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IN-SITU GROUNDWATER TREATMENT TECHNOLOGY USING BIODEGRADATION

by

Edward J. Bouwer, Principal Investigator

Gordon D. Cobb, Ph.D. Student

The Johns Hopkins University
34th and Charles Streets
Baltimore, Maryland 21218

for

U.S. Army Toxic and Hazardous Materials Agency
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In-situ biorestoration has great potential for the remediation of contaminated subsurface environments. Many hazardous organic micropollutants can be transformed by microorganisms under proper environmental conditions. This study sought to identify those environmental factors necessary for satisfactory implementation and control of in-situ treatment technologies. Several in-situ biorestoration schemes are discussed in detail. Qualitative performance criteria were established as a means of evaluating in-situ groundwater restoration schemes, due to the heterogeneous nature of the subsurface and the lack of basic knowledge on in-situ processes. Implementation of a generally applicable in-situ biorestoration strategy for all contaminated sites is not currently feasible. Continuation of laboratory experiments and implementation of site-specific pilot studies at well-characterized field sites are recommended.

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TABLE OF CONTENTS

Section	Page
LIST OF FIGURES	iv
LIST OF TABLES	v
1. ABSTRACT	1
2. INTRODUCTION	2
3. SUBSURFACE MICROBIOLOGY	5
3.1 Subsurface Biological Activity	5
3.2 Environmental Factors	5
3.2.1 Dissolved oxygen	5
3.2.2 pH	6
3.2.3 Temperature	6
3.2.4 Nutrients	6
3.2.5 Soil moisture content and salinity	7
3.2.6 Pollutant and organic concentrations	8
3.2.7 Electron acceptor conditions and redox potential	9
4. IN-SITU BIORESTORATION	18
4.1 Preliminary On-Site Investigation	18
4.2 Containment	19
4.3 Treatment Technology	19
4.3.1 Enhancement of indigenous microbial populations	22
4.3.2 Addition of acclimated organisms	22
4.3.3 Treatment trains	25
5. EXPERIMENTAL	28
5.1 Experimental Design	28
5.2 Experimental Results	28
6. PERFORMANCE CRITERIA	35
6.1 Capital Costs	35
6.2 Annual Operating Costs	36
6.3 Performance Effectiveness	36
6.4 Residuals Generated	37

6.5 Secondary Environmental Impact 37
6.6 Commercial Availability 38
6.7 Application To Groundwater 38
6.8 Operation And Maintenance Costs 38
6.9 Treatment Interference 38
6.10 General Applicability 39

7. CONCLUSIONS AND RECOMMENDATIONS 40
7.1 Conclusions 40
7.2 Recommendations 41

8. REFERENCES 45

LIST OF FIGURES

	Title	Page
3.1	Schematic for Possible Pathway for Conversion of TeCE to CO_2 through Reductive Dehalogenation	17
4.1	Proposed Aquifer Decontamination Scheme Using Above Ground Biological Reactor	23
4.2	Proposed Scheme to Establish an In-Situ Biological Treatment Zone Downgradient from a Contaminant Plume	26
5.1	Column Effluent Concentration Versus Time Data for Sulfate during the First Five Months	30
5.2	Column 1 Effluent Versus Time Data for Carbon Tetrachloride, Bromodichloromethane, and Bromoform during the First Five Months	34

LIST OF TABLES

	Title	Page
2.1	Potential Benefits of In-Situ Treatment of Contaminants	4
3.1	Removal of Halogenated Aliphatics in Methanogenic Biofilm Column with 2.5 Day Detention Time	10
3.2	Removal of Halogenated Aliphatics in Sulfate Reducing Biofilm Columns	10
3.3	Removal of Halogenated Aliphatics in Denitrifying Biofilm Columns	11
3.4	Biotransformation of Halogenated Aliphatic Compounds by Microorganisms	14
5.1	Fixed-Film Reactor Feed Composition for Columns 1 and 2	29
5.2	Average Removals of Organic Micropollutants in Biofilm Column Reactors	32
5.3	Average Removals after Six Months for Selected Organic Micropollutants in Columns 1 and 2	33
7.1	Favorable and Unfavorable Chemical and Hydrogeological Site Conditions for Implementation of In-Situ Biorestoration	43

1. ABSTRACT

In-situ biorestoration has great potential for the remediation of contaminated subsurface environments. Recent evidence suggests that microorganisms are capable of flourishing in a variety of subsurface environments. In particular, many hazardous organic micropollutants can be transformed by microorganisms under proper environmental conditions. This study sought to identify those environmental factors necessary for satisfactory implementation and control of in-situ treatment technologies. These factors include dissolved oxygen content, pH, temperature, redox potential, availability of mineral nutrients, salinity, soil moisture content, concentration of primary organic carbon sources, concentration of organic pollutants, and the predominant electron acceptor condition. Although many concepts for groundwater treatment and recovery are being investigated, the application of these specialized techniques at contaminated sites has been limited. Several in-situ biorestoration schemes are discussed in detail. Performance criteria were established as a means of evaluating suggested in-situ groundwater restoration schemes and to compare their effectiveness with current remedial measures. However, due to the heterogeneous nature of the subsurface and the lack of published data on in-situ processes, only a qualitative assessment of in-situ biorestoration performance can be undertaken. Biofilm processes are potentially important for transformations of organic micropollutants in subsurface environments. Laboratory biodegradation experiments indicate that biotransformation of numerous organic pollutants is possible under proper redox conditions. Implementation of a generally applicable in-situ biorestoration strategy for all contaminated sites is not feasible at the present time though, due to the site-specific nature of contamination incidents. Considerable research must still be performed in order to adequately characterize the hydrogeological, biological, chemical, and physical properties at contaminated sites so that the most effective treatment system can be developed.

2. INTRODUCTION

Pollution of soil and groundwater is a critical problem of national concern. Widespread subsurface contamination has resulted from Department of Defense (DoD) activities and production of chemical warfare materials. Surveys of many U.S. Army Material Development and Readiness Command (DARCOM) installations have identified groundwater contamination by solvents and heavy metals (Weston, 1983). Explosives and propellant contaminated soil has resulted from munitions production. Another major source of subsurface contamination at military bases has been rework operations such as degreasing, electroplating, and paint stripping.

The most commonly observed organic contaminants that have been identified under the Installation Restoration Program of DoD are volatile halogenated solvents, such as trichloroethylene, methylene chloride, 1,1,1-trichloroethane, and tetrachloroethylene, chlorinated benzenes, and nitrated aromatics (TNT and similar compounds) (Weston, 1983). Furthermore, contamination of groundwater by JP-5 jet fuel is frequently encountered in the vicinity of military airfields. The compounds above are relatively soluble in water and are quite mobile in aquifers. For this reason, these compounds can travel great distances in the subsurface and can cause widespread contamination. These substances are a potential threat to public health as several are known or suspected human carcinogens or mutagens, or are toxic to humans and aquatic organisms at high concentrations. Therefore, knowledge about the fate of these toxic organic chemicals in the environment and the development of economical control and treatment techniques is desirable.

Due to the relative inaccessability of many aquifers and the expense and complications of detailed monitoring and study, the extent of groundwater contamination is generally poorly quantified (Hutchins et al., 1985). Since groundwater environments do not have the natural cleansing mechanisms common in surface waters, once groundwater becomes contaminated, it may remain so for many years without additional treatment (McCarty et al., 1981). Therefore, there exists a need to develop cost-effective technologies for the treatment of contaminated groundwater at U.S. Army installations.

There are a variety of methods that may be employed to either permanently contain or sequester organic pollutants in the subsurface in order to minimize downgradient effects and at least partially cleanup the aquifer. These restoration methods require physical removal of polluted water for above ground physical, chemical, and/or biological treatment. Cleanup of contaminated soil is generally accomplished by costly excavation and transport to an approved hazardous waste landfill. A potential cost savings may be realized if contaminated groundwater and soil were treated in place.

In-situ biorestoration has great potential for the remediation of contaminated subsurface environments. In particular, recent research efforts have shown that many organic pollutants can be transformed by microorganisms under proper environmental conditions (Kobayashi and Rittmann, 1982). There are numerous advantages in employing in-situ biotransformation techniques to

contaminated groundwater environments (Table 2.1). The primary benefit of in-situ treatment is its potential cost-effectiveness in relation to other treatment schemes. In-situ biorestoration is one of the only methods available that can completely destroy an organic contaminant and the only method capable of degrading a contaminant at its source. The objective of this research is to assess the potential for using in-situ biological treatment for restoration of contaminated aquifers. This report summarizes our progress in determining conditions that must be met for biodegradation to occur, identifying the most feasible treatment approaches, and developing performance criteria to qualitatively evaluate the feasibility of various treatment options.

TABLE 2.1

Potential Benefits of In-Situ Treatment of Contaminants

* Cost-effective
* Indigenous microorganisms can, in many instances, bring about complete mineralization of many organic compounds on-site
* Indigenous microorganisms can destroy material sorbed to the soil matrix
* Destruction of contaminants can occur within a reasonable time frame
* Different microorganisms may act upon different parts of the same compound
* Different microorganisms can have alternative degradation pathways which allow contaminant utilization in a variety of environmental conditions
* Indigenous microorganisms can often adapt to new compounds or environmental conditions
* Treatment of contamination at its source is better than at individual withdrawal points, especially in rural environments, due to contaminant localization
* Minimal disturbance to the contaminated site
* Treatment moves with the groundwater
* Continued treatment occurs after shutdown of operation

3. SUBSURFACE MICROBIOLOGY

3.1 Subsurface Biological Activity

At one time, the presence and activity of subsurface microorganisms below the root zone of soil was believed to be either extremely limited or nonexistent. This inaccurate belief resulted from a general lack of scientific interest in this area, combined with inadequate or inappropriate methods for detecting subsurface biological activity (Wilson et al., 1986b). Due to newly developed techniques in microbial ecology and microscopy, several recent studies have indicated that microorganisms can actually be present and active at considerable depths in the subsurface (Wilson et al., 1983; Balkwill and Ghiorse, 1985; Webster et al., 1985). Deeper regions of the unsaturated zone and shallow, water-table aquifers have exhibited bacterial numbers around 10^6-10^7 organisms per gram of subsurface material. Two independent measures of metabolic activity indicate that between 1 to 10% of the total cell count is metabolically active (Webster et al, 1985). Morphologically, subsurface microorganisms differ very little from surface microorganisms except with respect to size. Many of the subsurface cells lack cytoplasmic constituents and contain polyhydroxybutyrate (PHB)-like storage inclusions, suggesting that they have adapted to oligotrophic environments (Poindexter, 1981). Despite having a smaller size adapted from exposure to more oligotrophic conditions, subsurface bacteria have a higher surface-to-volume ratio which allows them to effectively take up and utilize nutrients from dilute solutions. Although speciation of microbial populations in the subsurface varies from site to site depending on subsurface conditions, rates of biodegradation by natural populations are apparently fast enough to protect groundwater quality in many aquifers (Wilson et al., 1986b).

3.2 Environmental Factors

Although the physical, chemical, and biological mechanisms which affect the ultimate fate of organic pollutants in the subsurface are still not clearly understood, numerous environmental factors have been shown to influence the ability of indigenous microbial populations to degrade pollutants. These factors include dissolved oxygen content, pH, temperature, redox potential, availability of mineral nutrients, salinity, soil moisture content, concentration of organic pollutants, concentration of primary organic carbon sources, and the predominant electron acceptor condition (Freeze and Cherry, 1979; Wilson et al., 1986b). The remainder of this section will consider these factors in greater detail.

3.2.1 Dissolved oxygen

The dissolved oxygen concentration determines the speciation of microorganisms in the subsurface. Although oxygen is a vital substance for some microorganisms, under certain conditions it may be toxic to a number of species. In general, at high concentrations the subsurface is primarily populated by

obligate aerobic organisms which are unable to grow in the absence of oxygen. As the oxygen concentration depletes, facultative aerobes, microaerophilic aerobes, and aerotolerant (facultative) anaerobes may be found (Brock et al., 1984). Facultative aerobes may grow in the presence of oxygen or by fermentation processes in the absence of oxygen. Typically, fermentation will not occur in the presence of oxygen due to much smaller energy yields. Microaerophilic aerobes require oxygen for growth, but only survive at low partial pressures of oxygen due to toxic effects at atmospheric oxygen levels. Facultative anaerobes are able to grow in the presence or absence of oxygen and are not drastically harmed at lower oxygen concentrations. Finally, there are obligate anaerobes which cannot tolerate oxygen due to its toxic effects on their growth. Thus, the species of microorganisms found within an aquifer system will be directly related to the oxygen concentration profile.

3.2.2 pH

Microorganisms usually have a pH range within which growth is favorable. Most soil environments have pH values between 5 and 9, the range in which many microorganisms optimum growth occurs (Brock et al., 1984). Relatively few species can grow at pH values less than 2 or greater than 10. Therefore, if a contaminated site is characterized by an extreme pH, soil neutralization may be necessary in order to increase microbial activity and stimulate the growth of microorganisms which are capable of degrading the pollutants.

3.2.3 Temperature

As temperature increases, chemical and enzymatic processes in the cell proceed more rapidly. Above a certain temperature, though, cellular components become temperature sensitive and may be irreversibly inactivated. A U.S. Geological Survey data base on groundwater quality parameters indicates that the average temperature of aquifers is 15°C with a standard deviation of ±5°C. Within the subsurface, the temperature increases, on average, approximately 1°C for each 40 meters of depth (Freeze and Cherry, 1979). However, in the upper layer of soil, where most contamination occurs, diurnal and seasonal variations in air temperature and solar intensity create a transient thermal zone. Thus, the microorganisms likely to survive will be those whose favorable temperature growth range falls within the subsurface temperature variations at a particular site.

3.2.4 Nutrients

In order to maintain satisfactory rates of growth, microorganisms require a wide variety of nutrients, whose concentrations in the cell are related to their cellular function. Of the naturally occurring elements, only six make up approximately 99 percent of living cells (Curtis, 1979). These six, known as macronutrients, are carbon, hydrogen, nitrogen, oxygen, phosphorus, and sulfur. In addition, calcium, sodium, potassium, and magnesium are also thought of as

macronutrients, although their concentrations within the cell are usually much lower. Macronutrients comprise the major building blocks of all cells and are involved in primary metabolic processes. Besides these macronutrients, many other nutrients are only needed in trace concentrations. These nutrients, known as micronutrients, include elements such as iron, manganese, cobalt, nickel, molybdenum, and selenium. The growth of microorganisms can only proceed at a rate which is proportional to the lowest nutrient concentration in relation to the other nutrients. By adding a higher concentration of the rate-limiting nutrient, microbial growth rates will then be proportional to a new rate-limiting compound. Since the micronutrient concentrations in the environment are typically in excess of the amount required by microorganisms, they will rarely be rate-limiting. Therefore, it is important to analyze the soil nutrient content to ensure that the macronutrient concentrations are not rate-limiting. If a deficiency is detected, the addition of nutrients to the soil might be required in order to stimulate microbial growth. The addition of too high a concentration of nutrients, however, could elicit a toxic reaction by the microorganisms. As nutrient levels become too high, growth rates will begin to show a marked decline rather than an increase.

3.2.5 Soil moisture content and salinity

All organisms require water for metabolic processes. The amount of water in different environments varies; however, water availability does not depend solely on water content, but also on adsorptive and solution factors (Brock et al., 1984). Water adsorbed to the soil surface may or may not be available to microorganisms, depending on how strongly it is adsorbed and how efficient a microoganism is in removing it. In addition, when solutes are dissolved in water, they are hydrated in varying degrees, and the amount of hydration can change the availability of water to microorganisms. Thus, an increase in salt concentration in soil decreases the availability of water, known as water potential (Brock et al., 1984).

When a microorganism grows in a soil with a water potential lowered due to the presence of salts, it must devote energy to extract water from solution. This energy expenditure usually decreases the growth yield or lowers the growth rate (Brock et al., 1984). In most soils, the salt concentration is lower than that in the cytoplasm of cells, so that water tends to flow into the cell due to a concentration gradient. If the external salt concentration becomes greater than that inside the cell, the organism will either obtain water for metabolic processes by increasing its internal salt concentration to create a favorable concentration gradient or lose microbial activity due to internal water loss from osmotic pressure. Microorganisms vary in their ability to increase their internal salt concentrations. For most soil microorganisms, increases in internal salt concentrations create toxic problems which can inhibit microbial activity. These salinity problems are more likely to predominate in the unsaturated zone of soil as water containing dissolved solutes is adsorbed to the soil particles. Microorganisms will then have to deal with higher local salt concentrations. Although the need for concern about soil moisture content and salinity in most saturated subsurface environments is not as vital as

unsaturated areas, precautions should be taken, when withdrawing water in a treatment scheme, to ensure that recharge is sufficient to maintain relatively constant moisture levels.

3.2.6 Pollutant and organic concentrations

The pollutant concentration affects the ability of microorganisms to degrade them. At high concentrations, which are typically found near the contaminant source, the activity of the microorganisms may be inhibited by toxic effects related to the pollutants. At lower concentrations, which are usually detected downstream from the contaminant source on account of dilution effects, the toxicity associated with higher pollutant concentrations may dissipate, allowing them to be degraded. There are two types of pollutant degradation: primary or secondary utilization. In order to maintain themselves, microorganisms require a minimum amount of energy. Microorganisms can potentially utilize an organic compound as their primary energy source if its concentration is greater than a minimum threshold level, which depends primarily on the pollutant concentration, but also on the degradative and metabolic properties of the microorganisms. Even more energy and carbon would be required for significant cell growth and reproduction. Thus, when the pollutant concentration is below its minimum threshold, a residual amount would presumably not be utilized and would persist in the environment. However, simultaneous utilization of several different compounds is possible, such that microorganisms can sometimes metabolize trace concentrations of pollutants either in the presence of a primary organic substrate which is capable of meeting a microorganisms energy needs or in the absence of a primary substrate if the compounds can collectively meet the minimum energy requirements of a cell. The process of utilizing trace contaminants in the presence of a primary substrate is known as secondary utilization, which is a mechanism that allows microorganisms to biotransform organic pollutants that otherwise could not support them. Thus, despite the fact that many organic pollutants are found at low concentrations in the environment (ppb levels), biological mechanisms exist that are capable of transforming pollutants present below their detection limits.

Since most pollutants are not ubiquitous in the environment, microorganisms in unpolluted areas must utilize natural organic compounds as a primary source of both carbon and energy for growth and maintenance. Natural sources of primary organic compounds are quite diverse in subsurface environments. Their distribution is site specific and highly variable, so that they are often expressed as a sum of compounds such as total organic carbon (TOC), dissolved organic carbon (DOC), chemical oxygen demand (COD) or biochemical oxygen demand (BOD). Since recent studies have indicated that microorganisms can flourish in large numbers in the subsurface, there is obviously enough natural organic carbon available to support microbial growth. Given that microorganisms can grow in the subsurface on the organic material present, secondary utilization of contaminants has the potential to be a major mechanism in in-situ biorestoration.

3.2.7 Electron acceptor conditions and redox potential

Depending on the electron acceptor condition present, microorganisms can mediate the transformation of a wide variety of organic pollutants often found at groundwater contamination sites. In subsurface environments characterized by the presence of oxygen, aerobic microorganisms biotransform a diverse number of predominantly aromatic organic compounds. Examples of these biotransformations include chlorinated benzenes under simulated subsurface conditions in the laboratory (Bouwer and McCarty, 1982; 1984), in soils (Marinucci and Bartha, 1979), and in river bank filtration (Kuhn et al., 1985); benzene, toluene, xylenes, and other chlorinated benzenes leaked into groundwater via gasoline or solvent spills (Wilson et al., 1986a); naphthalene, dibenzofuran, fluorene, anthracene, and pentachlorophenol near a wood-creosoting plant (Lee et al., 1984); and chlorophenols (Baker et al., 1980). In addition, methylotrophic organisms have been shown to degrade dichloromethane (Brunner et al., 1980) and trichloroethylene in soil (Wilson and Wilson, 1985). Methanotrophic organisms were shown to utilize several chlorinated ethene compounds (Fogel et al., 1986). Finally, ethylene dibromide was shown to degrade in two types of surface soils at low concentrations (Pignatello, 1986).

The extent of biotransformation of these compounds is limited by the dissolved oxygen concentration. For pollutants present at high concentrations (ppm range) which can be utilized as a primary energy and carbon source, a constant source of dissolved oxygen would be required to continue the biotransformation process. Thus, when the concentration of organic contaminants is high, subsurface oxygen will quickly be depleted and aerobic metabolism will cease within a short distance in an aquifer. Fortunately, further biotransformations can often be mediated by anaerobic microorganisms in the more reducing, anoxic environments.

Although anaerobic conditions in the subsurface are usually typified by sequential conditions of denitrification, sulfate respiration, and methanogenesis, most controlled anaerobic experiments are carried out in highly reducing, methanogenic environments. Anaerobic microorganisms tend to biotransform halogenated aliphatic compounds that usually persist in aerobic environments. As examples, tetrachloroethylene (TeCE), trichloroethylene (TCE), dichloroethylenes (DCE), dibromochloromethane (DBCM), bromodichloromethane (BDCM), bromoform (BF), 1,1,1-trichloroethane (111-TCE), carbon tetrachloride (CT), 1,2-dibromo-3-chloropropane (DBCP), hexachloroethane (HCE), and chloroform (CF) have been shown to be biotransformed under methanogenic conditions in a variety of environmental experiments (Barrio-Lage et al, 1986; Bouwer et al.,1981; Bouwer and McCarty, 1983a; 1984; Bouwer and Wright, 1987; Kleopfer et al., 1985; Parsons and Lage, 1985; Vogel and McCarty, 1985; Wilson et al., 1986), while only BDCM, DBCM, CT, and BF were biotransformed under sulfate reducing conditions (Bouwer and Wright, 1987) and only CT and BF under denitrification conditions (Bouwer and McCarty, 1983b). The removal of selected halogenated aliphatic compounds in acetate supported biofilm columns under different anaerobic electron acceptor conditions is presented in Tables 3.1 through 3.3. Recent work has also shown that aromatic compounds, previously thought to require oxygen in order to initiate degradative processes, may also

TABLE 3.1

Removal of halogenated aliphatics in methanogenic biofilm column with 2.5 day detention time (from Bouwer and Wright, 1987).

Compound	Lag Period (weeks)	Influent* µg/L	Effluent* % Removal
TeCE	9-12	18 ± 3	86 ± 7
CF	9-12	17 ± 4	95 ± 3
111-TCE	9-12	10 ± 2	> 99
CT	9-12	7 ± 2	> 99
DBCP	9-12	17 ± 2	> 99
BDCM	2	13 ± 1	89 ± 8
BF	2	18 ± 3	> 99
EDB	2	20 ± 4	> 99
HCE#	0	4 ± 1	> 99

* One standard deviation of the mean values are given.
\# This compound was added to column feed for final two months of operation.

TABLE 3.2

Removal of halogenated aliphatics in sulfate reducing biofilm columns (from Bouwer and Wright, 1987).

	2.5 day detention time			1.5 hour detention time		
Compound	Lag Period	Influent* µg/L	Effluent* % Removal	Lag Period	Influent* µg/L	Effluent* % Removal
TeCE	------	8 ± 4	13 ± 45	------	19 ± 6	12 ± 42
CF	------	17 ± 3	-39 ± 37	------	75 ± 9	-11 ± 27
111-TCE	10 mos	9 ± 2	72 ± 8	------	59 ± 7	33 ± 19
CT	<2 wks	7 ± 1	> 99	3 mos	39 ± 10	> 99
DBCP	<2 wks	12 ± 4	98 ± 7	6 mos	82 ± 8	82 ± 8
BDCM	<2 wks	16 ± 2	96 ± 5	3 mos	74 ± 13	> 99
BF	<2 wks	19 ± 3	> 99	3 mos	68 ± 28	> 99
EDB	<2 wks	19 ± 3	63 ± 9	------	74 ± 18	30 ± 30
HCE#	none	7 ± 2	> 99			

* One standard deviation of the mean values are given.
\# This compound was added to column feed for final two months of operation.

TABLE 3.3

Removal of halogenated aliphatics in denitrifying biofilm columns (from Bouwer and Wright, 1987).

	2.5 day detention time			1.0 hour detention time		
Compound	Lag Period	Influent* µg/L	Effluent* % Removal	Lag Period	Influent* µg/L	Effluent* % Removal
TeCE	------	7 ± 2	0 ± 40	------	5 ± 2	40 ± 31
CF	------	28 ± 2	-13 ± 16	------	10 ± 2	30 ± 17
111-TCE	------	27 ± 3	30 ± 7	------	8 ± 2	37 ± 29
CT	<2 wks	17 ± 2	> 99	4 wks	11 ± 3	73 ± 5
DBCP	------	37 ± 3	14 ± 11	------	22 ± 3	23 ± 17
BDCM	<2 wks	24 ± 2	90 ± 8	------	13 ± 3	38 ± 21
BF	<2 wks	36 ± 2	> 99	------	18 ± 3	33 ± 20
EDB	------	30 ± 2	23 ± 7	------	11 ± 2	36 ± 22
HCE#	none	4 ± 2	80 ± 17			

* One standard deviation of the mean values are given.
\# This compound was added to column feed for final two months of operation.

be biotransformed in methanogenic environments. Some examples include chloro- and bromobenzoates (Horowitz et al., 1983; Suflita et al., 1983); benzene, ethylbenzene, o-xylene, and toluene (Wilson et al., 1986a); phenolic compounds, polynuclear aromatic hydrocarbons, and coal-tar distillates at a former wood-treating facility in Minnesota (Ehrlich et al., 1982); and eleven aromatic lignin derivatives (Healy Jr. and Young, 1982). In addition, ethylene dibromide was also shown to be degraded by methanogenic microorganisms (Bouwer and McCarty, 1985).

In general, organic compounds acting as electron donors undergo oxidation reactions. However, due to the highly electronegative character of halogen substituents, particularly on aliphatic compounds, many polyhalogenated compounds frequently behave as electron acceptors or oxidants and are reduced (Vogel et al., 1987). The more highly halogenated the compound, the faster the relative rate of reduction; while the less halogenated the compound, the faster the relative rate of oxidation (Vogel et al., 1987). Thus, halogenated compounds may undergo either oxidative or reductive transformation depending on their structure and environmental conditions.

In anoxic environments, observed reduction of both halogenated aliphatic and aromatic compounds has been attributed to reductive dehalogenation. Two basic mechanisms of biological reductive dehalogenation have been shown (Vogel et al., 1987):

$$(3.1) \quad RX + H^+ + 2e^- \text{-----}> RH + X^-$$

$$(3.2) \quad \underset{X\ X}{-\underset{|}{C}-\underset{|}{C}-} + 2e^- \text{-----}> C{=}C + 2X^-$$

Reaction (3.1) typically occurs for polyhalogenated aromatic (Suflita et al., 1982), methane, or alkene compounds (Vogel and McCarty, 1985). Reaction (3.2) occurs predominantly for polyhalogenated alkanes with vicinal halides.

The reductive mechanism involves the formation of either a carbon radical or a dihalocarbene complex with an enzyme such as cytochrome P-450 (Castro et al., 1985; Vogel et al., 1987). The stability of the carbon-halogen bond and the free radical intermediate influence the reactivity of halogenated compounds. The C-I, C-Br, C-Cl, and C-F bond dissociation energies for a methyl carbon are 56, 70, 84, and 108 kcal/mole, respectively; while the C-Br and C-Cl bond dissociation energies for an aromatic carbon are 72 and 86 kcal/mole, respectively (Morrison and Boyd, 1974). Consequently, the ease of reductive dehalogenation in a particular redox environment generally follows the order I > Br > Cl >> F.

Bond dissociation energies, especially for aliphatic compounds, tend to increase when a halogen is replaced with a hydrogen. The reactivity of products decreases during sequential reductive dehalogenation (e.g. CCl_4 > $CHCl_3$ > CH_2Cl_2 > CH_3Cl > CH_4 and CBr_4 > $CHBr_3$ > CH_2Br_2 > CH_3Br > CH_4). The observed persistence of 1,2-dichloroethylene and vinyl chloride from TeCE and TCE in reducing environments tends to support this hypothesis (Vogel and McCarty,

1985).

Although reactivity decreases with sequential dehalogenation, abiotic hydrolysis tends to increase as the number of halogen substituents decreases. Thus, a coupling of reductive dehalogenation with abiotic hydrolysis could lead to a faster overall rate of detoxification of organic compounds by creating dehalogenated intermediates that are capable of being utilized by other microorganisms for carbon and energy. For example, methanol (CH_3OH), a carbon and energy source for methylotrophic organisms, may be formed during the abiotic hydrolysis of chloromethane (CH_3Cl), which can be a reductive dehalogenation product of carbon tetrachloride (CCl_4) or chloroform ($CHCl_3$). Therefore, the determination of bond dissociation energies and electrochemical reduction behavior for halogenated organic contaminants would be useful in evaluating the potential reactivities of organics under redox conditions found in groundwater environments (Bouwer and Wright, 1987).

Long periods of acclimation may be necessary before biodegradation can occur. Acclimation often involves a readjustment of the levels and types of microbial enzymes (both inductive and derepressive) or microbial species in response to the presence of a new substrate (Healy Jr. and Daughton, 1986). The time and concentration required for acclimation of microorganisms to subsurface pollutants are unknown. Comparison of results presented in Tables 3.2 and 3.3 for different liquid detention times indicates an apparent increase in time required for acclimation and a decrease in extent of removal for shorter liquid detention times (Bouwer and Wright, 1987). Pre-adaptation or prior exposure of bacteria can often dramatically reduce the time for biodegradation of contaminants (Spain et al., 1980; Spain and van Veld, 1983) by shortening the acclimation lag time. The acclimation lag time reflects the time for slow mutations to be sufficiently expressed in a given population so that the ability to degrade xenobiotic compounds can effectively take place (Healy Jr. and Daughton, 1986). Acclimation apparently requires a minimum concentration for many contaminants, particularly xenobiotics. Spain and van Veld (1983) reported a threshold concentration of 10 ppb for microbial adaptation to p-nitrophenol in samples of sediments and natural waters. Organic compounds below their acclimation threshold concentration might not be biodegraded because a sufficient population of adapted microorganisms could not develop. However, an unacclimated compound could be degraded without microbial adaptation occurring if another compound induces enzymes capable of degrading the unacclimated compound. This is known as fortuitous degradation. Nevertheless, a long period without biodegradation cannot be solely attributed to lag time. Nutrient limitation or microbial populations with unsuitable degradative capabilities could exist in a given groundwater environment. Still, a more thorough understanding of acclimation processes may help to explain why some compounds tend to persist in the subsurface when they have been reported to degrade in laboratory cultures and natural water, sediment, and soil samples (Healy Jr. and Daughton, 1986).

Based upon the large number of compounds that may be broken down by microorganisms, biorestoration of contaminated sites would appear to be a viable treatment option in many contamination cases. Table 3.4 presents the products

TABLE 3.4

Biotransformation of Halogenated Aliphatic Compounds
by Microorganisms (from Vogel et al., 1987)

Compound[@]	Initial Concentration[#]	Product(s)[@]	System[*]	References
CM	1µM	CO2	O/P/Ex	Colby et al., 1977
	N/A	Formaldehyde	O/E/mo	Patel et al., 1982
DCM	2-5mM	CO2	O/P	Brunner et al., 1980
	1µM	CO2	O/P/Ex	Colby et al., 1977
	10µg-1mg/L	CO2	O/P	LaPat Polasko et al., 1984
	1-25mg/L	CO2	O/M	Rittmann and McCarty, 1980
	10mM	Formaldehyde	O/P/Ex	Stucki et al., 1981
CF	30µg/L	CO2,CH4	Am/M	Bouwer and McCarty, 1983a
	5.5µg/g	CO2	O/S	Strand and Shippert, 1986
CT	30-200µg/L	CO2,CH4	Am/M	Bouwer and McCarty, 1983a
	40-115µg/L	CO2,CF	Ad/M	Bouwer and McCarty, 1983b
	5-15µg/L	CO2	A/M	Bouwer and Wright, 1987
	1µM	CO2	O/P/Ex	Colby et al., 1977
BM	1µM	CO2	O/P/Ex	Colby et al., 1977
	N/A	Formaldehyde	O/E/mo	Patel et al., 1982
BCM	10mM	Formaldehyde	O/P/Ex	Stucki et al., 1981
DBM	10mM	Formaldehyde	O/P/Ex	Stucki et al., 1981
BF	20-40µg/L	DBM	A/M	Bouwer and Wright, 1987
12DCE	20µg/L	CO2,CH4	Am/M	Bouwer and McCarty, 1983a
	5mM	2-Chloroethanol	O/P/Ex	Janssen et al., 1985
	5mM	CO2	O/P	Stucki et al., 1983
111TCE	25-200µg/L	unidentified	Am/M	Bouwer and McCarty, 1983a
1122TeCE	30µg/L	112TCE	Am/M	Bouwer and McCarty, 1983a
HCE	6.5µg/L	TeCE	O/S/M	Criddle et al., 1986
BE	5mM	Ethanol	O/P/Ex	Janssen et al., 1985
EDB	20-30µg/L	CO2	Am/M	Bouwer and Wright, 1987
	20-30µg/L	CO2	As/M	Bouwer and Wright, 1987
	5.7mM	Ethene	O/S	Castro and Belser, 1968
	6-18µg/L	CO2	O/S	Pignatello 1986
VC	100µg/L	CO2	Am/M	Vogel and McCarty, 1985
DCE	N/A	CO2,VC	A/S/M	Parsons et al., 1984
	N/A	VC	Am/M	Vogel and McCarty, 1985
TCE	2mg/L	cDCE,tDCE	A/S	Kleopfer et al., 1985
	1µM	CO2	O/P	Nelson et al., 1986
	16.5µg/L	DCE	A/S/M	Parsons et al., 1984
	N/A	DCE	Am/M	Vogel and McCarty, 1985
	150µg/L	CO2	O/S	Wilson and Wilson, 1985
TeCE	30µg/L	CO2,TCE	Am/M	Bouwer and McCarty, 1983a
	1650µg/L	TCE	A/S/M	Parsons et al., 1984
	20µg-20mg/L	TCE	Am/M	Vogel and McCarty, 1985
1CP	5mM	1-Propanol	O/P/Ex	Janssen et al., 1985
DBCP	3.6mM	Propanol	O/S	Castro and Belser, 1968

@ Names for abbreviated compounds:

CM	- Chloromethane
DCM	- Methylene Chloride
CF	- Chloroform
CT	- Carbon Tetrachloride
BM	- Bromomethane
BCM	- Bromochloromethane
DBM	- Dibromomethane
BF	- Bromoform
11DCE	- 1,1-Dichloroethane
12DCE	- 1,2-Dichloroethane
111TCE	- 1,1,1-Trichloroethane
112TCE	- 1,1,2-Trichloroethane
1122TeCE	- 1,1,2,2-Tetrachloroethane
HCE	- Hexachloroethane
BE	- Bromoethane
EDB	- Ethylene Dibromide
VC	- Vinyl Chloride
DCE	- Dichloroethylene
cDCE	- cis-Dichloroethylene
tDCE	- trans-Dichloroethylene
TCE	- Trichloroethylene
TeCE	- Tetrachloroethylene
1CP	- 1-Chloropropane
DBCP	- 1,2-Dibromo-3-chloropropane
CO2	- Carbon Dioxide
CH4	- Methane

N/A - concentration was not specified

* O:aerobic; A:unspecified anaerobic; Am:methanogenic; As:sulfate reduction; Ad:denitrification; S:soil or aquifer material used as biological seed; M:mixed-culture; P:pure-culture; E:enzyme derived from microorganism; Ex:cell extract; mo:monooxygenase

of biotransformation for selected 1- to 3-carbon halogenated aliphatic compounds under different environmental conditions. One problem, however, is that microorganisms do not always mineralize organic pollutants to carbon dioxide and water. Occasionally the organic pollutants are transformed into more toxic compounds than the original compound. For example, TeCE has been shown to be transformed via reductive dehalogenation to vinyl chloride under methanogenic conditions, although evidence exists that vinyl chloride may be mineralized to carbon dioxide and water (Figure 3.1) (Vogel and McCarty, 1985). Thus, knowledge of potential end-products of biological subsurface reactions is a vital prerequisite when employing in-situ techniques.

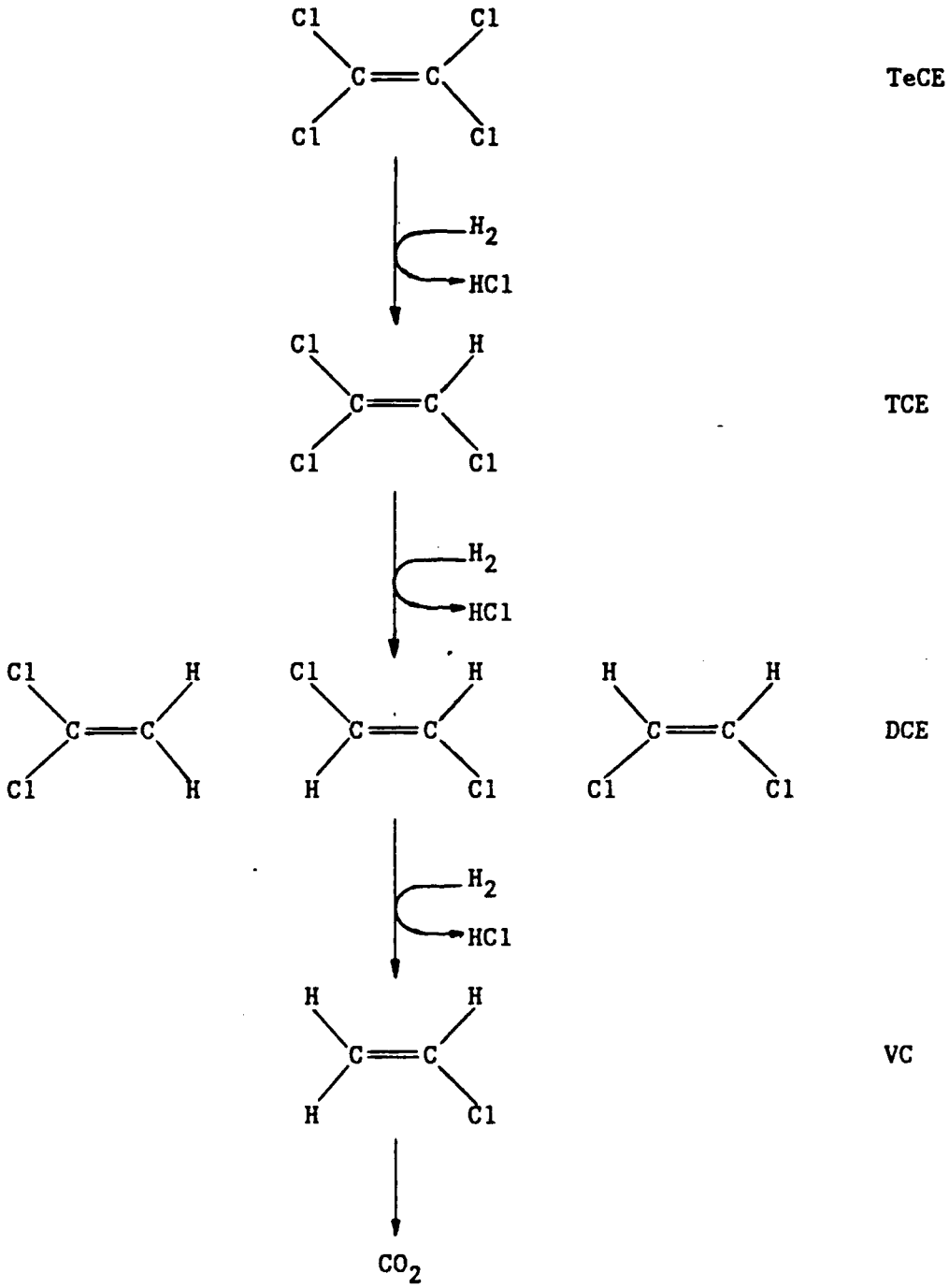


FIGURE 3.1
Schematic for possible pathway for conversion of TeCE to CO_2 through reductive dehalogenation (from Vogel and McCarty, 1985)

4. IN-SITU BIORESTORATION

4.1 Preliminary On-Site Investigation

Although many concepts for groundwater treatment and recovery are being investigated, the application of these specialized techniques at contaminated sites has been limited. The successful implementation of aquifer rehabilitation strategies requires an understanding of existing site conditions. Information regarding site geology and hydrology must be defined in order to properly determine the eventual location of the treatment system. Geological considerations should include stratigraphic effects such as horizontal extent of the aquifer and heterogeneity of the soil. Hydrogeological data includes porosity, permeability, and groundwater velocity, direction, and recharge/discharge (Freeze and Cherry, 1979). In addition, hydraulic connections between aquifers, potential recharge/discharge areas and water-table fluctuations must be considered.

After the detection of the contamination incident, determination of the quantity and hazardous characteristics of the contaminant(s) within the aquifer system is necessary in order to select the most appropriate restoration technique. Sources of contamination can vary from a one-time release associated with a chemical spill to a continuous release of hazardous materials, such as leachate migrating from a leaking landfill. If a continuous release is occurring, the initial procedure, where feasible, is to remove or abate the source of contamination to the aquifer. Although the complete removal of the source is not always physically feasible, the amount of time required to restore an aquifer will be greatly reduced if a continuous source of contamination is eliminated.

Chemical and physical properties of the contaminants identified must also be considered. A compound's physical or chemical characteristics, such as solubility, volatility, hydrophobicity, density, and octanol-water partition coefficient contribute to its availability in solution. Migration of contaminants within an aquifer system will depend in part on the character of the contaminants involved. The movement of organic materials that enter the subsurface environment is largely governed by sorption (McCarty et al., 1981). Sorption affects the rate of travel of organic material relative to that of water through subsurface systems. The extent of sorption is probably a function of the fraction of organic carbon within the subsurface soil matrix. The partitioning of organic contaminants between the water phase and the soil matrix can be estimated by their octanol/water partitioning coefficients (McCarty et al., 1981). For example, the movement of pollutants with high partition coefficients is retarded in the subsurface due to extensive partitioning onto the soil matrix, while those with low partition coefficients tend to move at about the same rate as infiltration in the unsaturated zone and groundwater velocity in the aquifer. In addition, the vertical distribution within an aquifer will depend on the density of the compounds. Contaminants such as oil or gasoline, which have densities less than 1 g/cm^3, will be immiscible with water, producing a plume of material which floats on the water table.

Conversely, contaminants heavier than water, such as chloroform (1.49 g/cm^3) or trichloroethylene (1.46 g/cm^3), will sink towards the bottom of an aquifer. Thus, water table fluctuations and soil heterogeneities, along with physical properties of the pollutants, will affect the extent of contamination both vertically and horizontally, which will affect the treatment scheme chosen.

4.2 Containment

After the initial detection of contamination, treatment efforts must be directed towards containment to minimize the migration of pollutants off-site. The type of containment will depend on the nature of the contaminant source, as described above, and the elapsed time since the contamination event. As time elapses, the aquifer rehabilitation becomes more complex. Two forms of containment are considered here: physical containment to prevent migration of contaminated ground water and hydrodynamic control, which is often associated with groundwater treatment strategies.

In the past, groundwater remediation often utilized physical containment systems, such as bentonite slurry trenches, grout curtains, sheet pilings, well points, and fixative injections, to impede groundwater flow (Josephson, 1980). Additionally, surface sealing to prevent infiltration of contaminants from the surface to the water table has also been employed. These methods have been used singly or in combination with varying degrees of success. Unfortunately, these containment systems are usually very expensive and rarely have produced satisfactory results (Josephson, 1980). In many instances, physical containment was employed as a temporary measure until a more permanent aquifer restoration method was developed.

Hydrodynamic control uses groundwater collection, such as pumping wells, to intercept contaminants and control local groundwater flow patterns. Aquifer recharge and discharge can be controlled by creating zones of depression and groundwater mounds which can often localize the contaminant plume. Hydrodynamic control is most commonly applied in conjunction with current aquifer restoration schemes which involve treatment and reuse of the contaminated groundwater. Several of these treatment technologies are considered in greater detail in the following section.

4.3 Treatment Technology

The first basic treatment step in any cleanup scheme is to employ physical methods, if possible, to recover any free product within the subsurface. The amount of available free product will be affected by its chemical and physical properties as well as by site heterogeneities. In addition, prior to the initiation of any in-situ biorestoration strategies, laboratory studies must be undertaken to determine the necessary requirements to effectively restore subsurface quality. Initially, a basic review of the microbiology and chemistry at the contaminated site is needed in order to ascertain nutrient availability, indigenous microbial populations, and the concentration and potential toxic

effects of the contaminants.

Groundwater microbiology and the environmental fate of groundwater pollutants have been primarily investigated under laboratory conditions. There are a number of factors which favor the use of laboratory-scale simulations of the groundwater environment, especially since they are easier and less expensive to implement. Lab-scale simulations are more flexible and allow a variety of controllable operating conditions for investigations of the effects of perturbations in a groundwater system. The ability to control experimental conditions in replicate systems permits the isolation of individual processes operating in groundwater environments and distinguishes them from other simultaneously occurring processes that affect contaminant fate (Healy Jr. and Daughton, 1986).

A major disadvantage of laboratory-scale experiments is that their operating conditions are often quite different from the native groundwater environment under consideration. Most early microbiological studies sought to determine the ability of a pure microbial culture to degrade a single substrate. Although pure culture studies have been useful in the investigation of basic microbial functions, recent laboratory-scale systems have emphasized the use of mixed-cultures of microorganisms in aquifer material under more realistic environmental conditions (Ehrlich et al., 1982; Herbes and Schwall, 1978; Kuhn et al., 1985; Lee et al., 1984; Parsons et al., 1984; Parsons and Lage, 1985; Pritchard and Bourquin, 1984; Spain et al., 1980; Wilson and Wilson, 1985). Still, there are problems associated with the use of mixed-culture systems. Since most laboratory simulations show little correlation when compared to groundwater environments, it is not known whether the data and kinetic models generated in the lab are reliable estimates of in-situ behavior (Healy Jr. and Daughton, 1986).

Since many different experimental designs have been employed to evaluate groundwater environments (e.g. batch studies, chemostats, microcosms, and continuous-flow reactors), it is difficult to compare results between two experimental setups, even when both are intended to simulate the same environmental location (van Veld and Spain, 1983). Low authenticity and lack of correlation add to the problems involved in estimating the margin of error in fate predictions based upon data obtained from laboratory studies (Healy Jr. and Daughton, 1986). If possible, laboratory studies should be verified with parallel field studies. Coordinated field and laboratory investigations could help in approximating the range in error associated with reliance on laboratory data (Healy Jr. and Daughton, 1986), although the correlation between the field and the laboratory will probably vary from site to site. Problems in scaling for in-situ biorestoration primarily involve physical and environmental factors rather than biological ones (Gulf Breeze Report, 1987).

An aquifer restoration project to document in-situ biodegradation of TCE is currently being operated at the Naval Air Station Moffett Field in Mountain View, California by researchers from the Stanford University Water Quality Laboratory. Five controlled stages of experimentation are planned. Stage one involves the injection of a conservative bromide ion tracer into an aquifer

uncontaminated by TCE to monitor natural flowrates and artificial groundwater patterns created by pumping from an extraction well. In stage two, bromide ion and TCE will be injected to monitor the fate of TCE with bromide acting again as a conservative tracer. This stage is intended to establish background levels of TCE removal. Stage three involves the addition of nutrients along with bromide ion and TCE to investigate whether an increased removal of TCE might occur due to stimulation of previously nutrient starved microorganisms. Since methanotrophs have been shown to degrade TCE under aerobic conditions (Wilson and Wilson, 1985), stage four will involve pulse injections of methane and oxygen in order to stimulate increases in methanotrophic population. In the final stage, a pulse addition of TCE along with methane and oxygen will investigate whether TCE is removed by methanotrophs more efficiently than background removal rates in earlier stages. Unfortunately, core samples that could provide data on any increases in subsurface biomass levels cannot be taken without disrupting the artificially induced groundwater flow pattern.

Most applications of in-situ biorestoration schemes have been to restore hydrocarbon-contaminated aquifers. Some of the sites implementing in-situ biorestoration have been cleaned to the point where hydrocarbons were no longer detectable within the aquifer and regulatory standards were satisfied (Wilson et al., 1986b). However, most of the published literature has lacked sufficient data to adequately support the assertion that in-situ biorestoration was the sole mechanism responsible (Healy Jr. and Daughton, 1986). In many cases, the proprietary nature of an investigation for a private company has been responsible for the lack of detailed information regarding specific procedures used and costs involved.

Selection of the biorestoration scheme to be used depends heavily on the environmental factors discussed previously. Any designed system must be specifically tailored to meet the needs of a particular site. The objective is to provide a local subsurface environment that is favorable for microbial growth by allowing sufficient contact and residence time of microorganisms acclimated to the contaminants. The most promising options for in-situ biorestoration, which are all based on the same basic concept of extraction of contaminated groundwater, treatment with nutrients, microorganisms, or both, and reinjection back into the soil, are considered in detail. These options are:

1) Enhancement of indigenous microbial populations;
2) Subsurface injection of acclimated microorganisms; and
3) Treatment trains

Since treatment schemes will always have to be tailored specifically to their sites, it is difficult to provide a detailed plan for any option. The following descriptions provide a basic overview of what the various treatment technologies seek to achieve.

4.3.1 Enhancement of indigenous microbial populations

Although reasonably large bacterial numbers are present in the subsurface, there are usually not enough to significantly affect the rate of biotransformation of organic contaminants. This is due, in part, to the oligotrophic nutrient conditions in many subsurface environments. The objective of this treatment scheme is to increase the indigenous microbial population in the subsurface in order to biotransform the contaminants at a faster rate. Previous research has shown that addition of nutrients to hydrocarbon contaminated soils has proven useful in increasing microbial degradation (Canter and Knox, 1985). In the past, enhancement of indigenous microorganisms has been utilized in a number of contamination cases, primarily involving hydrocarbons under aerobic conditions. However, laboratory research seems to indicate that enhancement should be a possible alternative for anaerobic sites as well, if proper nutrient status is attained.

The vast majority of microorganisms in the subsurface are attached to soil particles. As a result, nutrients must be brought to the microorganisms by advection or diffusion through the water or soil gas phases. Thus, this treatment's objective is best accomplished by withdrawing contaminated aquifer water, adding nutrients, and reinjecting via a series of wells back into the aquifer (see Figure 4.1). The type of nutrients required, either macronutrients, micronutrients, and/or an organic carbon source, depends on an analysis of the needs of the soil environment. If the contaminated water withdrawn has a contaminant concentration which might prove toxic to the microorganisms upon reinjection, water from an outside source might be necessary to dilute contaminant levels. A concern with this treatment scheme is that the water being recharged to the aquifer is not completely clean. The recharge water should not be reinjected outside of the drawdown zone of the central withdrawal well(s) or it could contaminate the aquifer downstream. In order to ensure that the recharge water does not extend beyond the drawdown zone, the amount of recharge water probably cannot exceed the amount withdrawn. Excess water will have to be discharged properly, especially if it contains detectable levels of contamination.

4.3.2 Addition of acclimated organisms

Rather than determining if the indigenous microbial population can effectively degrade the contaminants present, a different but unproven approach considers the addition of acclimated microorganisms to biodegrade the pollutants. Specific organisms may be inoculated into the subsurface environment or the environment may be altered to favor growth of a population with specific metabolic capabilities (Thomas et al., 1987). Populations specific for degrading particular contaminants may be isolated by sequential enrichment culture or genetic manipulation. Enrichment culturing seeks to isolate those microorganisms, either from water withdrawn from the aquifer or laboratory grown strains, that are capable of a response to the particular contaminants involved. Acclimation can result from an increase in the number of microorganisms that can degrade the contaminant, new metabolic capabilities that

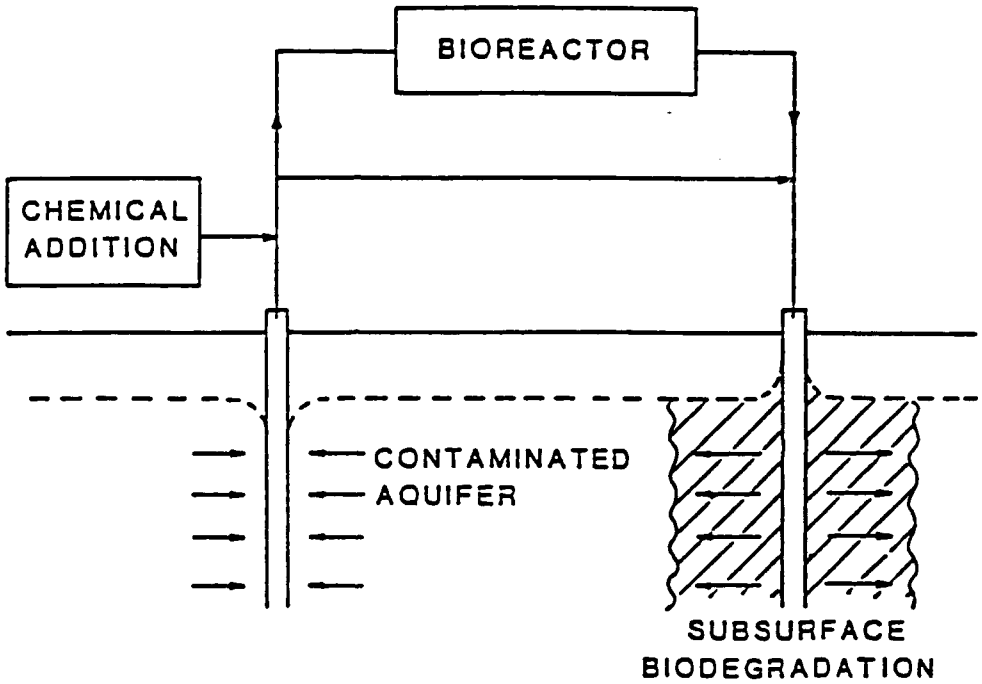


FIGURE 4.1 Proposed Aquifer Decontamination Scheme Using Above Ground Biological Reactor

result from genetic changes, or an increase in the quantity of the enzymes necessary for the transformation (Spain et al., 1980). The genetic changes include overproduction of enzymes, inactivation or alteration of regulatory gene control, or production of enzymes with altered specificities (Ghosal et al., 1985).

Genetic manipulation involves altering bacterial strains in hopes of producing new strains that can degrade contaminants more efficiently. Genetic manipulation can be accomplished by two different basic methods. In the first method, microorganisms are exposed to a mutagen such as ultraviolet light or nitrous oxide. A population with specialized degradative capabilities is then isolated by enrichment culturing; however, this may result in weakened strains because the mutagenic exposure is non-specific, affecting the entire microbial genome with little experimental control (Thomas et al., 1987). In the second method, recombinant DNA technology is used to change the genetic structure of microorganisms. Typically, a plasmid that codes for a specific degradative pathway is transferred from one microorganism to another (Kilbane, 1986). A plasmid is an extra-chromosomal DNA that can be transferred from one bacterium to another via conjugation, transduction, or transformation (Lehninger, 1975). Multiple degradative capabilities can be recombinantly incorporated within a single plasmid, possibly providing organisms with novel degradation pathways for a variety of compounds. Therefore, recombinant techniques allow for the synthesis of desirable genetic traits into a single organism.

A specific example of genetic manipulation involves the development of a bacterial strain capable of degrading 2,4,5-trichlorophenoxyacetic acid (2,4,5-T). Using a technique called plasmid-assisted molecular breeding, Kellogg et al. (1981) isolated a microorganism from mixed culture that used 2,4,5-T as its sole carbon and energy source. The technique requires inoculating a chemostat with both microorganisms from a variety of hazardous waste sites and microorganisms that carry plasmids which code for the degradation of specific xenobiotic compounds. Laboratory studies indicated that the isolated species, Pseudomonas cepacia AC1100, was capable of degrading a number of chlorophenols in addition to 2,4,5-T (Kilbane et al., 1983). However, due to environmental concerns over the use of genetically manipulated microorganisms, field tests to determine the effectiveness of this bacterial strain have not been conducted.

Currently, the EPA (Gulf Breeze Environmental Research Laboratory) and DoD are involved in a cooperative biodegradation research program. One aspect of this program considers the genetic manipulation of an aerobic, TCE-degrading microorganism called G4. Preliminary investigation suggests that a large plasmid may be involved in TCE degradation. In addition, an abiological factor is apparently required in order for TCE degradation to occur. This factor was initially identified as phenol, although toluene, o-cresol, or m-cresol can also be utilized. This abiotic compound seems to serve as an inducer of TCE-degrading enzymes.

One problem which might occur, however, is that the laboratory grown microorganisms, whether enriched for or genetically manipulated, may not be able

to effectively compete under the more hostile environmental conditions of the subsurface, an important aspect to consider when adding acclimated microorganisms. A second difficulty involves getting the added microorganisms to the location of the contaminants in the subsurface, since bacterial transport, especially in fine-grained materials, is often minimal. The wide dispersal of added microorganisms within the subsurface depends upon the permeability of the substrata to microorganisms, which has not been reliably documented in the literature (Healy Jr. and Daughton, 1986). In relatively permeable aquifers, care is required to maintain the permeability by controlling the inoculum size and the onset of rapid growth and biomass accumulation. An excessive concentration of microorganisms in the injection water or uncontrolled growth at the point of injection can create clogging problems that can effectively block further dispersal (McDowell-Boyer et al., 1986; Oberdorfer and Peterson, 1985). Given that the biotransformation of organic pollutants may be less favorable for shorter liquid detention times (Bouwer and Wright, 1987), channeling effects due to microbial clogging in the vicinity of injection or extraction wells may increase local groundwater velocities, shortening the local liquid detention times and reducing the overall rate of biotransformation. Still, an advantage of adding microorganisms to the subsurface is that they can be grown in greater numbers in an aboveground reactor on-site than can be grown within an aquifer. Although this treatment scheme can be used alone, it is probably better if used in conjunction with other in-situ strategies.

4.3.3 Treatment trains

In most contaminated hydrogeologic systems, the complexity of both contaminant interactions and heterogeneous subsurface conditions often precludes any one remediation scheme from achieving the necessary treatment requirements. Instead, several unit operations, in series or sometimes in parallel, must be merged into a single treatment process train in order to efficiently restore groundwater quality to a desired level (Wilson et al., 1986). For example, physical containment methods alone only act as temporary plume control measures without significantly affecting sorbed contaminant concentrations. On the other hand, in-situ biodegradation alone can affect sorbed contaminant levels, but does not prevent a contaminated plume from moving off-site.

Most remediation strategies utilizing in-situ biorestoration have often started by removing heavily contaminated soils and/or removing any free product floating on the groundwater table prior to instituting biorestoration procedures (Wilson et al., 1986). Contaminant removal helps to alleviate potential toxic problems to microorganisms associated with higher pollutant concentrations by diluting the contaminant plume and to reduce the effective time required to accomplish the in-situ biorestoration.

If the contaminant plume tends to flow in one direction, a small zone of intense biological activity could be developed, with the addition of nutrients and perhaps acclimated microorganisms, downstream of the leading edge of the plume (Figure 4.2). This zone would intercept the groundwater flow path and allow the microorganisms present to cleanse the contaminated water as it flows

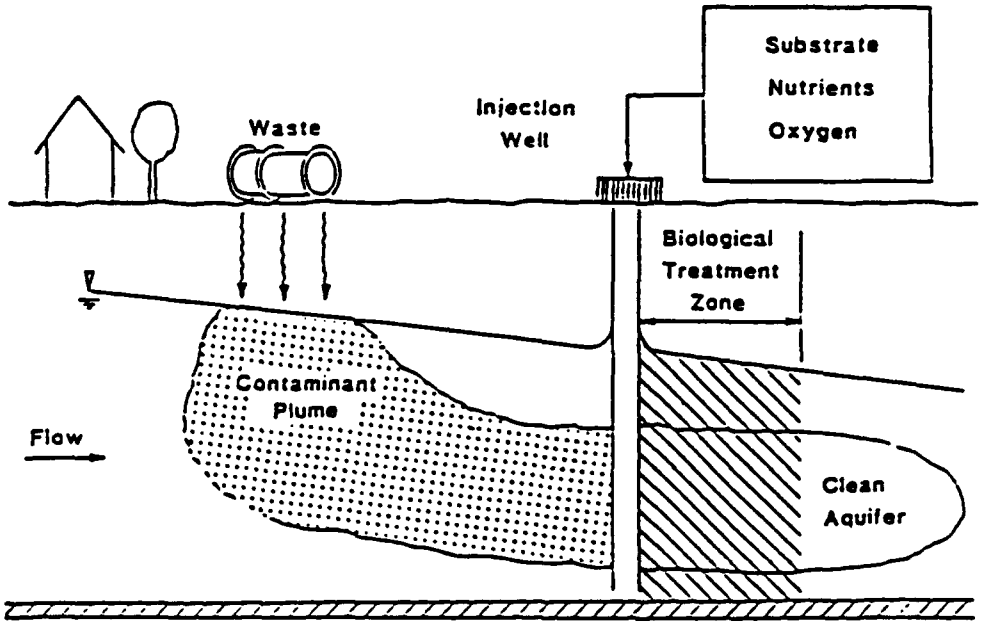


FIGURE 4.2 Proposed Scheme to Establish an In-Situ Biological Treatment Zone Downgradient from a Contamination Plume

past. Placement of a biologically active barrier at the property line of a contaminated site may prevent contaminated groundwater from moving off-site. A series of monitoring wells downstream of the biological zone would detect for contaminant breakthrough, in which case contaminated water could be withdrawn, treated, and reinjected upstream of the treatment zone.

There are numerous proven aboveground treatment processes available for handling a variety of organic and inorganic wastewaters. Still, the limiting factor is getting the contaminant or contaminated subsurface material to the desired treatment unit, or in the case of in-situ biorestoration, getting the treatment process to the contaminated material. Successful remediation requires a thorough understanding of the hydrogeologic, geochemical, and pollutant characteristics in order to predict the scheme for maximum remedial effectiveness which will also minimize remediation costs.

5. EXPERIMENTAL

5.1 Experimental Design

Two continuous-flow, laboratory biofilm column reactors with multiple electron acceptors were operated in order to monitor the efficiency with which microorganisms can biotransform organic pollutants at environmentally significant concentrations. One column was 100cm x 1.1cm I.D. and filled with 3mm glass beads (Column 1). The second column was 100cm x 2.5cm I.D. and filled with 6mm glass beads (Column 2). A defined sterile mineral salts solution (Table 5.1) containing acetate as the primary substrate, ammonia, oxygen, nitrate, and sulfate was continuously applied to both columns in an upflow mode. Glass beads were used as the biofilm support media in order to minimize sorptive effects, while simulating subsurface flow conditions. A group of organic priority pollutants, which included halogenated 1- to 3-carbon aliphatics, chlorinated benzenes, and alkylbenzenes were applied simultaneously at concentrations between 10 and 175μg/L. The columns were seeded with primary sewage to initiate bacterial growth and operated in the dark at a constant temperature (22±1°C) to prevent the growth of photosynthetic organisms. A peristaltic pump maintained a feed rate of 0.45ml/min for both columns, which resulted in a packed-bed detention time of 1.3 hours for Column 1 and 7.6 hours for Column 2.

Influent and effluent samples were collected frequently in continuous flow-through bottle reservoirs without headspace in order to prevent volatilization losses. Additional samples were collected from sampling ports located along the length of the column. The halogenated 1- to 3-carbon aliphatics and chlorinated benzenes were quantified by pentane-extraction gas chromatography with electron capture detection (Henderson et al., 1976). Chlorinated and alkylbenzenes were also assayed by closed-loop stripping analysis (Grob and Zurcher, 1976). Acetate, ammonia, nitrate, and sulfate were assayed by ion chromatography. Dissolved oxygen content and pH were analyzed by procedures given in Standard Methods (American Public Health Assoc., 1980).

5.2 Experimental Results

Within a day after starting the mixed solute feed into Columns 1 and 2, the effluent concentrations of dissolved oxygen and nitrate both decreased to below detection limits. Thus, evidence for acetate utilization by aerobic oxidation and denitrification was immediately established in the columns. Oxygen was depleted in both columns prior to their first sampling ports (2.5 and 3cm depths, respectively) and no nitrification activity was measured. Approximately two months were required for the aerobic and denitrifying biofilm growth to attain an apparent steady-state thickness. After several months of operation, sulfate started to be utilized in the columns, as conditions became more favorable for a sulfate reducing biofilm to develop (Figure 5.1). Around this time, "black" growth often associated with biological sulfide production was observed near the tail end of the denitrifying biofilm, providing visual

TABLE 5.1

Fixed-Film Reactor Feed Composition for Columns 1 and 2.

COMPOUND	CONCENTRATION*
Primary Substrate	
Acetate	60
Inorganic Nutrients	
NH_4^+	10
$MgSO_4 \cdot 7H_2O$	22.5
Na_2SO_4	1.85
$NaNO_3$	13.8
$CaCl_2$	27.5
$NaHCO_3$	20
Trace Elements (Fe, Co, Ni, Zn, Se, Al, Mn, B, Mo)	1
Buffer - pH 7.1	
KH_2PO_4	8.5
K_2HPO_4	21.8
$Na_2HPO_4 \cdot 7H_2O$	33.4
Electron Acceptors	
Oxygen	8**
Nitrate	10
Sulfate	10

*All concentrations are in mg/L

**This value represents the saturated dissolved oxygen concentration

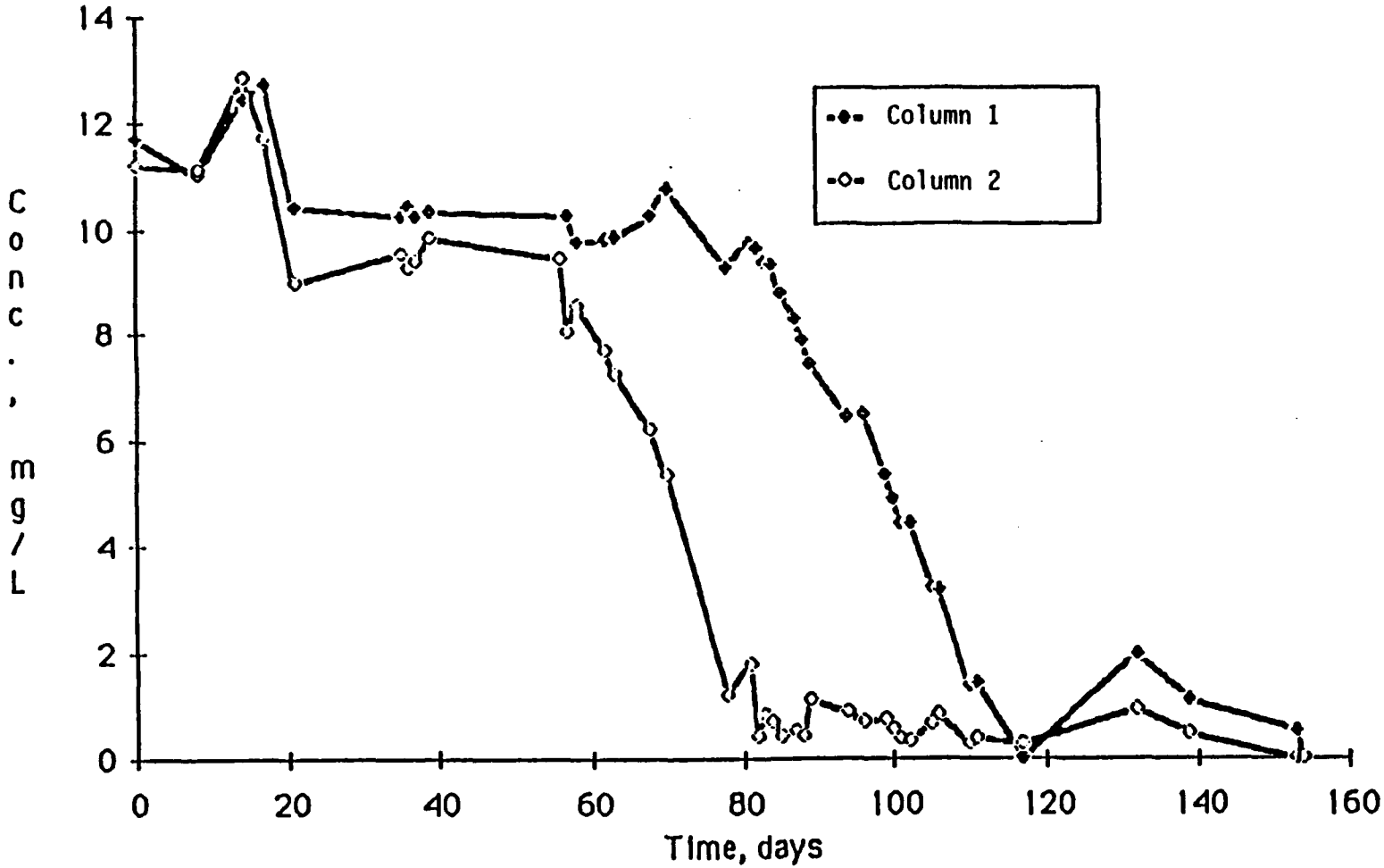


FIGURE 5.1 Column effluent concentration versus time data for sulfate during the first five months. Influent sulfate concentration remained at 11.0 ± 1.0 mg/L throughout the entire period.

confirmation that sulfate respiration was occurring. Although both columns were fed excess acetate, no evidence for methanogenic activity has been observed throughout this study, despite inoculating the anoxic portions of both columns with methanogenic organisms.

Previous work (Bouwer and McCarty, 1983a; Bouwer and Wright, 1987) has shown a high removal efficiency for most of the halogenated aliphatic compounds used in this study in methanogenic columns with a packed-bed detention time of 2.5 days. The less reducing conditions of denitrification and sulfate respiration present at the shorter detention times in this study have not been conducive to the complete removal of all the applied halogenated compounds (Table 5.2). The behavior is in qualitative agreement with observed removal efficiencies found for less reducing conditions in column studies with detention times of both 1 hour and 2.5 days (Bouwer and McCarty, 1983b; Bouwer and Wright, 1987). Comparing the results from this research at short detention times with previous studies, it appears that a longer detention time allows for a greater percentage removal of most halogenated aliphatics, chlorinated benzenes, and alkylbenzenes under favorable electron acceptor conditions, presumably because the micropollutants are in contact with the biomass for a longer time.

In the first several months of the project, little evidence was obtained for the biotransformation of most of the halogenated aliphatic compounds in the acetate-supported biofilm columns (Table 5.3). The observed removals of approximately 20 to 30% might have been due to initial sorption losses onto the biofilm. The initial persistence of these halogenated compounds agrees with results observed under denitrifying conditions, which favors the transformation of only a few halogenated micropollutants (Bouwer and McCarty, 1983b; Bouwer and Wright, 1987).

Around the same time that sulfate started to be utilized as an electron acceptor in the columns, significant transformation of CT, BF, BDCM, and DBCM was observed (Figure 5.2). This suggested that sulfate reduction was required for their biotransformation. In addition, DBCP also began to show significant removal in Column 1 after about a six-month lag period, which indicated that a biological mechanism was involved.

The secondary utilization of the applied micropollutants in the mixed electron acceptor system used in this research agrees with results observed in single electron acceptor biofilm column studies (Bouwer and McCarty, 1983a,1983b; Bouwer and Wright, 1987). The biotransformaiton of CT, BF, BDCM, and DBCM below their detection limit in Column 1 corresponded to the nearly complete removals of these compounds observed under both denitrification and sulfate respiration (Bouwer and McCarty, 1983b; Bouwer and Wright, 1987). The lesser removal of DBCP, EDB, and 111-TCE was similar to that observed in sulfate reducing environments (Bouwer and Wright, 1987).

TABLE 5.2

Average Removals of Organic Micropollutants in Biofilm Column Reactors

Compound	COLUMN 1 Influent* µg/L	COLUMN 1 % Removal	COLUMN 2 Influent* µg/L	COLUMN 2 % Removal
Aliphatics				
CF	134 ± 31	53 ± 13	111 ± 18	40 ± 14
CT	79 ± 22	> 99	68 ± 11	85 ± 7
BDCM	155 ± 36	> 99	131 ± 22	56 ± 15
DBCM	131 ± 30	> 99	112 ± 21	59 ± 12
BF	177 ± 37	> 99	154 ± 31	62 ± 11
111TCE	92 ± 22	58 ± 12	78 ± 13	38 ± 16
EDB	109 ± 23	58 ± 10	92 ± 19	46 ± 13
TeCE	46 ± 12	42 ± 20	35 ± 7	38 ± 18
DBCP	155 ± 34	90 ± 3	137 ± 33	44 ± 15
Aromatics				
12DCB@	72 ± 14	41 ± 14	63 ± 13	40 ± 15
13DCB@	52 ± 11	41 ± 15	44 ± 9	40 ± 15
14DCB@	56 ± 12	46 ± 14	47 ± 10	41 ± 16
124TCB@	34 ± 7	43 ± 14	28 ± 6	38 ± 18

* One standard deviation of the mean values are given.

@ The following abbreviations were not previously defined:

12DCB - 1,2-Dichlorobenzene
13DCB - 1,3-Dichlorobenzene
14DCB - 1,4-Dichlorobenzene
124TCB - 1,2,4-Trichlorobenzene

TABLE 5.3

Average Removals after Six Months for Selected Organic Micropollutants in Columns 1 and 2.

COMPOUND	COLUMN 1 INFLUENT* (ug/L)	COLUMN 1 % REMOVAL	COLUMN 2 INFLUENT* (ug/L)	COLUMN 2 % REMOVAL
ALIPHATICS				
Chloroform	80±11	34±20	68±14	24±23
1,1,1-Trichloroethane	73±8	41±9	57±12	25±20
Tetrachloroethylene	18±4	26±32	16±7	16±42
Ethylene Dibromide	58±7	31±19	50±10	22±24
Dibromochloropropane	79±31	44±29	73±27	40±29
AROMATICS				
1,2-Dichlorobenzene	49±13	32±25	42±11	23±25
1,3-Dichlorobenzene	33±8	31±25	28±7	24±22
1,4-Dichlorobenzene	37±10	30±27	32±8	23±23
1,2,4-Trichlorobenzene	18±4	31±27	15±4	21±28

*All values represent average concentrations and their standard deviations

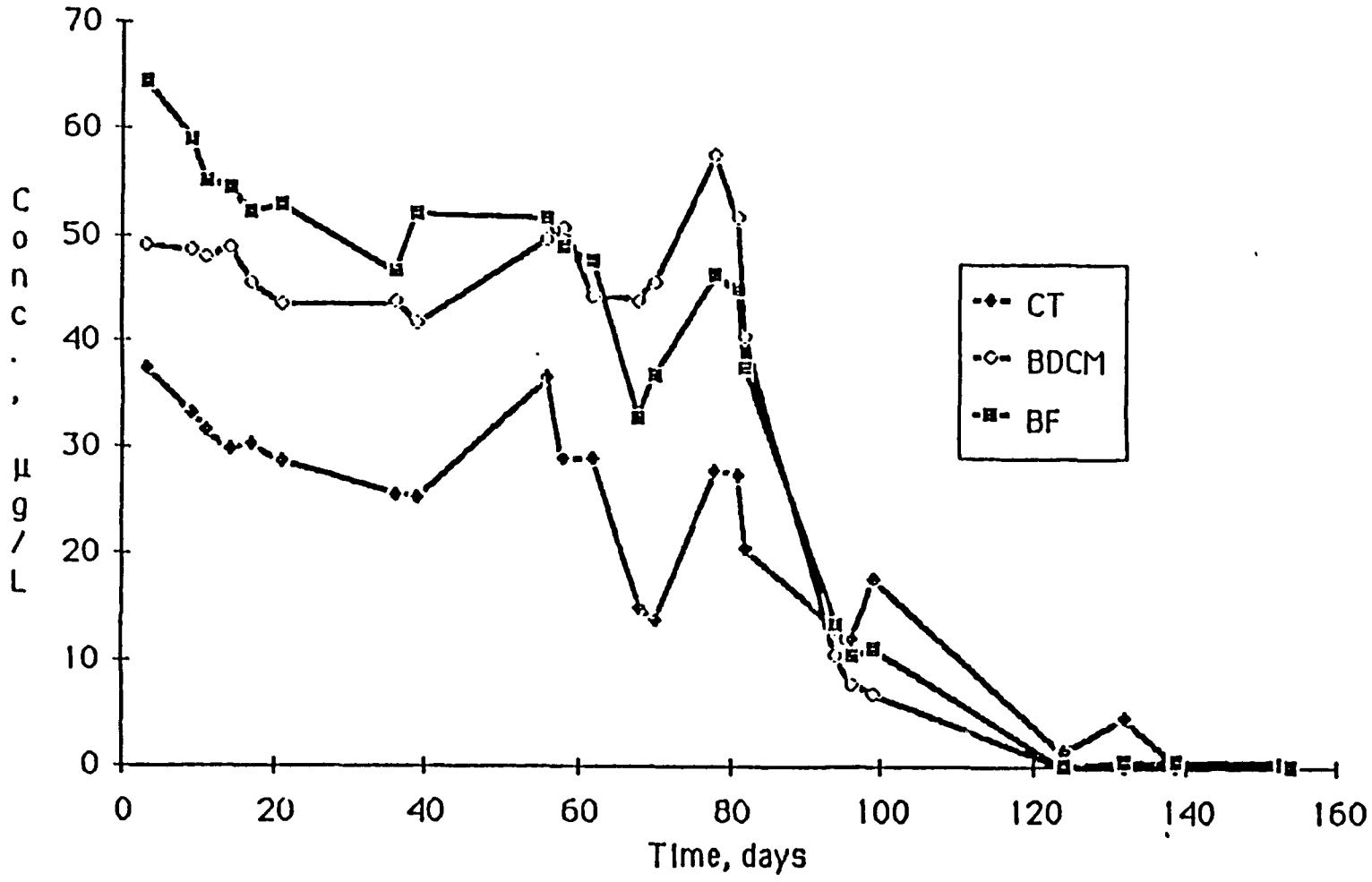


FIGURE 5.2 Column 1 effluent concentration versus time data for carbon tetrachloride (CT), bromodichloromethane (BDCM), and bromoform (BF) during the first five months. Influent concentrations remained at 46 ± 16 ug/L, 72 ± 13 ug/L, and 74 ± 24 ug/L, respectively, throughout the entire period.

6. PERFORMANCE CRITERIA

Currently, there is relatively little quantitative data regarding in-situ biorestoration of aquifers, especially with respect to costs associated with any particular process. This lack of information makes it difficult to adequately compare the cost effectiveness of the various schemes. Despite this, the remainder of this section will focus on the following performance criteria as a tool to qualitatively evaluate the feasibility of in-situ technology:

1) Capital costs;
2) Annual operating costs;
3) Performance effectiveness;
4) Residuals generated;
5) Secondary environmental impacts;
6) Commercial availability;
7) Previous experience and applications with contaminated groundwater;
8) Operation and maintenance requirements;
9) Treatment interference; and
10) General applicability;

Performance criteria would be difficult to compare quantitatively, even with accurate data, due to the highly site-specific nature of all contamination incidents.

6.1 Capital Costs

Costs for biological in-situ treatment are determined by the nature of the site geology and hydrogeology, the extent of contamination, the types and concentrations of contaminants, and the amount of groundwater and soil requiring treatment. There is no simple formula for in-situ cost estimation. Yet, costs provided for actual site cleanups indicate that in-situ biological treatment can be far more economical than excavation and removal or conventional pumping and treatment methods (Wagner et al., 1986). All in-situ biorestoration strategies would require the installation of both injection and withdrawal wells. The number of wells needed would depend on the design of the well field. For example, a central withdrawal well would probably be surrounded by a circular-type pattern of injection wells, while a biological treatment zone would possibly have a linear set of injection wells upgradient of the plume and a linear set of withdrawal wells downstream. Monitoring wells would also be needed in order to detect movement of contaminants off-site or out of the treatment zone. In addition, depending on the nutrient distribution system and whether organisms will be added, some type of above ground storage, mixing or biological reaction vessels will be necessary. Their sizing will be based on the expected volume of material to be handled. Wagner et al. (1986) provide detailed cost estimates for groundwater containment, removal, or diversion strategies used in conjunction with in-situ treatment.

6.2 Annual Operating Costs

This will vary with the chosen treatment scheme. Least costly would be an initial pumping procedure with nutrient addition to stimulate growth, after which natural biological processes take over under normal aquifer conditions. Higher costs would be associated with continuous pumping and nutrient addition to establish a subsurface region of active biological growth or the continuous addition of an adapted microbial population. A potential savings may be realized if periodic spikes of nutrients, substrate, and/or microorganisms are determined to be sufficient to maintain an effective subsurface microbial population. Costs will increase with increasing extent of contamination and length of time for cleanup.

6.3 Performance Effectiveness

The performance effectiveness is not a function of the treatment scheme as much as the environmental factors such as soil conditions and contaminant properties. Overall effectiveness is likely to be measured based on the rate of contamination decrease in the subsurface. Cleanup strategies taking less than 3 to 5 years would probably be considered satisfactory, depending on the amount of contamination, while processes that take longer would be unsatisfactory and additional treatment schemes other than in-situ processes would have to be employed.

Biotransformation is most easily documented under carefully controlled laboratory conditions that permit accurate mass balances. Due to the complexity of subsurface environments and the inability to conduct precise experiments in-situ, most investigations lack conclusive evidence for in-situ biotransformation. Complete mineralization or partial conversion of an organic substrate by microorganisms to its ultimate degradation products and cell mass can be easily monitored in a closed experimental system that allows for overall mass balances. Radiolabelled substrates can be used to obtain rigorous mass balances. The ultimate distribution of metabolites can then be quantified into their respective biotic or abiotic compartments (Healy, Jr. and Daughton, 1986). Although the introduction of radiolabelled compounds into groundwater is currently prohibited by both federal and state regulatory agencies, their usefulness in field situations is questionable since it is theoretically impossible to isolate a portion of the subsurface without affecting environmental processes that control the fate of contaminants. However, environmental responses unique to microbial activity can be used as circumstantial evidence that in-situ biotransformation is occurring. Examples of such activity include reduction of substrate concentration (assuming sorption is negligible) with a corresponding increase in biomass, the onset of metabolic behavior indicative of catabolism (e.g. acclimation of microbial populations to a particular compound), and production of catabolites indicative of biotransformation or compounds that result indirectly from catabolism (e.g. gas production or reduction of terminal electron acceptors).

6.4 Residuals Generated

A concern when increasing the bacterial numbers in the subsurface is the potential for clogging effects, due to the greater amounts of biomass, both in the pores of the soil and in well screens and casings. Clogging could lead to channeling effects which might decrease the treatment efficiency by bypassing contaminated zones within an aquifer. A slight possibility also exists that enhanced bacterial growth could lead to the generation of toxic by-products, which might be harmful to human health if produced in drinking water aquifers. However, it is highly unlikely that human pathogenic organisms, which comprise a negligible portion of total subsurface microorganisms, would be able to grow and produce toxins in a hostile subsurface environment, especially without a human host present. Research on rapid-infiltration of secondary wastewater effluent has shown that concentrations of fecal coliforms and viruses are reduced to below detectable limits within a short travel distance in the subsurface (Bouwer et al., 1980). Another concern is the incomplete transformation of organic products, especially when the intermediate products generated exhibit toxicity greater than that of the original product. Additionally, incomplete transformation necessarily means that aquifer cleanup has not been effective. If aboveground biological reaction vessels are needed, there will be biological sludge generated which must be disposed of properly.

6.5 Secondary Environmental Impact

There are a wide variety of secondary environmental impacts that potentially could occur. Since only a finite number of monitoring, injection, and withdrawal wells can be installed, movement of contaminated groundwater off-site is a potential problem. Even with complex well systems, heterogeneous soil properties, such as fractures, could remove groundwater outside of the area of detection. Given that these in-situ processes require increased microbial populations for faster treatment, possibly involving the addition of genetically manipulated microbes, the possibility exists that a microbial population could be modified within the subsurface, perhaps creating a harmful mutant strain. Unfortunately, it is nearly impossible to predict what effect the subsurface will have on reproduction and degradative ability of microorganisms. Several impacts are associated with the aboveground reactors as well. Volatilization losses in on-site biological reactors during aeration and mixing are a potential air pollution problem. The magnitude of the problem depends on the geographical location of the site and the amount of actual losses into the air. A final example would be the generation of odors from the biological reactors. This is likely to be a relatively minor problem, since the prevailing winds would tend to dissipate the odors away from the site. Other secondary impacts are likely to occur and their hazardous potential must be addressed when implementing treatment technologies.

6.6 Commercial Availability

The initial interest in biological restoration of aquifers began with the treatment of sites contaminated by hydrocarbon spills. Treatment usually consisted of methods to provide adequate oxygen and perhaps nutrients to the soil. Today, although there is very little packaged technology available, especially for anaerobic treatment, numerous companies have begun to develop and market treatment schemes for aquifer restoration (Nagel et al., 1982; Ohneck and Gardner, 1982; Quince and Gardner, 1982a; 1982b; Sylvester and Landon, 1982; and Yaniga, 1982). The future availability of these technologies will ultimately depend on their ability to efficiently degrade pollutants and restore the contaminated site.

6.7 Application to Groundwater

Applications of in-situ restoration have mainly been limited to hydrocarbon contamination incidents (Canter and Knox, 1985). In the recent literature, almost all documented cases have been concerned with maintaining oxygenated conditions within the subsurface, since hydrocarbons are degraded easily by microorganisms in the presence of oxygen. The treatment of solvents that have been shown to degrade well in anaerobic environments has usually been done aboveground by physical methods, such as air stripping or carbon adsorption (Canter and Knox, 1985; Nyer, 1985). As biological research efforts progress, more contaminated sites will probably utilize some form of in-situ treatment technology, under both aerobic and anaerobic conditions, as the primary method of aquifer restoration.

6.8 Operation and Maintenance Costs

A problem with in-situ technology is that control of the system may be quite difficult, especially given the current limited experience of in-situ treatment. The proper amount of pumping, nutrient addition, and microorganism addition will probably require strict controls and the attention of highly skilled operators. Operation and maintenance costs will depend largely on the areal extent of contamination and the type and amount of nutrients and/or microorganisms needed for effective treatment. Additional costs will be incurred from the monitoring program and the complexity of chemical analysis. As the cleanup procedure continues, operating and maintenance requirements should decrease due to a decrease in the overall extent of contamination and an increase in knowledge regarding site conditions.

6.9 Treatment Interference

Several independent factors, some of which have been previously discussed, could be expected to interfere with the efficiency of in-situ biorestoration of aquifers. Due to the complex, heterogeneous nature of soil, not all subsurface locations are likely to be restored equally. For example, fluctuations in the

water table might serve to trap pollutants under a clay lens or channel flow away from contaminated areas. Lack of nutrients could inhibit microbial activity which would slow the rate of biotransformation. High concentrations of heavy metals or pollutants might cause toxic effects which could inhibit biological metabolism. When designing an in-situ biorestoration scheme, attention must be given to potential treatment interferences so that steps may be taken to prevent them from significantly affecting the process efficiency.

6.10 General Applicability

Since the implementation of any treatment method, not only in-situ treatment, is so highly site-specific, the general applicability of in-situ biorestoration is hard to assess without considering the conditions present at the contaminated site. The applicability of in-situ treatment, as stated earlier, depends on both the soil conditions and contaminant properties. However, since microorganisms can mediate a wide variety of organic biotransformations, in-situ biorestoration would appear to be a feasible option, either by itself or perhaps in conjunction with other treatment schemes.

7. CONCLUSIONS AND RECOMMENDATIONS

7.1 Conclusions

In most contaminated subsurface systems, the restoration process is so complex with respect to contaminant behavior and site characteristics that no one treatment strategy is likely to meet all removal requirements. In all likelihood, it will be necessary to combine treatment processes into an overall scheme in order to restore ground water quality to a required level within a reasonable period of time. For example, containment, either physical barriers or hydrodynamic control, can only serve as a temporary measure for plume control, although hydrodynamic control will often be an integral part of any withdrawal and treatment or in-situ treatment scheme.

There are already proven above ground treatment processes available for treating a variety of contaminants. Yet, despite their proven success, these processes are ineffective if the contaminants cannot be extracted from the soil for treatment. In-situ biorestoration would provide a feasible option for attacking those contaminants which are difficult to remove from the soil matrix. In addition, biorestoration would also be effective in biotransforming compounds in areas where they are not necessarily detected by monitoring wells. However, in order to ensure successful treatment, the in-situ process must be able to reach the contaminated material. The key to successful cleanup is a thorough understanding of the hydrogeologic and chemical characteristics at a contaminated site, so that a proper integration of restoration strategies can be achieved which maximizes removal potential and minimizes operating and capital costs.

The laboratory biofilm column reactors demonstrated that a number of environmentally important organic micropollutants were transformed under sequential electron acceptor conditions. Secondary utilization, a degradative mechanism by which bacteria are capable of destroying trace concentrations of organic contaminants that otherwise could not support bacterial growth, may be an important means of pollutant removal in oligotrophic subsurface environments. In particular, the degree of biotransformation depended on the electron acceptor being used by the microorganisms in obtaining energy.

Based on current research in the laboratory and in the field, in-situ biological treatment has the potential to destroy organic contaminants, thereby restoring aquifers to their natural state. Present knowledge suggests that in-situ biorestoration is likely to be more cost-effective than other treatment approaches and should be an environmentally sound technology. Although investment in in-situ treatment offers a potentially large return, current techniques are still in the basic research phase. Many information gaps must be investigated prior to the implementation of a generally applicable treatment scheme. Fundamental information on factors affecting biotransformation and reaction rates is urgently needed. Other essential areas where further investigation is necessary include the determination of acclimation periods required by microorganisms, system engineering and design in order to provide

sufficient contact between contaminants and bacteria, and characterization of the subsurface geology and biological and chemical activity at contaminated sites. The interaction between biomass accumulation and hydraulic properties of an aquifer also needs to be established. Some of these areas will be examined in the second phase of this project. Additional field experience will also provide much needed information.

7.2 Recommendations

At the present time, there are no generally applicable, commercially available in-situ biorestoration strategies for the treatment of groundwater contamination. Although an abundance of material detailing the ability of microorganisms to degrade organic compounds under a variety of experimental conditions is available, there is little conclusive evidence, particularly in anoxic environments, for the in-situ biotransformation of organic pollutants. Considerable knowledge has been gained regarding the environmental factors necessary for optimizing subsurface microbiological activity; however, a combination of either regulatory restrictions or field heterogeneities often hinders the widespread implementation of these optimal factors. Performance criteria have been proposed in order to evaluate the potential cost- and treatment-effectiveness of different remediation strategies. Many research questions have been answered, but many more require further investigation. The following recommendations represent some of the general needs which must be addressed.

1) site characterization
The hydrogeological, biological, chemical, and physical properties of contaminated sites must be characterized more fully. This is particularly true when assessing the cost of treatment needs so that unnecessary equipment or materials are not purchased.

2) laboratory studies investigating relationship among environmental factors
Further investigation of the interactions between the different environmental factors is necessary in order to maximize in-situ treatment efficiency. Microcosm or column studies which simulate the subsurface environment provide valuable knowledge about environmental factors. Changes in biotransformation due to the variation of these environmental factors can be investigated easily under controlled conditions in laboratory studies. The manipulation of different combinations of factors can provide large amounts of information quickly.

3) selection of specific field sites for demonstration projects
Since laboratory studies are often conducted under "idealized" conditions, their results should only be taken as a general indicator of what might actually occur in the subsurface. Biotransformations at specific field sites need to be investigated in order to corroborate laboratory results with actual conditions. Selection of a particular remedial action will depend on the

nature and concentration of the contaminants and the requirements for successful microbial growth. A list of favorable and unfavorable chemical and hydrogeological conditions for implementation of in-situ biorestoration at a specific site appears in Table 7.1.

While in-situ biorestoration holds great promise for control of organic contamination, the principles involved are in the basic research phase. Many fundamental questions that have been posed in this report and the accompanying report "Hydrogeologic Effects of In-Situ Groundwater Treatment Using Biodegradation" by Grant Garven (Contract No. DAAG29-81-D-0100; Delivery Order 2378) need to be answered before this technique can be generally applied. The best course of action for the U.S. Army is to continue laboratory-scale evaluation and to select field sites with favorable characteristics (Table 7.1) for pilot testing of in-situ biorestoration approaches.

4) evaluate complex mixtures

Most laboratory studies tend to look at simplified environmental systems, such as pure cultures of bacteria degrading a single pollutant. The ability of a microorganism to adapt to a toxic compound at high concentration is encouraging, but more detailed studies are needed with mixed microbial cultures working cooperatively to degrade complex mixtures of compounds at typical environmental concentrations. With a mixture of compounds, the potential exists for synergism (an increase in the severity of toxicity) or antagonism (a decrease in the severity of toxicity). Although an individual pollutant may be biotransformable at certain concentrations, it could change from biodegradable to non-biodegradable and/or toxic when combined with other pollutants. This aspect of mixtures would be difficult to assess and would initially require the evaluation of a given mixture in the lab to investigate its potential biodegradability.

5) monitoring procedures

The installation of monitoring wells at field sites can potentially alter the groundwater flow pattern. However, in order to examine whether the selected in-situ strategy is effective, some type of monitoring must be performed. Procedures need to be outlined for the development and installation of monitoring wells within a contaminated aquifer which will not significantly affect the effectiveness of the remediation scheme.

Table 7.1 Favorable and unfavorable chemical and hydrogeological site conditions for implementation of in-situ biorestoration

FAVORABLE

CHEMICAL CHARACTERISTICS

small number of organic contaminants

non-toxic concentration

diverse microbial populations

suitable electron acceptor condition

pH 6 to 8

HYDROGEOLOGICAL CHARACTERISTICS

granular porous media

high permeability

uniform mineralogy

homogeneous media

saturated media

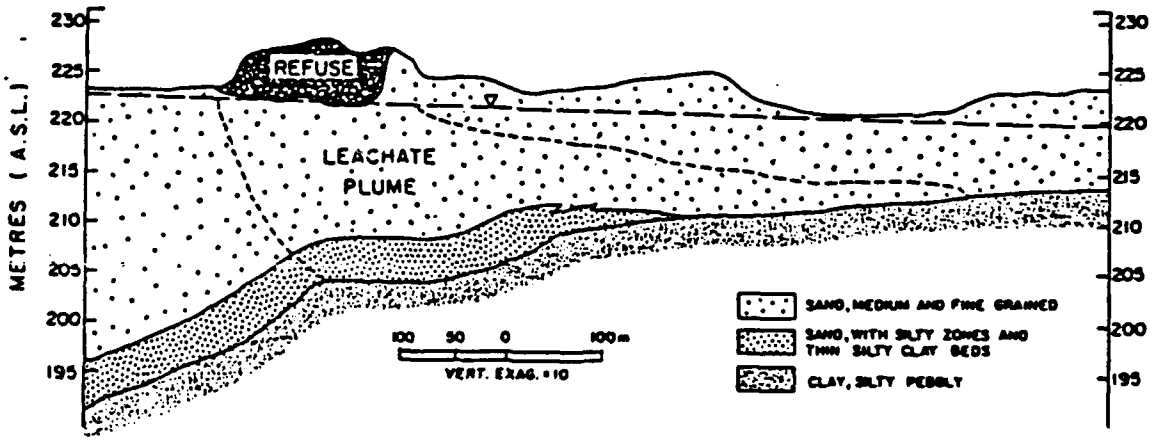


Table 7.1 (continued)

UNFAVORABLE

CHEMICAL CHARACTERISTICS

numerous contaminants

complex mixture of inorganic and organic compounds

toxic concentrations

sparse microbial activity

absence of appropriate electron acceptors

pH extremes

HYDROGEOLOGICAL CHARACTERISTICS

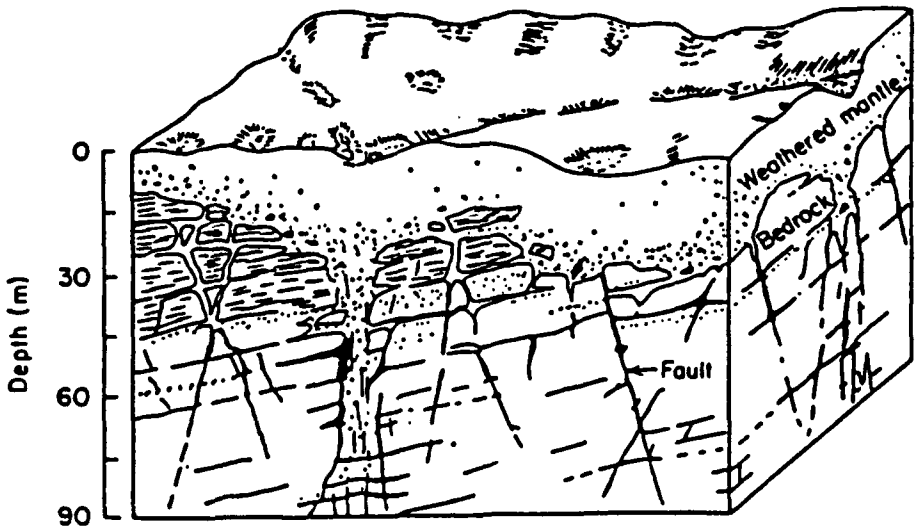
fractured rock

low permeability

complex mineralogy

heterogeneous media

unsaturated-saturated conditions



8. REFERENCES

American Public Health Association (1980). Standard Methods for the Examination of Water and Wastewater, 15th ed., Washington D.C.

Baker, M.D., Mayfield, C.I. and Inniss, W.E. (1980). Degradation of Chlorophenols in Soil, Sediment and Water at Low Temperature. Water Res., 14(12): 1765-1771.

Balkwill, D.L. and Ghiorse, W.C. (1985). Characterization of Subsurface Bacteria Associated with Two Shallow Aquifers in Oklahoma. Appl. Environ. Microbiol., 50(3): 580-588.

Barrio-Lage, G., Parsons, F.Z., Nassar, R.S. and Lorenzo, P.A. (1986). Sequential Dehalogenation of Chlorinated Ethenes. Environ. Sci. Tech., 20(1): 96-99.

Bouwer, E.J., Rittmann, B.E. and McCarty, P.L. (1981). Anaerobic Degradation of Halogenated 1- and 2-Carbon Organic Compounds. Environ. Sci. Tech., 15(5): 596-599.

Bouwer, E.J. and McCarty, P.L. (1982). Removal of Trace Chlorinated Organic Compounds by Activated Carbon and Fixed-Film Bacteria. Environ. Sci. Tech., 16(12): 836-843.

Bouwer, E.J. and McCarty, P.L. (1983a). Transformations of 1- and 2-Carbon Halogenated Aliphatic Organic Compounds under Methanogenic Conditions. Appl. Environ. Microbiol., 45(4): 1286-1294.

Bouwer, E.J. and McCarty, P.L. (1983b). Transformations of Halogenated Organic Compounds under Denitrification Conditions. Appl. Environ. Microbiol., 45(4): 1295-1299.

Bouwer, E.J. and McCarty, P.L. (1984). Modeling of Trace Organics Biotransformation in the Subsurface. Ground Water, 22(4): 433-440.

Bouwer, E.J. and McCarty, P.L. (1985). Ethylene Dibromide Transformations under Methanogenic Conditions. Appl. Environ. Microbiol., 50(2): 527-528.

Bouwer, E.J. and Wright, J.P. (1987). Transformations of Trace Halogenated Aliphatics in Subsurface Microcosms with Anoxic Biofilms. Submitted to the Journal of Contaminant Hydrology.

Bouwer, H., Rice, R.C., Lance, J.C. and Gilbert, R.G. (1980). Rapid-Infiltration Research at Flushing Meadows Project, Arizona. J. Water Poll. Ctrl. Fed., 52(10): 2457-2470.

Brock, T.D., Smith, D.W. and Madigan, M.T. (1984). Biology of Microorganisms, 4th ed., Prentice-Hall, Inc., Englewood Cliffs, NJ.

Brunner, W., Staub, D. and Leisinger, T. (1980). Bacterial Degradation of Dichloromethane. Appl. Environ. Microbiol., 40(5): 950-958.

Canter, L.W. and Knox, R.C. (1985). Ground Water Pollution Control, Lewis Publishers, Inc., Chelsea, MI.

Castro, C.E. and Belser, N.O. (1968). Biodehalogenation. Reductive Dehalogenation of the Biocides Ethylene Dibromide, 1,2-Dibromo-3-Chloropropane, and 2,3-Dibromobutane in Soil. Environ. Sci. Tech., 2(10): 779-783.

Castro, C.E., Wade, R.S. and Belser, N.O. (1985). Biodehalogenation: Reactions of Cytochrome P-450 with Polyhalomethanes. Biochemistry, 24: 204-210.

Criddle, C.S., McCarty, P.L., Elliott, M.C. and Barker, J.F. (1986). Reduction of Hexachloroethane to Tetrachloroethylene in Groundwater. J. Contam. Hydrology, 1(1/2): 133-142.

Curtis, H. (1979). Biology, 3rd ed., Worth Publishers, Inc., New York, NY.

Ehrlich, G.G., Goerlitz, D.F., Godsy, E.M. and Hult, M.F. (1982). Degradation of Phenolic Contaminants in Ground Water by Anaerobic Bacteria: St. Louis Park, Minnesota. Ground Water, 20(6): 703-710.

Fogel, M.M., Taddeo, A.R. and Fogel, S. (1986). Biodegradation of Chlorinated Ethenes by a Methane-Utilizing Mixed Culture. Appl. Environ. Microbiol., 51(4): 720-724.

Freeze, R.A. and Cherry, J.A. (1979). Ground Water. Prentice-Hall, Inc.. Englewood Cliffs, NJ.

Ghosal, D., You, L.S., and Chakrabarty, A.M. (1985). Microbial Degradation of Halogenated Compounds. Science, 228: 135-142.

Grob, K. and Zurcher, F. (1976). Stripping of Trace Organic Substrates from Water: Equipment and Procedure. J. Chromatog., 117: 285-294.

Healy Jr., J.B. and Young, L.Y. (1979). Anaerobic Biodegradation of Eleven Aromatic Compounds to Methane. Appl. Environ. Microbiol., 38(1): 84-89.

Healy Jr., J.B. and Daughton, C.G. (1986). Issues Relevant to Biodegradation of Energy-Related Compounds in Ground Water: A Literature Review. Sanitary Engineering and Environmental Health Research Laboratory, College of Engineering, School of Public Health, University of California Berkeley. UCB/SEEHRL 86-10.

Henderson, J.E., Peyton, G.R. and Glaze, W.H. (1976). A Convenient Liquid-Liquid Extraction Method for the Determination of Halomethanes in Water at the Part-Per-Billion-Level. In Identification and Analysis of Organic Pollutants in Water, Keith, C.H., Ed. Ann Arbor Science Publishers, Inc., Ann Arbor, MI, pp. 105-112.

Herbes, S.E. and Schwall, L.R. (1978). Microbial Transformations of Polycyclic Aromatic Hydrocarbons in Pristine and Petroleum-Contaminated Sediments. Appl. Environ. Microbiol., 35(2): 306-316.

Horowitz, A., Suflita, J.M. and Tiedje, J.M. (1983). Reductive Dehalogenations of Halobenzoates by Anaerobic Lake Sediment Microorganisms. Appl. Environ. Microbiol., 45(4): 1459-1465.

Hutchins, S.R., Tomson, M.B., Bedient, P.B. and Ward, C.H. (1985). Fate of Trace Organics during Land Application of Municipal Wastewater. CRC Crit. Rev. Environ. Ctrl., 15: 355-413.

Janssen, D.B., Scheper, A., Dijkhuizen, L. and Witholt, B. (1985). Degradation of Halogenated Aliphatic Compounds by Xanthobacter autotrophicus GJ10. Appl. Environ. Microbiol., 49(3): 673-677.

Josephson, J. (1980). Groundwater Strategies. Environ. Sci. Tech., 14(9): 1030-1035.

Kellogg, S.T., Chatterjee, D.K., and Chakrabarty, A.M. (1981). Plasmid-assisted molecular breeding: New Techniques for Enhanced Biodegradation of Persistent Toxic Chemicals. Science, 214: 1133-1135.

Kilbane, J.J., Chatterjee, D.K. and Chakrabarty, A.M. (1983). Detoxification of 2,4,5-Trichlorophenoxyacetic Acid from Contaminated Soil by Pseudomonas cepacia. Appl. Environ. Microbiol., 45(5): 1697-1700.

Kilbane, J.J. (1986). Genetic Aspects of Toxic Chemical Degradation. Microb. Ecol., 12(1): 135-145.

Kleopfer, R.D., Easley, D.M., Haas Jr., B.B., Deihl, T.G., Jackson, D.E. and Wurrey, C.J. (1985). Anaerobic Degradation of Trichloroethylene in Soil. Environ. Sci. Tech., 19(3): 277-280.

Kobayashi, H. and Rittmann, B.E. (1982). Microbial Removal of Hazardous Organic Compounds. Environ. Sci. Tech., 16(3): 170A-183A.

Kuhn, E.P., Colberg, P.J., Schnoor, S.L., Wanner, O., Zehnder, A.J.B. and Schwarzenbach, R.P. (1985). Microbial Transformations of Substituted Benzenes during Infiltration of River Water to Groundwater: Laboratory Column Studies. Environ. Sci. Tech., 19(10): 961-968.

Lee, M.D., Wilson, J.T. and Ward, C.H. (1984). Microbial Degradation of Selected Aromatics in a Hazardous Waste Site. Dev. Ind. Microbiol., 25: 557-565.

Lehninger, A.L. (1975). Biochemistry, 2nd ed. Worth Publishers, Inc., New York, NY.

Marinucci, A.C. and Bartha, R. (1979). Biodegradation of 1,2,3- and

1,2,4-Trichlorobenzene in Soil and Liquid Enrichment Culture. Appl. Environ. Microbiol., 38(5): 811-817.

McCarty, P.L., Reinhard, M. and Rittmann, B.E. (1981). Trace Organics in Groundwater. Environ. Sci. Tech., 15(1): 40-51.

McDowell-Boyer, L.M., Hunt, J.R. and Sitar, N. (1986). Particle Transport through Porous Media. Water Res. Res., 22(13): 1901-1921.

Morrison, R.T. and Boyd, R.N. (1974). Organic Chemistry, 3rd ed. Allyn and Bacon, Inc., Boston, MA.

Nagel, G., Kuehn, W., Werner, P. and Sontheimer, H. (1982). Sanitation of Groundwater by Infiltration of Ozone Treated Water. GWF-Wasser/Abwasser, 123(8): 399-407.

Nelson, M.J.K., Montgomery, S.O., O'Neill, E.J. and Pritchard, P.H. (1986). Aerobic Metabolism of Trichloroethylene by a Bacterial Isolate. Appl. Environ. Microbiol., 52(2): 383-384.

Nyer, E.K. (1985). Groundwater Treatment Technology. Van Nostrand Reinhold Company Inc., New York, NY.

Oberdorfer, J.A. and Peterson, F.L. (1985). Waste-Water Injection: Geochemical and Biogeochemical Clogging Processes. Ground Water, 23(6): 753-761.

Ohneck, R.J. and Gardner, G.L. (1982). Restoration of an Aquifer Contaminated by an Accidental Spill of Organic Chemicals. Ground Water Monit. Rev., 2(4): 50-53.

Parsons, F., Wood, P.R. and DeMarco, J. (1984). Transformations of Tetrachloroethene and Trichloroethene in Microcosms and Groundwater. JAWWA, 76(2): 56-59.

Parsons, F. and Lage, G.B. (1985). Chlorinated Organics in Simulated Groundwater Environments. JAWWA, 77(5): 52-59.

Patel, R.N., Hou, C.T., Laskin, A.I. and Felix, A. (1982). Microbial Oxidation of Hydrocarbons: Properties of a Soluble Methane Monooxygenase from a Facultative Methane-Utilizing Organism, Methylobacterium sp. Strain CRL-26. Appl. Environ. Microbiol., 44(5): 1130-1137.

Pignatello, J.J. (1986). Ethylene Dibromide Mineralization in Soils under Aerobic Conditions. Appl. Environ. Microbiol., 51(3): 588-592.

Poindexter, J.S. (1981). Oligotrophy: Feast and Famine Existence. Adv. Microbial Ecology, 5: 63-89.

Pritchard, P.H. and Bourquin, A.W. (1984). The Use of Microcosms for Evaluation of Interactions between Pollutants and Microorganisms. Adv. Microbial Ecology,

7: 133-215.

Quince, J.R. and Gardner, G.L. (1982a). Recovery and Treatment of Contaminated Ground Water: Part I. Ground Water Monit. Rev., 2(3): 18-22.

Quince, J.R. and Gardner, G.L. (1982b). Recovery and Treatment of Contaminated Ground Water: Part II. Ground Water Monit. Rev., 2(4): 18-25.

Rittmann, B.E. and McCarty, P.L. (1980). Utilization of Dichloromethane by Suspended and Fixed-Film Bacteria. Appl. Environ. Microbiol., 39(6): 1225-1226.

Spain, J.C., Pritchard, P.H. and Bourquin, A.W. (1980). Effects of Adaptation on Biodegradation Rates in Sediment/Water Cores from Estuarine and Freshwater Environments. Appl. Environ. Microbiol., 40(4): 726-734.

Spain, J.C. and van Veld, P.A. (1983). Adaptation of Natural Microbial Communities to Degradation of Xenobiotic Compounds: Effects of Concentration, Exposure Time, Inoculum, and Chemical Structure. Appl. Environ. Microbiol., 45(2): 428-435.

Strand, S.E. and Shippert, L. (1986). Oxidation of Chloroform in an Aerobic Soil Exposed to Natural Gas. Appl. Environ. Microbiol., 52(1): 203-205.

Stucki, G., Galli, R., Ebersold, H.-R., and Leisinger, T. (1981). Dehalogenation of Dichloromethane by Cell Extracts of Hyphomicrobium DM2. Arch Microbiol., 130(5): 366-371.

Stucki, G., Krebser, U. and Leisinger, T. (1983). Bacterial Growth on 1,2-Dichloroethane. Experientia, 39(11): 1271-1273.

Suflita, J.M., Horowitz, A., Sheldon, D.R. and Tiedje, J.M. (1982). Dehalogenation: A Novel Pathway for the Anaerobic Biodegradation of Haloaromatic Compounds. Science, 218: 1115-1117.

Suflita, J.M., Robinson, J.A. and Tiedje, J.M. (1983). Kinetics of Microbial Dehalogenation of Haloaromatic Substrates in Methanogenic Environments. Appl. Environ. Microbiol., 45(5): 1466-1473.

Sylvester, K.A. and Landon, R.A. (1982). Subsurface Oil Contamination. Ground Water Monit. Rev., 2(4): 54-56.

Thomas, J.M., Lee, M.D., Bedient, P.B., Borden, R.C., Canter, L.W. and Ward, C.H. (1987). Leaking Underground Storage Tanks: Remediation with Emphasis on In-Situ Biorestoration. National Center for Ground Water Research, Oklahoma. EPA/600/2-87/008.

van Veld, P.A. and Spain, J.C. (1983). Degradation of Selected Xenobiotic Compounds in Three Types of Aquatic Test Systems. Chemosphere, 12: 1291-1305.

Vogel, T.M. and McCarty, P.L. (1985). Biotransformation of Tetrachloroethylene

to Trichloroethylene, Dichloroethylene, Vinyl Chloride, and Carbon Dioxide under Methanogenic Conditions. Appl. Environ. Microbiol., 49(5): 1080-1083.

Vogel, T.M., Criddle, C.S. and McCarty, P.L. (1987). Transformations of Halogenated Aliphatic Compounds. Submitted to Environmental Science and Technology.

Wagner, K., Boyer, K., Claff, R., Evans, M., Henry, S., Hodge, V., Mahmud, S., Sarno, D., Scopino, E. and Spooner, P. (1986). Remedial Action Technology for Waste Disposal Sites, Second Edition. Noyes Data Corporation, Park Ridge, NJ.

Webster, J.J., Hampton, G.J., Wilson, J.T., Ghiorse, W.C. and Leach, F.R. (1985). Determination of Microbial Cell Numbers in Subsurface Samples. Ground Water, 23(1): 17-25.

Weston, R.F., Inc. (1983). Installation Restoration General Environmental Technology Development. Task 1. Solvent and Heavy Metals Removal from Groundwater. Report No. DRXTH-TE-CR-82176.

Wilson, B.H., Smith, G.B. and Rees, J.F. (1986). Biotransformations of Selected Alkylbenzenes and Halogenated Aliphatic Hydrocarbons in Methanogenic Aquifer Material: A Microcosm Study. Environ. Sci. Tech., 20(10): 997-1002.

Wilson, J.T., McNabb, J.F., Balkwill, D.L. and Ghiorse, W.C. (1983). Enumeration and Characterization of Bacteria Indigenous to a Shallow Water-Table Aquifer. Ground Water, 21(2): 134-142.

Wilson, J.T. and Wilson, B.H. (1985). Biotransformation of Trichloroethylene in Soil. Appl. Environ. Microbiol., 49(1): 242-243.

Wilson, J.T., Miller, G.D., Ghiorse, W.C. and Leach, F.R. (1986a). Relationship Between the ATP Content of Subsurface Material and the Rate of Biodegradation of Alkylbenzenes and Chlorobenzene. J. Contam. Hydrology, 1(1/2): 163-170.

Wilson. J.T., Leach, L.E., Henson, M. and Jones, J.N. (1986b). In Situ Biorestoration as a Ground Water Remediation Technique. Ground Water Monit. Rev., 6(4): 56-64.

Yaniga, P.M. (1982). Alternatives in Decontamination for Hydrocarbon-Contaminated Aquifers. Ground Water Monit. Rev., 2(4): 40-49.