic variability. Two samples were taken through one of those two probes to evaluate the within-probe variability of results.

Results and Discussion

Table 2 lists the depth to ground water, pH, organic carbon, and inorganic carbon concentrations, along with the measured soil-gas carbon dioxide concentrations and the carbon dioxide concentrations calculated from the ground water inorganic carbon and pH values. Linear regression of the soil-gas carbon dioxide concentrations on the ground water organic carbon concentrations results in a correlation coefficient (r) of 0.81. This indicates a linear soil-gas correlation above 95 percent statistical significance for 6 data points (McGhee 1985). Carbon dioxide concentrations of two samples from the same probe (645A) were 0.35 percent and 0.37 percent, while the result 1 meter away (645B) was 0.35 percent.

Assuming the data from near well 631 represent background ground water inorganic carbon and soil-gas CO_2 concentrations, increased soil-gas carbon dioxide correlates well with organic contamination of ground water at this site. There is a general correlation between ground water inorganic carbon and organic carbon concentrations ($r=0.98$, $n=6$). This finding is in agreement with several observations of increased ground water inorganic carbon concentrations due to subsurface oxidation of organic material (Bishop et al. 1966, Haas et al. 1983, Krauskopf 1967, Matthess 1982). Carbonate minerals in the aquifer are not the source of variations in the measured carbon dioxide concentrations (Krauskopf 1967) because the aquifer materials are consistent throughout the sampling locations (Soil Conservation Service 1985) and a mineral source of dissolved inorganic carbon would result in a marked rise in pH along with dissolved organic carbon, which is not the case (see Table 2). The fact that the pH at the well with the lowest dissolved inorganic carbon concentrations (631) is higher than at the well with the highest dissolved inorganic carbon concentration (645) is evidence for a source of inorganic carbon other than carbonate minerals.

A number of factors could have contributed variability to the observed correlations. Because we did not perform a total characterization of the organic ground water contaminants present, it is possible that varying contributions to the measured organic carbon ground water concentrations occurred from organic compounds that are not susceptible to subsurface oxidation. Also, soil-gas samples were taken at a constant depth below the ground surface and not at constant depths above the water table or at depths equal to a constant fraction of the vadose zone thickness. In addition, at the lower concentration locations the relative magnitude of the contributions of plant respiration to the soil-gas CO_2 concentrations could be substantial.

In earlier studies at this site, benzene and chlorobenzene were found to be absent from soil gases overlying

TABLE 2
Ground Water and Soil-Gas Data

	Ground Water				Soil-Gas	
Well[a]	Depth to GW (m)	pH	Organic Carbon (mg/L)	Inorganic Carbon (mg/L)	Measured Soil-Gas CO_2 (%)	Calculated Equilibrium CO_2 (%)[b]
631	5.5	7.10	2.52	40.8	0.09	0.6
641	2.4	6.95	13.02	76.0	0.45	1.6
643	2.2	7.08	12.27	73.2	0.32	1.2
645A[c]	2.8	7.02	14.79	76.8	0.36[d]	1.4
645B[c]	2.8	7.02	14.79	76.8	0.35	1.4
647	2.7	6.98	12.54	73.6	0.41	1.5
649	2.9	7.20	9.20	57.9	0.10	0.7

[a] Well numbers indicate position on east-west ordinate in 32-meter (100 foot) increments

[b] Calculated from dissolved inorganic carbon and pH measurements for ionic strength of 0.7 molal

[c] Probes separated by 1 meter

[d] Mean of duplicate samples of 0.35% and 0.37%

high concentrations of these compounds in the ground water (Kerfoot and Barrows 1987, Kerfoot and Mayer 1987). Both benzene and chlorobenzene have been shown to undergo aerobic biooxidation in laboratory simulations of aquifer conditions (Bishop et al. 1966, Jamison et al. 1976, Kobayashi and Rittman 1982). Although the data obtained from the study described here do not prove a relationship between ground water dissolved organic carbon and soil-gas carbon dioxide concentrations, they show a correlation at this site that is consistent with subsurface hydrocarbon oxidation.

Because rapid biooxidation of organic compounds has been observed for very low volatility compounds (Wilson et al., 1985) it is anticipated that this technique may be of value for application in cases where conventional soil-gas surveying can be of only limited utility due to low volatility of contaminants (e.g., Jet A fuel, diesel fuel, heating oils) and their tendency to undergo subsurface oxidation. Carbon dioxide monitoring could also be used as a quality control procedure to detect possible false negatives obtained from routine soil-gas surveying for volatile organic compounds prone to subsurface oxidation. In addition, the technique may serve as a means for differentiating between non-aqueous petroleum liquids contamination having very limited oxygen or water supply and thus limited CO_2 production, and dissolved fuel contamination where the availability of water allows CO_2 production from dissolved hydrocarbons. Further development of the technology to test its applicability under different geohydrologic and meteorologic conditions and for different contaminants and contaminant concentrations is required before it can be applied generally.

Notice

Although the research described in this article has been supported by the United States Environmental Protection Agency under contract 68-03-3245 to LEMSCO, it has not been subjected to Agency review and therefore does not necessarily reflect the views of the Agency and no official endorsement should be inferred.

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Biographical Sketches

Henry B. Kerfoot is a project manager in the Advanced Monitoring Systems Department of Lockheed Engineering and Management Services Co. Inc. (1050 E. Flamingo Rd., Suite 120, Las Vegas, Nevada 89119). He holds degrees in chemistry from the Johns Hopkins University and Florida State University. His current research interests include vadose zone techniques for detection of subsurface contamination, indirect indicators of ground water contamination, and soil-gas analysis for process control in cleanups.

Cynthia L. Mayer is a scientist in the Advanced Monitoring Systems Department of Lockheed Engineering and Management Services Co. Inc. (1050 E. Flamingo Rd., Las Vegas, Nevada 89119). She received a B.A. in molecular biology and an M.S. in engineering science from the University of California, Berkeley. She is involved in research on soil-gas surveying techniques.

Philip B. Durgin is a hydrogeologist in the Advanced Monitoring Systems Division of U.S. EPA's Environmental Monitoring Systems Laboratory (Las Vegas, Nevada 89114). He has received a Ph.D. in geology from the University of Idaho and is responsible for directing EPA's research in soil gas and external monitoring of underground storage tanks.

Joseph J. D'Lugosz is chief of the aquatic and Subsurface Monitoring Branch of U.S. EPA's Environmental Monitoring Systems Laboratory (Las Vegas, Nevada 89114). He has received an M.S. in geology from Texas Tech and directs research on several aspects of hydrologic monitoring.

PRESENTATION BY DR. GEORGE PICKWELL

NOSC

ENVIRONMENTAL SCIENCES DIVISION
CHEMISTRY/BIOCHEMISTRY BRANCH
CODE 521

ENVIRONMENTAL BIOTECHNOLOGY

MARINE BIOMONITORING

BIOFIX (BIOlogical Field Investigations of toXicity) * * * * * * *

- Lysozymes - acute response to toxic stress (e.g. from heavy metal ions such as Cu^{2+})
- Metal-binding Proteins - chronic/acute accomodation to heavy metal stress (e.g. elevated thioneins)
- Mixed Function Oxygenase, Cytochromes P-450 - elevated activity in response to organic xenobiotics
- Heat Shock Proteins - general acute toxic stress (e.g. hsp induction, protein synthesis shuts down)
- ATP-ase, ATP Synthetase Complex - acute/chronic respiratory distress (e.g. system is interdicted by TBT)

ENVIRONMENTAL BIOTECHNOLOGY

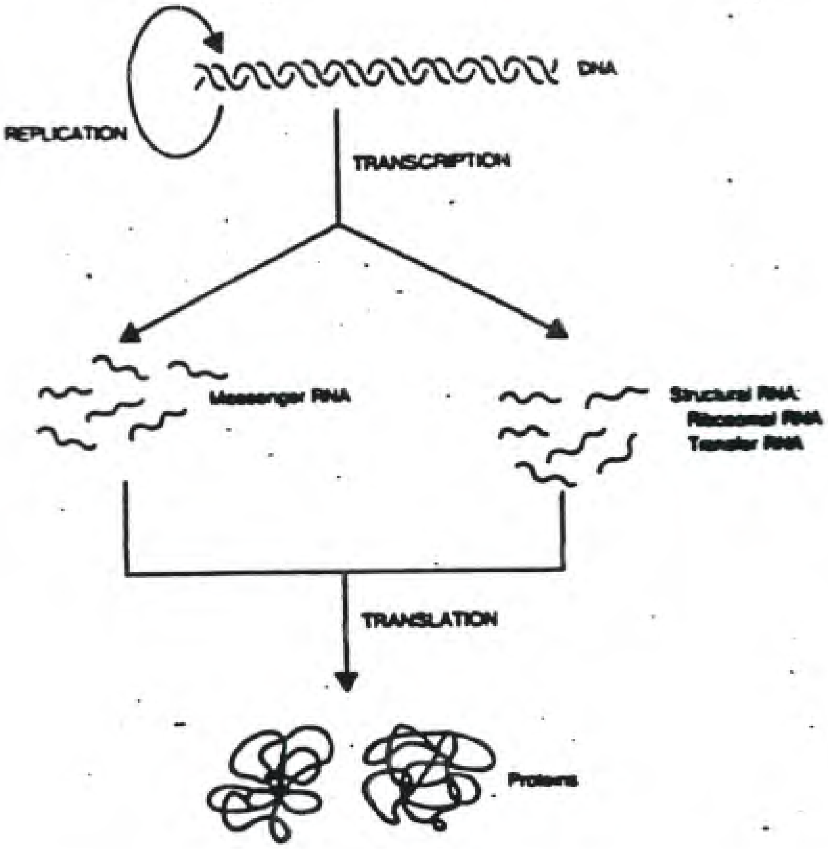
BIOREACTOR SYSTEMS

Project	Metal Recovery Bioreactors	Portable Bioreactor for Hazardous Waste Detoxification	Site-Specific Bioreactors for Hazardous Waste Detoxification
Main Objective	Recovery of Toxic and Strategic Metals	Optimized Detox of Refractory Toxicants Using Microbes	Optimized On-Site Detox using Native Microbes
Approach	Use Metal-Binding Proteins Produced by Genetic Engineering	Use Lab Cultured Microbes Engineered or Mutated to Break Down Specific Toxicants	Find and Stimulate Native Degradative Microbes On-Site

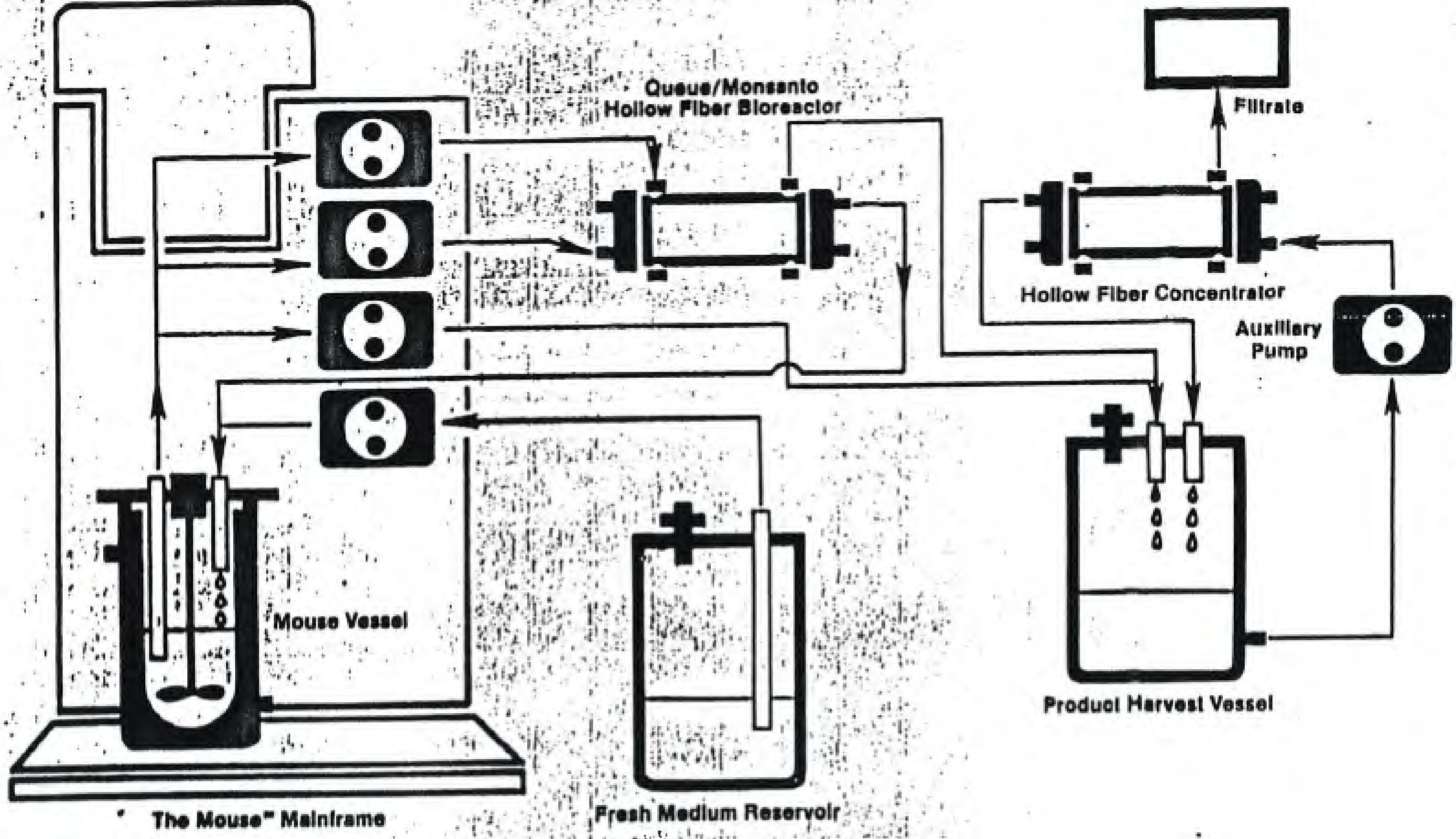
Metal Ions Known to be Bound by Metallothioneins (partial list)

MT Source	# of Metal Atoms Bound / Molecule MT	Metals Bound
Vertebrates	7	Bi(III), Cd(II), Co(II), Hg(II), In(III), Ni(II), Pb(II), Pt(II), Sb(III), & Zn(II)
Invertebrates	6	
Vertebrates	?	Au(I & III), Cu(II), Hg(I), Ir(III), Os(III), Pd(II), Pt(IV), Re(V), Rh(III), Ru(III), Sn(II), & Tl(III)
Vertebrates	12	Ag(I) & Cu(I)

? Stoichiometry not known.


REPLICATION
DNA
TRANSCRIPTION
Messenger RNA
Structural RNA:
Ribosomal RNA
Transfer RNA
TRANSLATION
Proteins

[illegible]



System With Mouse
Automated continuous production and concentration of product utilizing the Queue Mouse and Queue/ Monsanto hollow fiber bioreactor and ancillary hollow fiber concentration equipment.

TECHNICAL OBJECTIVES-

-Facilitate breakdown/disposal of Navy HW.- emphasis on refractory materials.

-Implement breakdown in bioreactors established as portable systems or permanent on-site systems where HW discharge is continuous.

OPERATIONAL PROBLEM-

-Hazardous Waste (HW) accumulation/discharge at Navy sites e.g. near shore, harbor, estuarine.

-Very slow to zero breakdown of some important Navy-specific HW, e.g. organotins, otto fuels.

-Possible environmental litigation resulting from HW accumulation/discharge.

-Fleet operational readiness receives negative impact from actual or threatened court actions.

TECHNICAL APPROACH-

-Isolate and grow microbes capable of breaking down specific HW.

-Optimize growth conditions for most active microbes.

-Isolate and clone genes coding for HW breakdown enzymes.

-Produce large quanities of the active genes.

-"Engineer" multiplied genes back into suitable microbes for accelerated HW breakdown.

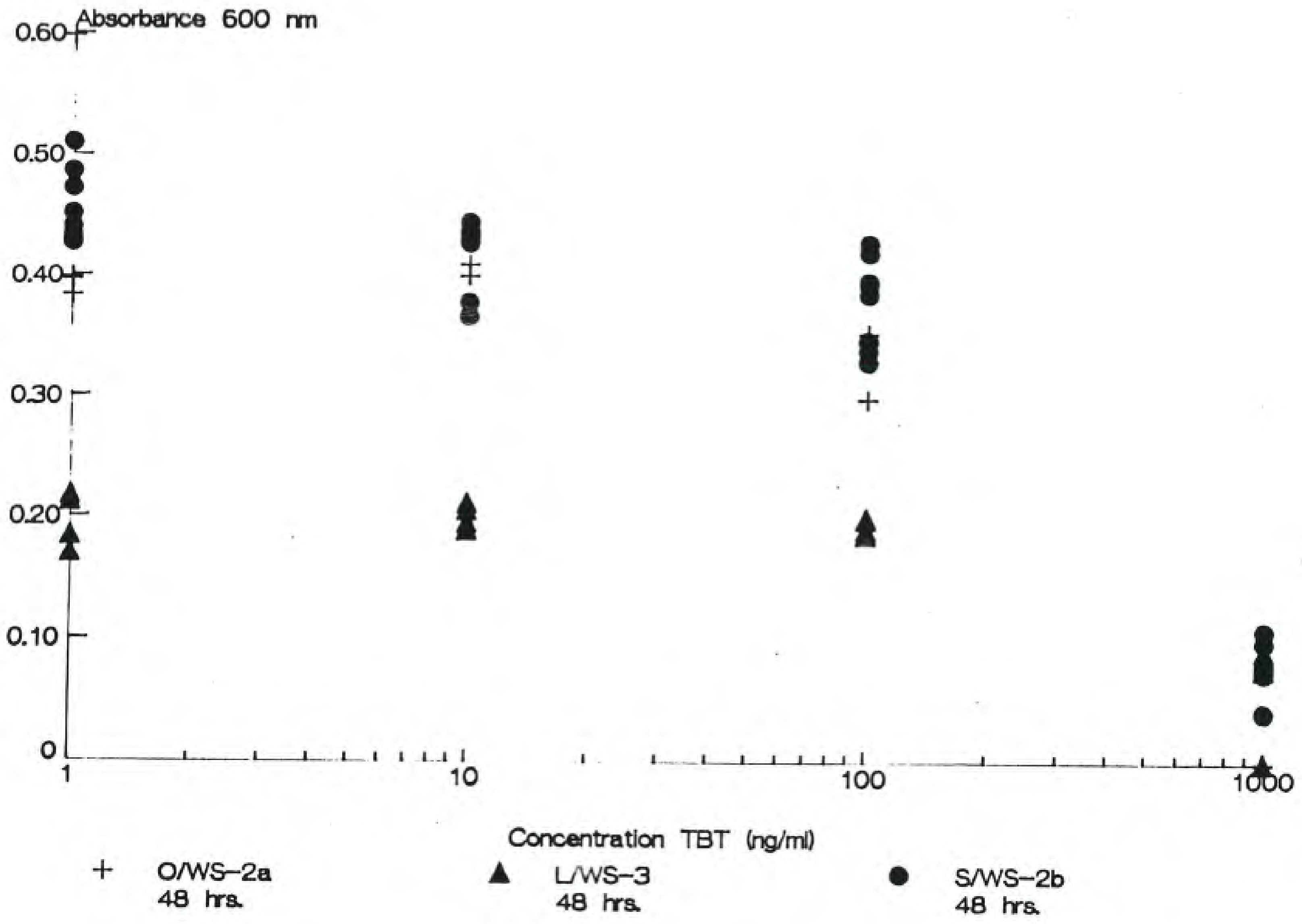
REQUIREMENTS FOR THIS TECHNICAL APPROACH

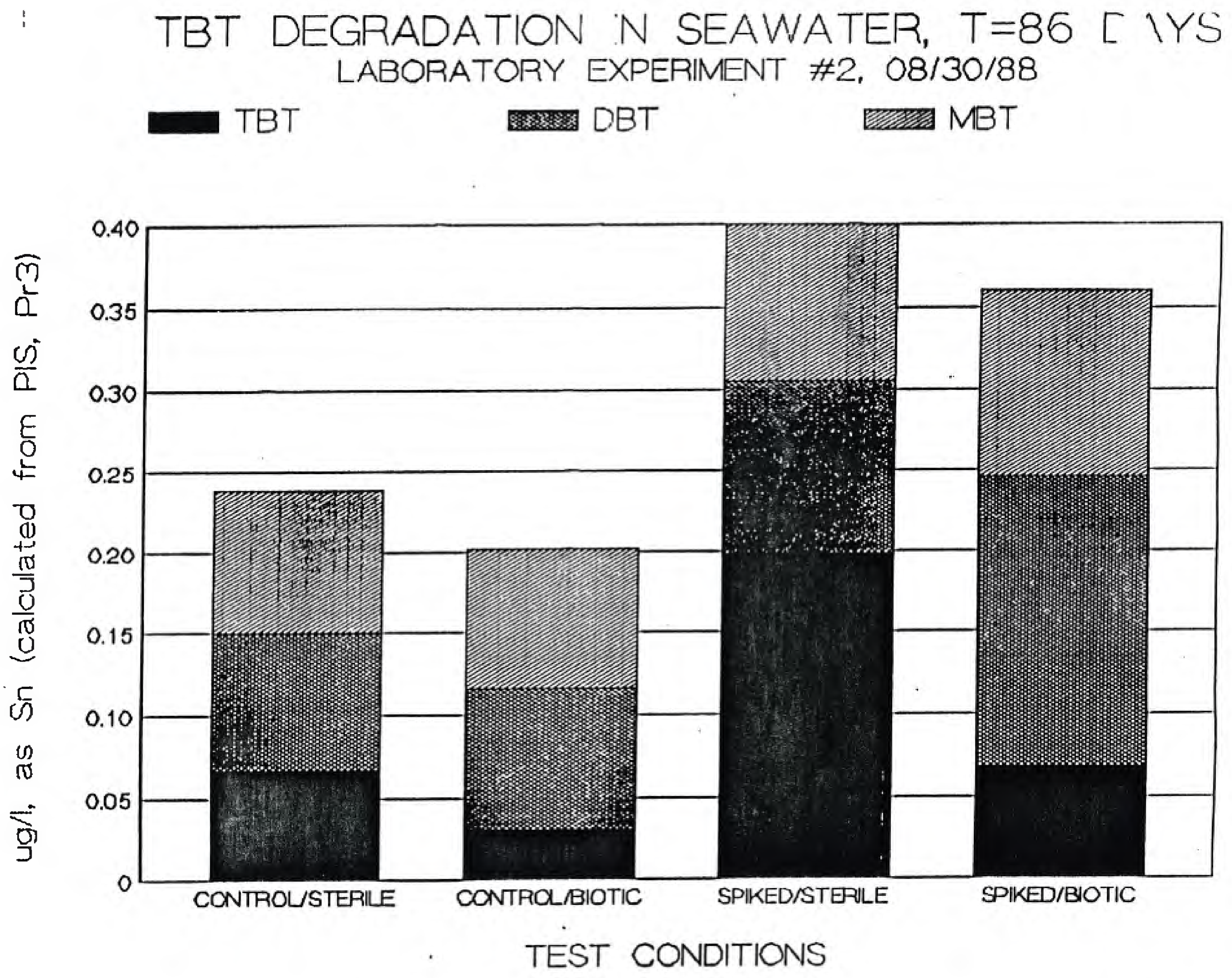
–Analytical Capabilities: Chemical (GC, GC–MS, FTIR)
Biochemical (2–D electrophoresis)

–Microbiological Capabilities: Cell culture of marine microbes

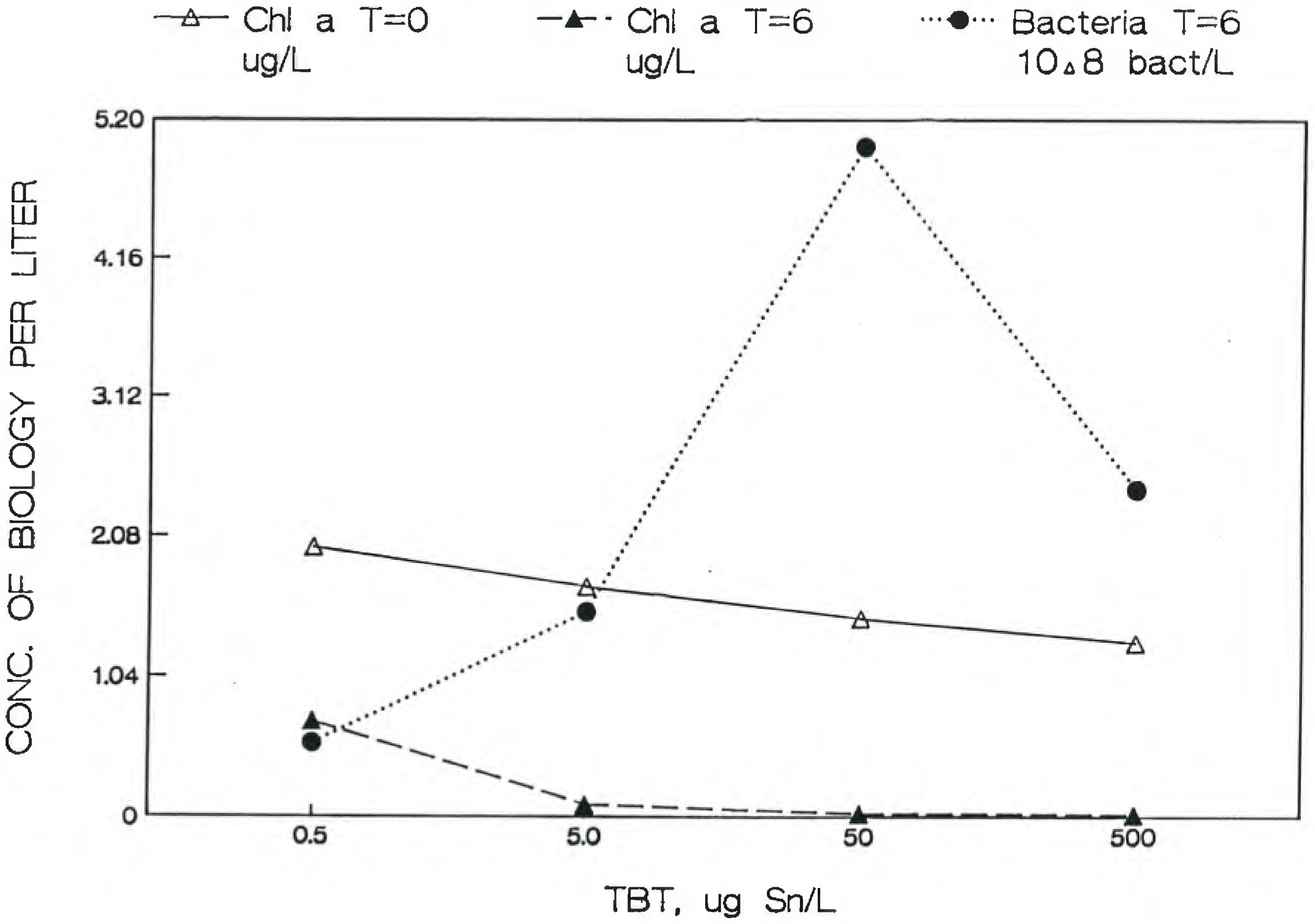
–Biotechnological Capabilities: Recombinant DNA methods.
Plasmid purification/amplification

Comparison of the Sensitivity of Strains
O, L , & S to TBT. (48 hrs. growth)

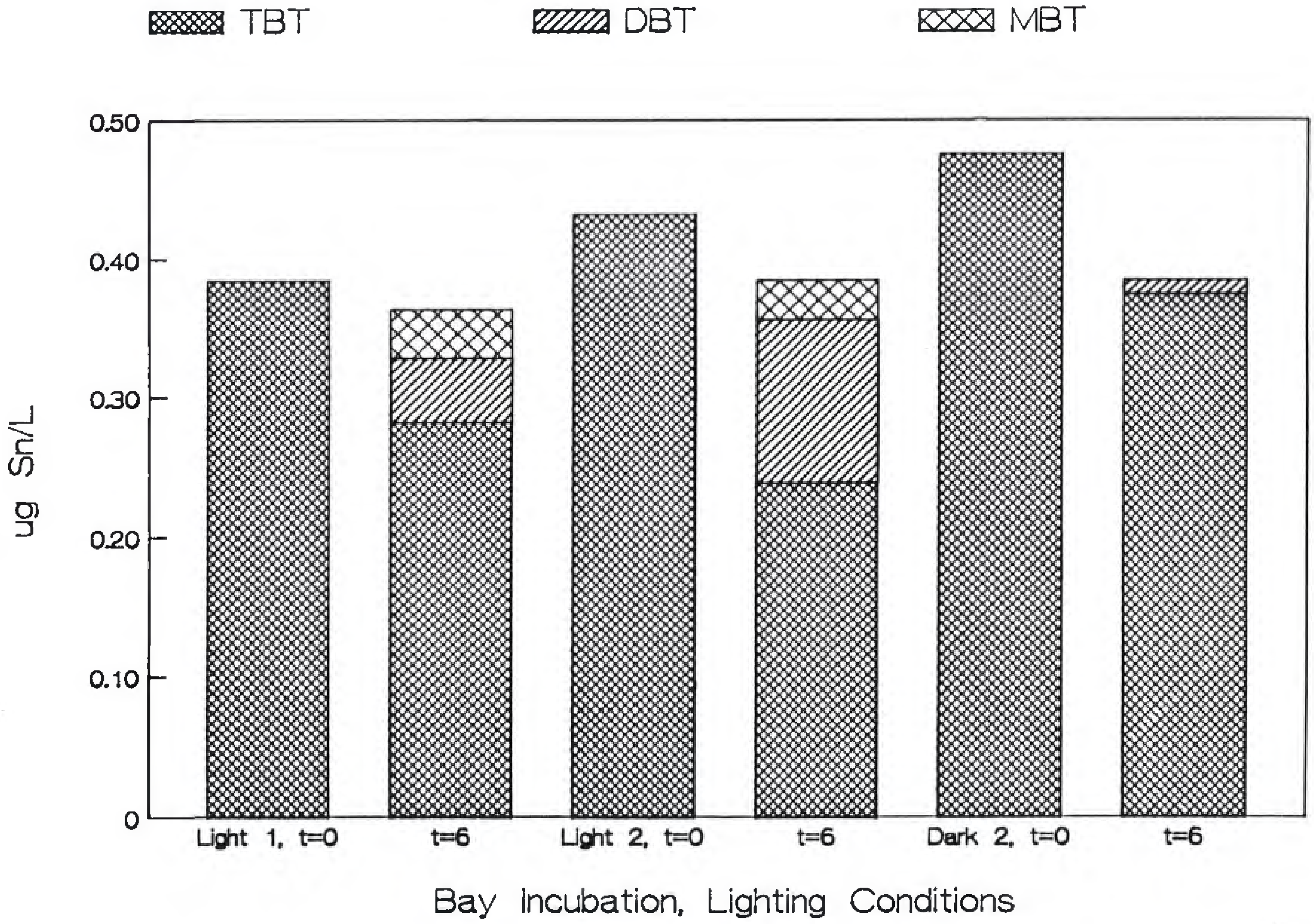


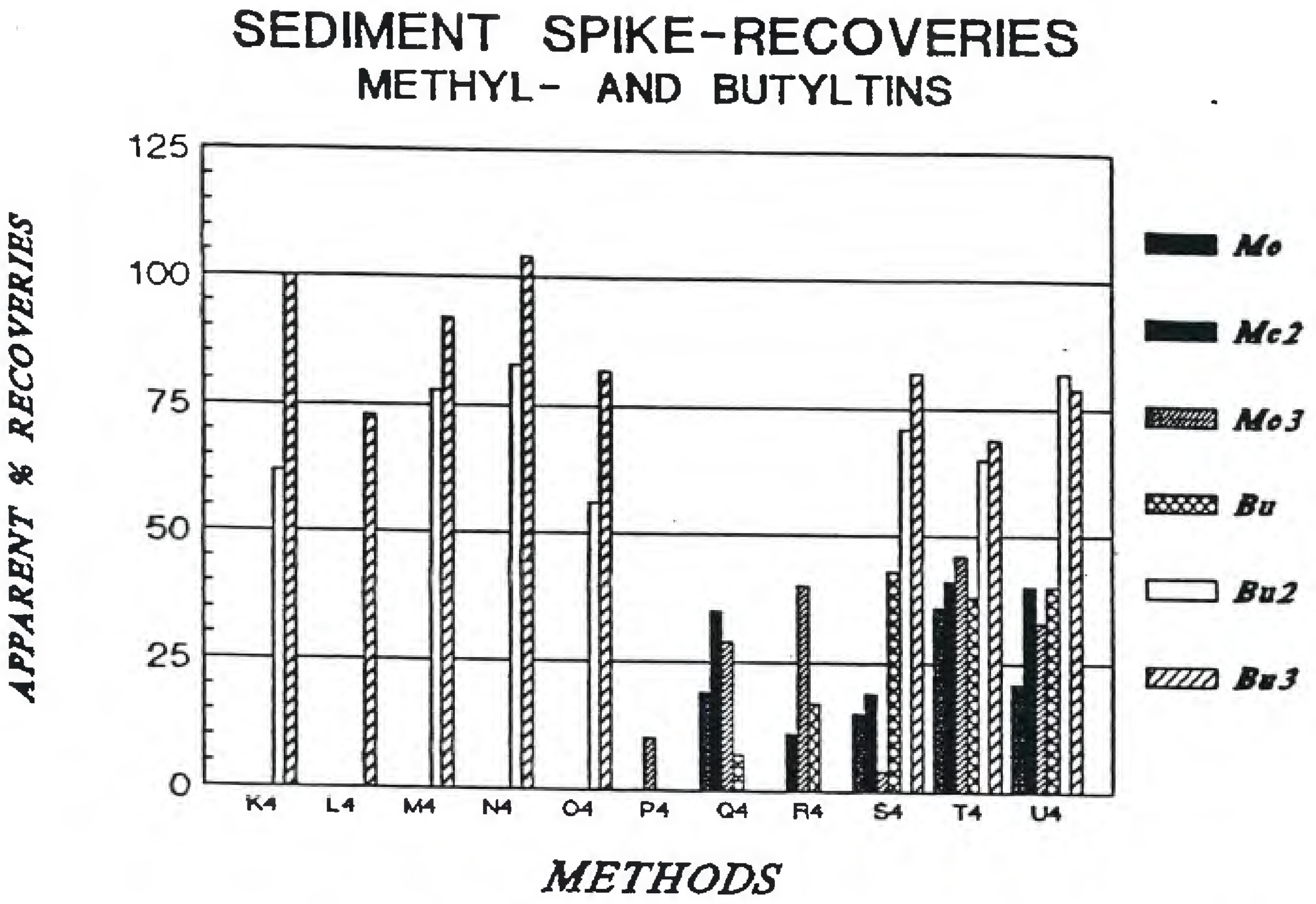

TBT DEGRADATION IN SEAWATER, T=86 DAYS
LABORATORY EXPERIMENT #2, 08/30/88
TBT
DBT
MBT
ug/l, as Sn (calculated from PIS, Pr3)
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0
CONTROL/STERILE
CONTROL/BIOTIC
SPIKED/STERILE
SPIKED/BIOTIC
TEST CONDITIONS

Effect of TBT on Chlorophyll a and Bacteria Levels in Natural Bay Water



Degradation of Labelled TBT in Light and Dark Conditions, in San Diego Bay Water





NOTE: Methods P4, Q4, and R4 did not include spikes of Bu_3Sn or Bu_2Sn, therefore there are no recovery data from these methods for these two compounds.

GRAPH LEGEND:

METHOD	ACID	SALT	SOLVENT SYSTEM
K4	50% Acetic	NaCl	MeOH/Hexane/Tropolone
L4	Conc. Acetic	NaCl	MeOH/Hexane/Tropolone
M4	50% Perchloric	NaCl	MeOH/Hexane/Tropolone
N4	Conc. Perchloric	NaCl	MeOH/Hexane/Tropolone
O4	None	NaCl	MeOH/Hexane/Tropolone
P4	20% Hydrochloric	CaCl2	MeOH/Hexane/Tropolone
Q4	50% Hydrochloric	NaCl	Ether/Tropolone
R4	50% Hydrochloric	NaCl	Toluene/Tropolone
S4	50% Hydrochloric	NaCl	Ether/Toluene/Tropolone
T4	50% Hydrochloric	NaCl	Ether/Toluene/Tropolone*
U4	50% Hydrochloric	NaCl	Hexane/Ether/Toluene/Tropolone*

* Successive extractions with these tropolone-containing solvents, as opposed to being mixed-solvent systems (as in methods K4 through S4).

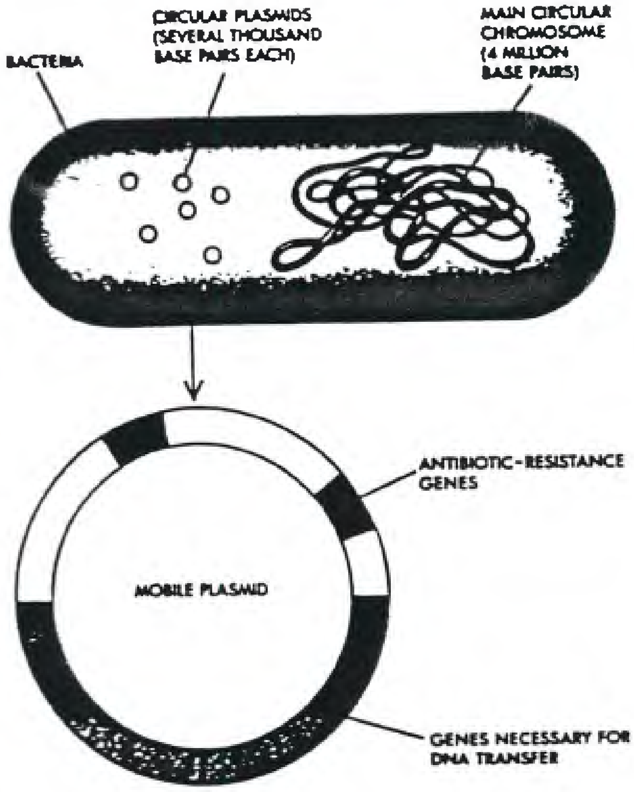
PLANS-

—Isolate O—tin metabolizing microbes.

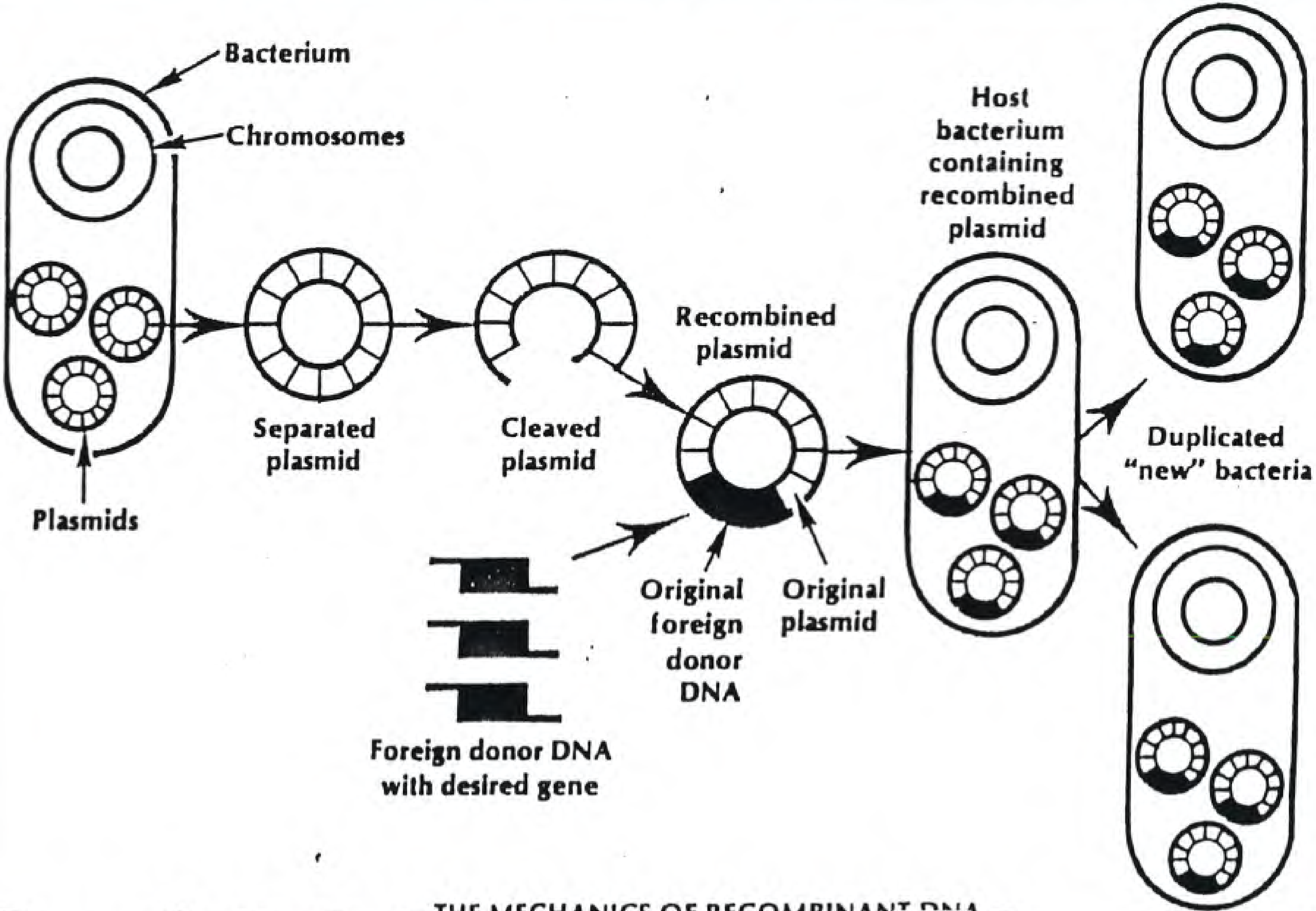
—Isolate plasmids from these microbes and locate site of gene (DNA) coding for O—tin metabolizing enzyme.

—Clone this enzyme's DNA (i.e. amplify gene copies).

—Place gene and plasmid back into a suitable microbial host and grow in large numbers for use in O—tin clean—up bioreactors.



Plasmids are small, autonomously replicating bacterial DNA molecules containing genes conveying resistance to specific antibiotics. Most plasmids are mobile, but nonmobilizable plasmids also exist.



THE MECHANICS OF RECOMBINANT DNA

SUMMARY -

1) Refractory HW build-up from Navy sources in coastal regions, harbors, and estuaries.

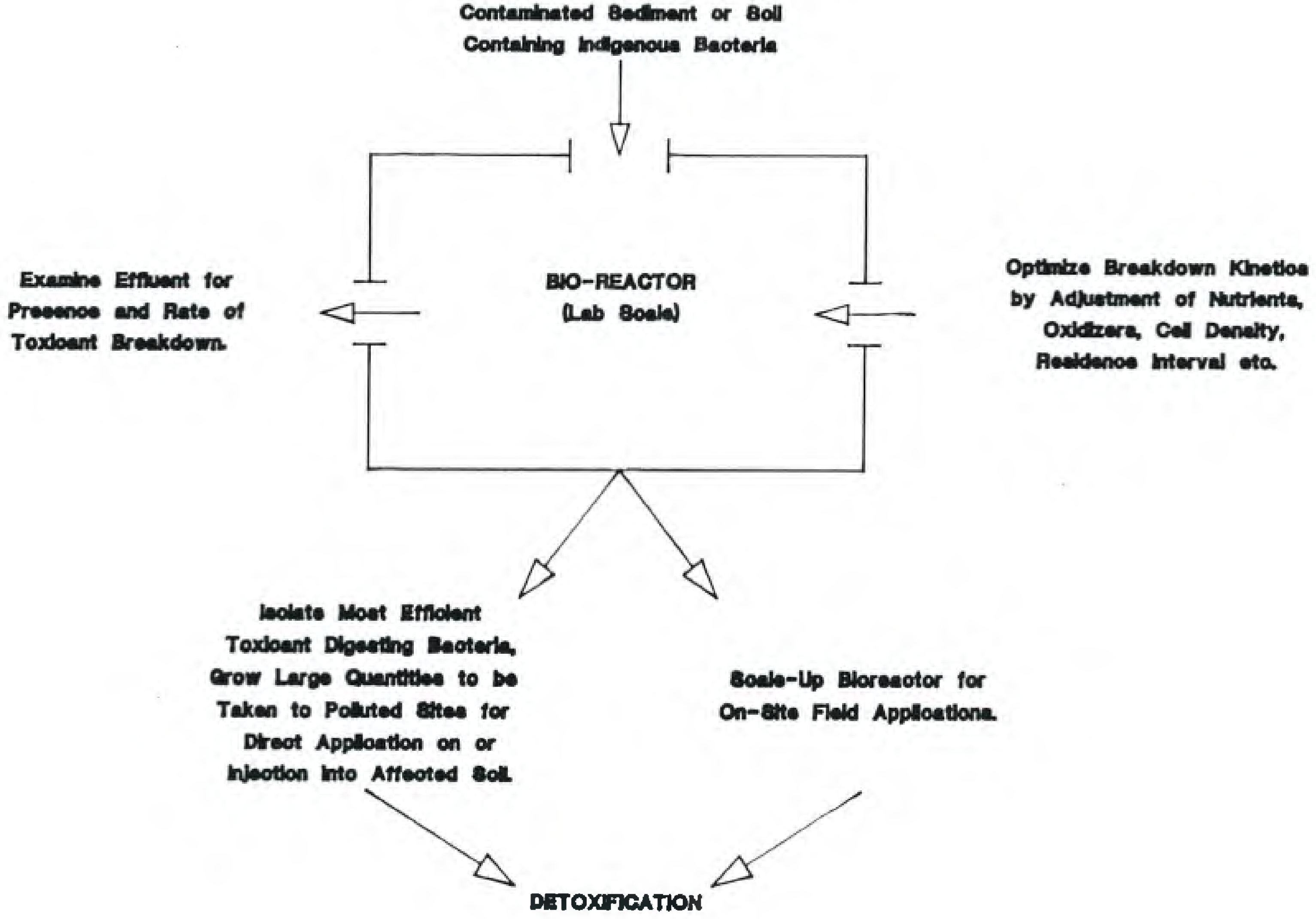
2) Fleet readiness impacted by real or potential threat of litigation may halt important activities connected with HW source.

3) Facilitate and expedite HW clean-up by use of bioreactors, either mobile or permanent.

4) Engineer and amplify microbes for detox of specific HWs.

5) Microbes retained in bioreactors thus avoiding environmental impact.

ENVIRONMENTAL BIOTECHNOLOGY



Contaminated Sediment or Soil
Containing Indigenous Bacteria

BIO-REACTOR
(Lab Scale)

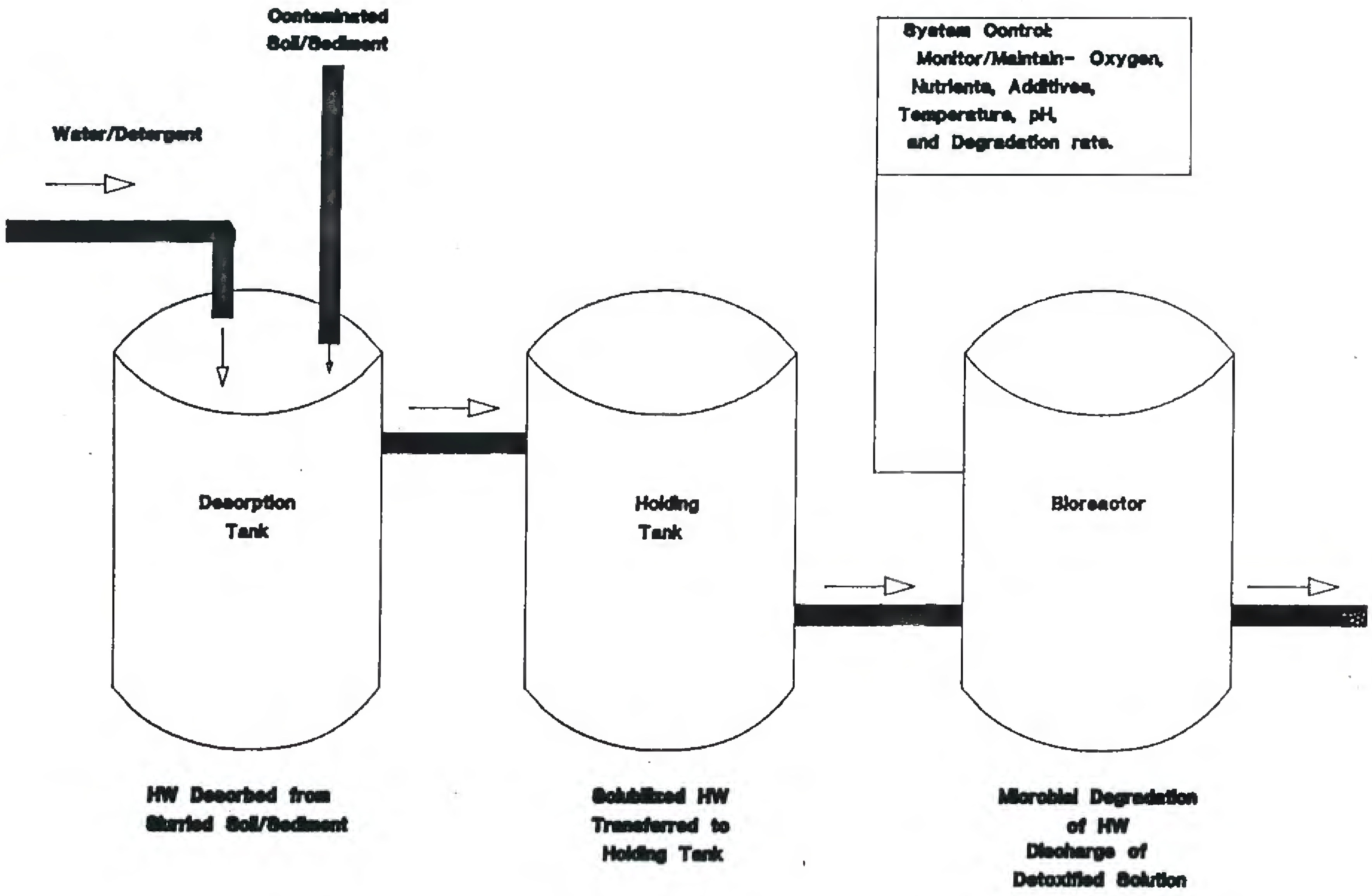
Examine Effluent for
Presence and Rate of
Toxicant Breakdown.

Optimize Breakdown Kinetics
by Adjustment of Nutrients,
Oxidizers, Cell Density,
Residence Interval etc.

Isolate Most Efficient
Toxicant Digesting Bacteria,
Grow Large Quantities to be
Taken to Polluted Sites for
Direct Application on or
Injection into Affected Soil.

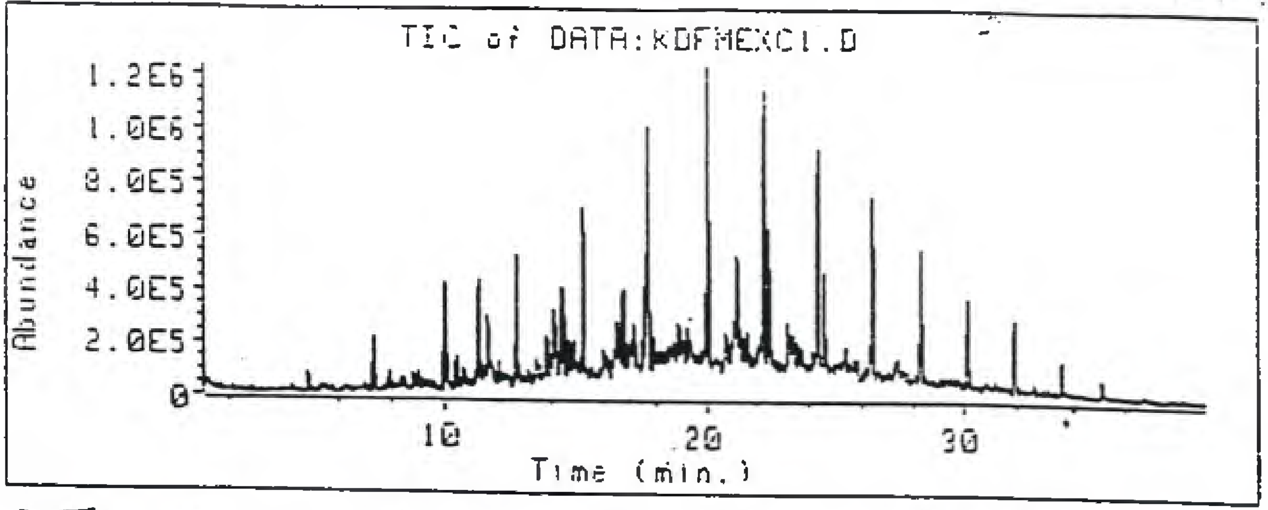
Scale-Up Bioreactor for
On-Site Field Applications.

DETOXIFICATION



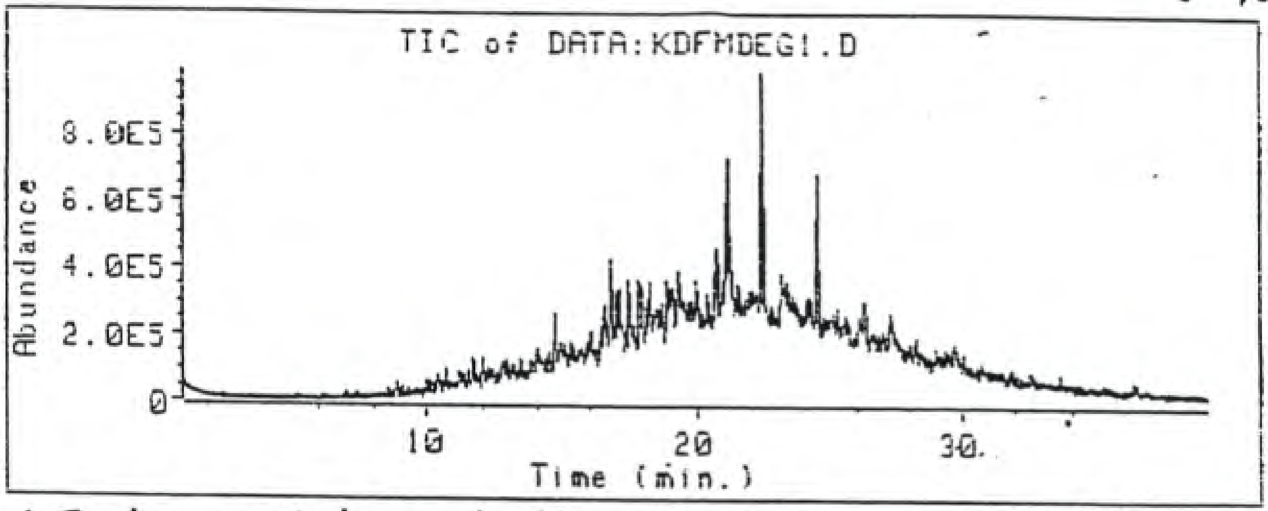
Hazardous Waste Decontamination System

8-12-92

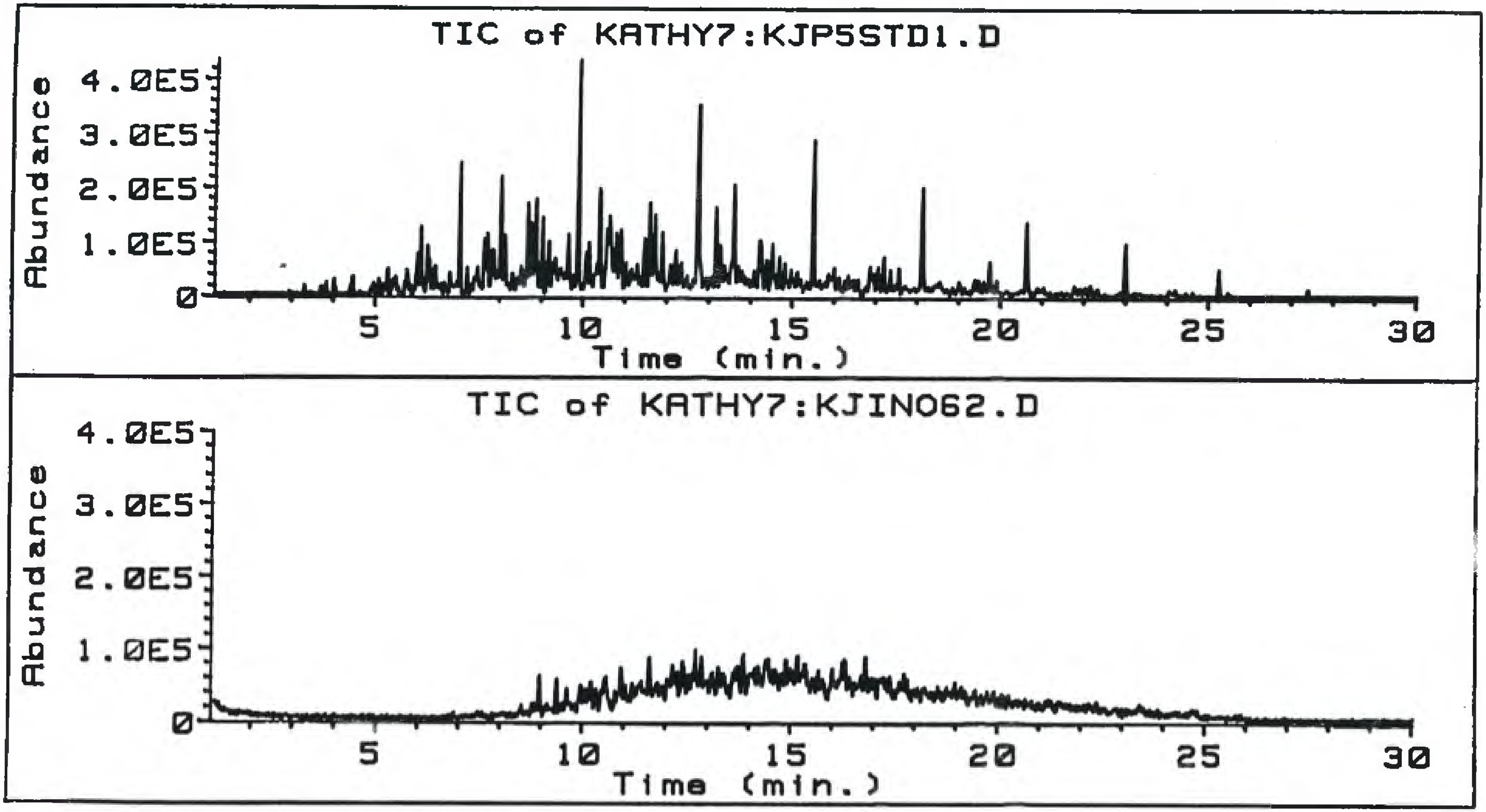


DFM extracted from SeaWater with hexane at T=0

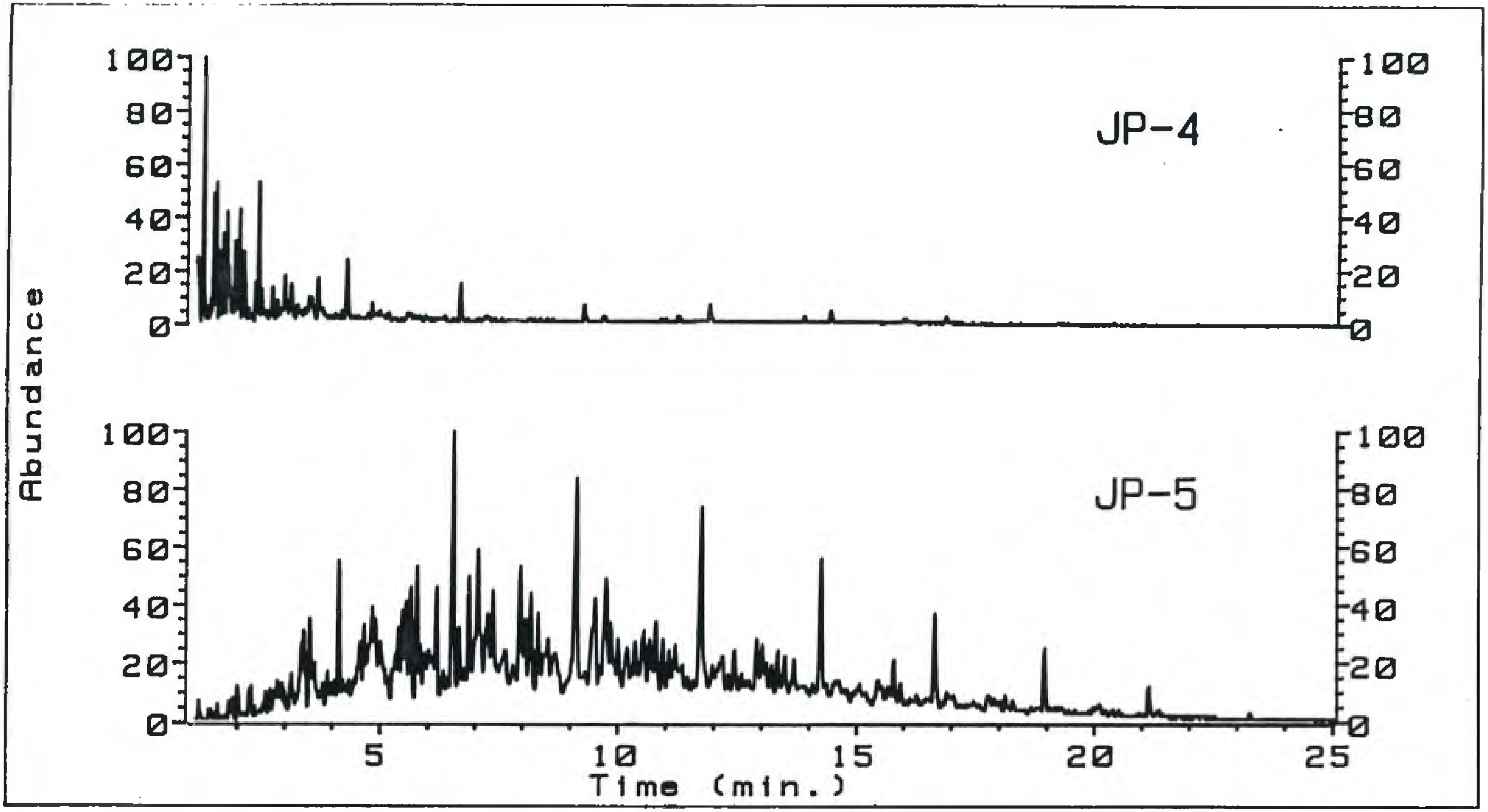
8-12-92



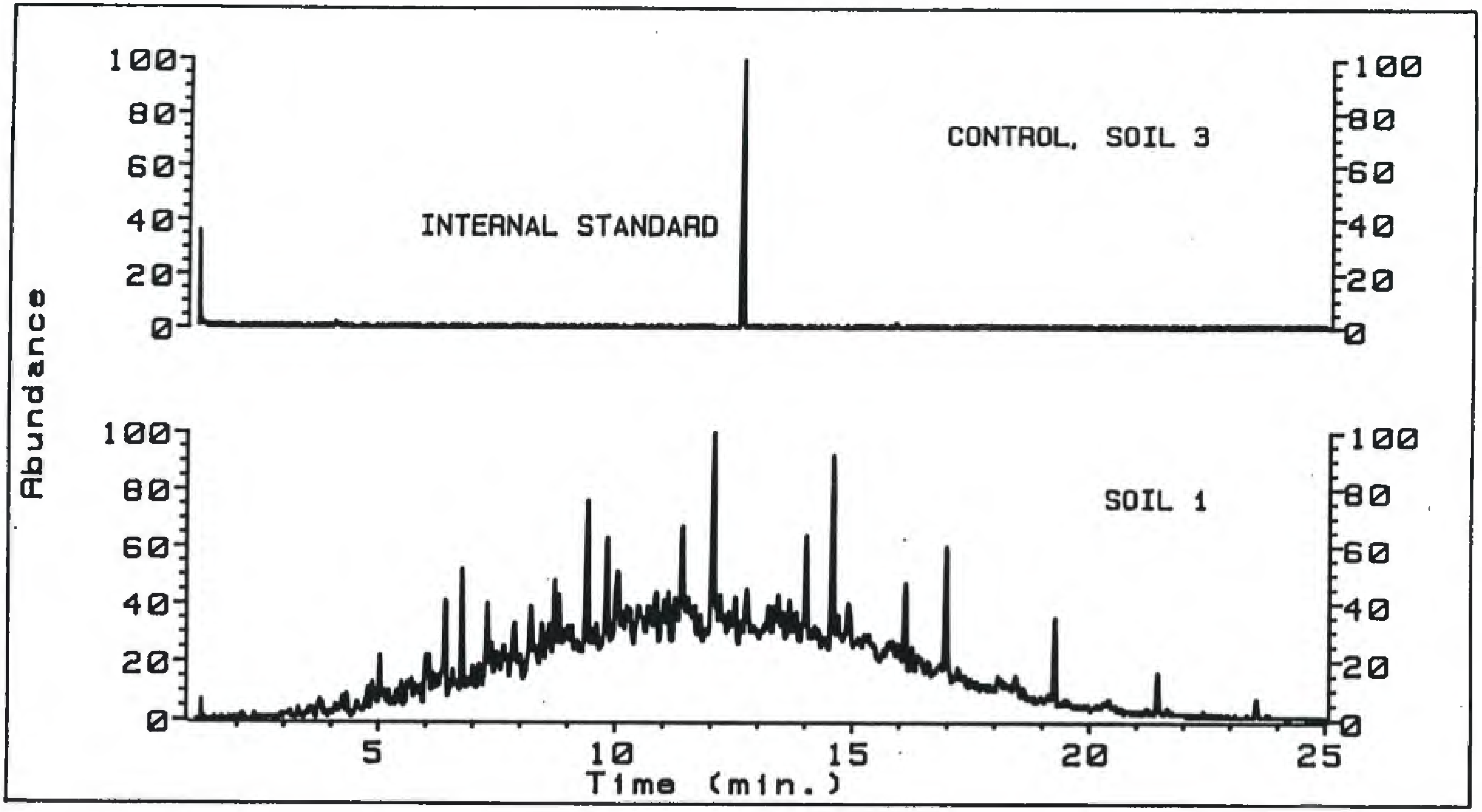
DFM 63 days of degradation in SW amended with NH_4NO_3 and KH_2PO_4



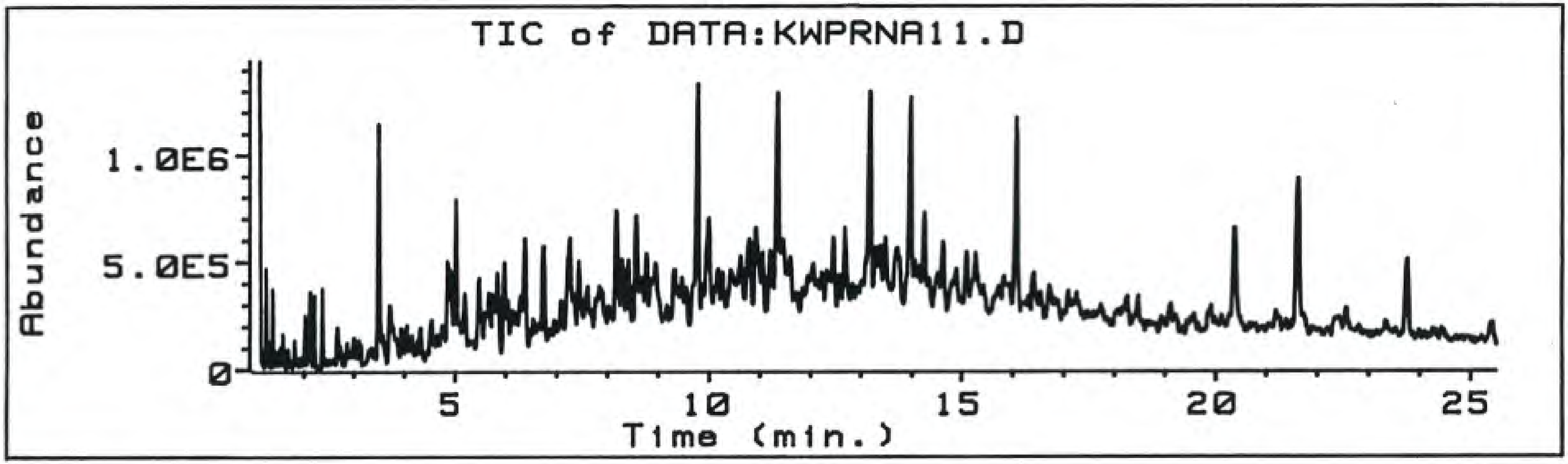
JP-5 DEGRADATION, T=0 AND T=40 DAYS



CHROMATOGRAMS OF JP-4 AND JP-5

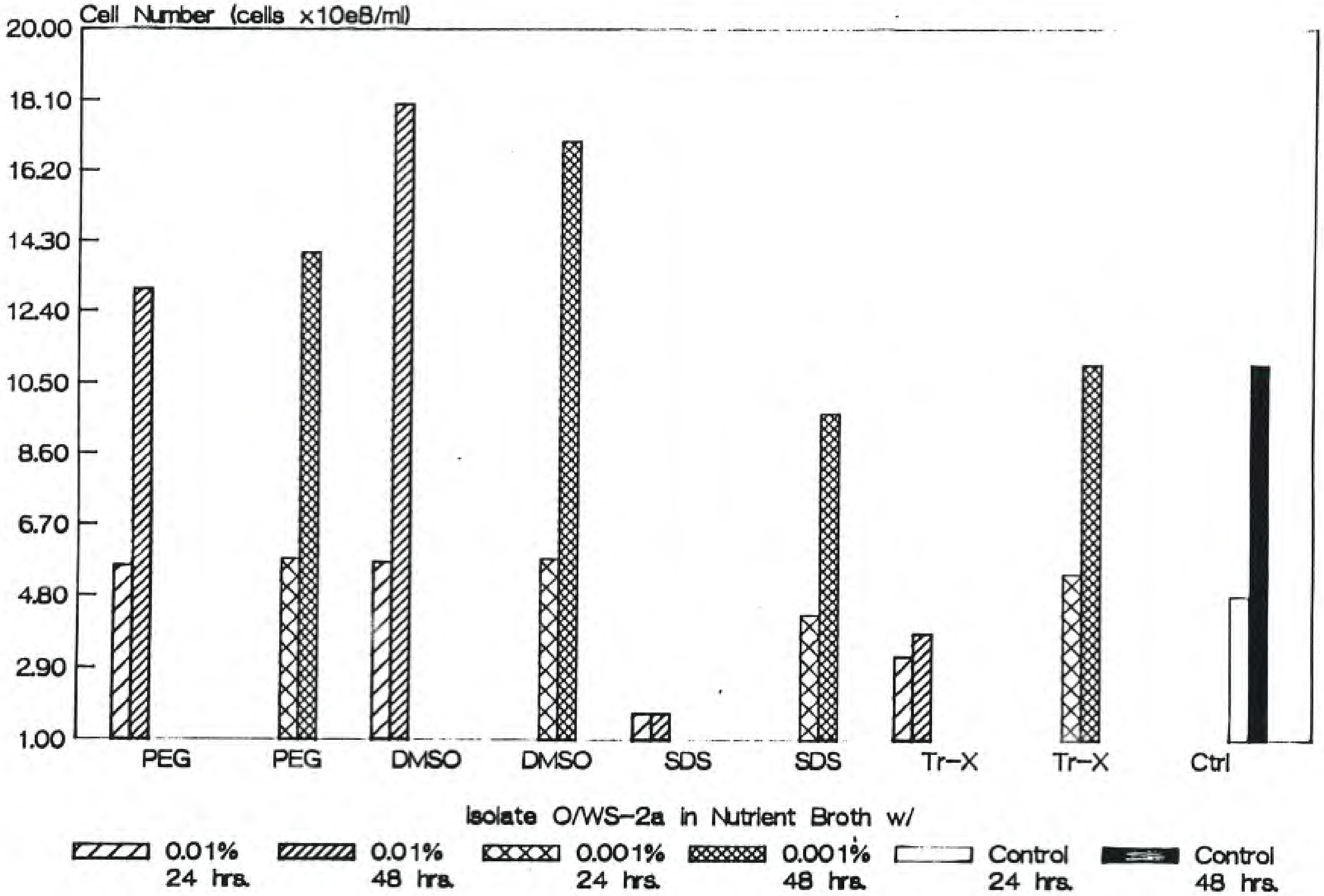


CHROMATOGRAMS OF EL CENTRO SOIL EXTRACTS

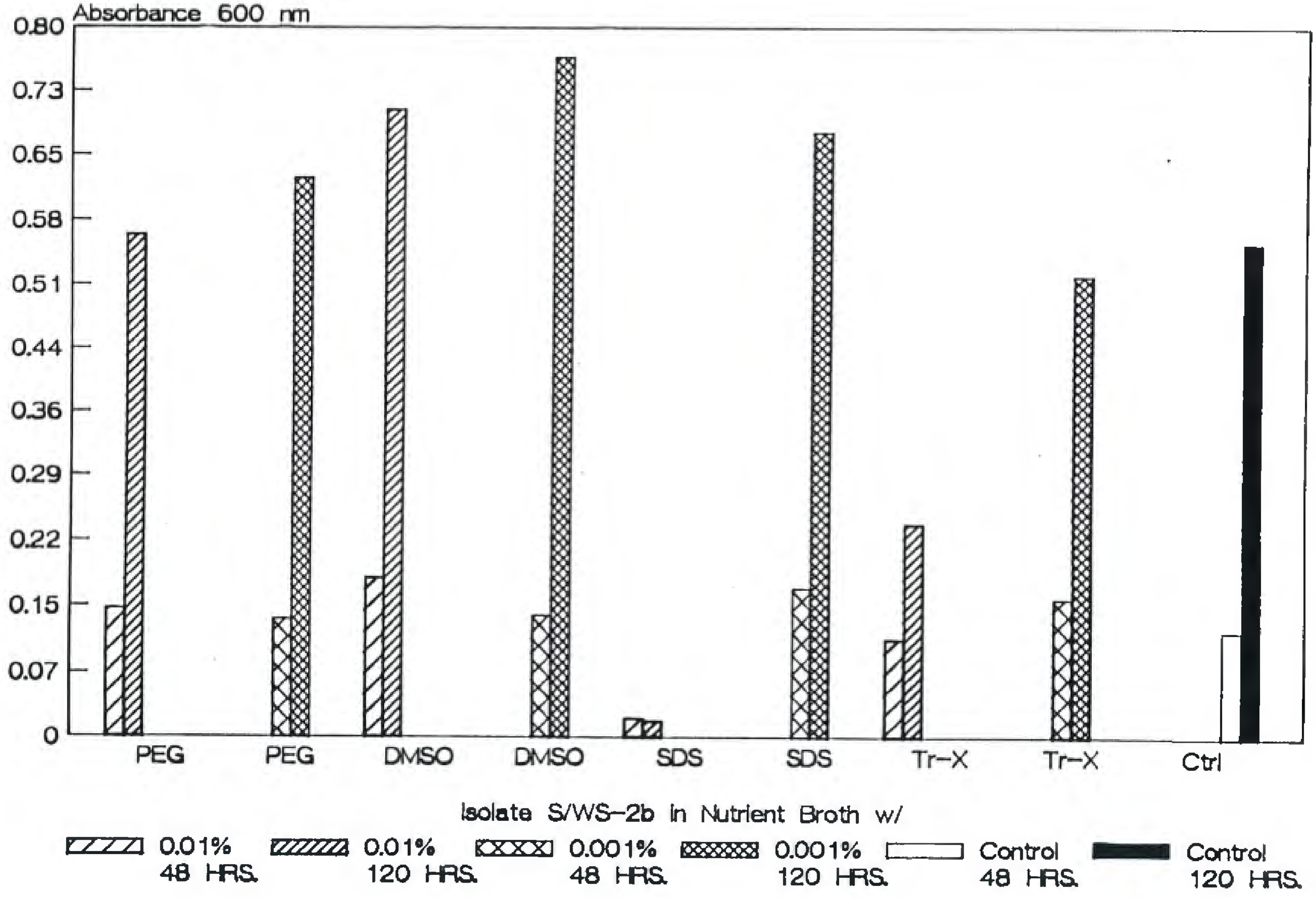


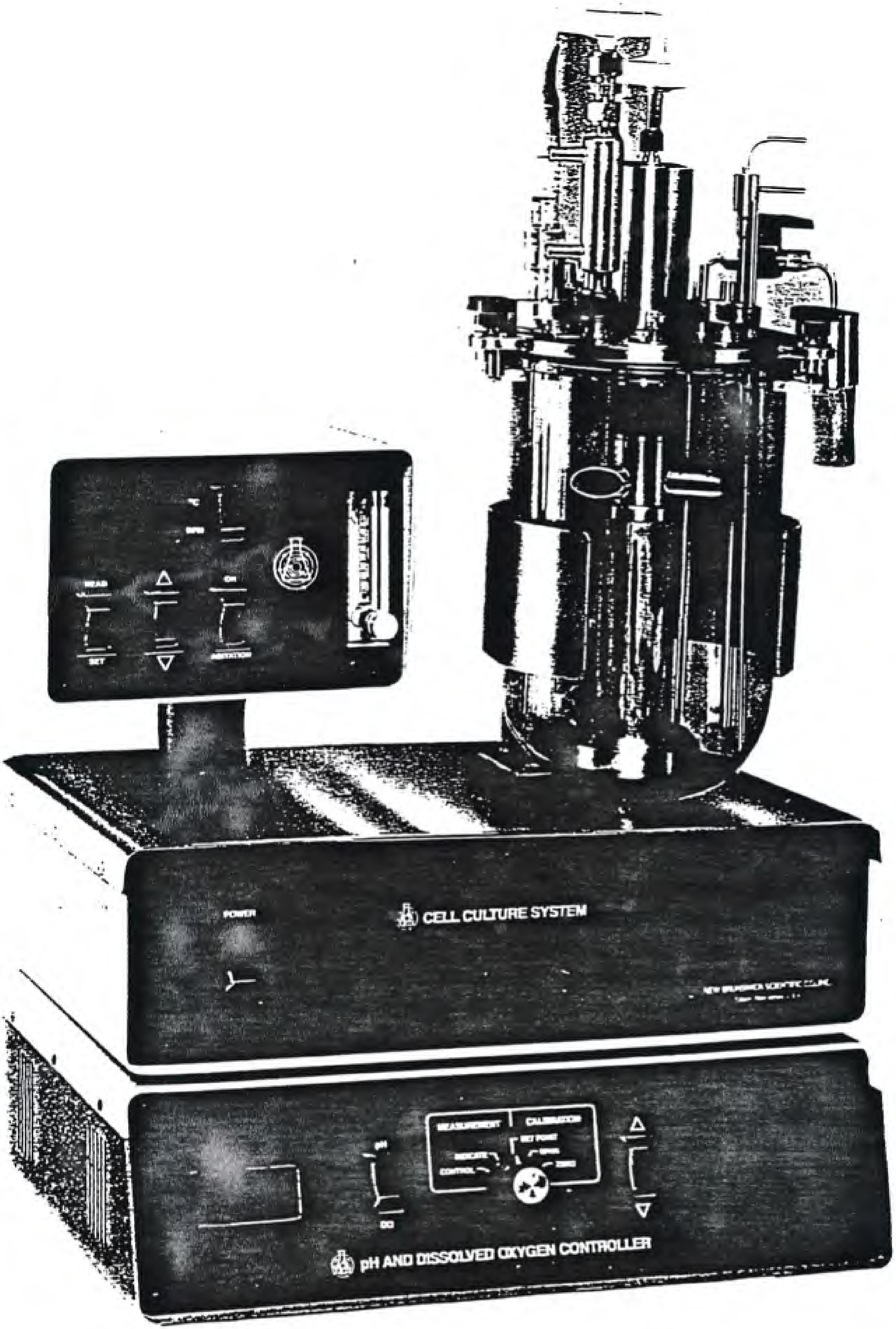
PATUXENT RIVER SEDIMENT EXTRACT

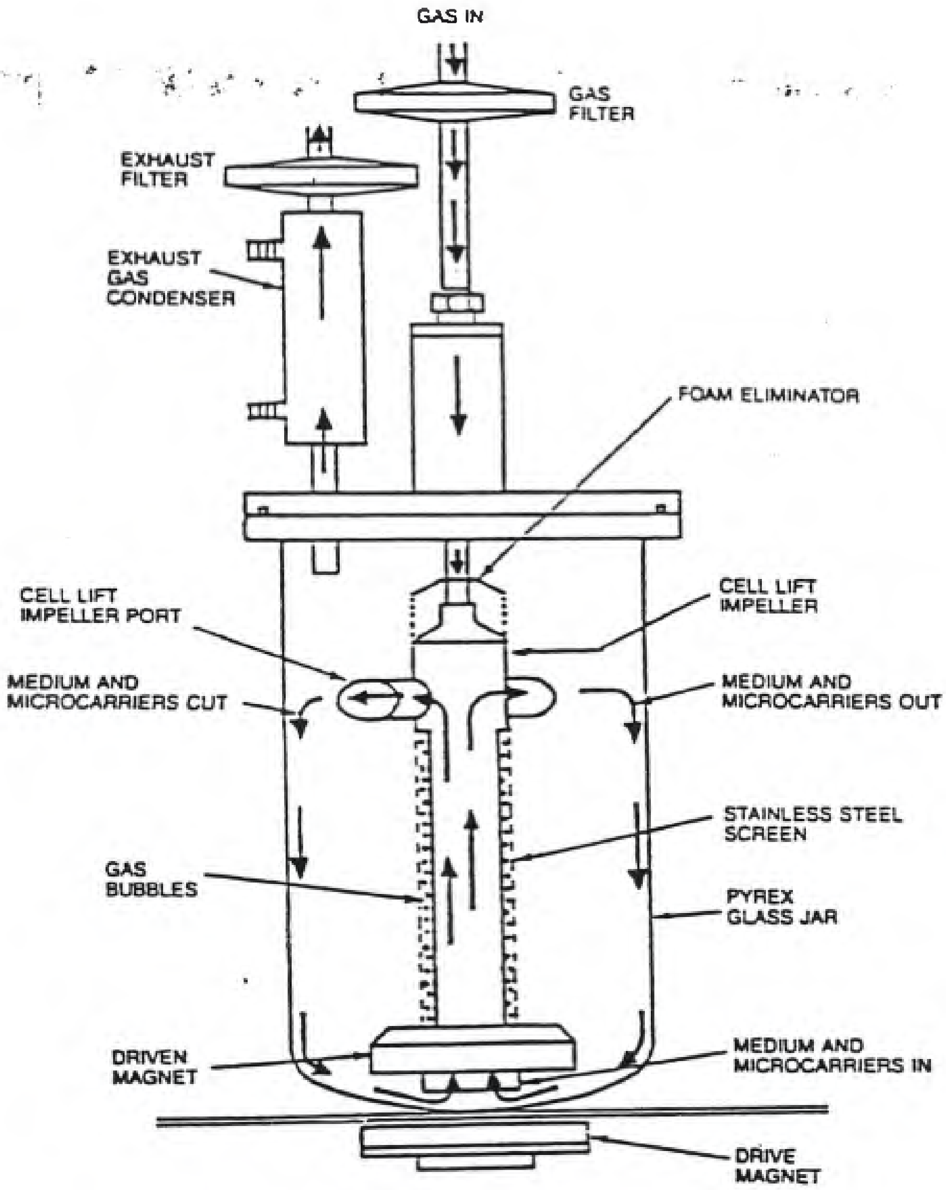
EFFECT ON BACTERIAL GROWTH
OF PEG, DMSO, SDS, AND TRITON X-100



EFFECT ON BACTERIAL GROWTH
OF PEG, DMSO, SDS, AND TRITON X-100

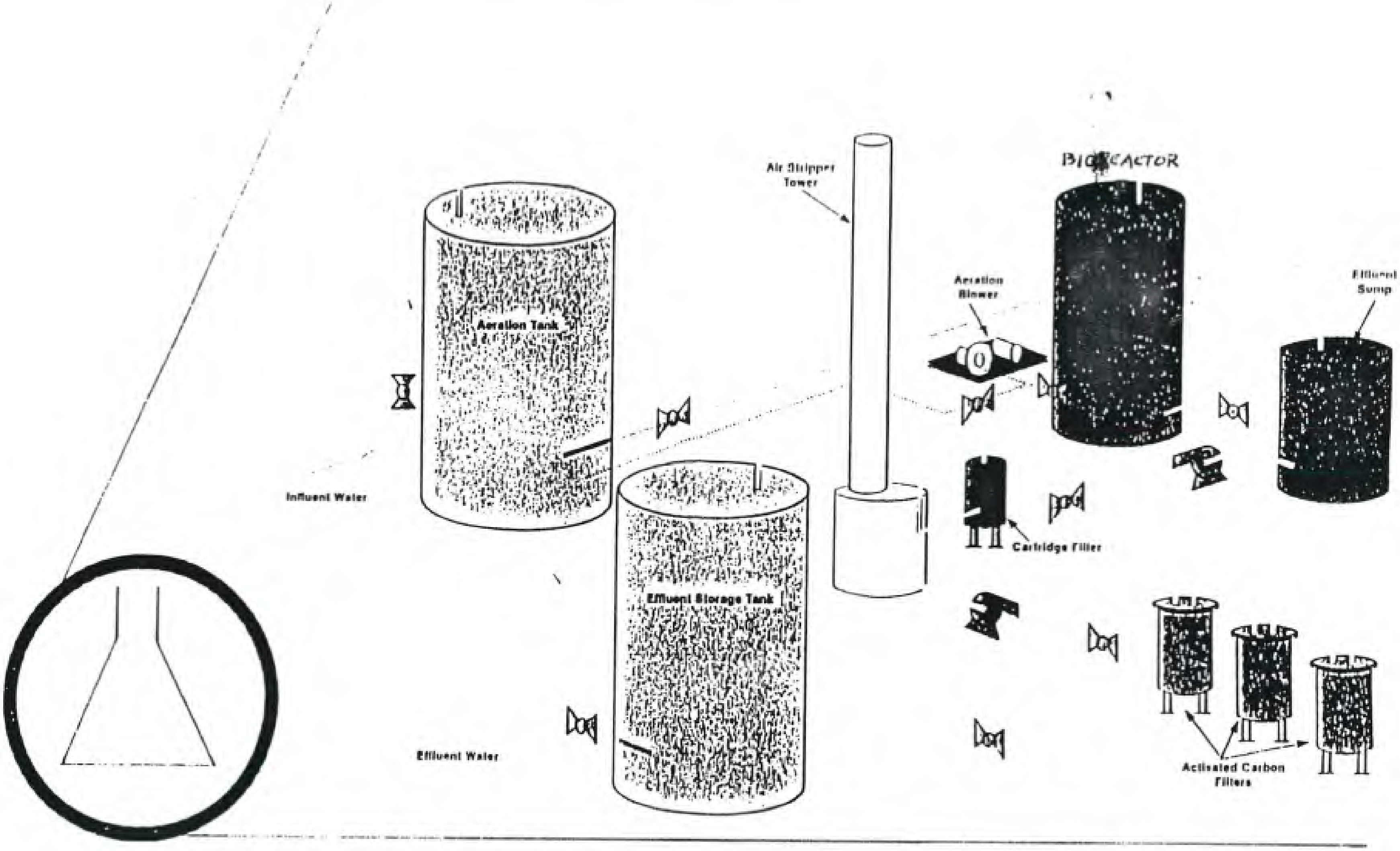



READ
SET
ON
AGITATION
POWER
CELL CULTURE SYSTEM
NEW BRUNSWICK SCIENTIFIC CO., INC.
MEASUREMENT
CALIBRATION
SET POINT
SPAN
ZERO
INDICATE
CONTROL
pH
DO
pH AND DISSOLVED OXYGEN CONTROLLER



Fermenter vessel equipped with a cell lift impeller.

BIOREACTOR SCALE-UP



Lab Scale ——▷ Industrial Scale

PRESENTATION BY DR. PAUL ROBERTS

Presented to the DOD Biotechnology Work Group, Monterey, California, 22 February 1989.

A Field and Modeling Study of Enhanced Oxidation of Chlorinated Aliphatic Compounds by Methanotrophic Bacteria

Lewis Semprini, Gary D. Hopkins, Paul V. Roberts, and Perry McCarty,
Department of Civil Engineering, Stanford University,
Stanford, California 94305.

INTRODUCTION

The in-situ bioremediation of aquifers contaminated with halogenated compounds, commonly used as solvents, is a promising alternative in the efforts to protect groundwater quality. This paper presents the results of a field demonstration at the Moffett Naval Air Station, Mountain View CA, that evaluated the feasibility of in-situ biodegradation of trichloroethylene (TCE), cis-and trans-1,2-dichloroethylene (cis-DCE, trans-DCE) and vinyl chloride (VC).

The method tested is an aerobic process that relies on the ability of methanotrophic bacteria to degrade the chlorinated aliphatics as secondary substrates. Wilson and Wilson (1985) demonstrated the oxidation of TCE to CO_2 in a methane-utilizing soil column. In laboratory mixed culture studies Fogel et al. (1986) and Henson et al. (1988) found a range of double and single bonded chlorinated compounds were degraded by methane-utilizing bacteria. The field investigation was undertaken to determine if an indigenous population of methane-utilizing bacteria could be stimulated under real aquifer conditions, and to quantify enhanced biotransformation of a range of chlorinated organics.

An understanding of key microbial and transport processes is required in designing and performing the experiments. Information on the transport processes of advection, dispersion, and sorption was obtained in tracer experiments. Sorption of the chlorinated organics onto aquifer solids was also investigated in laboratory studies. Basic microbial processes were studied in detail in laboratory experiments. A mathematical model that incorporated these key processes was adapted to simulate the in-situ biotransformation process. Results of both the field and modeling studies will be presented.

EXPERIMENTAL METHODOLOGY

The pilot scale tests were performed in a shallow, semi-confined aquifer consisting of sands and gravels (Semprini et. al., 1988). The experiments were performed as a series of stimulus-response tests, where the stimulus was the injection of chemicals in interest into the test zone, and the response was measured as chemical concentrations at observation wells. Experiments were performed under induced-gradient conditions created by injecting groundwater amended with the chemicals of interest into a fully penetrating injection well, and extracting at a fully penetrating extraction well located six meters away. Observation wells were located in between at spacing of 1, 2.2, and 3.8 meters from the extraction well. The chemical concentrations of the injected and extracted fluids, and the three monitoring wells, were measured using an automated data acquisition and control system located at the field site. The system continuously monitored samples for DO and methane, bromide as a conservative tracer, and the chlorinated aliphatics of interest. A complete analysis was obtained every 45 minutes, yielding eight analyses at each sampling location per day.

MODEL DESCRIPTION

A non-steady-state biotransfromation model was developed that incorporated key microbial and transport processes determined in the laboratory and field experiments. Monod kinetics were used to model microbial growth, utilization of methane as an electron donor, and oxygen as an electron acceptor. A dual-term formulation was used, in which either methane or oxygen could limit microbial growth. The microbes were assumed to be present as an attached shallow biofilm,i.e., substrate concentrations in the biofilm are assumed equal to the local concentration in the pore water. Advection and dispersion were modeled as 1-D uniform flow, based on the results of tracer tests and 2-D simulations. Biotransformation kinetics were modeled using a competitive inhibition model, based on laboratory and field results, where rates were inhibited by the presence of methane. Sorption and desorption of the chlorinated organics onto the aquifer solids was modeled using a first order kinetic model, which was also selected based on the laboratory and field results. The resulting set of non-linear partial differential equations were solved numerically, by finite differencing the spatial derivatives, and numerically integrating the resulting set of ordinary differential equations.

RESULTS OF THE FIELD AND MODELING STUDIES

The field experiments were performed over a three year period. The first year's experiments included bromide tracer tests to characterize advective-dispersive transport, initial biostimulation of a methanotrophic biomass, and initial studies of TCE transformation. Figure 1 shows the field observations and model simulations of the methane and DO response at the S2 observation after the introduction of methane and DO. The good match between the model and the field results indicates the basic processes included in the model represent those occurring in the field. The model also simulates the transient response that resulted from the initiation of the injection of 4 and 8 hr alternate pulses of methane and DO, respectively. The long pulses were started in order to keep methane and oxygen separated near the injection well, while mixing through dispersive processes in the test zone. Model simulations indicated that pulsing distributed the microbial growth over a larger region around the injection well and minimized excessive local growth that can lead to clogging.

In the second and third season's of field testing the chlorinated organics were added to the test zone before it was biostimulated. In the second year, trans-DCE, cis-DCE, and TCE were continuously injected, while in the third year vinyl chloride (VC) was also injected. In both years nearly complete breakthrough of the organics was observed, before the test zone was biostimulated, indicating limited transformation due to abiotic or biotic processes. Upon addition, methane and DO were observed to be rapidly consumed with essentially no lag, indicating that methanotrophs previously stimulated were still present in the test zone to initiate uptake. In order to achieve model matches to the transient uptake of DO and methane a much higher initial microbial mass in the test zone was required compared to the first year's simulations shown in Figure 1.

A comparison of model simulations of the degradation of VC, trans-DCE, and cis-DCE in the third season are shown in Figure 2. The rapid decrease in concentration of the chlorinated organics in response to the initiation of methane and DO directly demonstrates biotransformation in response to biostimulation. In order to simulate the rate of VC transformation shown in Figure 2, the second order rate coefficient was approximately half that of methane, while TCE (not shown) rate was approximately two orders of magnitude lower than methane. Simulations for the observation well closest to the injection well indicated oscillations in VC and trans-DCE concentrations, that were positively correlated with methane concentration, could be explained by competitive inhibition kinetics and kinetically limited sorption-desorption from the aquifer solids.

The extents of transformation after about 400 hrs were: VC > 95%, trans-DCE > 90%, cis-DCE > 45% and TCE about 20%. Similar observed and simulated responses and degrees of transformations were obtained for trans-DCE, cis-DCE, and TCE in the second year's test.

The rate parameters used for methanotrophic growth, methane utilization, and transformation of the halogenated organics were in good agreement with published values and with results of our laboratory studies. The solid-water partition coefficients as well as the first order rate parameters for transfer between phases generally agreed with values determined in our batch laboratory studies.

The results of this work provide a quantitative foundation for design of aquifer restoration operations based on aerobic biotransformations by methanotrophic bacteria. The effectiveness of such treatment was shown to be the greatest for vinyl chloride among the halogenated alkenes studied. A mathematical model that incorporates key microbial and transport processes accurately simulates the transient response of the system to enhanced in-situ biotransformation. Models of this type will be of much use in the design of restoration systems.

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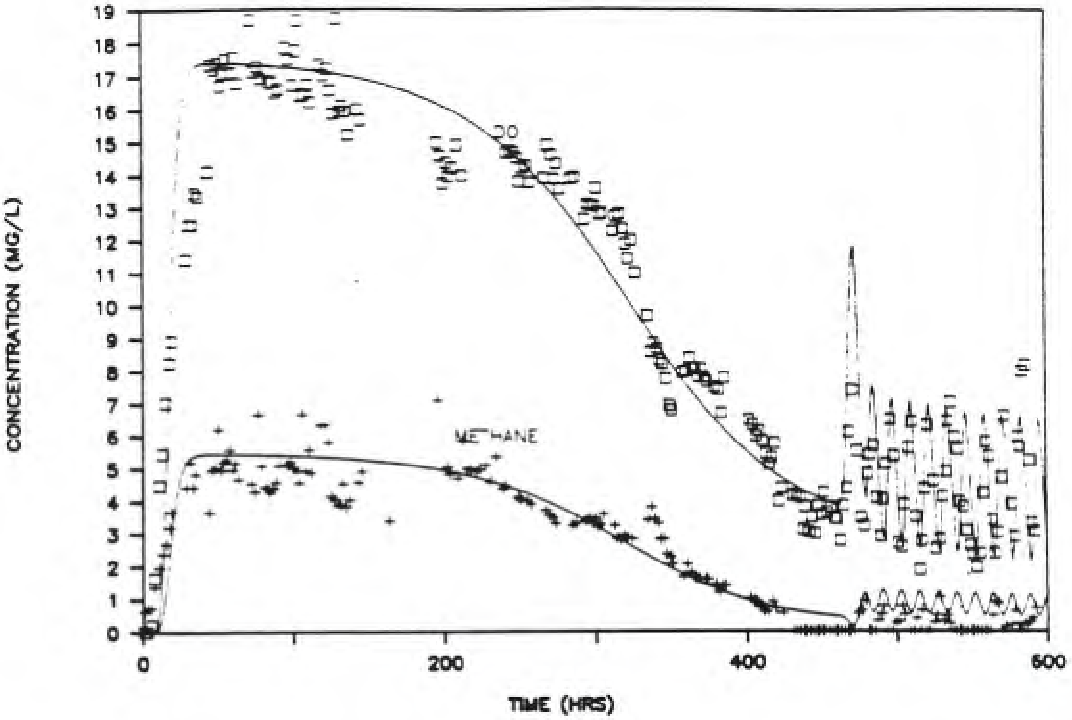

Figure 1 - Model simulation and field results of methanotrophic population response to biostimulation.

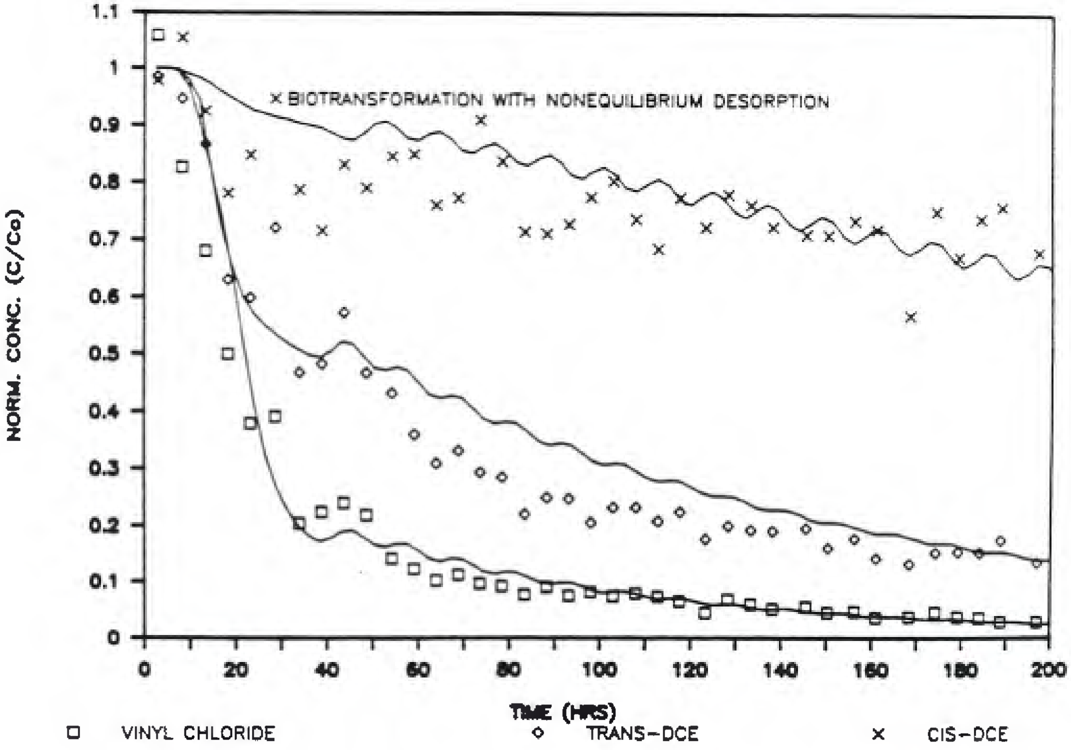

Figure 2 Model simulation and field results for methanotrophic degradation of cis-DCE, trans-DCE, and VC.

Technical Report No. 302
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Lewis Semprini, Paul V. Roberts, Gary D. Hopkins, and Douglas M. Mackay

Department of Civil Engineering
Stanford University
Stanford, California 94305-4020

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ABSTRACT

The in-situ remediation of aquifers contaminated with halogenated aliphatic compounds, commonly known in water supply as chlorinated solvents, is a promising alternative in efforts to protect groundwater quality. Biotransformation of the contaminants, by enhancing an indigenous microbial population capable of degrading the contaminants, has potential as an effective method for in-situ treatment.

This report presents the experimental methodology and the initial results of a field experiment evaluating the feasibility of in-situ biotransformation of TCE and related compounds. The method being tested relies on the experimentally proven ability of methane fed mixed cultures of bacteria to degrade these contaminants to stable, non-toxic, end products. Controlled experiments are performed in the subsurface in the presence and absence of biostimulation to evaluate the degree of biodegradation.

The field site is located at the Moffett Naval Air Station, Mountain View, Ca. The test zone is a shallow, confined aquifer composed of coarse grained alluvial sediments. The test zone has the following favorable characteristics: 1) high transmissivity, 2) an inorganic chemistry that will not inhibit aerobic microbial growth, 3) a background contamination with chlorinated solvents, and 4) the presence of methane-oxidizing bacteria. To create the test zone, an extraction well and injection wells were installed six meters apart, with three intermediate monitoring wells. A real time automated data acquisition and control system was developed which continuously monitors the concentrations of halogenated organic compounds, methane, oxygen, and bromide as a conservative tracer.

Bromide and TCE transport experiments were performed under induced flow conditions before the test zone was biostimulated. The bromide tracer tests indicated hydraulic residence times on the order of 0.5 to 2 days between the injection well and the observation and extraction wells. TCE was observed to be retarded compared to bromide, due to sorption onto the aquifer solids. Mass balances indicated that the injected TCE was recovered at the extraction well to the same extent as bromide, indicating little transformation of TCE before biostimulation.

Biostimulation of the test zone was achieved by injecting groundwater containing methane and oxygen in alternating pulses. Complete methane utilization was observed within a few weeks, confirming the presence of indigenous methanotrophic bacteria. By using pulse cycles of 8 to 12 hours, the biogrowth was distributed in the test zone, preventing biofouling of the area close to the injection well.

Under the influence of active biostimulation approximately 20 - 30% of the TCE was degraded within 2 meters of travel in the test zone, corresponding to the zone of methane utilization. The limited transformation most likely results from 1) a slow rate of degradation due to the high degree of chlorination of the TCE molecule, and 2) the limited enhancement of methane-utilizing population due to the limited quantity of methane and oxygen injected under saturated conditions. Laboratory experiments indicate compounds which are less chlorinated (i.e vinyl chloride and cis-and trans-DCE) are degraded more rapidly than TCE. In the second phase of field testing the biotransformation of several of these compounds, along with TCE, will be evaluated.

CONTENTS

FIGURES

TABLES

LIST OF ABBREVIATIONS AND SYMBOLS

ABBREVIATIONS

C/Co -- normalized concentration (measured/injected)
cm -- centimeter
cm^3 -- cubic centimeter
conc -- concentration
d -- day
DAC -- data acquisition and control
DCA -- dichloroethane
DCE -- dichloroethylene
d.l. -- detection limit
DO -- dissolved oxygen
f_{oc} -- fraction of organic carbon
ft -- feet
g -- gram
gpd -- gallons per day
hr -- hour
kg -- kilogram
m -- meters
m^2 -- square meters
meq -- milliequivalent
meq/l -- milliequivalents per liter
mg -- milligram
mg/l -- milligrams per liter
min -- minute
PCE -- tetrachloroethylene
TCA -- trichloroethane
TCE -- trichloroethylene
ug -- microgram
ug/l -- micrograms per liter

SYMBOLS

D -- dispersion coefficient (m^2/d)
K -- hydraulic conductivity (m/d)
n -- porosity (cm^3/cm^3)
K_d -- distribution coefficient (cm^3/g)
P_b -- bulk density (g/cm^3)
Pe -- Peclet number (u x/D)
r -- radius (m)
R -- retardation factor (dimensionless)
r/L -- leakage factor (dimensionless)
T -- transmissivity (gpd/ft)
u -- pore fluid velocity (m/d)
x -- distance (m)

ACKNOWLEDGMENTS

The authors thank the personnel of the U.S. Navy, especially the Public Works Department at the Moffett Naval Air Station for allowing the Field Site to be located on their base. They have cooperated fully in helping us solve the many logistical problems associated with performing a field study of this type.

We would also like to thank the staff at the Oakland Office of the California Regional Water Quality Control Board for permitting us to perform these experiments. Thomas Berkins and Steve Morse have provided helpful suggestions which have aided in the design of the experiments.

Members of the EPA Kerr Laboratory have also provide input to the experimental design, the characterization of the test zone, and have conducted laboratory studies which have help guide the field experiments. They include Jack Keeley, John Wilson, Micheal Henson, Barbara Wilson, Joseph Keely (now at the Oregon Graduate Center), and Burt Bledsoe.

At Stanford Perry McCarty and Dunja Grbic-Galic, co-principal investigators involved in laboratory aspects of the project, have provided valuable input to the field project. Barton Thompson, a recent graduate (now with the EPA), made significant contributions to the development and characterization of the field site. Also assisting were Willam Ball, Christoph Buehler, Costas Chrysikopoulos, Helen Dawson, Meredith Durant (now with the Hewlett Packard Corp.), Thomas Harmon, Susan Henry, Robert Johns, Nancy Lanzarone, and Kevin Mayer.

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SECTION 1

INTRODUCTION

The in-situ remediation of aquifers contaminated with halogenated aliphatic contaminants, commonly known in water supply as chlorinated solvents, is a promising alternative in efforts to protect groundwater quality. Chlorinated aliphatic compounds are frequently observed in groundwater. In a survey of 945 water supplies, Westrick et al. (1984) found trichloroethylene (TCE), tetrachloroethylene (PCE), cis- and/or trans-1,2-dichloroethylene (DCE), and 1,1-dichloroethylene to be the most frequently appearing compounds other than trihalomethanes. Approaches for the restoration of aquifers contaminated by these compounds based on extracting the contaminated groundwater by pumping and subsequently treating at the surface have been shown to be effective, but often entail great expense and also a risk of transferring the contaminants to another medium, i.e., the atmosphere. To circumvent these difficulties, in-situ treatment of the contaminants has come to be considered a potentially favorable alternative, with investigations centering on promoting biotransformation of the contaminants.

Our group at Stanford University is assessing under field conditions the capacity of native microorganisms, i.e., bacteria indigenous to the subsurface environment, to metabolize halogenated synthetic organic contaminants, when proper conditions are provided to enhance microbial growth. Specifically, the growth of a consortium of methane-utilizing bacteria is being stimulated in a field situation by providing ample supplies of dissolved methane and oxygen. Under biostimulation conditions, the transformation of representative halogenated organic contaminants, such as trichloroethylene (TCE), is assessed by means of controlled addition, frequent sampling, quantitative analysis, and mass balance comparisons.

The field demonstration study is being conducted at Moffett Naval Air Station, Mountain View, CA, with the support of the Kerr Environmental Research Laboratory of the U.S. Environmental Protection Agency, and with the cooperation of the U.S. Navy. To provide guidance for and confirmation of the field work, laboratory experiments and analyses are also being conducted, both at Stanford University's Water Quality Control Research Laboratory and at the Kerr Laboratory.

This report summarizes the experimental approach taken in the field study, the characterization of the test zone before the initiation of the evaluation experiments, and the results of the first phase of the field evaluation.

BACKGROUND

The in-situ restoration of aquifers contaminated with hydrocarbons is not a new idea. Raymond (1974) pioneered the development of the process for the in-situ reclamation of aquifers contaminated by liquid fuels. This work indicated that after promoting the proper conditions in the subsurface (i.e by the addition of oxygen and nutrients), a native population of microorganisms was stimulated that degraded the hydrocarbon contaminants. The microorganisms used the hydrocarbon contaminants as primary substrates for growth.

In-situ biorestoration of aquifers contaminated by halogenated aliphatic compounds requires a somewhat different approach, since in most cases the halogenated aliphatic compounds can not be utilized by native microorganisms as primary substrates for growth. However, they can be degraded as secondary substrates by microorganisms which utilize another primary substrate for growth. The in-situ degradation of these compounds is therefore promoted by the stimulation of a particular class of native microorganisms through the introduction of the appropriate primary substrate for growth (electron donor) and electron acceptor into the treatment zone.

The method being evaluated relies on the transformation of the chlorinated aliphatic compounds by methane-utilizing bacteria (methanotrophs). These bacteria grow on methane as a sole carbon source under aerobic conditions. The chlorinated aliphatic compounds are thought to be transformed by the methane monooxygenase enzyme, an enzyme with a broad range of specificity, that is produced by the methanotrophic bacteria.

The transformation has been demonstrated in laboratory studies using soil columns and mixed cultures. In experiments performed in an unsaturated soil column with an atmosphere of 0.6% natural gas and air, Wilson and Wilson (1985) found microorganisms were stimulated which degraded TCE fairly completely to carbon dioxide and cell material. Fogel et al. (1986) using mixed cultures of methane-oxidizing bacteria found TCE, vinyl chloride, vinylidene chloride, and cis- and trans-1,2-dichloroethylene to be rapidly degraded. Henson et al. (1987) found a range of both single and double carbon compounds to be degraded by mixed cultures. The rate of transformation was reported to be faster the less substituted the molecule with chlorine atoms and the more evenly distributed the chlorines on the molecule.

RESEARCH OBJECTIVES

The overall objective of this work is to assess the efficacy of a the proposed method for enhancing the in-situ degradation of the halogenated aliphatic compounds. The specific objectives of the field study are:

1) To demonstrate whether the proposed method of promoting the microbial decomposition of trichloroethylene and related compounds is effective under controlled experiments performed in-situ, in an aquifer representing conditions typical of groundwater environments;

2) To quantify the rate of decomposition and to identify intermediate transformation products, if any; and

3) To bracket the range of conditions under which the method is effective, and to establish criteria for dependable treatment of a real contamination incident.

SECTION 2

CONCLUSIONS

This reports summarizes the results of the first phase of the field experimentation evaluating in-situ methodologies for the restoration of aquifers contaminated with halogenated aliphatic compounds. The conclusions which can be drawn from these results are as follows:

1) In order to create a test zone in the subsurface in which controlled experiments can be conducted, detailed characterization of the zone must be performed, including:

    a) hydrogeology of the test zone based on coring, well logs, pump tests, piezometric measurements and published information on the local area;

    b) groundwater chemistry, including both major and minor inorganic compounds and trace organic compounds;

    c) aquifer solids analysis for microbial activity and sorption behavior of selected organic solutes; and

    d) hydraulics based on natural-gradient and induced-flow tracer tests.

2) The real time automated data acquisition and control system which was developed permits frequent sampling and reproducible analyses which are required for evaluation experiments of this type.

3) Tracer experiments provide valuable information on the test zone, including hydraulic residence times, dispersion, the degree of capture, and the retardation of TCE compared to bromide due to sorption on the aquifer solids. The tracer tests were found to be quite reproducible, which was required for the systematic, objective comparisons with the biostimulation results. Tracer tests before biostimulation indicated little transformation of TCE.

4) Indigenous methane-oxidizing bacteria were easily stimulated in the test zone within a few weeks by the pulsed addition of methane and oxygen. No nutrient addition was required to stimulate growth. Rapid growth kinetics were observed, with the microbial population increasing near the injection well such that all the methane was consumed within 1 meter of travel. Long pulse cycles of up to 12 hours were successfully used to distribute the bacterial growth, and to prevent biofouling of the aquifer.

5) Under active biostimulation conditions, 20 to 30 % of the TCE added to the test zone was degraded. Similar estimates of the degree of degradation were obtained using mass balances and comparisons with bromide as a conservative tracer. Degradation occurred within the test zone where methane was being utilized.

6) The limited degree of TCE transformation is attributed to the following factors:

   a) the high degree of chlorination of the TCE molecule, resulting in a slow rate of oxidation,

   b) the limited methane-oxidizing population, which can be stimulated with the amounts of methane and oxygen that can be delivered under saturated conditions, and

   c) possible competitive inhibition of TCE degradation by methane.

7) In the second phase of the field evaluation, other compounds which are less chlorinated, i.e. dichloroethylene isomers, will be tested along with TCE. Experiments will be performed to determine whether competitive inhibition is an important process and to assess the effect of the pulsed injection method on the rate of degradation.

SECTION 3

RECOMMENDATIONS

The limited degradation of TCE observed during the first phase of the field evaluation experiments indicates that more information is required before this process can be used on a larger scale for treatment of a real contamination incident. More basic laboratory research and pilot scale testing in the field are required. Laboratory investigations should address the factors which affect the rate of aerobic biotransformation of TCE and related compounds. The pilot scale field experimentation should determine whether the factors which enhance transformation in the laboratory can be successfully implemented in the field.

Important questions which must be addressed in laboratory experiments are:

1) How do the rates of transformation depend on the structure of the compound that is being degraded?
2) Do the conditions of biostimulation and maintenance of the microbial population affect the rate of transformation of different compounds?
3) Is there competitive inhibition between the methane and selected organic solutes which slows the rate of transformation?
4) Does biostimulation using different primary substrates or electron acceptors result in more effective degradation?
5) Is the addition of minor nutrients an important factor?
6) How does the sorption of the organics onto the aquifer solids affect the rate of biodegradation?

The pilot scale field tests should be continued, with new experiments being designed based on the results of the laboratory investigations. These pilot scale tests will help establish criteria for dependable treatment of real contamination incidents. Important criteria include; 1) the type of aquifers for which the process is best suited, 2) the range of environmental conditions in which the process may be applied, and 3) the most effective means of biostimulating the aquifer to achieve effective biotransformation.

Continuing both laboratory and pilot scale field studies at a well characterized site provides a basis for determining what information is needed for the design of in-situ restoration schemes at different sites. For instance, the comparison of field and laboratory results will evaluate how successfully parameters, which are generated in laboratory studies, predict biotransformation in the field. If parameters determined by laboratory studies, such as soil microcosm experiments, are of value, it would provide a low cost means of obtaining the necessary information to implement the treatment method at different sites.

Finally, pilot studies which evaluate this process in the unsaturated zone should be performed. Effective transformation, which requires the stimulation of a large microbial population, may be achieved by supplying ample quantities of methane and oxygen. This should be easier in the unsaturated zone compared to the saturated zone.

SECTION 4

FIELD EXPERIMENT METHODOLOGY

The experimental methodology developed to meet the goals of the field study is as follows:

1. Select a representative demonstration site based on available information regarding regional hydrology and geochemistry, and considering practical and institutional constraints;
2. Characterize the site by means of coring, pump tests, sampling and analysis of the native groundwater;
3. Construct a system of wells for injection, extraction, and monitoring of water at the site;
4. Design and install an automated system for sampling and analysis of the groundwater at the demonstration site;
5. Determine the velocity and direction of groundwater flow under natural gradient conditions, by means of bromide tracer tests;
6. Assess the mobility of trichloroethylene, relative to bromide tracer, at the demonstration site and quantify residence times in the system under injection/extraction conditions;
7. Stimulate the growth of native methane-oxidizing organisms by injecting dissolved methane and oxygen (biostimulation mode); and
8. Assess the transformation of trichloroethylene under biostimulation conditions.

This methodology provides a staged approach for evaluating the proposed technology. The initial stages of the study (1-5) focus on selecting the field site and characterizing its physical, chemical, microbiological and hydraulic properties. The latter stages of the experiment involve biostimulating methane-oxidizing bacteria in the test zone and evaluating the degree of transformation of a specific contaminant of interest.

The information obtained during the early stages of the experiments is critical to the success of subsequent evaluation experiments, which are dependent on the ability to run controlled experiments in the subsurface. The hydraulic information obtained in pump tests and tracer experiments is required in order to design a fluid injection and extraction system that creates an in-situ reaction zone. The chemical, physical and microbiological characteristics of the test zone also indicate whether favorable conditions exist for the biostimulation of a native population of methane-oxidizing bacteria. These data are necessary in determining whether a controlled evaluation of the proposed technology can be performed at the selected site.

The basic approach of the evaluation experiments is to create a test zone in the subsurface. The conceptual model for this approach is shown in Figure 1. A series of injection, extraction, and monitoring wells are installed within a confined aquifer. An induced flow field is created by the injection and extraction of fluid. The chemicals of interest for a specific experiment are metered into a stream comprising 10 to 15 percent of the extracted groundwater and then reinjected. The concentrations of the specific chemicals are monitored at several locations, including the injected fluid, the three monitoring wells, and the extracted fluid. The spatial and temporal responses of the chemicals in the test zone are determined by frequent monitoring, using an automated data acquisition and control system located at the site.

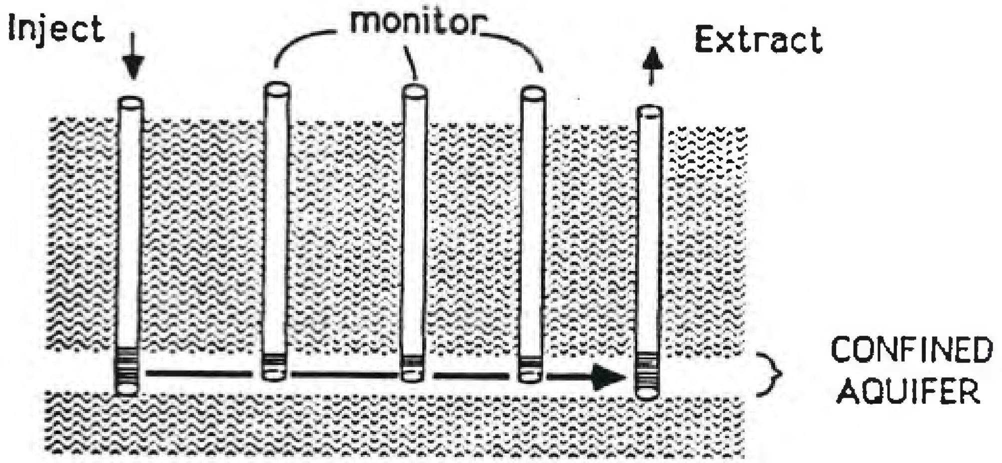

Figure 1. Conceptual model for the creation of the subsurface test zone.

The sequence of field experiments using this approach is outlined in Table 1. The initial experiments study the transport of bromide ion as a conservative tracer. The experiments determine fluid residence times in the system, the degree of dispersion, and the recovery of the injected fluid at the extraction well. In later experiments, bromide, dissolved oxygen and the chlorinated aliphatic compounds of interest are injected simultaneously. The retardation factors of the different chemicals with respect to bromide, owing to sorption, are determined. The transformation of the chlorinated aliphatic compounds in these experiments is evaluated based on comparisons with the bromide tracer. Two criteria are used: 1) the degree of steady-state fractional breakthrough achieved at the monitoring wells, and 2) mass balances on the amounts injected and extracted. These tracer experiments therefore, serve as pseudo controls, permitting a comparison of the observed responses before and after the test zone is biostimulated.

TABLE 1. SEQUENCE OF EXPERIMENTS AND PROCESSES STUDIED DURING THE FIRST PHASE OF THE FIELD EVALUATION.

Injected Chemicals	Process Studied
1) Br^-	Advection/Dispersion
2) $Br^- + O_2$	Retardation/Dispersion (TCA - Elution)
3) Br^-+TCE + O_2	Retardation (Transformation)
4) $CH_4 + O_2$+(nutrients)	Biostimulation
5) $CH_4 + O_2$+(nutrients)+ TCE	Biotransformation

The biostimulation experiments (Stage 4) involve the addition of methane, oxygen, and nutrients (if required), to stimulate the growth of methane-consuming bacteria in the test zone. The transient response of the different chemical components is monitored, as previously discussed. This experiment determines: 1) how easily the methane-oxidizing bacteria are stimulated and whether nutrients are required, 2) stoichiometric requirements of oxygen to methane, 3) information on the kinetics and the rate of growth, and 4) the areal extent over which biostimulation is achieved.

The degree of biotransformation of the chlorinated aliphatic compound (TCE) is evaluated in the final stage (Stage 5) of the experiment. Known quantities of TCE are introduced into the biostimulated zone along with methane, oxygen and bromide. The extent of transformation of TCE is determined based on both mass balances and steady-state breakthrough concentrations at monitoring points, compared to those of bromide as a conservative tracer. The results are also compared with those obtained during the earlier pseudo-control experiments (Stage3) before the test zone was biostimulated.

SECTION 5

SELECTION AND OF THE CHARACTERIZATION FIELD SITE

FIELD SITE DESCRIPTION

After a reconnaissance study of several sites, a location at the Moffett Naval Air Station, Mountain View, Ca., was chosen (Figure 2). The site, designated SU-39, located on the lower part of the Stevens Creek alluvial fan is approximately 3 km south of the southwest extremity of San Francisco Bay. The surface elevation at the site is 8.5 m above mean sea level.

The experimental site is located in a region where the groundwater is contaminated with several organic solutes for which this biorestoration method might be applied. The area of groundwater contamination shown in Figure 2 represents the 1 mg/l TCE contour of the "A" Aquifer delineated in January, 1983 (Canonie Engineers, 1983). The plume contains concentrations of 1,1,1-trichloroethane (TCA) and trichloroethylene (TCE) of up to 100 mg/l, measured at points 700 and 1000 meters from the SU-39 site. Thus, if effective, the treatment method may have direct use in the area where it was evaluated.

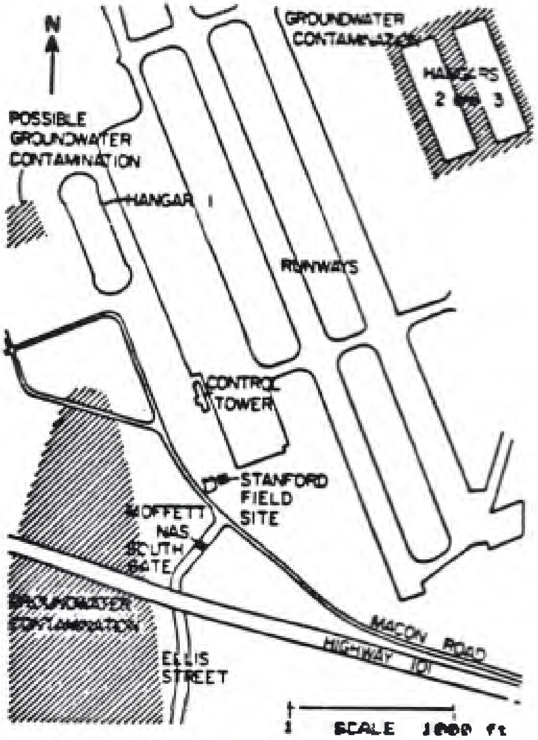

Figure 2. Location of the field site, SU-39, at the Moffett Naval Air Station, Mountain View, California.

GEOLOGIC CHARACTERISTICS

The geologic characteristics of the test zone have been examined using cores samples and well cuttings. Figure 3 shows the location of the wells installed at the test site to date. A series of exploratory wells (1,SI,3,4,5,6) were installed in July 1985, using the hollow stem auger drilling method. Cores were obtained using 2 inch pitcher barrels that were pushed ahead of the drill bit. The 6 test drillings identified a shallow, confined aquifer which is known as the "A" Aquifer, the shallowest of several in the region. Well logs indicate the aquifer is approximately 1.2 meters thick with a top 4.4 to 4.6 meters below the ground surface; the bottom ranges from 5.3 to 5.7 meters below the surface. The aquifer is confined between silt clay layers.

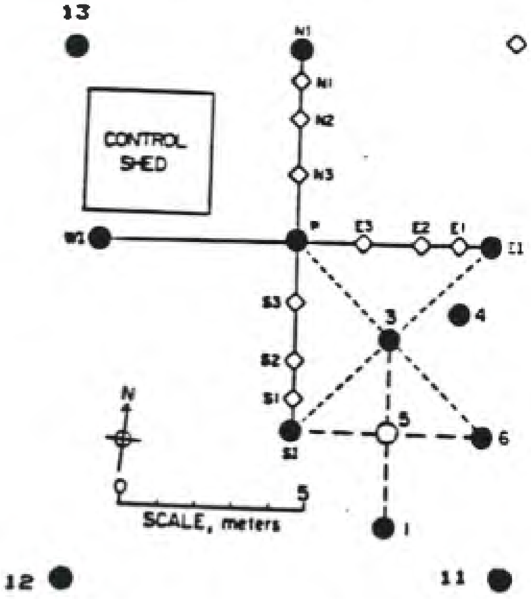

Figure 3. Map of the well field installed at the field site.

Figure 4 was constructed from the core well logs of the fully penetrating wells SI, P, NI. Well SI is the injection well and P is the extraction well used in the experiments. The three well logs have similar lithologic profiles. The uppermost 2 ft consists of silty sand with pebbles up to 8 cm in diameter. This surface layer is underlain by approximately 12 ft of silt and clay of a brownish-black to olive gray color, indicating that the sediment contains organic material. The bottom of this sequence is marked by a clayey sand that commonly separates the silt and clay overburden from the underlying aquifer.

The aquifer consists of fine- to coarse-grained sand and appears well bedded in most cores. Gravel lenses with pebbles up to 2.5 cm in diameter occur in some cores within the sand layers. Due to the presence of gravel, intact cores were difficult to obtain. Cores were often lost over the depth interval from 15.5 to 17 ft below the surface. This zone is considered to have the highest gravel fraction.

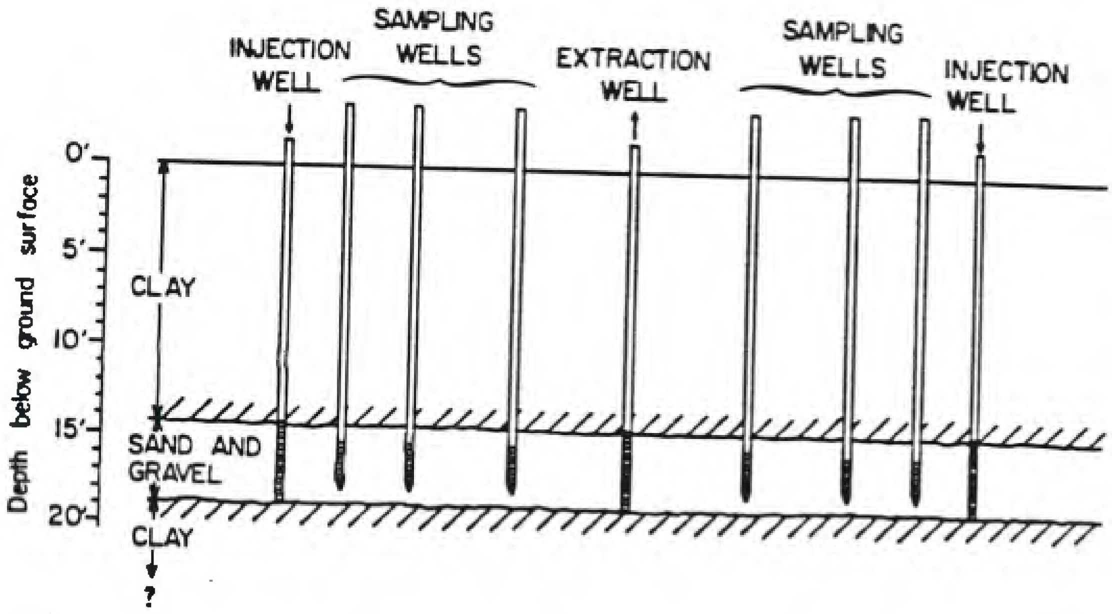

Figure 4. Vertical section of the test zone.

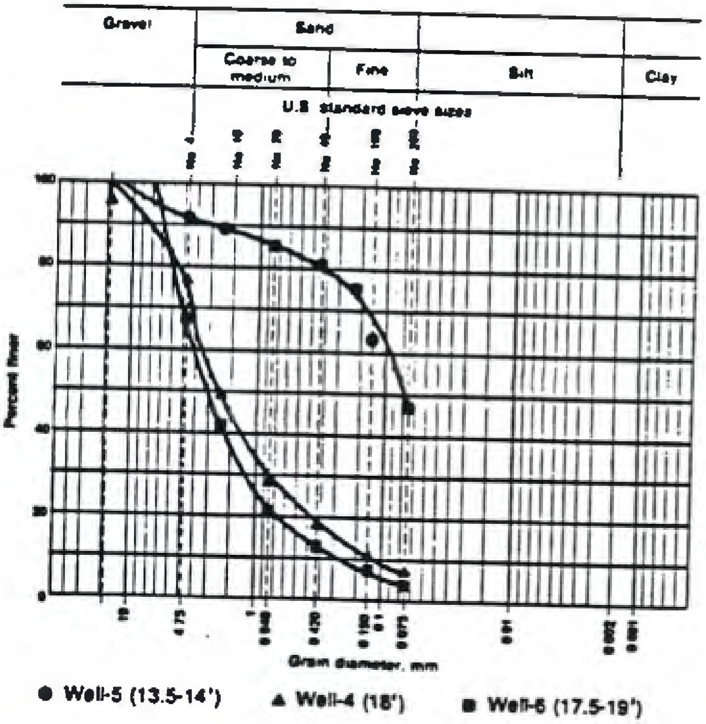

Figure 5. Particle size distribution of the aquifer core samples based on standard sieve analysis.

A layer of dark greenish-gray silty clay underlies the aquifer (top at 19 ft below the surface). While no well was drilled through this clay/silt layer at the project site, other studies in the vicinity have shown that this layer is approximately 20 ft thick and is underlain by another aquifer (Canonie, 1983).

The particle size distributions of aquifer cores are shown in Figure 5. Core samples taken from wells 4 and 6 at a depth of 18 ft and 17.5 - 18 ft respectively, have similar distributions of particle sizes, with a large fraction of the solids being coarse to medium sands and gravel. The core sample from well 5 at a depth 13.5 - 14 ft has a greater fraction of fine sand and silt, which is consistent with well log observations. Petrographic analysis shows the aquifer solids consist of rock fragments of the parent rock of the Santa Cruz Mountains. These include include graywackes, cherts, and volcanics of eugeosynclinal (slope) origin (Franciscan Series).

The observations at the test site are consistent with geologic studies in the region. The interlayering of coarse and fine sediments in the Santa Clara Valley results from changes in sea level caused by world-wide climate fluctuations (Atwater, et al, 1977). During times of high sea level (warm periods), fine grained estuarine sediments were deposited in the valley, resulting in the clay and silt aquitards. During times of low sea level (glaciation in northern latitudes), these sediments were covered by coarser grained alluvial deposits that form the aquifers. At the study site, the aquifer consists of alluvial sediments deposited during the last 5000 years. The test zone appears to have the structure of a buried stream channel containing sand and gravel. This structure is common in alluvial aquifers that are characterized by deposition from multiple channels with constantly shifting loci of deposition, resulting in discontinuous lenses of sand and gravel (Press and Siever, 1974).

HYDRAULIC CHARACTERISTICS

Hydraulic Gradient

Maps of the regional piezometric surface of the "A" Aquifer have been reported by Canonie Engineers (1983). The hydraulic gradient is northward at about 4.5 meters per kilometer. Piezometric measurements made with the original wells of the test zone, indicated that the aquifer was confined with a piezometric surface 2.5 meters above the top confining layer (21 ft above mean sea level). The magnitude and direction of the gradient in the test zone was in the range of regional values. The original gradient estimates had a large level of uncertainty due to the short spatial resolution. Wells 11, 12, 13, installed in August 1986, provide a more accurate estimate of the local gradient due to the greater distances between wells. A gradient of 0.0032 in a northerly direction was estimated using these wells.

Pump Tests

Numerous pump tests were performed to determine hydraulic properties of the aquifer. The tests determined the transmissivity, which permitted estimates of the hydraulic conductivity and the natural gradient groundwater velocity. The possible influence of leakiness, barriers and abnormalities was also examined. Finally, the steady-state drawdown was investigated in a long term pump test.

The drawdown pump tests were performed using a SE 200 A well test device, obtained on loan from the Robert S. Kerr Environmental Research Laboratory, USEPA; Joseph Keely of the Kerr Laboratory provided guidance in the use of the instrument. The equipment consisted of a central mini-computer and downhole pressure transducers. During the tests, six transducers were placed in the wells (SI, P, NI, P1, EI, 6). In all but one test, water was pumped from well P at a steady rate, while drawdown versus time was measured in other wells. To explore for directional variability, one test was run by extracting from well EI.

The pump tests were analyzed by standard methods as described in Freeze and Cherry (1979). A direct method for calculating transmissivity and the storage coefficient is based on semi-log method based on the equation of Cooper and Jacob (1946). The second method used is based on the method of log-log type curve matching.

The log-log plots of drawdown versus time showed that the responses matched type curves for a leaky aquifer (Walton, 1960). Freeze and Cherry (1979) indicate that when production wells are screened only in a single aquifer (as is the case of the test zone well) it is quite usual for the aquifer to receive flow from the adjacent beds. Thus leakage through the confining layers is likely occurring in region of the test zone. Matches were therefore made to the leaky aquifer type curves.

The results show good agreement between the semi-log method and the more rigorous type curve method. A semi-log method yielded an average transmissivity of 12600 gpd/ft. An average value of 11100 gpd/ft was obtained based on the type curve method. The match method gave an average value of the storage coefficient of 0.0013 and an average r/L value of approximately 0.05, indicating the aquitards are not very leaky. This result may explain the good agreement between the match method and the semi-log method, which assumes the system is confined system.

A summary of the results of the pump tests is presented in Table 2. The transmissivity values were fairly reproducible from test to test. The transmissivity values show no significant differences based on the location of the observations wells or the pumping well. Anisotropies in transmissivity in the horizontal plane were not indicated by the tests. However, a more detailed analysis of the data is currently being performed using a computer code which uses a non-linear least squares routine to estimate best fit parameters, for a given solution. These analyses will more accurately determine if anisotropies in transmissivity exist.

The high transmissivity results in an estimated hydraulic conductivity of 100 m/d (based on an aquifer thickness of 1.4 meters). The hydraulic conductivity is in the range of values given by Bouwer (1978) for coarse sand (20-100 m/d), gravel (100-1000 m/d) and sand-gravel mixes (20-100 m/d), which is consistent with the aquifer cores as indicated by the particle size distributions shown in Figure 5.

TABLE 2. SUMMARY OF THE PUMP TEST RESULTS

Pump Test	Duration (min)	Rate (gpm)	T(avg)[1] (gpd/ft)	K(avg)[2] (m/d)
5	90	5	13654	122
6	456	5	11272	101
7	495	5	9440	84
8	600	5	9625	86
9	3471	4	11505	102
		Average	11100	100
		std	1500	15

[1] match to leaky aquifer type curves
[2] based on an average aquifer thickness of 1.4 m

The long-term pump tests show that steady-state drawdowns were achieved, and that the aquifer was capable of supplying water at rates required for the experiments, with less than a 1 meter drawdown at the extraction well. The long-term pump tests did not detect any abrupt barriers to flow.

The pump tests indicated that the site had several favorable hydraulic features: 1) high transmissivity should permit the required pumping and injection of fluids into the test zone; 2) loss of permeability by clogging due to biological growth or chemical precipitation, would be limited, due to the original high permeability; 3) the aquifer is semi-confined, thus the test zone is fairly well bounded in vertical direction; and 4) the aquifer was capable of supplying groundwater at rates required for the experiments with less than one meter of drawdown at the extraction well.

One potential problem with the high hydraulic conductivity is that the velocity of the groundwater under natural gradient conditions is high. A velocity of 1 m/d was estimated based on the hydraulic conductivity of 100 m/d, the measured hydraulic gradient across the field of 0.0032, and an estimated porosity of 0.33. This high groundwater velocity limits the control of fluid residence times, since the induced flow field must be operated in such a manner as to overcome the natural flow in order to assure capture of the injected solutes.

CHEMICAL CHARACTERISTICS

Samples of the groundwater from the A-aquifer were obtained during the pump test program to determine the the background concentrations of inorganic and organic components. The analyses provided information on the quality of the groundwater in the area of the test zone and determined whether the aquifer was contaminated with chlorinated aliphatics of interest.

Inorganic Composition

Table 3 presents the major anions and cations, along with other parameters. The charge balance, as well as the measured and calculated TDS, indicate that all of the major ions have been identified. The major cations in decreasing milliequilavent concentrations are as follows: calcium > magnesium > sodium > potassium. The major anions are, in declining order: sulfate > bicarbonate > chloride > nitrate. The groundwater hardness is 920 mg/l, based on the calcium and magnesium concentrations, and would be classified as very hard water. Bicarbonate is the major form of alkalinity at the measured groundwater pH of 6.5. The dissolved oxygen content of the groundwater is below 0.2 mg/l.

The analysis of the major chemical components indicates that the test zone is suitable for the experiments. The chemical composition, including the pH, is suitable for the microbial growth. However, because the concentration of dissolved oxygen in the groundwater is very low, all of the oxygen required for microbial growth must be added to the test zone. The presence of high nitrate and low ammonia concentrations indicate that the aquifer is not anaerobic. Thus, major problems associated with the change in the oxidation state by the addition of oxygen are not anticipated, at least from the microbiological point of view. The high calcium concentrations may present problems, e.g., the precipitation of sulfates and carbonates with changes in fluid chemistry. The chemical composition of the groundwater indicates that the fluid phase concentrations are close to the solubility limits of gypsum ($CaSO_4$) and calcite ($CaCO_3$). Owing to the high sulfate concentration, the groundwater is not considered of drinking water quality, which facilitated obtaining regulatory approval to perform the experiments.

Trace Chemical Analysis

Analysis for trace element composition was performed by Inductively-Coupled Argon Plasma Spectrometry at the Robert S. Kerr Environmental Research Laboratory (Bledsoe, 1985, unpublished data). Table 4 shows concentrations of all inorganic elements were below 1000 ug/l, and in most cases below the detection limit of the analysis. Concentrations were below levels that would be considered toxic to microorganisms, and indicate that the addition of trace nutrients may be required to promote effective microbial growth.

TABLE 3. GROUNDWATER CHEMISTRY: MAJOR IONS AND OTHER PARAMETERS

MAJOR IONS	Concentrations		Milliequivalents Calculated from Lab 1 results
	(mg/l)	(mg/l)	(meq/l)
CATIONS	Lab 1*	Lab 2*	
Na^+	53.	44.	2.3
K^+	2.6	1.5	<0.1
Ca^{++}	200.	216.	10.0
Mg^{++}	100.	93.	8.2
NH_4^+	<0.1	nd	<0.1
TOTAL	356.	355.	20.5
ANIONS	Lab 1*	Lab 3*	
Cl^-	42.	39.	1.2
Br^-	0.6	<0.2	<0.1
HCO_3^-	270.	227.	4.4
NO_3^-	6.9	14.9	<0.1
PO_4^{3-}	0.1	nd	<0.1
SO_4^{2-}	750.	699.	15.6
TOTAL	1070.	980.	21.3

CHARGE BALANCE ERROR = 2%

OTHER PARAMETERS

Total Dissolved Solids (TDS, mg/l)
Measured = 1456 ± 15 (by gravimetric analysis)
Calculated = 1426 (from major ion analyses)
Estimated = 1000-1400 (from specific conductance)

pH = 6.5 (measured in the field)

DO < 0.2 mg/l
Temperature = 18°C (measured in the field)

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*Major ion analyses conducted by different laboratories. Lab 1, Lab 2, and Lab 3 refer to Sequoia Analytical Laboratory, Kerr Environmental Research Laboratory, and Stanford University Civil Engineering Laboratory, respectively.

TABLE 4. TRACE CHEMICAL COMPOSITION OF THE GROUNDWATER FROM THE SU-39 SITE.

TRACE INORGANIC CONSTITUENTS*

Element	DISSOLVED (ug/l)	TOTAL (ug/l)
Fe	nd	540
Mn	300	310
B	150	200
Zn	10	30
Sr	67	76
Ba	20	20
Al	<100	<100
As	<30	<30
Be	<3	<3
Ag	<10	<10
Cd	<3	<3
Co	<10	<10
Cr	<10	<10
Cu	<10	<10
Hg	<30	<30
Li	<10	<10
Mo	<10	<10
Ni	<10	<10
Pb	<20	<20
Ti	<100	<100
Se	<30	<30
Tl	<20	<20
V	<10	<10

TRACE ORGANIC CONSTITUENTS**

Chemical	Concentration (ug/l)
1,1-dichloroethylene (1,1 DCE)	14
1,1-dichloroethane (1,1 DCA)	0.5
1,1,2-trichloro-1,2,2-trifluroethane (Freon113)	9.4
1,1,1-trichloroethane (TCA)	97.4 ± 30

* determined by Inductively-Coupled Argon Plasma Spectrometry; when results were below detection limit (d.l.), the results are listed as less than (<) the d.l. for the method

** determined by gas chromatography or gas chromatography/mass spectrometry. Values listed are averages of duplicate determinations, except for TCA. TCA analyses were conducted on seven samples taken during the period 7/9/85-11/10/85; the TCA concentrations in the samples ranged in concentration from 56 to 131 ug/l.

Analyses were conducted to determine the type and concentrations of trace organic compounds at the field site. Four volatile organic compounds were detected, as shown in Table 4. The highest concentrations in the native ground water were found for 1,1,1-trichloroethane (TCA), which is present at a concentration on the order of 100 ug/l, varying over a range of 56-131 ug/l for analyses conducted over several months. Trace amounts of other halogenated compounds are present, as shown in Table 4. Trichloroethylene (TCE) was not detected in these samples.

Analyses were as performed for purgeable aromatics. No such compounds (e.g., benzene, xylene, toluene, chlorinated aromatics), were detected. Total (non-purgeable) organic carbon was determined to be approximately 2 mg/l, within the range of 0.1 - 10 mg/l reported for groundwaters due the presence of natural humic and fulvic acids (Freeze and Cherry, 1979).

These analyses showed that the native groundwater in the test zone had the following important characteristics with respect to chlorinated compounds:

1) It was contaminated with halogenated organics at low concentrations. This was considered an important criterion for obtaining regulatory approval to conduct the experiments. The concentrations, however, were low and would not be toxic to the native bacteria.

2) The TCE concentration was below the detection limit. Thus, controlled experiments can be performed by adding small but measureable quantities of TCE to the test zone.

The results of the initial inorganic and organic analyses indicated that the groundwater was of a suitable chemical composition for performing the experiments. The chemical composition would not inhibit the stimulation of the methanotrophic bacteria, and it appears feasible to inject and transport dissolved oxygen in the test zone without undue consumptive losses.

AQUIFER SOLIDS ANALYSIS

Core samples of the aquifer material were obtained in order to characterize the aquifer material's physical, chemical, and microbiological properties. Some of the core material was to be used for microbiological studies in the laboratory. Aseptic procedures as outlined by Wilson et al. (1983) were used for obtaining the cores samples and transferring the materials to storage containers.

Microbial Enumeration

The acridine orange-epifluorescence procedure of Ghiorse and Balkwill (1983) was used to enumerate the active bacteria attached to solid samples from the test zone. The analysis indicated that the microorganisms were typically attached to particles of organic matter. The bacterial numbers per gram of dry solids varied from 2 - 39 x 10^6 within the range of values of 1 - 50 x 10^6 obtained in subsurface investigations of Ghiorse and Balkwill (1983), Wilson et al (1983), and Webster et al. (1985). No apparent trend with depth was indicated. The highest value, however, was observed in the sand and gravel zone, 17 -

17.5 ft below the surface. The bacteria counts may be associated with the high permeability of this zone and a corresponding greater flux of nutrients.

The presence of methanotrophic bacteria was not established using this enumeration procedure, since the method is not type specific. The presence of methane-consuming bacteria on aquifer solids was, however, demonstrated in columns studies discussed by Wilson et al. (1987). In these studies, columns were packed with core solids obtained from well SI. After exchanging the pore water with water containing methane and oxygen, oxygen and methane consumption was observed. This study and the bacteria enumeration study indicated that the test zone had an indigenous microbial population that could be successfully biostimulated.

Organic Carbon Content

The organic carbon content of the Moffett aquifer material was determined by measurement on a Dohrmann DC-80 organic carbon analyzer following pretreatment consisting of acidification with H_3PO_4 and heating under vacuum to remove carbonate, addition of $K_2S_2O_8$, and autoclaving at 121°C for 4 hours in sealed ampules to oxidize the organic matter to CO_2. The ampules were then broken into the oxygen stream of the DC-80 analyzer, and the CO_2 production was quantified by a Horiba nondispersive IR spectrometer. Coarse-grained samples were preground for 10 seconds in a tungsten carbide mill to facilitate complete removal of inorganic carbon and complete recovery of the organic carbon.

Results are summarized in Table 5. For the bulk material, the average value was 0.11 percent carbon, with no significant influence of pregrinding. The organic matter appeared to be concentrated in the clay fraction, with organic carbon contents six times that of the bulk material, whereas the coarse-grained fractions have organic carbon contents as much as 40 percent less than the bulk average.

TABLE 5. ORGANIC CARBON CONTENT OF MOFFETT AQUIFER SOLIDS

Size Fraction	Organic Carbon Content Percent, mean ± std. dev. ground	 not ground
Bulk	0.112 ± 0.020#	0.110 ± 0.014
Clay-top		0.649 ± 0.039
Clay-bottom		0.638 ± 0.090
U.S. Std. Mesh		
<200		0.161 ± 0.014##
-100+200	0.113 ± 0.009	
-60+100	0.087 ± 0.005	
-40-60	0.100 ± 0.008	
-20+40	0.062 ± 0.005	
-8+20	0.095 ± 0.009	
-4+8	0.082 ± 0.007	

* 4 replicates, unless otherwise noted.
\# 3 replicates
\## 6 replicates

Based on these measurements, it appeared likely that the Moffett aquifer material would exhibit substantial sorption capacity, significantly greater than observed at the Borden site in our previous field experiment (Roberts et al., 1986; Curtis et al, 1986), where the organic carbon content was measured as 0.02 percent.

Sorption onto Aquifer Solids

The degree of sorption of several chlorinated aliphatic compounds onto aquifer solid samples was determined in batch sorption experiments. The procedure used in the batch sorption experiments is that described by Curtis et al. (1986). The size fraction studied was that which passed through a #10 U.S Standard sieve, i.e particle diameters less than 2 mm. This fraction represents approximately 33% of the particle mass present in cores as shown in Figure 5.

The results of 3-day batch experiments for PCE, TCE and 1,1,1-TCA are plotted in Figure 6, with the corresponding fit to a Freundlich isotherm. It is evident that TCA sorbs less that TCE, while PCE sorbs most strongly. The isotherms are fairly linear, as indicated by the exponent term being greater than 0.90. Linear fits resulted in K_d values of 0.42, 1.4 and 4.0 cm^3/g for TCA, TCE, and PCE respectively.

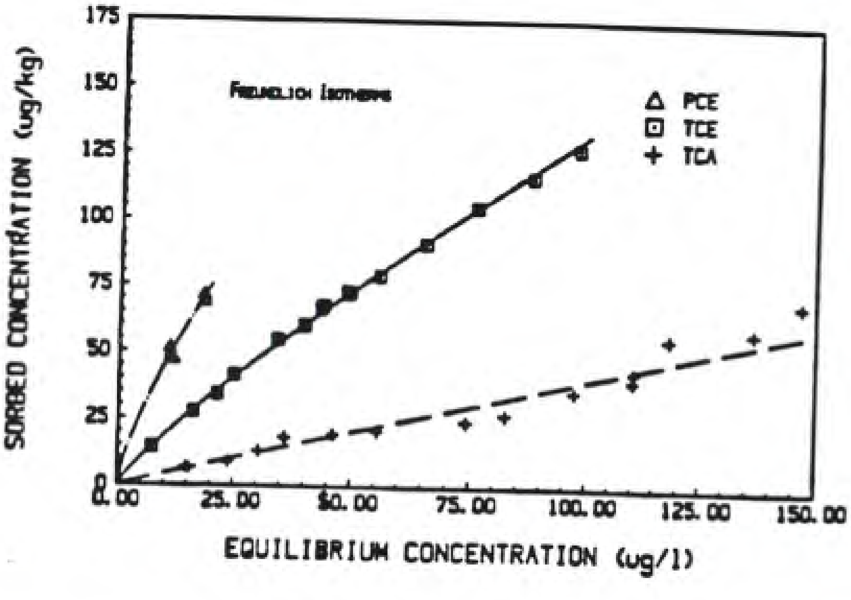

Figure 6. Freundlich isotherms for PCE, TCE and 1,1,1-TCA based on 3 day batch sorption experiments onto aquifer solids.

Estimates of the K_d values were made based on the empirical relationship of Karickhoff et al. (1979), where K_d is dependent on the fraction organic carbon of the solid and the sorbing solute's solubility in water. Solubility data tabulated in Horvath (1982) was used in the estimates. The organic carbon content of the bulk solids used in the sorption studies was 0.001. The estimated K_d values were 0.266, 0.318, and 1.06 cm^3/g for TCA, TCE and PCE, respectively. The measured values show a trend similar to that predicted from partitioning theory, with higher K_d values for the less soluble compounds. However, the estimated K_d values are consistently lower, by factors 1.6, 4.4, and 3.8 for TCA, TCE and PCE, respectively. Similar results were obtained for low carbon content materials by Curtis et al. (1986b) and Schwarzenbach and Westall (1981), who explained the larger measured partition coefficients in terms of sorption to mineral surfaces.

TABLE 6. MEASURED AND PREDICTED K_d VALUES FOR PCE, TCE, AND 1,1,1-TCA, AND ESTIMATED RETARDATION FACTORS.

Compound	Measured Sorption Coefficient K_d (cm^3/g)	Predicted Sorption^2 Coefficient K_d (cm^3/g)	Retardation^3 Factor R
TCA	0.42	0.27	2.5-3
TCE	1.4	0.32	6.5-8.5
PCE	4.0	1.06	17-22

1) based on measured linear sorption isotherm
2) based on the empirical correlation with water solubility of Karickhoff et. al. (1979) and the measured f_{oc} = 0.001
3) based on Eq 1. with p_b = (1.6-1.9 g/cm^3), and n = (0.3-0.4)

Estimates of the degree of retardation of the sorbing solutes relative to a nonsorbing solutes were made based on the retardation factor as described by Freeze and Cherry (1979), given by

$$R = 1 + p_b K_d / n \qquad (1)$$

where p_b is the bulk density of the aquifer material (g/cm^3); n is the porosity (cm^3/cm^3); and K_d is the equilibrium distribution coefficient. The estimated retardation factors are presented in Table 6. Based on these estimates, the movement of TCE through the test zone would be expected to be retarded by a factor of 6.5 to 8.5. This has important implications for the time required to establish steady-state concentrations during the tests, and the effect the sorption process may have on the biotransformation of the TCE.

SECTION 6

SITE INSTRUMENTATION

THE WELL FIELD

Figure 4 presents a vertical section of the test zone and the well field used in the experiments. The well field was designed to permit simultaneous experiments by creating two test zones through the injection of fluids at both the south (SI) and north (NI) injection wells, and extraction at the central extraction well (P). The operation of the extraction well is intended to dominate the regional flow field in the study area in an approximation of radial flow.

The injection wells are located 6 meters from the extraction well. The monitoring wells are located 1.0, 2,2 and 4.0 meters from the injection wells. This spacing should result in roughly equivalent fluid residence times between monitoring wells if radial flow conditions exist. The extraction and injection wells are constructed of 2" PVC wellstock which is slotted over a 5 ft screened section. The screened section is positioned 14 ft to 19 ft below the surface in order to fully penetrate the aquifer. After installation with a hollow stem auger, the borehole around the screened section was back filled with sand (Monterey #8). The monitoring wells are 1.75" O.D. stainless steel well casing with a 2 ft screen drive point (Johnson Wirewound #35 slot). The wells were installed with minimal disturbance of the aquifer by augering to within one foot of the aquifer top and hand-driving the wellpoint into the middle of the aquifer. The 2 ft screen section was placed to intercept what was considered to be the most permeable zone consisting of sands and gravels.

In order to prevent losses by volatilization and sorption, the fluid injection and sampling lines are 1/4 inch O.D. stainless steel tubing. The tubing runs from the well bottom to inside the control shed, with a maximum length of approximately 16 meters. The tubing has a series of orifices along the well's slotted interval, in order to collect a representative fluid sample from the formation.

THE AUTOMATED DATA ACQUISITION AND CONTROL SYSTEM

An automated data acquisition system has been devised at the site to implement the field experiments. The system permits the continuous measurement of the experiment's principal parameters: the concentrations of the bromide ion tracer, methane, halogenated aliphatic compounds of interest, and dissolved oxygen, as well as pH. The methods of analysis and the practical detection limit for each parameter under field conditions are summarized in Table 7.

A schematic of the system is shown in Figure 7. The system is driven by a microcomputer. A data acquisition and control program (DAC) has been designed and programmed that can be operated in either manual or automated mode. Manual mode permits selection of samples, creation of a sample sequence for automated operation, calibration of various instruments, and graphing the results as the sampling proceeds. During automated operation, the DAC selects the sample to be analyzed, opens the proper solenoid, and activates a peristaltic sampling pump, located in the control building. After withdrawing approximately 1.2 liters of

TABLE 7. METHODS OF ANALYSIS AND PRACTICAL DETECTION LIMITS FOR EACH PARAMETER UNDER FIELD CONDITIONS.

Parameter	Method	Detection Limit
Dissolved Oxygen	Probe	0.1 mg/l
pH	Probe	NA
Anions (Cl, Br, NO_3, SO_4)	Ion Chromatography	0.5 mg/l
Halogenated Organics (Freon 113, TCA, TCE)	Gas Chromatography-ECD	1.0 ug/l
Methane	Gas Chromatography-FID	0.2 mg/l

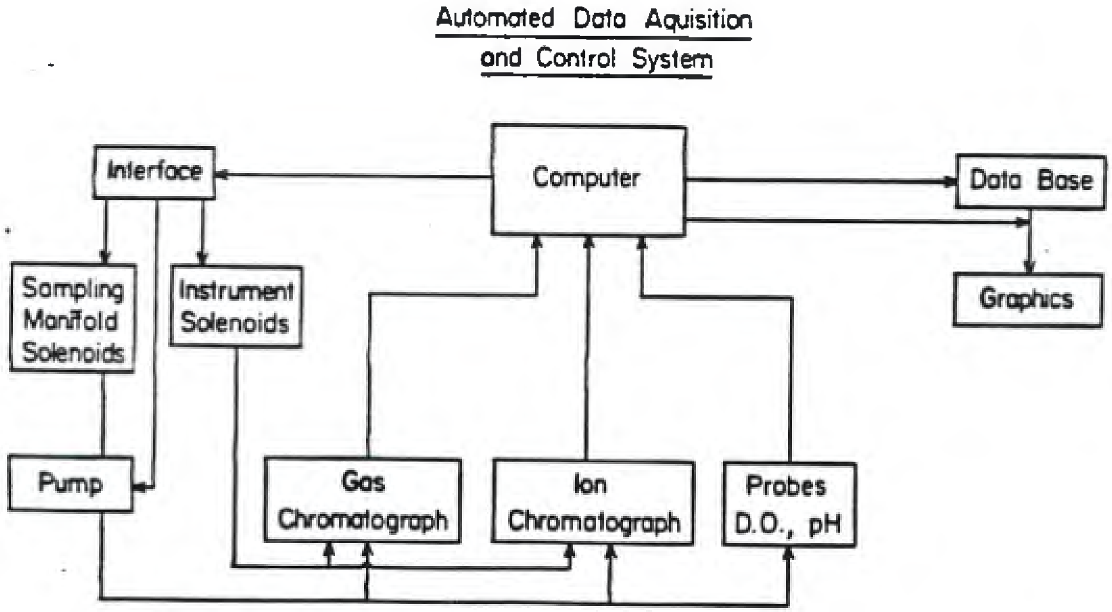

Figure 7 Schematic of the automated data acquisition and control system.

sample, pumping is stopped and samples are analyzed using the methods given in Table 7. After completion of the data acquisition cycle, the DAC integrates the chromatogram (in the case of ion and gas chromatography), calculates and stores the results, and proceeds with the next sample.

In order to realize real-time control and interpretation, measurements are made continually throughout a period of several weeks or months at a frequency corresponding to approximately two per hour. The sampling points are typically six in number, the injected fluid, extracted fluid, three intermediate monitoring points, and the effluent from the air stripper (for monitoring the groundwater discharged to a storm sewer). In order to obtain precise and reproducible measurements during an experiment, the instruments are calibrated daily.

A series of experiments were performed using the DAC system to study the transport characteristics of the test zone under a variety of flow conditions. Natural gradient tests were performed in order to estimate the groundwater velocity and direction at the site. Induced flow tests were performed, in which groundwater was injected and extracted, to study transport under conditions similar to those used in the biostimulation and biodegradation stages of the experiment. The DAC system was found to work reliably and generated more than enough data to observed the transient responses at observation locations. The results of these tracer tests will be presented in the following section.

SECTION 7

RESULTS OF TRACER TESTS

NATURAL GRADIENT TRACER TESTS

Two natural gradient tracer tests were performed, Tracer2 and Tracer3. The tests were performed as follows: a slug of 460 liters of bromide tracer was injected over a period of 3 to 4 hrs into a well along the main line of wells SI through NI, and then allowed to drift under natural gradient conditions. Responses at monitoring wells encompassed both the the breakthrough and the elution of the bromide tracer. In the Tracer2 test, well P was used to inject the tracer and wells N3, N2, and N1 were used as monitoring wells. In the Tracer3 test, well SI was used to inject the tracer, and all the wells along south to north legs were monitored.

The experiments indicated that the groundwater flow was primarily in a northerly direction. Figure 8 shows responses at the monitoring wells for the Tracer2 and Tracer3 tests, respectively. The response curves are skewed in shape, with a sharp rise in concentration followed by a gradual decrease, or tailing, to background concentrations. The areas under the response curves are shown to be reduced as the distance from the injection well increases, especially for the Tracer3 test. The maximum concentrations are significantly lower than the injected concentrations. The decrease in area with distance and the low maximum concentrations suggest either a flow direction that deviates slightly from being parallel to the line of the observation wells and/or a large amount of lateral dispersion.

Table 8 summarizes the results from the natural gradient tracer tests. The skewed shape of the response curves are indicated by the greater time associated with the center of mass of the response curves

TABLE 8. ESTIMATES OF REGIONAL VELOCITIES BASED ON THE RESULTS OF THE NATURAL GRADIENT TRACER EXPERIMENTS.

	Well	Distance from the Inj. Well (m)	Time Max Conc. (hrs)	Time Center of Mass (hrs)	Velocity[1] (m/hr)	Area Under Response Curve (mg-hr/1)
Tracer2	N3	2.0	8.8	17.9	0.11	1555
	N2	3.8	27.8	38.6	0.10	1059
	N1	5.0	32.8	50.5	0.10	1250
Tracer3	S1	1.0	16.4	32.9	0.7	3658
	S2	2.2	32.5	44.3	1.2	2131
	S3	3.8	12.9	20.0	4.8	1019

[1] Velocity based on center of mass

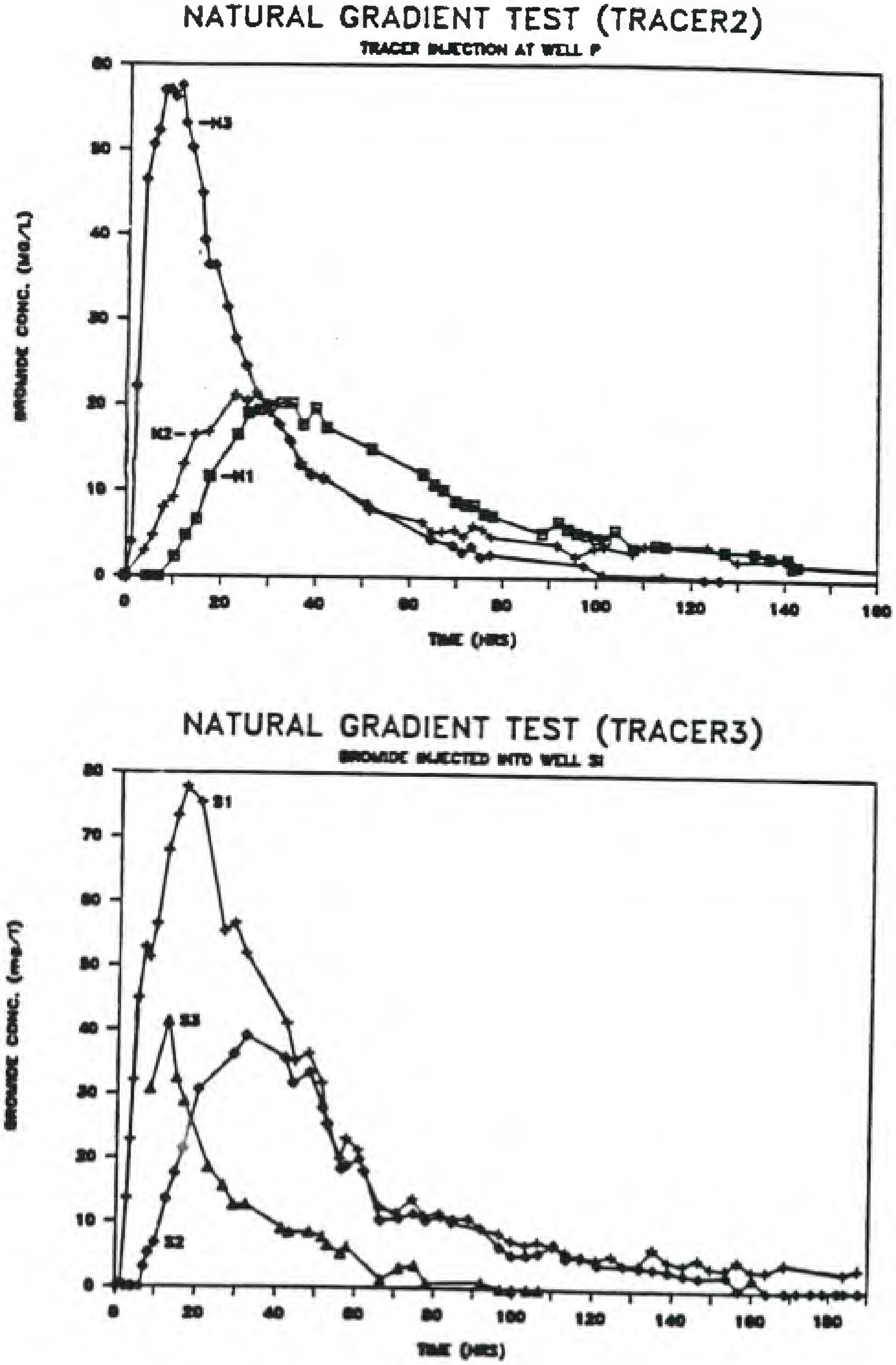

Figure 8. Results of the natural gradient tracer tests (Tracer2 and Tracer3)

compared to the time to the maximum observed concentration. The groundwater velocity estimates based on the time corresponding to the center of mass of the response curve are in good agreement for the Tracer2 test. An average value of 2.6 m/d was obtained. The results obtained from the Tracer3 test are more variable, with higher values obtained the farther the observation well is from the injection well. The higher velocities are seen to be associated with a decrease in area under the response curves.

The rapid transport in the test zone is typified by the initial response at the S3 monitoring well, which precedes that of the S2 well, even though the latter well is located closer to the injection well for this test. This earlier breakthrough is reproduced in all the tracer experiments performed to date. These data suggest that the aquifer is quite heterogeneous, with high permeability zones rapidly conveying the tracer to the distant wells, while the responses at observation wells closest to the injection well represent contributions from a range of permeability zones. The observation wells are not fully penetrating. Thus, if there is layering and vertical structure in the test zone, the monitoring wells may be sampling different zones, especially along the south experimental leg, where the variations in estimated velocity are great. The extensive tailing in the response curves would also suggest multi-permeability zones, as discussed by Moltz et al. (1986).

The results of the two natural gradient tests indicate a fairly high groundwater velocity at the site: approximately 2.4 m/d. The velocity is somewhat higher than the 1 m/d value obtained from the measured gradient and hydraulic conductivity estimated from pump tests, but nonetheless of the same order of magnitude. The hydraulic conductivity, however, is based on an aquifer thickness of 1.5 meter. If the thickness were less, higher estimates of groundwater velocity would result.

INDUCED FLOW TRACER TESTS

Two induced flow tracer experiments -- Tracer4 and Tracer5 -- were performed under the conditions used in the later evaluation experiments. The Tracer4 experiment studied the transport of bromide ion and dissolved oxygen through the test zone. The Tracer5 experiment studied the transport of bromide ion and TCE. The south experimental leg was chosen for the experiments, with fluid being injected into the SI well and extracted at well P. This configuration results in an induced gradient which is superimposed on the natural gradient, thus creating conditions for the effective capture of the injected fluid at the extraction well.

The induced-flow tracer tests were performed as follows: groundwater was extracted at a rate of 8 l/min and reinjected at a constant rate of 1 l/min and 0.6 l/min in the Tracer4 and Tracer5 experiments, respectively. The groundwater was air stripped prior to reinjection to remove background concentrations of organics and to oxygenate the groundwater to a DO concentration of 8 mg/l. Bromide (Tracer4) and bromide and TCE (Tracer5) were added to the air-stripped ground water to achieve the desired concentration and injected at a constant rate. The injection of tracers was performed as a broad pulse. Bromide was added at an average concentration of 120 mg/l for 107 hrs in the Tracer4 test and at a concentration of 230 mg/l for 250 hrs in the Tracer5 experiment. In the Tracer5 experiment TCE was injected concurrently with bromide at an average concentration of 160 ug/l for 350 hrs. The tracer breakthroughs as well as their elution from the test zone were continuously measured at the monitoring wells S1, S2 ,S3 and the extraction well.

Figure 9 shows the DO responses observed in the Tracer4 experiment. The data show a tightly spaced temporal response over four days, with approximately ten samples at each observation point per day. The results show a rapid breakthrough at the S1 observation well. The breakthrough at the S3 observation well, located 4 meters from the injection well, preceded that at well S2, 2.2 meters from the injection well, which indicates short circuiting of flow resulting from aquifer heterogeneities. Steady-state concentrations were achieved at S1 after a period of injection of approximately 50 hrs, while 80 hrs were required to achieved steady-state values at well S3.

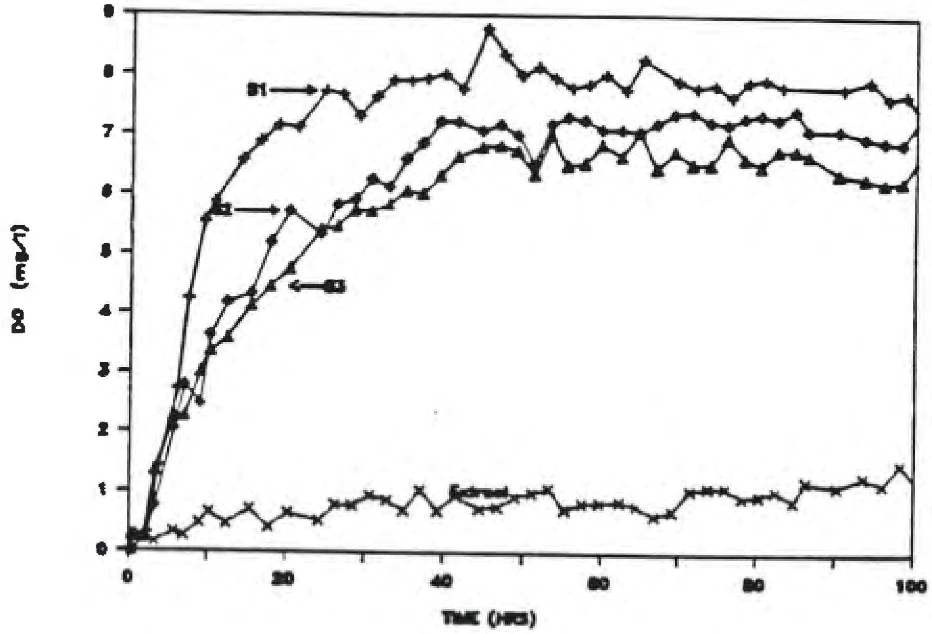

Figure 9. Response of DO at the observation locations in the induced flow tracer test (Tracer4).

The steady-state values show lower concentration values the greater the distance from the injection well. This probably results from one of the following factors: 1) a small degree of DO consumption along the flow path, or 2) some dilution of the injected water by native groundwater having a low DO concentration. The extraction well definitely shows the effect of mixing with the native groundwater, owing to the injection rate (1 l/min) being 1/8 of the extraction rate (8 l/min). The maximum extraction well concentration was approximately 12 percent of the injection concentration, consistent with dilution estimates. The breakthrough of DO at these concentrations at the extraction well indicated that little utilization of DO occurred during transport through the aquifer. Thus, the ability to transport DO through the aquifer, which is required during the biostimulation phase of the experiment, was demonstrated.

Figure 10 shows the bromide responses for the same experiment, normalized to the injection concentration. Both the initial breakthrough and the elution from the test zone after ceasing bromide injection are shown. The bromide breakthroughs have the same characteristics as the DO breakthroughs, discussed above. The decrease in steady-state bromide concentrations with distance from the injection well indicates dilution by the native groundwater. Thus, with the injection-extraction conditions used, the test zone was not being completely dominated by the injected fluid.

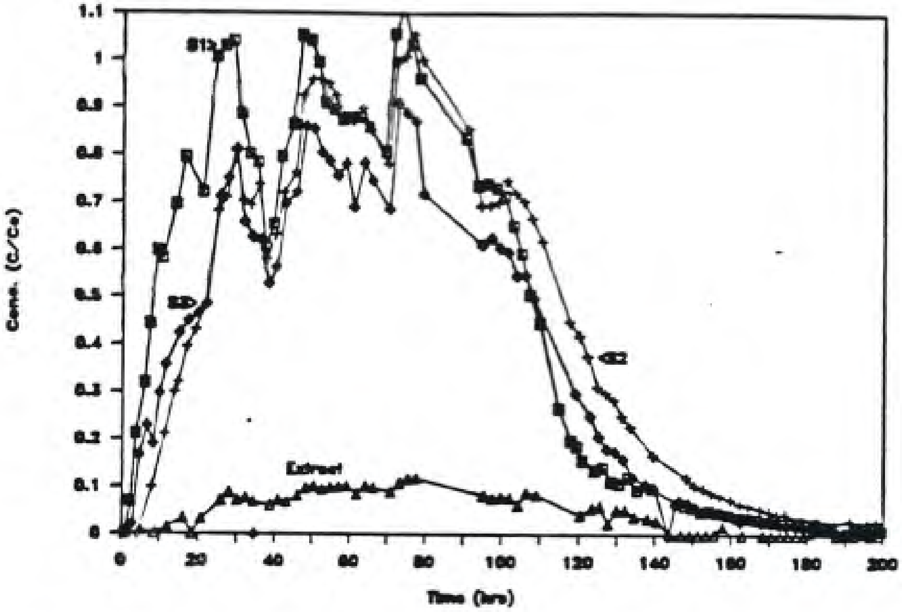

Figure 10. Normalized response of bromide in the Tracer4 test.

Figure 11 shows the response of both bromide and TCE at the S1 observation well, during the early stages of the Tracer5 experiment. The movement of TCE is shown to be retarded with respect to bromide, with a more gradual approach to the injected concentration. The shapes of the breakthrough curves do not conform to that predicted from transport theory for homogeneous media, assuming local equilibrium sorption. These observations suggest: 1) the influence of rate limitation effects on sorption, or 2) the effects of multi-conductivity zones in the aquifer. The observations during the elution phase of the experiment, after the TCE addition was stopped, show extended tailing as shown in Figure 12. The extended tailing is is another indication of the processes described above.

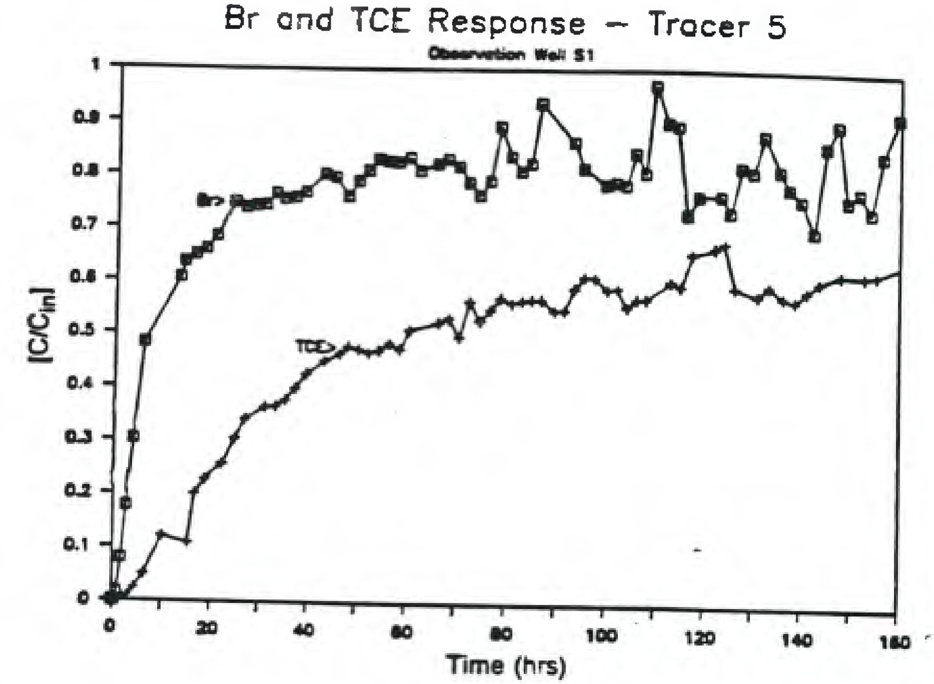

Figure 11. Normalized response of bromide and TCE at the S1 observation well in the Tracer5 experiment.

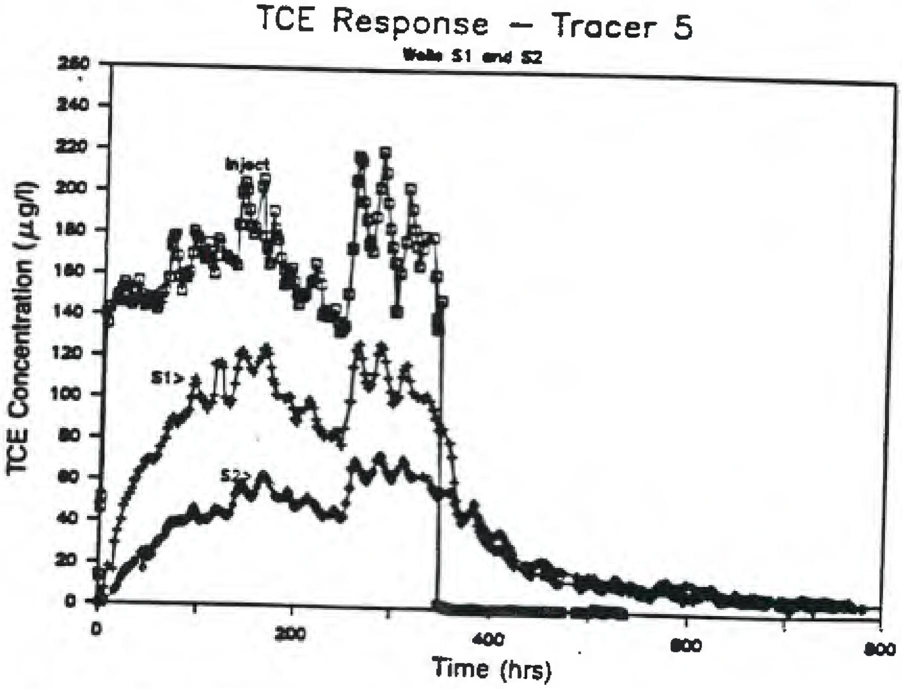

Figure 12. The TCE responses at observation locations in the Tracer5 experiment.

Mass balances based on the Tracer5 results indicated that 61 percent of the injected bromide was recovered by the extraction well, whereas 55 percent percent of the TCE was recovered, over the 800 hr period during which continuous observations were made. Due to the slow elution from the test zone, the total TCE recovered with continued pumping is probably equivalent to that of bromide. The mass balances indicate that the injected fluid is not completely recovered at the extraction well under the injection-extraction conditions used during the first year of the field testing. The recovery of the TCE is similar to that obtained for bromide, indicating that the loss of TCE results primarily from the flow conditions, and not degradation. Thus, the Tracer5 test serves as a pseudo control experiment to which the biotransformation studies can be compared.

Estimated Transport Times

The average fluid residence times from the injection to the observation wells and corresponding fluid velocities were estimated based on the results of the tracer experiments and the initial biostimulation experiment. The estimates are based on the time required to achieve 50% of the steady-state breakthrough concentrations. During the injection period of 350 hrs, the steady-state TCE concentrations, however, were not obtained, due possibly to the slow rate of sorption onto the aquifer solids. The long term steady-state fractional breakthroughs for TCE were therefore assumed equal to that achieved by the bromide tracer. Transport times were also estimated for TCA, based on its elution from the test zone during the Tracer4 experiment. The elution resulted since TCA is present as a background contaminant in the groundwater and air-stripped groundwater, such that the TCA concentration injected during the experiment was always lower than that of the native groundwater.

Table 9 presents the average residence times for the S1 and S2 observation wells. These two wells are presented since they will be discussed in most detail in the latter evaluation experiments, and the shape of their breakthrough response is relatively symmetrical such that the 50% value is fairly representative of the center of mass of the response curve. Several important transport characteristics are indicated by the results. The transport times and corresponding velocities are shown to be very reproducible from test to test. The average fluid residence times based on the bromide tracer are 7.3 hrs and 16.0 hrs from the injection well to the S1 and S2 observation wells, respectively. This corresponds to an average fluid velocity of 0.14 m/hr in both cases. Methane and DO analyses were found to yield similar residence time estimates as obtained using the bromide. This result indicates that these dissolved gases are easily transported through the test zone and are not retarded.

TABLE 9. RESIDENCE TIMES AND TRANSPORT VELOCITIES OF DIFFERENT COMPOUNDS IN INDUCED FLOW EXPERIMENTS.

Test	Compound	Obs. Well	Residence Time (hrs)	Velocity (m/hr)
Tracer4	Bromide	S1	7.6	0.130
Tracer5	Bromide	S1	7.7	0.132
Biostm1	Bromide	S1	6.7	0.149
Tracer4	Bromide	S2	17.9	0.122
Tracer5	Bromide	S2	14.8	0.148
Biostm1	Bromide	S2	15.4	0.143
Tracer4	DO	S1	7.2	0.138
Biostm1	Methane	S1	6.0	0.167
Tracer4	DO	S2	16.7	0.131
Biostm1	Methane	S2	16.1	0.136
Tracer4	TCA	S1	10.0	0.100
Tracer5	TCE	S1	42.5	0.024
Tracer4	TCA	S2	30.0	0.073
Tracer5	TCE	S2	156	0.014

The data for TCA and TCE indicate that these compounds are retarded. The residence times for transport from the injection well to the S1 observation well are 10 hrs and 42 hrs, for TCA and TCE, respectively, compared with 7.3 hrs for bromide. The resulting retardation factors are 1.4 for TCA and 5.75 for TCE. Estimates based on the S2 well data yield retardation values of 1.9 and 9.8 for TCA and TCE, respectively. The values are in good agreement with those predicted from the batch experiments performed in the laboratory (Table 6). The data show an increase in the retardation value, with a greater residence time in the test zone. This may result from a rate-limited sorption process as discussed by Roberts et al. (1986).

Summary of Tracer Test Results

The results of the tracer experiments indicate that reproducible transport experiments can be performed in the test zone. The fluid residence times in the test zone are fairly short, on the order of 8 hrs to the first observation well to 30 hrs at the extraction well. Owing to the high groundwater velocity under natural gradient conditions, longer transport times are not possible, since an extraction rate of at least 8 l/min is required to ensure effective recovery of the injected fluid at the extraction well. The tracer experiments indicated recovery of 60 to 75 of the bromide injected. TCE was recovered to the same degree as bromide, indicating negligible loss of TCE. There is some dilution of the injected groundwater by the native groundwater with the degree of dilution increasing with distance from the injection well. Evidence of aquifer heterogeneities were observed, for example, with tracer being rapidly transported to well S3. Observation wells S1 and S2 yield similar transport velocities and, based on modeling discussed in detail later, conform to the behavior expected for the case of an induced flow field superimposed upon a natural potential field. TCE was found to be retarded due to sorption onto the aquifer solids. The degree of retardation was within the range of values predicted based on batch sorption experiments with aquifer solids performed in the laboratory.

Modeling the Tracer Test Results

Preliminary modeling of the results of the tracer experiments has been performed using 1-D and 2-D models. The semi-analytical model, RESSQ, developed by Lawrence Berkeley Laboratory and described by Javandel, Doughty, and Tsang (1984) was used to simulate 2-D advective transport under the injection, extraction and natural gradient conditions of the tracer experiments. 1-D analytical solutions were used to estimate dispersion coefficients and to determine if a 1-D modeling approach could be used in the development of a numerical model to simulate the biostimulation and biotransformation processes.

The RESSQ model was used to estimate: 1) the areal extent of the injected fluid front that develops around the injection well and observation wells, 2) the fluid residence times from the injection well to the observation wells, and 3) the degree of recovery of the injected fluid at the extraction well.

Simulations were performed to illustrate the original design of the well field to permit simultaneous experiments along three experimental legs. The model input parameters were: fluid injection at a rate of 0.5 l/min at three wells; an extraction rate of 8 l/min; no regional flow velocity; a porosity of 0.35; and an aquifer thickness of 1.2 meters. Figure 13 shows the results of the simulations. An injected fluid front of uniform size develops around each of the three experimental legs. The maximum width of the front is approximately 1.6 meters around the S1 and S2 observation wells.

Figure 14 shows the fronts that develop when a regional groundwater velocity of 300 m/yr in a northerly direction is imposed on the simulation discussed above. The front around the east injection well is shifted northward due to the groundwater flow. The regional flow also results in a thinning of the front along the southern leg and a broadening along the northern leg. These results indicated that the southern leg (SI,S1,S2,S3) should be used in the experiments for the following reasons: 1) the injected fluid supplying the nutrients becomes less dispersed, and hence a more dense microbial population can be stimulated, and 2) by injecting upstream of the natural groundwater flow, the injected tracers and chlorinated hydrocarbons can be most effectively recovered at the extraction well. The area dominated by the injected fluid does become smaller, however, which helps explain the dilution of the injected fluid by the native ground water that was observed in the tracer experiments.

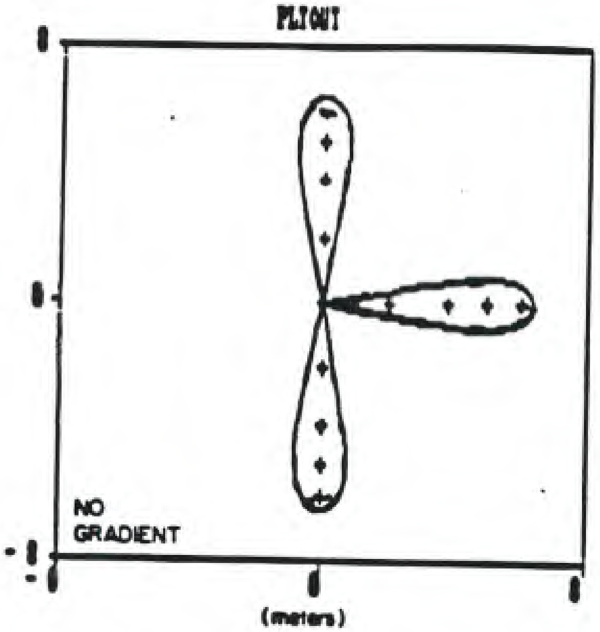

Figure 13. RESSQ simulations of the injected fluid fronts which develop under induced flow conditions of the tracer experiments with no regional flow.

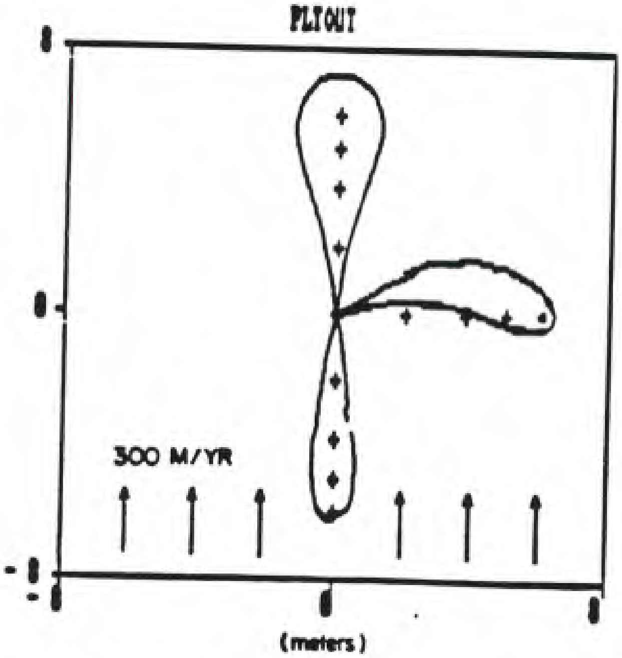

Figure 14. RESSQ simulations of the injected fluid fronts which develop under induced flow conditions of the tracer experiments with a regional flow of 300 m/yr.

Simulations were performed with the RESSQ model to determine whether the predicted fluid residence times are in the range of values estimated by the tracer tests. The model predicted fluid residence times of 8 hrs and 21 hrs for wells S1 and S2, respectively, in fairly good agreement with the Tracer4 test values of 7.6 and 18 hours. Aquifer properties used in the simulation were a regional fluid velocity of 300 m/yr, a porosity of 0.35, and an aquifer thickness of 1.25 m, which are in good agreement the measured and estimated values. The simulations indicate that the injected fluid should be totally captured by the extraction well under these conditions. The tracer tests, however, indicated that only 70 percent of the bromide was captured. The reason for this lower degree of capture is not known, but heterogeneities in aquifer properties are likely responsible.

The simulations indicate that the region near the injection well does not conform to uniform flow, but that the flow is fairly uniform at distances more than approximately 0.5 meters from the point of injection, and hence in the region of the observation wells. To determine the degree of dispersion required to fit the observed breakthrough response at the S1 and S2 wells, 1-D simulations were performed. The non-linear least squares fitting program described by van Genuchten (1981) was used in fitting the data to the solution to the 1-D convective-dispersive transport equation.

Figure 15 shows the fit to the DO breakthrough response at the S-2 observation well in the Tracer4 experiment. A fairly good fit is

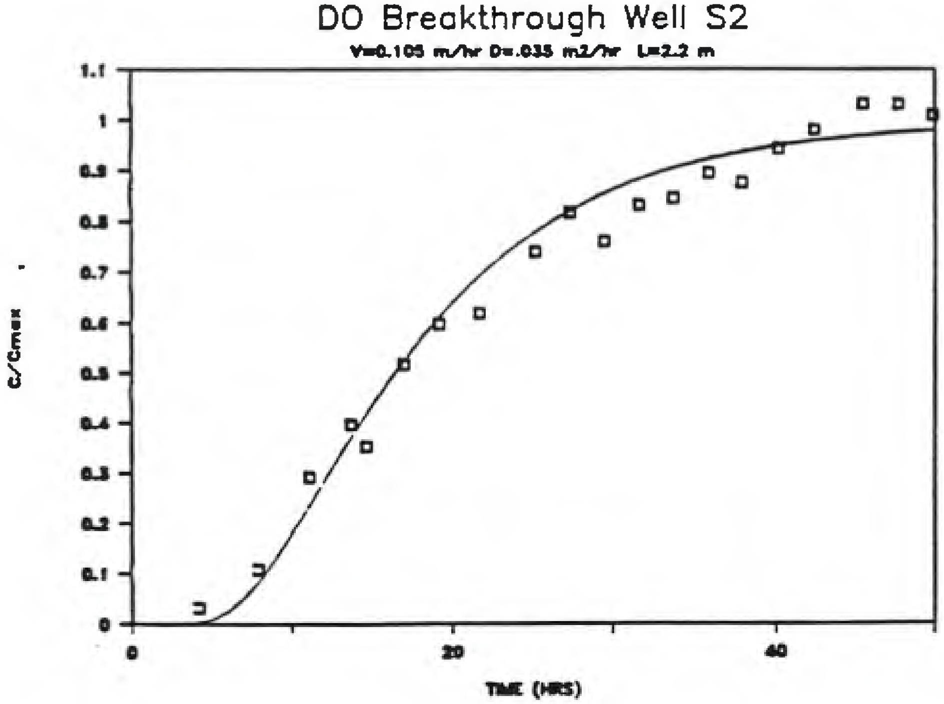

Figure 15. Fit of the 1-D advective-dispersion transport model to the breakthrough of DO at the S2 observation well during the Tracer4 test.

obtained with the 1-D model, with a resulting Peclet number (Pe) of 6.6, which corresponds to an aquifer dispersivity of 0.33 m (Length/Pe). Model fits to the different experiments and for Br, DO, and methane were performed for the S1 and S2 wells. The best-fit Peclet number based on the S1 well ranged 2.7 to 4.0, with an average value of 3.1. The values based on the S2 well ranged from 3.4 to 6.6 with an average value of 4.4. The resulting average dispersivities were 0.32 and 0.50 meters based on the S1 and S2 wells, respectively. The 1-D analysis resulted in best fit dispersivity values that are in a similar range from the analysis S1 and S2 data. The results indicate that 1-D transport modeling is of value in the initial stages of experimental design and data interpretation, when complex biostimulation and biotransformation processes must be taken into consideration. A more detailed analysis of the tracer data is currently being performed with more complex 1-D and 2-D transport models.

PULSED INJECTION

To enhance the effectiveness of biostimulation, it was decided to introduce the methane (primary substrate) and oxygen (electron acceptor) as alternating, timed pulses. This decision was reached based upon consideration of two crucial requirements: 1) the need to avoid clogging of the injection well and borehole interface, and 2) the need to achieve as uniform a distribution of microbial growth as possible throughout a substantial portion of the aquifer. Failure to fulfill the first requirement would cause loss of hydraulic capacity and premature termination of our experiments, as the drastic chemical measures such as chlorination or strong acid treatment that are customarily employed to rejuvenate clogged wells would interfere with biostimulation. Failure to satisfy the second requirement would lead to conditions of extremely high microbial densities near the injection point and low bacterial populations elsewhere, which would not be conducive to secondary substrate utilization as needed to degrade halogenated aliphatic compounds by methanotrophs. It was thought that introducing the two essential additives, methane and oxygen, as alternating timed pulses would assure their separation in the injection well and borehole, thus discouraging biological growth in that critical region. The methane and oxygen would then mix gradually, owing to the action of hydrodynamic dispersion and associated mixing processes, during transport through the aquifer, stimulating the growth of methanotrophic bacteria over the zone in which mixing occurs. In designing the pulsed injection system, two important variables had to be selected: 1) the ratio of the individual pulses of methane and oxygen, and 2) the overall pulse length.

The ratio of the individual pulses of methane and oxygen can be estimated approximately from knowledge of the stoichiometry of methane oxidation. The oxygen requirement for complete oxidation of methane is 2 moles oxygen per mole methane, which corresponds to a mass ratio of 4 g O_2 per g methane. In choosing the pulse lengths, the concentrations achieved by the saturation columns for oxygen and methane also must be taken into account.

The overall pulse length was evaluated by employing a transport model that incorporates a periodic input (Valocchi and Roberts, 1983). The form of periodic input that corresponds most closely to the case of alternating inputs of methane and oxygen is the rectangular pulse, or saw-toothed wave. The model of Valocchi and Roberts (1983) takes into account the effects of advection, dispersion, and sorption on transport and mixing of rectangular pulses under conditions of uniform flow. Although the situation at the Moffett field site certainly differs appreciably from the simple case of uniform flow in a homogeneous medium, the model computations based on the idealized case are instructive in exploring the effects of pulse length on mixing, and serve as a point of departure for experimental design.

In the absence of reaction, the normalized amplitude ratio is the most convenient measure of the degree to which the pulses remain separated during transport, or conversely the degree to which mixing has occurred. The amplitude ratio is the ratio of observed magnitude of concentration fluctuations measured at an observation a distance X removed from the injection point to the magnitude of the fluctuations measured at the injection point. The amplitude ratio varies from zero to unity: a value near unity means that concentration fluctuations are damped nearly completely, and signifies virtually complete mixing over the distance traversed, whereas a value near zero implies negligible mixing.

Model computations were conducted under conditions simulating those at the Moffett site. The important variables were the integral distance, x; the pore water velocity, u; and the Peclet Number for dispersion, Pe. The values for the simulation were chosen as x = 1 m, u = 0.12 m/h, and Pe = 5 (dimensionless), to correspond to the results at the nearest observation, S1, based on the results of the early tracer tests., i.e., the dissolved oxygen breakthrough in the initial stages of the Tracer4 set. The computation's results (Figure 16) indicated that substantial mixing over a transport distance of 1 m (the distance from SI to S1) would be attained using a pulse length on the order of several hours, and that pulse lengths on the order of several tens of hours would prevent adequate mixing prior to the first observation well.

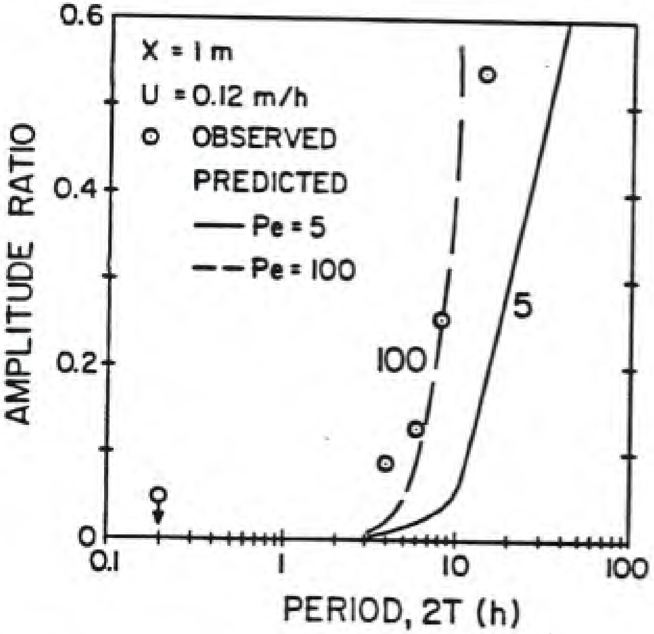

Figure 16. Comparison of predicted and observed effects of dissolved oxygen pulsing

To test the model, toward the end of the Tracer 5 experiment the dissolved oxygen injection was switched to an on/off mode, with pulse lengths chosen to span the range of potential choices for experimental operation, i.e., less than one hour to 12 hours. The observed values are shown in Figure 16 as open circles.

The observations show qualitatively the kind of trend predicted by the model: with short pulses (<1 hour), the mixing is complete within the first meter, but, as the pulse period is increased beyond a critical value of several hours, substantial concentration fluctuations begin to appear at the observation well, indicating that mixing is less than complete. The prediction does not agree quantitatively with the data, as the onset of substantial observed concentration fluctuations occurs at a lower critical value of the pulse period. Indeed, the value of the Peclet Number must be chosen as 100, rather than the observed value of 5, to simulate the pulsing data satisfactorily. These deviations may well be caused by deviations from the model assumptions of uniform flow in a homogeneous medium. Nonetheless, the qualitative agreement between predicted and measured values for the effect of pulsing was deemed adequate as a framework for experimental design of the biostimulation phase.

SECTION 8

BIOSTIMULATION AND BIOTRANSFORMATION EXPERIMENTS

The biostimulation and biotransformation experiments in the 1986 field season were conducted in two stages. First, the test zone was biostimulated by the pulse injection of methane and oxygen into the test zone. After the zone had been successfully stimulated, TCE was continuously injected as previously discussed.

Figure 17 shows the injection system used in the biostimulation and biodegradation experiments. The system uses two counter-current columns to sorb the methane and oxygen to approximately 80 percent saturation,

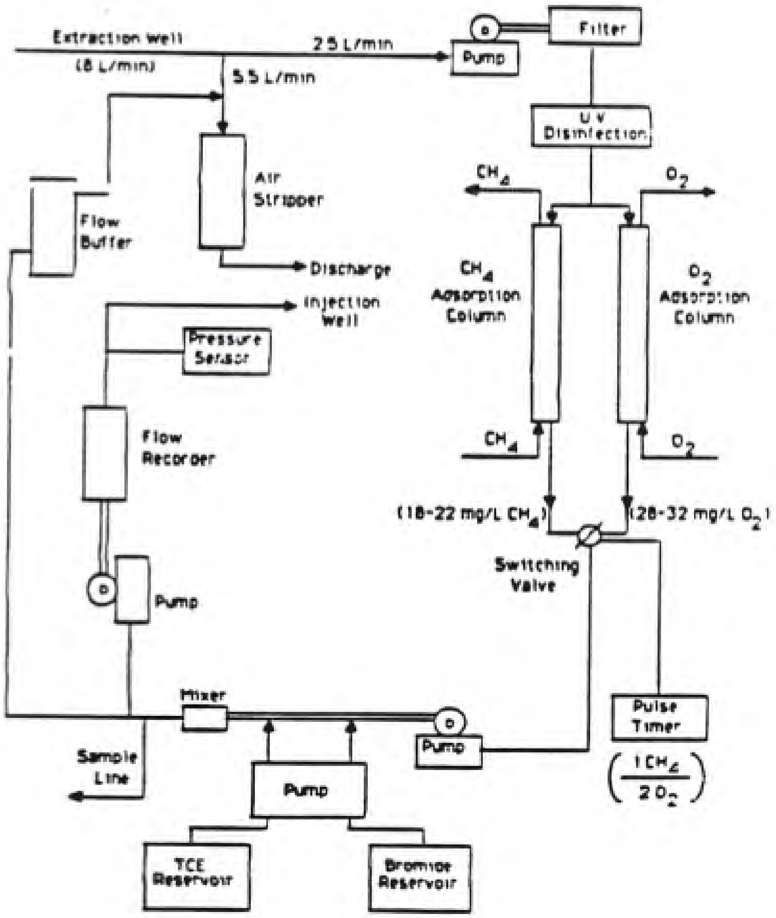

Figure 17. Schematic of the injection system used in the biostimulation and biodegradation experiments.

resulting in concentrations that are approximately 20 mg/l for CH_4 and 32 mg/l for DO. These solutions are alternately pulsed, with a pulse time ratio of about 2:1 (methane:oxygen), based on the stoichiometric requirements. A pulse timer permits the ratio and the length of the pulses to be varied. The other components of the injection system permit the accurate and continuous addition of the bromide tracer and TCE into the injection stream, the monitoring of the injection rates, and the sampling of the injection fluid, while maintaining a constant rate of injection.

BIOSTIMULATION EXPERIMENT

The biostimulation experiment was performed under same induced flow conditions as the earlier tracer tests. The pulse cycle for the injection of either methane or oxygen containing groundwater was varied during the course of the experiment, from less than 1 hr during start-up to ensure the pulsing would not interfere with growth, to a 12 hr period during the later stages to distribute growth in the test zone. No additional nutrients were added to the groundwater.

Figure 18 shows the dissolved oxygen concentration as a function of time at the three observation wells and the extraction well. The consumption of oxygen increases with time, indicating the stimulation of a microbial population. During the initial stages of the experiment (0-50 hrs) there is little evidence of oxygen consumption. The maximum DO concentrations of 19, 17, and 14 mg/l at wells S1, S2, and S3, respectively, result from the combined effects of the pulsed injection of DO water and dilution by the native groundwater. At this stage of the experiment, the microbial population in the test zone was sufficiently low so that the DO consumption was not observable.

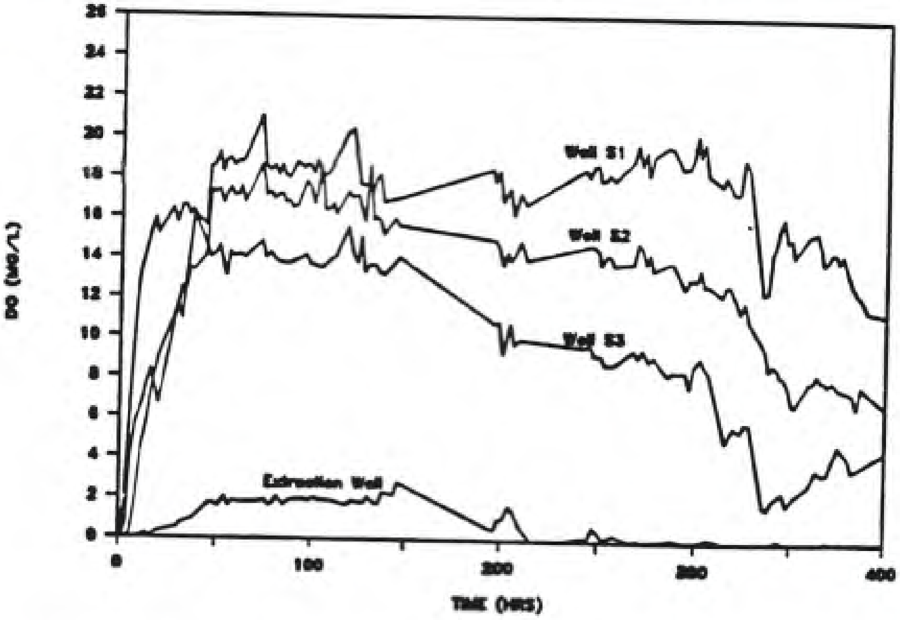

Figure 18. DO response during the biostimulation experiment.

The first signs of consumption were observed in the extraction well and the S3 observation well after approximately 200 hrs of injection. The concentration at the extraction well decreased below the detection limit after 300 hrs of injection. Owing to the continuous removal by microorganisms, the decrease in DO was greater the longer the travel paths through the aquifer. As time proceeds, the increase in the growth of microbial population throughout the test zone results in an increase in the DO consumption along the flow path.

The methane response was similar to that observed for the DO, which is expected, as methane is the electron donor and oxygen the electron acceptor for microbial growth. Figure 19 shows the response of the methane and DO at the S2 observation well. The fairly rapid decrease in the methane concentration over the period of 200 to 400 hrs indicates fairly rapid growth kinetics typical of aerobic microorganisms. A significant amount of methane substrate is also incorporated into cells. Based on the concentration values during the period of 350 - 375 hrs, the ratio of oxygen to methane consumed was 2.25 mg O_2/ mg CH_4, which is significantly lower than the ratio of 4 required for complete oxidation. The lower value suggests incorporation of the methane substrate into the cell mass, with a yield coefficient of approximately 0.5 mg cells per mg CH_4, in the range values for methane-utilizing bacteria reported by Anthony (1977).

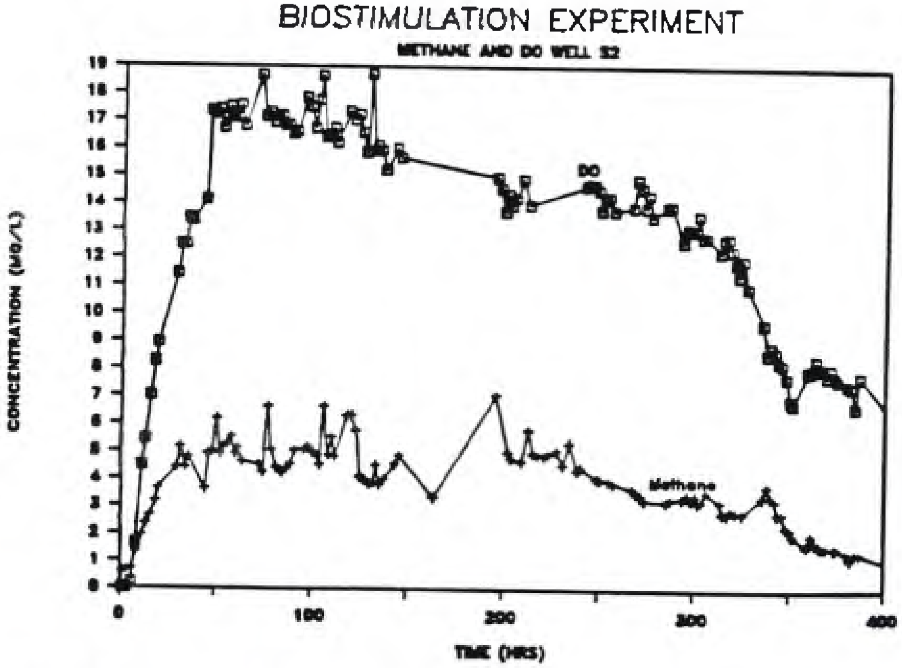

Figure 19. The response of methane and DO at the S2 observation well during the biostimulation of the test zone.

After 450 hrs of injection, the methane concentration at the S2 observation well decreased below the detection limit, indicating that the microbial mass was increasing near the injection well. The pulse cycles were therefore lengthened to 12 hrs in order to prevent biofouling near the wellbore. Figure 20 shows the response of the system to the pulsing at the S2 observation well. Peak methane values are shown to increase from below detection to approximately 1mg/l, as a result of the longer pulse duration. Peak methane concentrations are noted to occur when minimum DO concentrations are observed, which is anticipated based on transport theory.

Long pulse cycles were continued throughout the biostimulation and biodegradation experiments, with durations ranging from 6 hrs to 12 hrs. Based on continued methane breakthrough at the observation wells, the pulsing is believed to have helped to distribute the microbial population in the test zone and prevented biofouling of the aquifer.

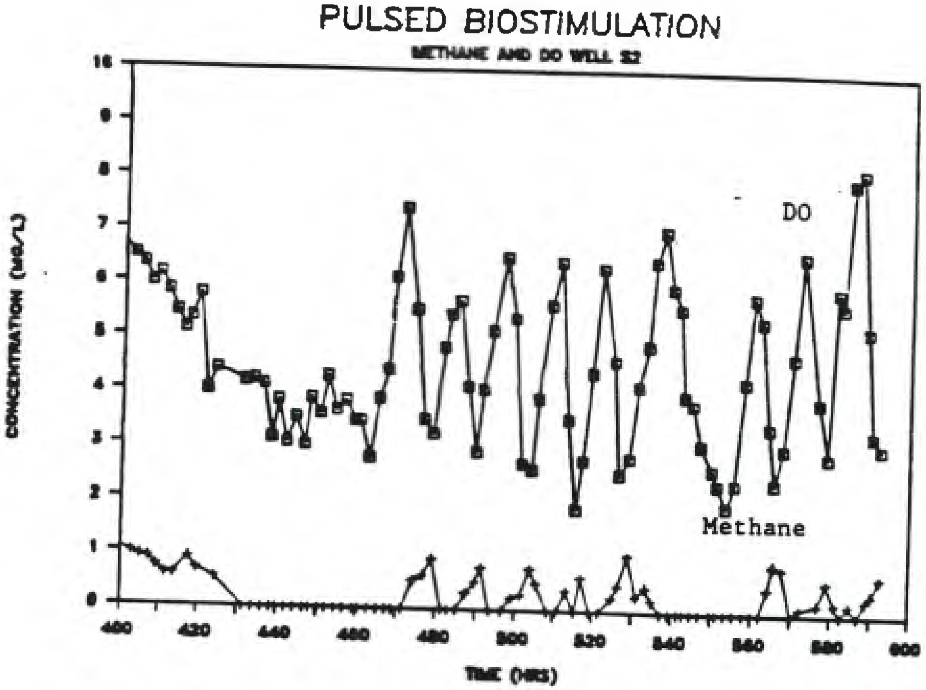

Figure 20. The effect of long term pulsing of DO and methane on the response at the S2 observation well.

The biostimulation experiment demonstrated that methane-oxidizing bacteria could be successfully established in the test zone. No additional nutrients were required to stimulate growth. The transient methane and DO responses indicated that a population was stimulated which grew closer to the injection well with time. The response indicates that microorganisms have fairly rapid growth kinetics, typical of aerobic organisms. Thus, pulsing was required to distribute the growth in the test zone and to prevent biofouling of the aquifer.

BIOTRANSFORMATION EXPERIMENTS

Biotransformation experiments were performed after the test zone was biostimulated. TCE was continuously injected over a three month period. During the initial stages TCE was injected at an average concentration of 100 ug/l. During the later stages, the concentration was lowered to 60 ug/l. Methane and oxygen (no nutrients) were continuously pulse-injected during this period to support the methane-oxidizing microorganisms which had been biostimulated.

During the initial phase of the experiment, the TCE concentrations slowly approached steady-state values. The normalized breakthroughs are presented in Figure 21. The response at the S1 well is very similar to that observed in the Tracer5 experiment, shown in Figure 11. Both experiments show fractional breakthroughs of approximately 60 percent of the injection concentration after 100-150 hrs of injection. The similar response indicates that little degradation of TCE was occurring in the biostimulated zone, within 1 meter from the injection well. This is further supported by the long term steady-state values of 80 percent of the injected concentration that are obtained after 400 hrs of injection.

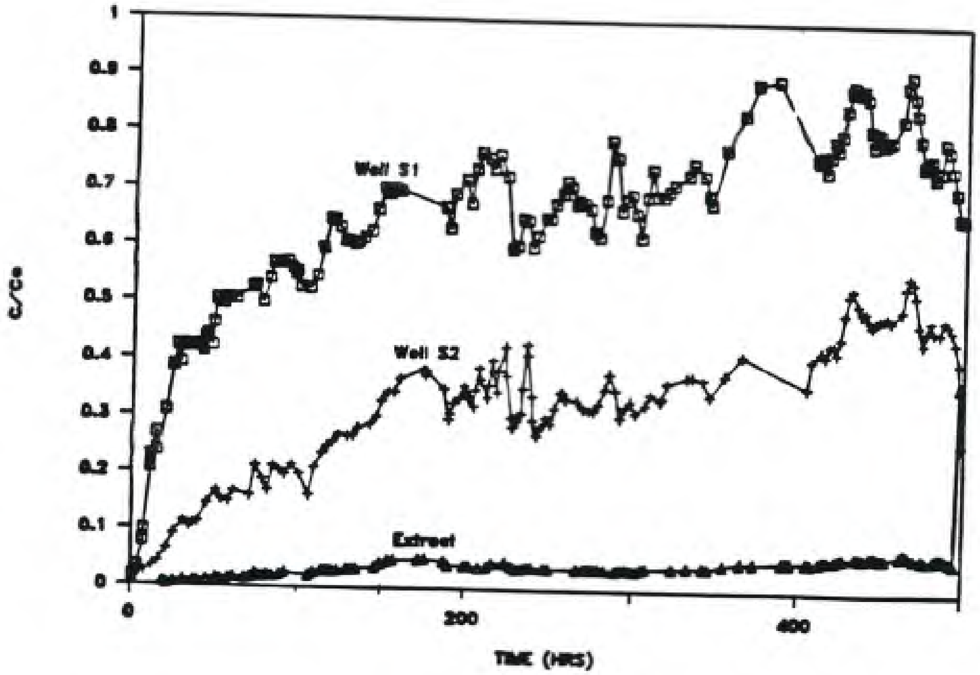

Figure 21. Normalized breakthrough of TCE at observation locations during the initial stage of the biotransformation experiment.

The breakthrough at the S2 observation well indicates that some degradation was occurring during the early stages of TCE addition. In the pseudo control experiment (Tracer5), the TCE reached approximately 30 percent of the injected concentration at the S2 well after 100 hrs of addition, while in the biostimulation experiment the concentration reached only 20 percent. The early breakthrough results indicated that degradation may be as high as 30 percent.

Comparisons of mass balances of the amount injected and extracted in the two experiments also suggests some degree of degradation was occurring during the early stages of the biodegradation experiment. In the Tracer5 experiment, 2.24 g of TCE were injected over a 338 hrs period, of which 0.9 g were recovered over the same period, representing a recovery of 39 percent at the extraction well. In the biotransformation experiment, 2.09 g of TCE were injected over a 347 hr period, of which 0.53 g were recovered in the extracted water, representing a recovery of 25 percent. The 11 percent lower recovery, or 35 percent on a relative basis, indicates that partial degradation occurred.

Toward the end of the biotransformation experiment, the TCE injection concentration was lowered from 100 to 60 ug/l, to ensure that no sorptive losses of TCE onto the aquifer solids would occur and that maximum steady-state concentrations were being achieved. This permitted an estimation of the degree of degradation based on the steady-state concentrations of TCE compared to bromide, a non-reactive conservative tracer.

Figure 22 shows bromide tracer results during a period when TCE concentrations were at steady-state (Figure 23). The fractional breakthroughs of bromide are shown to be significantly higher than those obtained by TCE. This comparison indicates that the lower normalized concentration of TCE results from degradation.

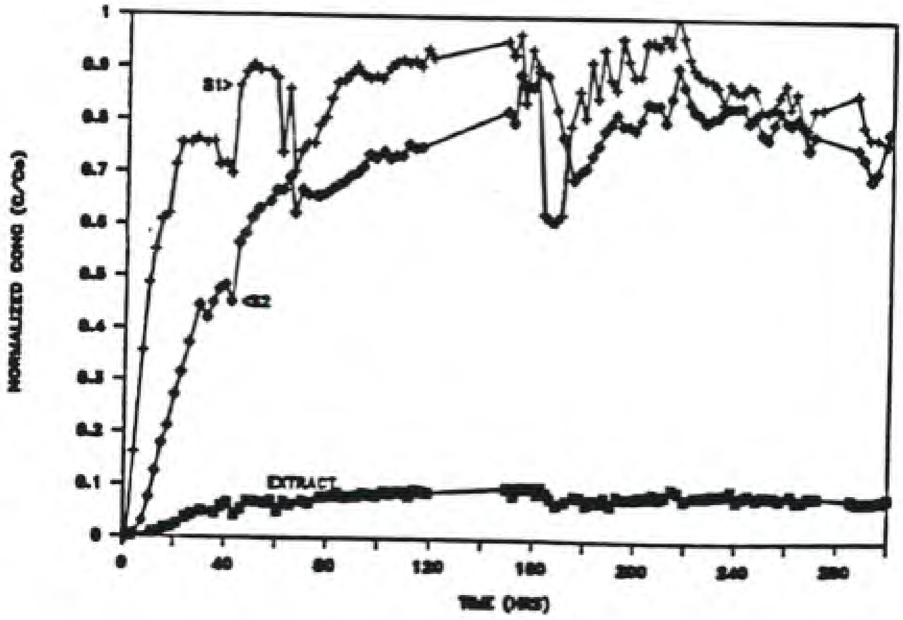

Figure 22. Normalized bromide tracer response during a steady-state period of the TCE biotransformation experiment.

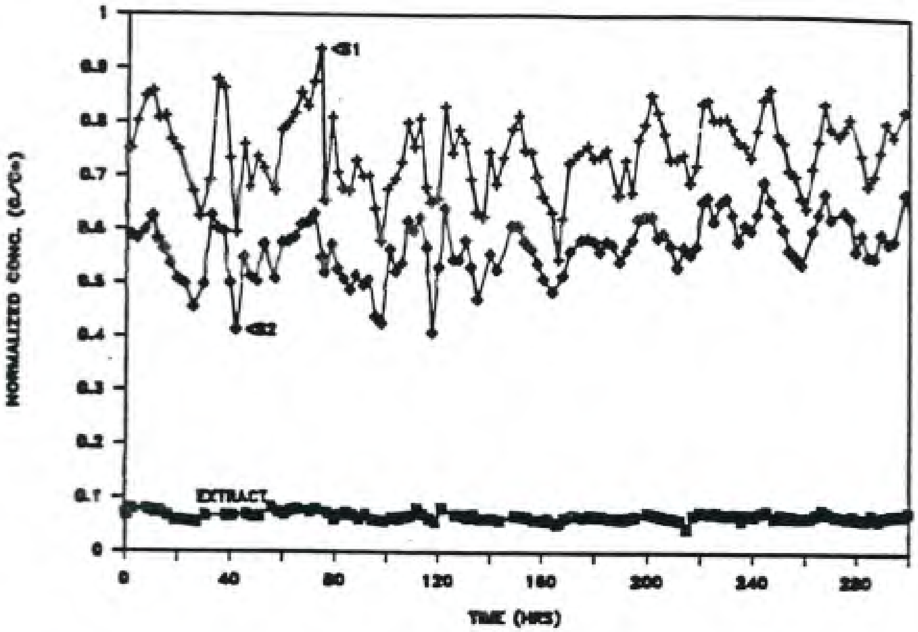

Figure 23. Steady-state TCE concentrations corresponding to the same time period as the bromide data in Figure 22.

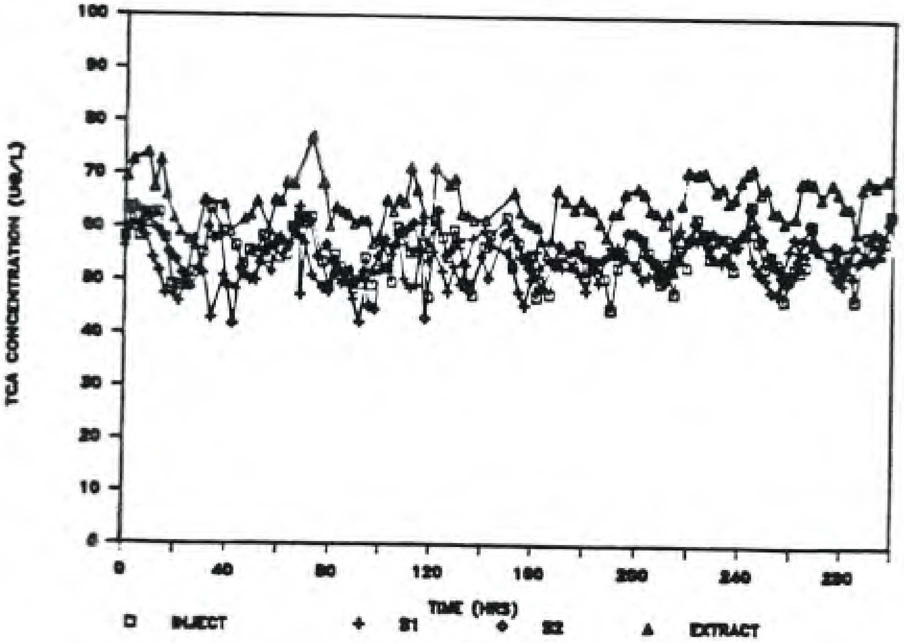

Figure 24. Steady state TCA concentrations corresponding to the same time period as the bromide and TCE data in Figures 22 and 23.

The concentrations of TCA during this steady-state phase of the experiment are presented in Figure 24, permitting comparison with the TCE results. TCA is present as a background contaminant, as indicated by the extraction well concentration which has an average concentration of 65 ug/l. The concentrations in the injected fluid and at the S1 and S2 monitoring wells are essentially equal with average values of 55.6, 53.3 and 55.3 ug/l, respectively. Little biotransformation of TCA occurred during transport through the test zone. After normalizing for the degree of mixing of the injected fluid with the native fluid, over 95% of the estimated TCA concentration was observed at the S1 and S2 monitoring locations.

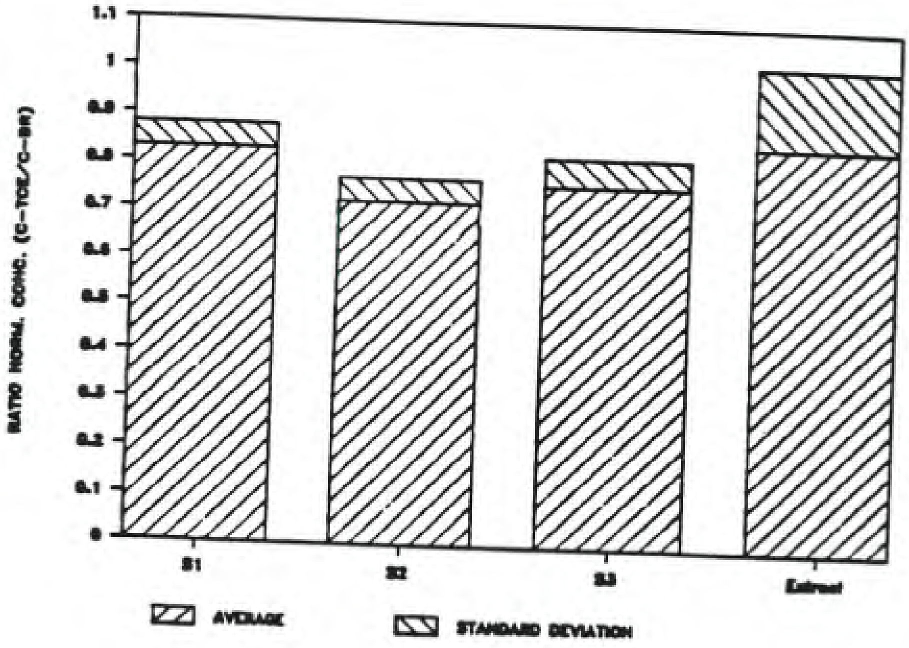

Figure 25. Estimated TCE biotransformation based on comparisons with bromide as a conservative tracer.

Figure 25 represents a summary of these experiments, where the fractional breakthroughs of TCE relative to bromide ion (TCE/Br) at the observation wells are compared. The ratios range from 70 percent to 80 percent, indicating a maximum degree of degradation of 30 percent. Degradation is noted to occur in the area of the test zone in which methane is present to support the methane-oxidizing bacteria. In the area between S2-S3 and the extraction well, no methane is present to support the bacteria, and accordingly no additional degradation of TCE is observed.

The degree of degradation indicated in this experiment is consistent with the earlier results, conducted approximately 100 days earlier. This suggests that the extent of degradation did not increase with time. Thus, acclimation to TCE, resulting in an enhancement in biodegradation, was not observed in the experimental period of 100 days.

The different methods of assessing the degree of degradation -- including mass balances, comparison of TCE breakthrough concentrations with the pseudo control experiment, and comparisons with bromide concentrations at steady-state within an experiment -- yield similar estimates of the degree of TCE degradation in the test zone. The degree of degradation is in the range of 20 to 30%. The results demonstrate that, if sufficient care is taken in obtaining the experimental data, quantitative evidence of degradation can be obtained in a real aquifer situation.

Discussion

The degree of TCE transformation observed in the test zone during the first field season is less than complete, and indeed barely within our ability to reach quantitative conclusions. Several factors may have contributed to the limited degree of transformation:

1) low solubility of methane and oxygen in water limits the TCE-degrading microbial population which can be stimulated under saturated conditions.

2) methane and TCE compete for the methane monooxygenase enzyme, such that the presence of methane can inhibit TCE degradation.

3) the high degree of chlorine substitution in the TCE molecule leads to a relatively low rate of aerobic degradation, relative to the rate of methane utilization.

4) extended acclimation to TCE is required before degradation begins to occur at a rapid rate.

5) minor nutrients are required for effective degradation.

6) the bacteria that were stimulated were not of the type that effectively degrades TCE.

7) the sorption of TCE onto the aquifer solids affects the rate of transformation.

The first three factors appear to be most important, based on the results of other studies. In the experiments of Wilson and Wilson (1985) discussed earlier, an unsaturated medium was exposed to a constant atmosphere of about 0.6% methane. A constant flux of methane was available to develop a high microbial population of methane-oxidizing bacteria. The population, however, developed under conditions of low methane concentrations in solution due to the low partial pressure of methane in the gas phase. Thus, the competition between methane and TCE for the methane monooxygenase enzyme was limited. In these experiments, the high microbial population and the lack of inhibition may have resulted in the high degree of degradation achieved.

Based on several experimental studies, Wilson and White (1986) developed an empirical correlation which relates the degree of TCE transformation to the amount of methane consumed. Based on their correlation, a reduction in TCE concentration of 35 percent is predicted when 6 mg/l of methane is consumed, the amount present in the biostimulation experiments. The estimate is in good agreement with the field results and indicates that the degradation is limited by the amount of methane and oxygen which can be delivered under saturated conditions.

TCE may have slow degradation kinetics, owing to its high degree of substitution by chlorine. Studies of Fogel et al. (1986) and Henson et al. (1987) indicate increasingly rapid disappearance of chlorinated aliphatics with decreasing degree of chlorine substitution. Recently Vogel et al. (1987), and McCarty (1987) indicated that the rate of degradation under aerobic or anaerobic conditions is related to the degree of oxidation of the compound. Vogel et al. (1987) indicate that, based on the available data, the rate of oxidation is higher for compounds containing fewer chlorine atoms per carbon atom, while for anaerobic conditions the reverse is true. Thus vinyl chloride and DCE would be expected to degrade faster than TCE or PCE. Also, the lack of degradation of TCA is consistent with the laboratory results of Henson et al. (1987), as well as with the arguments of Vogel et al. (1987).

Insufficient data are available to determine if acclimation to TCE is required to achieve effective degradation. The test zone was not previously contaminated with TCE. The test zone was contaminated with TCA, however, for which no degradation was observed. The results indicate that the rate of degradation is more highly dependent on structure than acclimation. TCE degradation has been observed in the laboratory cultures isolated from sediments not exposed to TCE. Whether a previous exposure to the chemical of interest has an effect on the rate of transformation is not clear at the present time.

Not enough is currently known about the final three possibilities listed above to determine their relative importance. Research work is currently being performed in our laboratory and elsewhere to gain a better understanding of how importantly these factors may effect the rate of degradation of TCE by methane-utilizing mixed cultures of bacteria.

The results indicate that the degradation resulted from the stimulation of methane-utilizing bacteria, which promoted TCE transformation. Another possible explanation is that the TCE was degraded anaerobically through the creation of anaerobic microzones resulting from the decrease of DO concentration with biostimulation. However, anaerobic degradation is considered unlikely owing to the presence of high nitrate concentrations (50 mg/l) throughout the test zone during the biotransformation experiments. Bouwer and McCarty (1983, 1985) found 1,1,1-TCA to be degraded under methanogenic conditions but not under denitrification conditions. Based on theoretical considerations presented by Vogel et al. (1987) TCE should behave similarly with little degradation under denitrification conditions. If methanogenic conditions existed, transformation of 1,1,1-TCA as well as TCE should be observed (Vogel et al. 1987). There, however, was no evidence of biotransformation of 1,1,1- TCA in the field experiment.

ELUTION OF TCE FROM THE TEST ZONE

After the first phase of the biostimulation experiment was completed, TCE injection was terminated, and the TCE remaining in the test zone was eluted by continuing to extract at the same flow rate as before (8 l/min). Methane and oxygen addition was also stopped and the injection well was used as a monitoring location. Figure 26 presents the monitoring data over the 2400 hour period during which this purging operation was continued; the gradual decrease in TCE concentrations at the observation wells and in the extracted water is easily seen. During the elution period, 50 to 100 pore volumes of water passed through the test zone, resulting in concentration decreases by a factor of 5 to 20.

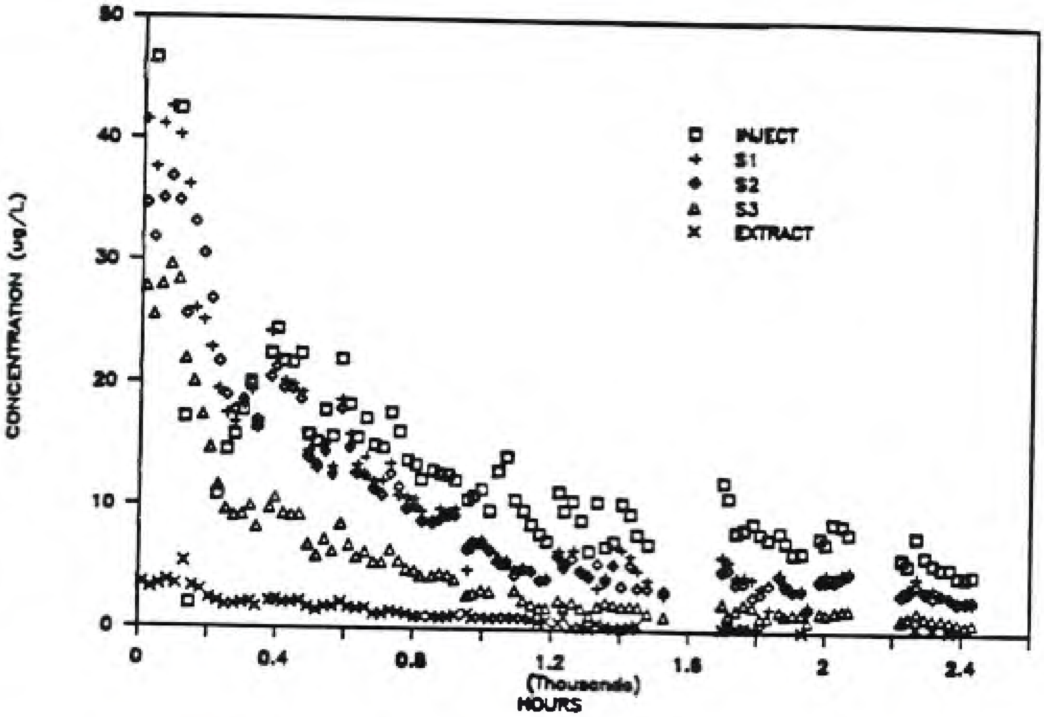

Figure 26. Elution of TCE from the test zone under induced flow conditions after stopping biostimulation and TCE addition.

A mass balance for TCE over the course of the TCE biostimulation experiment shows that of the total 10.1 g that were injected during the course of this experiment, 4.5 g were recovered in the water pumped from the extraction well, representing a recovery of 45 percent. During this same overall period, 65 percent of the bromide tracer was recovered. The lower recovery of TCE supports the conclusion that 25 to 30 percent of the injected TCE was degraded, consistent with our interpretation of the monitoring well data shown in Figure 25.

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PROVIDED AS BACKGROUND FROM DR. ED BRYAN

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Submitted for publication in Proceedings of the International Conference on Physiochemical and Biological Detoxification of Hazardous Wastes, held in Atlantic City, New Jersey in May of 1988.

INTERNATIONAL CONFERENCE ON PHYSIOCHEMICAL AND BIOLOGICAL DETOXIFICATION OF HAZARDOUS WASTES

Session on Research Needs*

Introduction

This session was held at the International Conference on Physiochemical and Biological Detoxification of Hazardous Wastes on May 4, 1988 in Atlantic City, New Jersey. Discussion, subject of this section of the Conference Proceedings, followed formal papers presented by John C. Crittenden, C.P. Leslie Grady, Jr. and Richard G. Luthy. Drafts of questions and panelist-response were circulated to the panelists for potential revision, the material was then edited by the Session Chairman and circulated to the panelists for their further consideration before being edited and submitted for publication as part of the Conference Proceedings.

Discussion by: James V. Basilico, Office of Research and
Development, U.S. Environmental Protection Agency,
Washington, D. C. 20460

Dr. Luthy's work is extremely important and could play a big role in Superfund groundwater cleanup which has been for the most part conducted without the benefit of the kinetic information he is working on. About 40% of the cleanup decisions at Superfund sites involved pump and treat technology based on a predicted duration of up to five years. However, failure to understand the processes controlling contaminant transport can result in extremely long pumping periods and costly and inefficient remediation. Annual operating and maintenance costs of a few hundred thousand to a few million dollars stretched over five years or more is considerable. Our research at the Ada Groundwater Research Lab suggests that groundwater remediation decisions would benefit from the type of information being sought by Dr. Luthy. We need to consider absorption/desorption data in designing our aquifer remedial actions correlated with the soil type and carbon content. Research on these topics could have a major economic impact on the way we clean up groundwater. The results are needed now.

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* Session Chairman was Edward H. Bryan, Ph.D., Program Director for Environmental Engineering, National Science Foundation, Washington, D. C. 20550. Opinions expressed are those of the participants and do not constitute an official position of the National Science Foundation or any other Federal agency.

Regarding Dr. Grady's comprehensive presentation, one of the more important things that I see needed is a standard data base. Perhaps a clearing house needs to be established to collect and collate information on research under way in toxics in hazardous waste treatability. Acceleration of research over the past five years has not been matched by an ability to store and retrieve the results. Perhaps a center such as the one at the New Jersey Institute of Technology could form a planning committee that would develop a protocol for reporting the types of information suggested by Dr. Grady.

Regarding Dr. Grady's suggestion for research on staged reactor systems with different microbial communities, I feel that sludge digestion is an excellent candidate for potential improvement over the way we've been doing it for the past 50 years. The objective would be to reduce the amount of residual organic solids from 50% to perhaps as little as 5% through use of multi-stage biological reactors. This would have a tremendous effect on sludge disposal options and cost.

Discussion by: Dr. J. K. Bewtra, Dept. of Civil Engineering
University of Windsor, Windsor N9B 3P4, Canada

Several points that complement the valuable suggestions already made include the concept described by the word "toxicity". It is very important that we all understand what we mean by toxicity and what we mean by toxic waste. Are all hazardous wastes toxic? If they are toxic, are they toxic to fish, aquatic life or to humans by the process of biocumulation? Do toxic substances have threshold values? Are we only interested in the toxicity to a particular biological process or intermediate products toxic during biological degradation? Both Drs. Luthy and Grady discussed thoroughly this aspect of toxicity and Dr. Crittenden rightly suggested that we should not tolerate cross-media transfer of pollutants.

Regarding the use of the terms removal, reduction, treatment, detoxification, and now destruction, it is important that they be understood and used properly. For example, in the sedimentation process, there is a physical separation of toxic metals from the liquid but they are concentrated in the sludge. And in the air stripping process mentioned earlier, the volatile organic compounds are being transferred from one phase to another. As far as the total environment is concerned, we still have the same amount of these toxics present but they become concentrated in the sludge or in the air. While they may be considered to have been removed, they have actually only been separated. I agree with the point made by a previous speaker that we should aim for complete destruction of toxics rather than simply achieve their separation. Dr. Crittenden's paper on removal of volatile organics using activated carbon, leaves open the question regarding regeneration of the carbon, that is whether more toxics are produced or liberated to the environment during regeneration.

Finally, we should determine the behavior, the impact and the environmental fate of all new synthetic chemicals before using them. By that, I mean that we should have an extensive data base, as was suggested by Dr. Grady on the environmental behavior of any new synthetic organic chemical proposed for use. Then, a standard procedure should be established to analyze the data as a step toward understanding their significance and leading toward a common understanding of what is actually happening. We went through a similar experience on a smaller scale in the sixties when we found that the hard detergents were accumulating in the environment. This led to a procedure for defining and testing detergents for biodegradability and eventual displacement of the hard by soft detergents in the market place. Of course, we ended up with the even bigger problem of phosphate pollution. I believe it is time that we come up with standard procedures for testing the environmental impacts of synthetic organic chemicals.

Discussion by: Dr. Leonard Dauerman, Dept. of Chemical
Engineering, New Jersey Institute of Technology,
Newark, NJ 07102

At the request of the industrial sponsors, no papers have been presented at this conference on our research leading to development of a comprehensive hazardous waste treatment technology based on the application of low-powered density microwave radiation because patent applications are being filed.

I view the remediation of hazardous waste sites from the perspective of the chemical engineer, scaling up a process. This is the perspective that industry will be forced to follow as the RCRA amendments and SARA superfund legislation are implemented. Excavation and capping will be the routes of last resort.

On-site remediation should follow the usual process by which a chemical process is scaled up involving contacts with process and equipment vendors with concepts that appear to be promising. After consideration of alternatives, the engineer decides which method is optimal and recommends its implementation.

We have to view the process of on-site remediation as a series of unit operations. I believe that research must be tied closely to implementation. Mature bench scale technologies should be Federally financed. Samples from remediation sites should be sent to vendors to determine the optimal combination of processes to bring about the desired result. This approach would be superior to the present approach, which I think is anachronistic, that is, placing one technology on a very complex remediation site that requires a combination of many different unit operations and expecting it to be a "cure-all".

I also believe that, after this initial step, utilization will determine the value of particular technology, with the marketplace determining its ultimate success or failure. In other words, the market should determine whether or not the research and the technology should continue. Research without rapid implementation will sour the funding agencies, industry and the public on the value of hazardous waste research. After 30 years in research, I have seen many research programs die from lack of drive to utilize the results or determine its applicability.

I am currently endeavoring to set up a pilot plan with the Toxic Waste Institute involving concept evaluation by use of pilot plants. We expect to implement this within the next few months on our microwave technology which will also be made available for testing the applicability of technology-specific problems, the idea being to determine whether the concept works or doesn't work.

Discussion by: Dr. Joseph D. Rosen, Dept. of Food Science,
Rutgers, the State University, New Brunswick, New Jersey 08930

The speakers made excellent presentations and exhibited wisdom touching on subjects for which we have good examples. Dr. Crittenden made a plea for determining material balance and the toxicity of the materials that are being formed in the various processes that we are devising. We have recently done a photo-oxidation of benzopyrene. In many cases, photo-oxidation and metabolism give the same products.

Many of the compounds that we're trying to get rid of are carcinogens because they go through a metabolic oxidative process. Similarly, photochemical oxidation sometimes results in production of carcinogenic material. In the case of benzopyrene, compounds are produced that are closely related to the extremely mutagenic benzopyrene diol epoxide. Since GC/MS cannot identify materials that are so polar, newer techniques such as LC/MS and MS/MS will have to be used.

Dr. Grady also touched upon something which is important...that we should not judge detoxification solely by evolution of carbon dioxide. In our experience, the polar mutagenic photo-oxidation products were bound to the soil very strongly and were effectively removed.

Discussion by: John Glaser, U.S. Environmental Protection Agency,
Hazardous Waste Engineering Research Laboratory,
Cincinnati, Ohio 45268

My reaction to the issues being considered at this conference is that conventional wisdom cannot always be applied to many of the problems because the problems are not conventional, and the wisdom base needed is non-existent at this stage of development pointing basically to the complexity of the overall problem of both soil and liquid remediation at hazardous waste sites. Throughout this conference I've observed a "model mentality" being developed, that is, a philosophy that can be characterized as being myopic with respect to the specifics of a model under consideration and ignoring the depths of knowledge available from other disciplines such as microbial ecology. Communication linkages between engineers, ecologists and microbiologists need to be strengthened.

A data base is certainly quite important to the overall considerations of this effort. However, one of the problems that I see with a data base is that we too often look specifically at single compound correlations and then extend those to rather complex mixtures that we encounter as waste in the environment. Obviously, there's a lack of predictive capability in that type of extrapolation.

Regarding Dr. Luthy's paper, I question the possibility of adsorption on dead biomass that may be developed during the experimentation. Work done by Jim Blacksburg some years ago studied that issue with specific references to activated sludge systems.

Other speakers at this session raised the idea of loss of identity of the target substrate and that's clearly insufficient for the overall detoxification purposes. We need a greater interrelation between those practicing and developing treatment technology with those people specifically more knowledgeable in toxicological testing. We are developing a program to include a very close relationship with our human toxicology people in the evaluation of processes.

Research needs exist in utilizing soil as a treatment medium, in determining how to deal with persistent environmental contaminants such as the PCB's and in combining physical, chemical and biological processes into a responsive system. A single system may not be completely adequate for all the wastes that we have to face. An overall strategy for attacking wastes is obviously something that we need.

Regarding new organism discovery and study, we've considered the possibility of having some form of a depository but we're not willing to go too far down the line at this time except to note that it certainly makes some good sense. Problems that are affecting the overall output of work in this area include the concept of intellectual rights that are most commonly-defined as patents. It's fairly clear that the patenting activity in this area will increase with time.

One area that we that we've had problems with is permit restrictions. EPA has to face the very same permit restrictions that you all do. We do not get any free ride just because we're part of the agency. We've gone through a lot of agony in getting our treatability permits.

One other point that I'd like to make in closing is to tell you, that within the agency we do have a new research program called the Biosystem Technology Development Program, basically funded from the Office of Research and Development which is currently exploring bringing together six different OR&D laboratories to use their resources to develop these kinds of technologies to answer the kind of problems that we're about today.

Discussion by: Wayne G. Landis, U. S. Army Chemical Research
Development, Engineering Center, Aberdeen Proving
Grounds, Maryland.

Dr. Grady suggested we place more emphasis on microbial ecology-the interactions and symbiosis that occurs in the competition between types of organisms. It's been mentioned that sometimes the detoxified effluent is mutagenic. Well, sure, because these organisms are in there trying to live and could care less about what we put them in for. One of the requirements for an organism to live is that it can compete with its fellow organisms. And so a lot of organisms produce halogenated organic compounds. It's not surprising that some of those find their ways into effluent.

Questions we have may be basic microbial population genetics as to why some organisms can degrade material where apparently an identical organism somewhere else can not. It may just be missing a certain part of the gene, a certain plasmid which isn't there yet. So a lot of this may just simply be microbial population genetics. And compared to those that you find for droposphila and other organisms, we really haven't done a whole lot with respect to the population of genetics of microbial organisms.

We tend to be really procaryotic chauvinists - tending to only deal with the heterotrophs. Other organisms that may be used include the photosynthetic bacteria, the eucarotic microorganisms, the fungi, yeasts and protozoa. There are lots of organisms that are microscopic with an amazing array of metabolite capabilities, which we almost never consider using.

So far today, all we've really talked about are microorganisms. Microorganisms have inside of them enzyme systems which could be a lot more efficient if they were removed immobilized and placed on a substrate... a bioreactor made of the enzymes, using enzymes not necessarily native to bacteria. The concept of using immobilized enzymes in a bioreator that is not limited to using enzymes from bacteria.

If we understand how detoxification occurs, we could possibly manufacture the equivalent, an organic catalyst that is not an enzyme and which could cost much less. There are already examples of this called the "moss reagents" that are capable of detoxifying organophosphates by mimicking the activity of enzymes that break down organophosphates. Their activity is not as spectacular as enzymes, but their molecular weight is only 200. They are compact, inexpensive and easily made.

While mentioned previously, I emphasize the need to examine the effects of our treated wastes on the receiving environment not just human health. This would include the remainder of the original waste stream components and the metabolic byproducts of the treatment system. Finally, we need to consider the possibility of in situ degradation of hazardous substances as an alternative to removing them from their environmental location, treating them, returning them or placing them someplace else. In order to do that , we're really going to have to understand microbial ecology.

Discussion by: Michael M. Martinson, BioTrol, Inc.
Chaska, Minnesota 55318

Our basic research supported by EPA on the biodegradation of pentachlorophenol required expertise in toxicity bioassay to determine the fate of the toxicant in the natural aquatic environment; chemistry to understand the chemical reactions that affect the fate of the toxicant and intermediates throughout the natural ecosystem; chemical engineering processes like photolyses, volatilization, adsorption and absorption; biological transformations; and microbiology to assess the impact of the microbial ecology and the species diversity.

This study allowed some very important observations of what's happening to a toxicant in an effluent or in a bioreactor. We found novel bacteria that were capable of using pentachlorphenols as a sole carbon source. We then tried some simple experiments to determine if the bacteria isolated from the natural environment could cause the disappearance of the added toxicant and to obtain an understanding of the mechanisms involved including the enzymology and the biochemical pathway. This work, conducted at the University of Minnesota led to potential commercialization of the concept. Now, there is a more widespread approval and acceptance of biological systems which means that it will not be necessary to conduct similar prolonged studies to understand best how to use biological systems, what products are going to be produced, and how we characterize the effluent.

Discussion by: Dr. Al W. Bourquin, Vice President, Ecova
Corporation, 3820 159th Avenue N.E., Redmond,
Washington 98052

Whenever any type of remediation is proposed, the public has a "right to know" what is proposed and how health and the environment will be protected. This is usually done in a public hearing. Ecova has been involved in public hearings on bioremediation and for the most part, the public has been very much in favor of biological treatment. In fact, in one case, when the alternative was incineration, the public was far more favorable towards biodegradation relating that process to familiar systems in treatment of sewage and processing of food products.

I've had considerable experience with biotechnology risk assessment program in EPA. Here, again, I think the problem is primarily one of public perception as compared to significant environmental risks. A review of what actions EPA has taken indicates the agency has approved for release almost every genetically altered organism that's come to them. That release, however, has been stopped in most cases at a local level principally because of a lack of proper information getting to the public. It is very important to address the general public with facts and to prevent the use of scare tactics by opponents. We could divert all the funds for biological treatment system development into defining all the possible intermediates of all possible organic chemicals and never progress any further than we are now in environmental cleanup.

Regarding metabolic intermediates, we must rely on existing information of microbial metabolism to tell us what to expect in breakdown products from many chemicals. Surely we don't need to redevelop all the basic biochemistry of the last 20 years.

Current emphasis on microbial genetics is beginning to better define functionally processes in microbial ecosystems. Knowledge of microbial physiology can provide the basis for prediction of metabolites produced, accumulated byproducts or residual toxicity. However, based on my experience in fate-testing of pesticides, I suggest we not put undue emphasis on the potential byproducts or toxic byproducts that might be developed by biological processes.

One of the things that has really hampered bioremediation from moving forward is the claim that "anything can be degraded." It really is important that we base remediation concepts on scientific principles, integrating chemical, physical and biological processes. There are compounds that are resistant to biodegradation that may require physical and chemical approaches. Comments on research needs include an observation that most of our design and control techniques are based on steady state analysis of the process while the actual conditions involve variability of the input, suggesting that research be directed toward process control with variable input, measurement techniques and use of statistics to determine the extent of detoxification.

General Discussion:

Panelists then responded to questions and issues that were brought to their attention by session participants.

Basilico responded to comments about the need for research to develop instrumentation by noting the large fraction of Superfund funds now being used to make measurements that need to be conducted in laboratories at high cost. He noted the current emphasis in the EPA Las Vegas Laboratory is on devices to conduct on-site, in situ measurements based on use of fiber optic sensors.

In response to a question regarding instrumentation based on use of biosensors analogous to those used in medical laboratories sensing presence of a "polyclonal monoclone antibody," Luthy indicated that "there is a problem in that most immuno-chemical techniques are robust only for molecules that are structurally or chemically complex. They work for pentachlorophenol but not for benzene. The simpler the molecule the less specific the technique with field samples. Immuno-chemical assays work best in well-defined, invariant matrices. There is usually a lack of specificity with the environmental samples compared to controlled tests in the laboratory.

Responding to a question relating to the offset between absorption and adsorption isotherms, Luthy identified and characterized the soil with which he worked as "undisturbed, subhumid grassland soil of the Barnes-Hamerly association from Cass County, North Dakota, obtained by W.S. Dahnke at the Agricultural Experiment Station, North Dakota State University. The organic content was 2.9% by weight."

In response to a comment asking that a "modern glossary or thesaurus" be developed in the toxic waste treatment field, another participant suggested utilizing services of professional organizations like the American Society for Testing of Materials and the National Sanitation Foundation for developing a suitable glossary and set of definitions.

General discussion regarding the ability to conduct remedial actions without necessarily being able to precisely describe all metabolic products and by products led to comments by Lewis that relate this to the utility of fermentation before understanding the complete physiological process involved in this reaction.

Panelist response included an observation that the application of a concept for remediation in absence of complete knowledge regarding products and byproducts carries with it an implicit judgement of risk, a topic of interest to persons in the field of ecological toxicology. Absence of knowledge and the burden of an assumption of the risk associated with decisions to use a particular concept or process for remediation is what tends to make persons "overcautious" when making those decisions.

Another comment about remediation in liquid media versus soil led to observation that the latter are likely to be "far more complex" with the problem not being as "straightforward" for soil remediation as it is in liquid media.

Eric Ivan from the Department of Environmental Engineering at the Technical University in Denmark expressed concern regarding a total lack of quality guidelines for hazardous waste contaminated soils, indicating with respect to European practice what he characterized as "complete confusion," leading to a lot of unnecessary things where people are transporting contaminated soil to other areas and putting it on dumps. And a lot of unnecessary things are done simply more for psychological reasons." His question regarding the "American position" on quality standards for hazardous waste and hazardous contaminated soil and research to set them up stimulated discussion by panelists centering around the question: How clean is clean? The census of response was that this really isn't the correct question to be asked. The level of safety essentially having to be related to the proximity of the contained soil to persons potentially at risk, giving due consideration to potential biopathways by which the contaminants could exert their deleterious effects which is the domain of the "ecologist toxicologist" and involves persons knowledgeable in risk assessment.

Discussion then turned to leachate treatment from hazardous waste disposal sites and the concept of "terminal destruction," e. g. high temperature incineration for recalcitrant organic substances. Personnel from the U. S. Environmental Protection Agency's laboratory in Cincinnati described elements of the extensive incineration research and development program underway there, calling attention to papers that had been presented previously at this conference, especially by Bishop.

Pignatello from the Connecticut Agricultural Station commented about the long-term persistence of compounds that are low in molecular weight like carbon tetrachloride, trichloroethylene and ethylene dibromide, the latter persisting "...in soil for many, many years..." and suggested that we need to examine carefully our analytical techniques for environmental surveys as well and methods for removal of compounds from contaminated soil.

Discussion then returned to biological methods, public reaction to remediation procedures and their right to access information relevant to the decision - making process. Adams of Monsanto made a plea for "credibility" with regard to claims made with respect to what can and cannot be achieved through use of biological processes.

Adele Buckley with Solarchem in Toronto, Canada concluded the session with a plea for considering photo-oxidation along with biological detoxification and incineration suggesting that photo-oxidation is being advanced as the secret method of the future, "but...in fact is a viable method immediately."

APPENDIX 4

SUBGROUP ASSIGNMENTS

WORKGROUP ASSIGNMENTS

Management & Policy Subgroup

Mr. Douglas Downey
Senior Research Engineer
HQAFESC-RDV

Mr. Brian J. Hausknecht
Naval Energy and Environmental
Support Activity

Ms. Mary Lingua
Naval Civil Engineering Laboratory

Mr. James McNabb
Environmental Protection Agency
Robert S. Kerr Environmental
Research Laboratory

Dr. Clemens Meyer
U.S. Army Corp. of Engineers

Mr. Steve Ragone
USGS

Dr. Ed Smith
USA CERL

Ms. Elizabeth Wilkins
Navy Facilities Engineering Command

Ordnance Subgroup

Mr. Bob Bartell
USATHAMA

Dr. C. Richard Lee
USAE-WES

Mr. Paul Schatzberg
Annapolis Laboratory, David Taylor

Fuels Subgroup

Dr. John Armstrong

Dr. Mick Arthur
Battelle Laboratories
Columbus Division

Dr. Susan Landon-Arnold
Northern State College

Dr. Gaylen Brubaker

Dr. Rob Hinchee
Battelle Columbus Laboratories

Dr. Ronald Hoeppel
Naval Civil Engineering Laboratory

Mr. Henry Kerfoot
Lockheed

Dr. Martin Reinhard
Stanford University

Dr. Ronald Simms
Utah State University

Dr. Rick Watts
University of Nevada at Reno

Inorganic/Heavy Metals

Dr. Alex Iskander
USA CRREL

Mr. Walter Mikucki
USA CERL

Dr. Nate Revis
Oak Ridge Research Institute

Dr. Sachio Yamamoto
Naval Ocean Systems Center

Chlorinated Solvents Subgroup

Dr. Dick Brown
Groundwater Technology, Inc.

Dr. D. B. Chan
Naval Civil Engineering Laboratory

Dr. H. G. Monbouquette
University of California at Los Angeles

Dr. George Pickwell
Naval Ocean Systems Center

Mr. Wayne Sisk
USATHAMA

Captain Cathy Vogel
University of Arizona

DEPARTMENT OF THE NAVY

Naval Civil Engineering Laboratory
Port Hueneme, CA 93043-5003