Final Report

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Field Implementation of Bioremediation at Indiana Department of Transportation Facilities

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16. Abstract

Bioremediation is often the most cost-effective and successful technique available for the remediation of soils and groundwater contaminated with organic pollutants (e.g. petroleum). The goal of bioremediation is to stimulate naturally occurring microorganisms to biodegrade the contaminants to harmless products. To be in compliance with EPA regulations all underground fuel storage tanks must have spill, leak and corrosion protection. Many older obsolete tanks had deteriorated to the extent that petroleum products had leaked into the environment. In the past, petroleum contaminated soils were typically excavated, followed by landfilling of the contaminated material. The continued use of landfills for disposal of petroleum contaminated soils is not desirable. An alternative technology is bioremediation. The objectives of this study were to increase utilization of bioremediation by INDOT and other agencies for the remediation of petroleum contaminated sites, reduce uncertainty associated with the design and implementation of bioremediation systems, reduce reliance on landfills for disposal of contaminated soils, reduce long-term liability associated with hazardous waste, and to improve the quality of engineering science utilized for the design of bioremediation systems. In addition, a guidance manual for remediation decision makers was developed. The benefits of achieving these objectives will be to decrease costs associated with Leaking Underground Storage Tank (LUST) remediation, improve environmental quality, and to improve public and environmental health. To achieve the objectives outlined above several bioremediation field demonstrations were successfully developed. A bioremediation field demonstration using Monitored Natural Attenuation was implemented and is an ongoing project at Linton, Indiana. A land farming field demonstration using excavated low hydraulic conductivity soils has been completed at Chrisney, Indiana. An engineered bioremediation field demonstration at a site with contaminated groundwater using a combined Air Sparging-Soil Vapor Extraction system is underway at Shoals, Indiana.

17. Key Words

Bioremediation, Petroleum, Fuel, BTEX, UST, LUST, Contamination

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A significant number of INDOT's petroleum contaminated sites have yet to undergo remediation. Several recommendations are suggested which would greatly assist the bioremediation implementation effort.

Specific Recommendations

A Corrective Action Plan (CAP) which outlines the remediation problem and proposed solution must be submitted to IDEM for approval prior to any site remediation. CAPs are prepared by remediation engineering consulting firms and reviewed by Pre-Engineering and Environment and then submitted to IDEM. Different approaches to the preparation and content of CAPs, design of bioremediation systems, and the level of detail provided in CAPs are taken by each specific firm. Format and content of the CAPs are not standardized. Information about the nature and extent of contamination, justification of recommendations, or remediation design details are often missing from CAPs. Pre-Engineering & Environment has requested assistance in reviewing CAPs and developing some standardization of the format and content.

CAPs submitted by consultants sometimes recommend the utilization of a relatively new product called oxygen releasing compound (ORC). ORC is a commercially available proprietary mixture of solid peroxides. Upon exposure to moisture ORC decomposes to oxygen and unidentified products. Extensive favorable information about ORC is available from the vendor which markets ORC, yet little independent evaluation has occurred. The use of ORC at INDOT sites, overall, has not been successful. For example, ORC reacts rapidly with iron which is abundant in Indiana soils. This reaction apparently significantly reduces ORC's effectiveness. Many CAPs submitted to Pre-Engineering and Environment continue to recommend the utilization of ORC. Pre-Engineering & Environment has requested assistance in evaluating the performance of ORC and developing guidelines for the acceptable utilization of ORC.

INDOT could benefit significantly from managing their own landfarming operations for treatment of excavated petroleum contaminated soils. During road construction projects it is common to discover petroleum contamination from old underground storage tanks which had been abandoned. In order to keep construction on schedule, excavation of the contaminated soil is necessary. Disposal of the contaminated soil is the responsibility of the construction contractor, however, the disposal costs are paid by INDOT. Significant amounts of money could be saved during road construction projects if the excavated soils were treated by landfarming at INDOT facilities rather than disposal in a landfill. Landfarming is an ex-situ bioremediation technique. Not only would cost-savings be realized but INDOT's long-term liability would be eliminated by landfarming treatment. Appropriate INDOT property must be identified for the potential location of petroleum landfarms. A series of workshops should be organized to train INDOT personnel about the regulations, design, construction, maintenance, operation, and closure of petroleum landfarms.

CHAPTER 2 INTRODUCTION

Due to EPA regulations, all underground storage tanks must have spill, leak and corrosion protection by the year 1999. Obsolete tanks, which existed at most Department of Transportation vehicle maintenance facilities prior to 1999, were required to be taken out of service. Many of these obsolete tanks had deteriorated to the extent that petroleum products stored in them had leaked into the surrounding soil. These contaminated soils require remediation, which in the past typically has involved excavation and landfilling of the contaminated material. For many reasons which include, long-term liability, economics and health risks, continued use of landfills for disposal of petroleum contaminated soils is not desirable. Furthermore, reduced reliance on landfilling is a goal of the State of Indiana, as exemplified by passage of House Bill 1240 in 1990, which established Solid Waste Districts for each county with the responsibility for implementing waste reduction and recycling programs to address this goal.

Bioremediation: An Environmentally Beneficial Procedure

An alternative, but under-utilized technology is bioremediation. Bioremediation is often the most cost-effective and successful technique available for the remediation of soils and groundwater contaminated with organic pollutants (e.g. petroleum). Bioremediation is the enhancement, acceleration and manipulation of naturally occurring biodegradation processes. Usually, the contaminated soils are left in place (in-situ) during treatment, which minimizes disruption of normal activities at the site (vehicle maintenance) and minimizes exposure to contaminants (no excavation required), while maximizing cost savings. The goal of bioremediation is to stimulate naturally occurring microorganisms to utilize organic pollutants as a food source. This is normally accomplished by adding oxygen and nutrient supplements (nitrogen and/or phosphorus). Pollutants are them transformed by microorganisms into harmless products, such as carbon dioxide and water. Several carefully controlled successful field demonstrations of bioremediation have taken place in recent years.

Implementation of bioremediation is currently hindered by a general lack of knowledge about the technology. Uncertainties associated with applicability and performance of bioremediation results in under-utilization of the technology. Currently there are no existing "design codes" or "standard practices" for the design, implementation or monitoring of bioremediation projects. Contractors implementing bioremediation must be certain that their design is justified by sound science and engineering principles.

However, some bioremediation strategies are only marginally justified by the research literature, and often not justified at all by the contractor's own data. Implementation of successful bioremediation systems would be enhanced by the development of a code of "standards of good practice" for use by bioremediation contractors.

National Needs and Priorities

Benzene and toluene are major components of gasoline and have known acute and chronic health hazards. Benzene is a known carcinogen. Contamination of groundwater used for drinking water supplies is a major route of exposure of humans to environmental pollutants. It is estimated that there are approximately 290,000 petroleum contaminated sites in the US. Remediation of these contaminated sites is a national need and priority. Remediation of petroleum contaminated soils is ALSO an INDOT priority.

Bioremediation: A National Focus Area for PTP

- Bioremediation is an innovative technology.
- Bioremediation is commercially available but under-utilized.
- Remediation of sites with leaking underground storage tanks is a priority issue for INDOT
- This initiative will form a partnership between FHWA, industry (bioremediation contractors), Purdue University and INDOT.
- This initiative will be implemented in 1995.
- Bioremediation provides measurable and understandable benefits.

Benefits of Bioremediation

- Potential direct cost savings of 2 - 4 million dollars over the currently utilized remediation method of excavation and landfilling of contaminated soils, for remediation of INDOT facilities alone.
- Reduced long-term liability due to the conversion of pollutants into harmless by-products rather than long-term storage in a landfill.
- Cleaner environment.
- Reduced health risks for citizens, state and federal employees due to reduced exposure to pollutants.

This study will result in more effective site remediation and significant cost savings to INDOT through application of bioremediation where appropriate. Development of a

"Bioremediation Guidance Manual" (appendix A) for bioremediation will reduce uncertainties when dealing with contractors and setting performance criteria. Developing techniques for expanding bioremediation application to low permeability soils will result in additional cost savings for INDOT site remediation. This initiative will be evaluated primarily by the cost-savings resulting from the increased utilization of bioremediation over current methods at INDOT facilities. Currently, remediation of petroleum contaminated soil costs approximately \$65 per yd^3.

Summary

Implementation and utilization of bioremediation is not widespread for many reasons. Since bioremediation is a relatively new and innovative technology, rules and standards of good practice have not been adopted by practitioners. Therefore, bioremediation is often viewed as an "art" rather than as an engineering science. Development of accepted standards of good practice and implementation of well designed demonstration projects will accelerate the deployment of this effective and economical procedure. In addition, remediation of petroleum contaminated sites will be completed with an environmentally friendly technology which solves a critical national problem.

CHAPTER 3 FUNDAMENTAL PRINCIPLES OF BIOREMEDIATION

Introduction

The objective of this chapter is to present the basic principles and concepts of biodegradation as they relate to bioremediation. Understanding of these processes is necessary to effectively manipulate subsurface conditions to enhance natural biodegradation processes. The application of these basic principles to the restoration of petroleum contaminated soil and groundwater is a field now commonly called "bioremediation" (Lee *et al.*, 1988; National Research Council, 1993; Thomas and Ward, 1989)

Subsurface Microbiology and Geochemistry

Microorganisms mediate the biotransformation of many different chemicals in the subsurface. Historically there has been much scientific debate over the relative contribution of abiotic and biotic chemical processes in the subsurface. Fifty years ago, very little was known about microorganisms in the environment, and thus abiotic transformations were thought to dominate. However, more recent research has contributed new knowledge about microbial life in the subsurface and this view has changed. While our understanding of subsurface microbiology and biogeochemistry is far from complete, biologically mediated reactions of both inorganic and organic compounds in the subsurface are known to be significant, and in most cases control groundwater chemistry (Chapelle, 1993). With respect to the biotransformation of organic pollutants, microbially mediated reactions are by far the most important. The abiotic transformation of pollutants does occur (e.g. dehalogenation, polymerization, and hydrolysis reactions), however, the environmental significance of these abiotic reactions relative to microbially mediated reactions is difficult to assess. This is partly due to the difficulty in distinguishing between abiotic and biotic processes in situ. Most importantly, many abiotic reactions are dependent on two environmental parameters which are controlled by microbial processes, redox and pH. Thus these types of abiotic reactions are therefore indirectly mediated by microorganisms as well.

Microorganisms can be classified in a number of different ways such as phylogenetically, morphologically, physiologically, or other characteristics (Table 1). For the purpose of understanding biodegradation it is most useful to begin the characterization of microorganisms according to their source of energy, and their source of carbon for cell growth. Potential energy sources are organic carbon, inorganic compounds, and sunlight.

Ideally for petroleum hydrocarbon bioremediation microorganisms will utilize them for carbon and energy. Microbes which oxidize organic compounds to obtain energy are organotrophs and those that oxidize inorganic compounds are lithotrophs. Potential sources of carbon for cell growth are either organic carbon or inorganic carbon (HCO_3^-, CO_2). Microbes which degrade organic compounds to obtain carbon for the synthesis of cellular constituents are heterotrophs and those that utilize inorganic carbon are autotrophs. Logically, most lithotrophs are also autotrophs and most organotrophs are also heterotrophs. Photoautotrophs and photoheterotrophs may exist in groundwater, but their utilization of photometabolism is obviously limited by access to sunlight (Brock *et al.*, 1997; Gottschalk, 1986).

Table 1. Microbial Characterization

Metabolism
energy source
carbon source
nitrogen source
type of metabolism
products formed

Morphology
shape
size
colony appearance
flagella
capsule/endospore
pigmentation

Genetics
DNA composition
16s RNA
information

Biochemical
cell wall
cell membrane
pigments
antigens

Physiology
temperature tolerance
O_2 tolerance
pH tolerance
salt tolerance
antibiotic sensitivity

The types of microorganisms found in the subsurface include protozoa, fungi, bacteria, and viruses. Bacteria are ubiquitous in groundwater, and in addition, they have very diverse catabolic abilities, morphology, physiology, and biochemical constituents. This diversity allows bacteria to survive in some of the most extreme environments on earth. Bacteria have been found up to 2.8 kilometers below the surface (Fredrickson and Onstott, 1996). Although they are microscopic, bacteria are the dominant biodegraders and drivers of biogeochemical cycling in groundwater. Bacteria live attached to subsurface particles as individuals or in colonies and also as unattached motile organisms. Attached microbes conserve energy while removing nutrients from the surrounding water, as well as deriving some protection from predation. The growth and survival of attached organisms is dependent on obtaining nutrients by transport through groundwater. Motile organisms are known to move in response to chemical gradients (chemotaxis), moving towards higher concentrations of nutrients and away from toxics. Bacterial transport in groundwater and environmental factors which induce bacteria to exhibit attached or motile phenotypes have been studied (Corapcioglu and Haridas, 1984; Corapcioglu and Haridas, 1985; Dawson *et al.*, 1981; Fletcher and Marshall, 1982; Harvey *et al.*, 1989). However, our ability to control and influence these processes in the subsurface in order to promote biodegradation is still under development.

Basic Metabolism

All living organisms obtain energy by mediating oxidation/reduction reactions (Figure 1). During respiration reduced organic and inorganic compounds serve as the reductant (electron donor) in the reaction. Oxidized, usually inorganic, species serve as the oxidant (electron acceptor). Aerobic respiring organisms utilize oxygen, which is the most ubiquitous electron acceptor in the environment, as an oxidant. However, the solubility of oxygen in water is relatively low (~8 mg/L @ 20°C) and the oxygen diffusion rate from the atmosphere through saturated and unsaturated soil is slow. Therefore, the availability of oxygen in groundwater is significantly limited by mass transfer.

Respiration

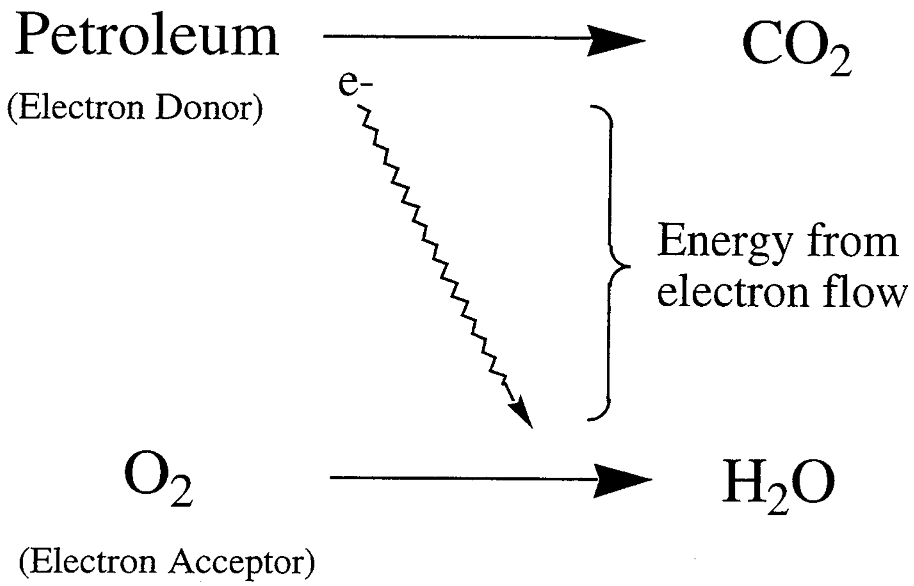


Figure 1 Respiration Model

A variation of typical oxidation/reduction reactions occurs when a fraction of a compound is oxidized and the remaining fraction is reduced (Figure 2). This type of catabolism is called fermentation, in contrast to respiration. Almost all fermenting bacteria cannot tolerate oxygen and are found in anaerobic environments living in close association with sulfate reducing or methanogenic bacteria (Brock *et al.*, 1997; Chapelle, 1993; Gottschalk, 1986).

Fermentation

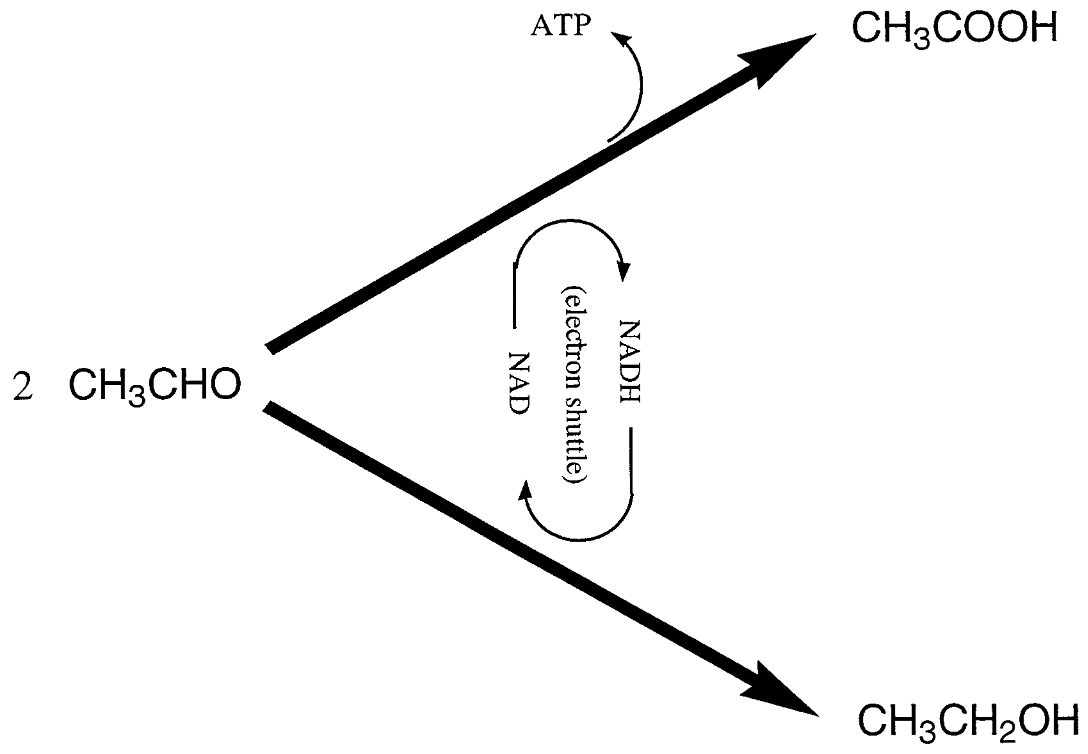


Figure 2 Fermentation Model

Organisms have evolved elegant mechanisms for converting the electron transfer which occurs during oxidation/reduction reactions into energy. Respiring organisms oxidize their energy source, stripping off the high energy electrons. The electrons are shuttled on electron carriers (NADH) to the cell membranes where electron transport phosphorylation occurs (Figure 3). Respiring organisms use electron transport to pump protons across the membrane to create a pH differential (charge and proton gradient) between the inside and outside of the cell. In this energized state, protons are driven by the gradient through specialized enzymes which convert the "proton motive force" into stored chemical energy, adenosine triphosphate (ATP). Driving the proton pumps consumes the electron's energy. At the terminal end of electron transport chain the now low energy electrons are transferred to a terminal electron acceptor.

Fermenting bacteria make ATP through the direct conversion of chemical bond energy, in a process called "substrate level phosphorylation (SLP)." The energy producing steps primarily occur during oxidation of carbonyl groups (Figure 2). Since no external electron acceptor is utilized, and biological electron carriers are in limited supply and must be recycled, the electrons generated from the energy producing oxidations of SLP must be discarded. This is accomplished by reducing some of the substrate, producing alcohols, or by reducing protons, producing hydrogen. Thus fermenters produce a mixture of both oxidized and reduced products such as CO_2, carboxylic acids, alcohols, and H_2.

Anaerobic respiration occurs when oxygen is depleted and other suitable oxidized species are available to act as an electron acceptor. Common anaerobic electron acceptors are oxidized nitrogen compounds (nitrate, nitrite), iron(III), oxidized sulfur compounds (sulfate, sulfite, S°), and CO_2. Other oxidized inorganic species are utilized in anaerobic respiration as well, and in addition, some organic compounds, such as fumarate, may also serve as electron acceptors (Zehnder, 1988). Bacteria obtain less energy from using electron acceptors other than oxygen. The amount of energy obtained from the oxidation of a given substrate is proportional to the reduction potential ($E^{\circ\prime}$) of the electron acceptor (Table 2). Electron acceptors which yield the largest amount of energy (e.g. oxygen) tend to be utilized preferentially over others that are available, probably because bacteria with access to more energy can grow faster and compete for resources more successfully (Figure 4).

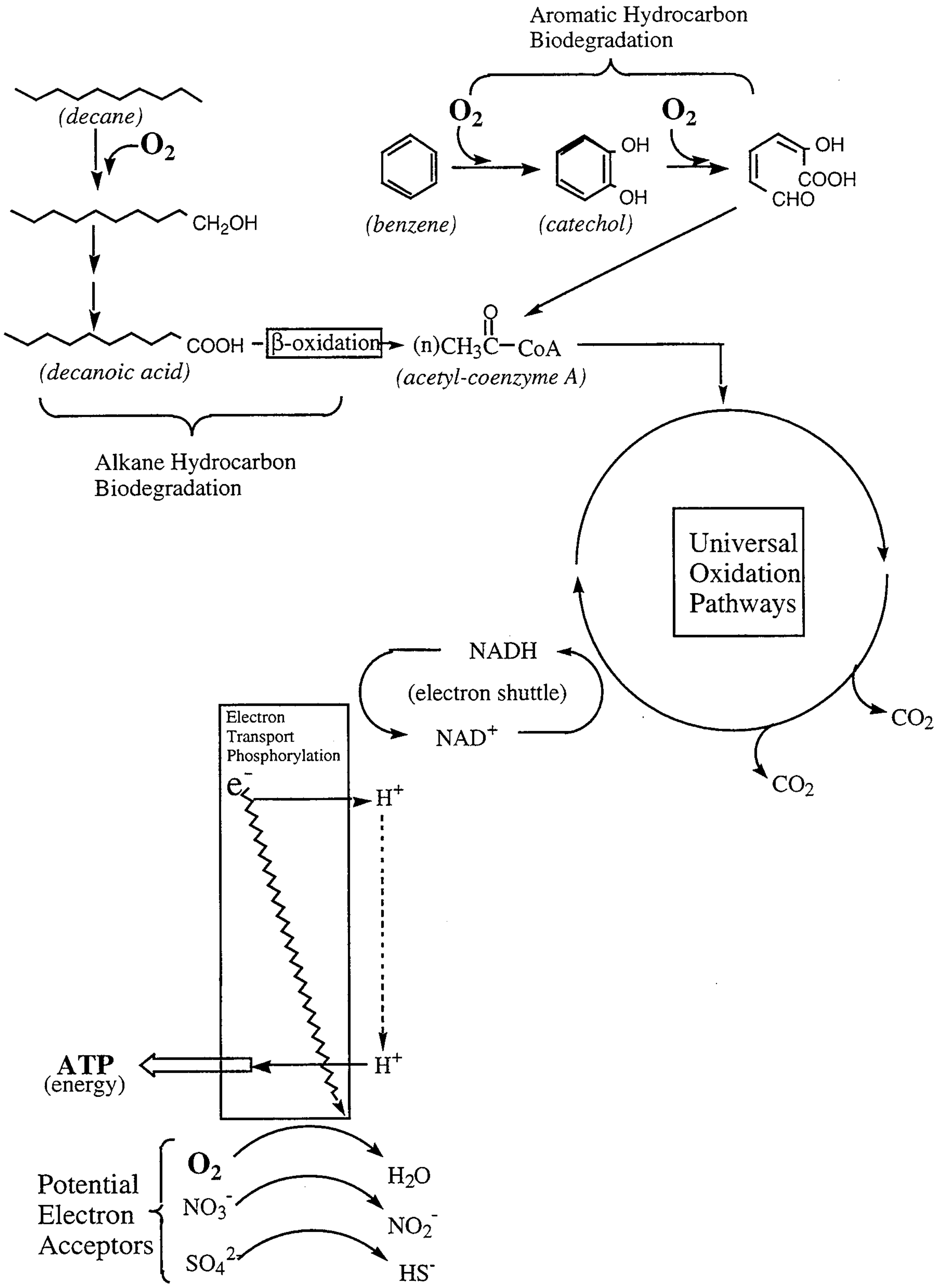


Figure 3 Model of Overall Catabolism

In addition, biochemical mechanisms exist which maximize energy production. For example, facultative aerobes which can use both oxygen and nitrate as electron acceptor are common. The presence of oxygen inhibits synthesis of the enzyme at the terminus of the electron transport chain which transfers electrons to nitrate (nitrate reductase), thus ensuring more energetically favorable oxygen utilization whenever possible.

Table 2. Reduction Potentials of Important Biological Oxidants (electron acceptors)

	$E^{\circ\prime}$(Volts)
$\frac{1}{4}O_2(g) + H^+ + e^- = \frac{1}{2}H_2O$	0.820
$\frac{1}{2}MnO_2(s) + \frac{1}{2}HCO_3^-(10^{-3}) + \frac{3}{2}H^+ + e^- = \frac{1}{2}MnCO_3(s) + H_2O$	0.527
$\frac{1}{2}NO_3^- + H^+ + e^- = \frac{1}{2}NO_2^- + \frac{1}{2}H_2O$	0.423
$\frac{1}{6}NO_2^- + \frac{4}{3}H^+ + e^- = \frac{1}{6}NH_4^+ + \frac{1}{3}H_2O$	0.344
$FeOOH(s) + HCO_3^-(10^{-3}) + 2H^+ + e^- = FeCO3(s) + 2H_2O$	$^-0.047$
$\frac{1}{6}SO_4^{2-} + \frac{4}{3}H^+ + e^- = \frac{1}{6}S(s) + \frac{2}{3}H_2O$	$^-0.195$
$\frac{1}{2}S(s) + H^+ + e^- = \frac{1}{2}H_2S(g)$	$^-0.243$
$\frac{1}{8}CO_2(g) + H^+ + e^- = \frac{1}{8}CH_4(g) + \frac{1}{4}H_2O$	$^-0.244$
$H^+ + e^- = \frac{1}{2}H_2$	$^-0.414$

Values apply for unit activity (1M or 1atm) in water at pH 7.0 and 25°C, except $HCO_3^- = 10^{-3}M$ which more typically represents environmental conditions.

$\Delta G^{\circ\prime} = -nFE^{\circ\prime}$, where n is the number of electrons transferred and F is Faraday's constant.
F = 96.5 kJ/volt-mole.

Adapted from (Brock *et al.*, 1997; Stumm and Morgan, 1981; Thauer *et al.*, 1977)

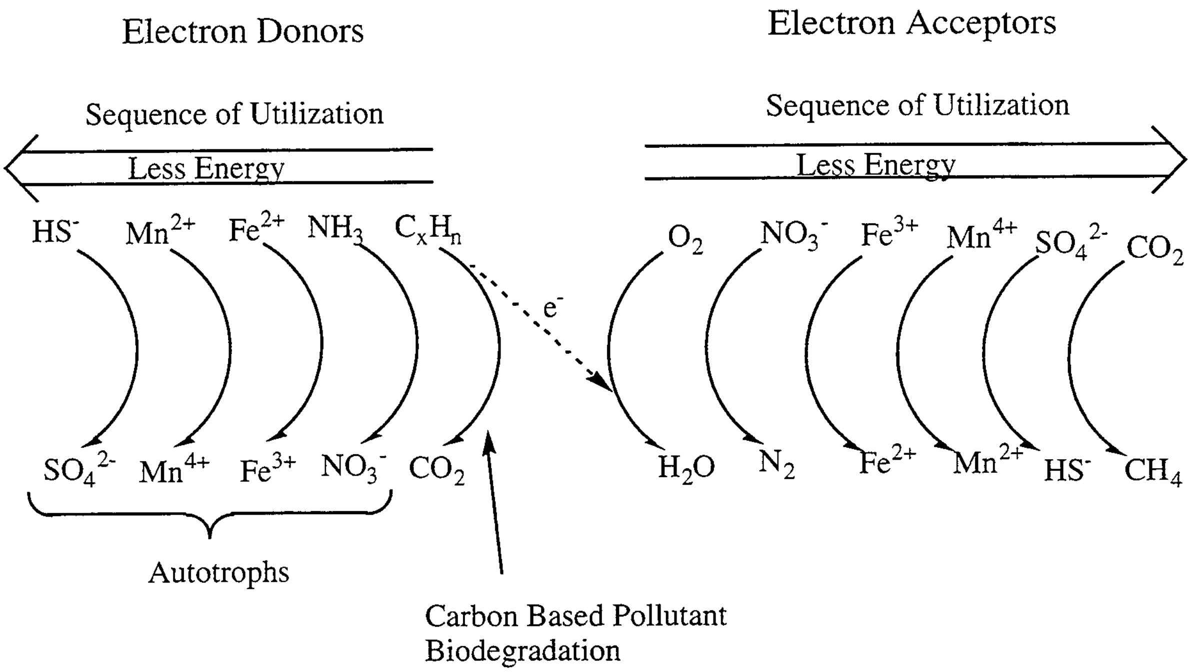


Figure 4 Sequence of Oxidation - Reduction Reactions

During biodegradation of organic compounds, large molecules are broken down into small molecules, which are oxidized, yielding electrons for energy production. For example, aromatic rings are cleaved open to form aliphatic chains, which are then cleaved into two-carbon pieces, which are subsequently oxidized to CO_2 (Figure 1). Likewise, long alkanes are cleaved into two-carbon pieces prior to oxidation to CO_2. However, the initiation of biodegradation of many compounds requires "activation" by the insertion of molecular oxygen (O_2) into the hydrocarbon, forming mono- and dihydroxylated compounds. Oxygenase enzymes perform this function. Once activated by oxygenase enzymes, further biodegradation of hydrocarbons often does not require molecular oxygen. An exception is the biodegradation of aromatic rings, during which oxygen is used as a reactant for ring activation and ring cleavage. Thus, it should be observed that oxygen can be utilized by bacteria as both electron acceptor and as a reacting co-substrate for initiating biodegradation reactions. A critical issue in biodegradation is that there are many alternatives to oxygen as an electron acceptor, while there are few alternatives to oxygen as a reactant for initiating hydrocarbon biodegradation. For this reason, when more rapid and complete biodegradation of certain hydrocarbons is desired, aerobic conditions are preferred. The bioremediation of groundwater aquifers which are contaminated with hydrocarbons is often limited by insufficient oxygen. Hydrogen peroxide, which decomposes to oxygen, is often used as a highly soluble source of oxygen for groundwater systems (Pardieck *et al.*, 1990; Pardieck *et al.*, 1992). Proprietary mixtures of solid peroxides are also being marketed as oxygen releasing enhancements for bioremediation.

One good alternative to oxygen as an electron acceptor is nitrate (NO_3^-). Significant research has also been devoted to the feasibility using nitrate as a supplemental electron acceptor (Dolfing *et al.*, 1990; Evans *et al.*, 1991; Hutchins, 1991a; Hutchins, 1991b). Nitrate is more soluble in water than oxygen, is a strong oxidant, and could potentially be more readily introduced into groundwater aquifers through injection wells. Interestingly, many monoaromatic compounds (BTEX) are biodegraded under anaerobic nitrate reducing conditions, although unlike oxygen, the nitrate is used only as an electron acceptor and not as a reactant in biotransformation reactions. Nitrate has relatively low toxicity, but does have known health hazards (methemoglobinemia) and is a regulated drinking water contaminant, and therefore, is used as a supplemental electron acceptor only under carefully controlled situations.

Environmental Conditions which Influence Biodegradation

The activity and types of microorganisms present in groundwater is greatly influenced by subsurface physical and chemical properties (Alexander, 1994). Biodegradation kinetics are generally highly dependent on temperature because enzyme function is temperature sensitive. Bacteria can be classified by the range of temperature within which they can grow. At optimum temperatures biodegradation kinetics reach maximum rates, while slightly above optimum temperature, cell constituents usually begin to degrade. As temperatures decrease below the optimum, biodegradation rates decrease until enzymes function at rates too slow to support growth. Temperature extremes will select for climate-adapted organisms. For example, cold-loving bacteria (psychrophiles) can grow at temperatures below 0°C and generally die at temperatures greater than 20°C. Mesophilic bacteria thrive at temperatures which are comfortable to humans. Although the vast majority of biodegradation studies have been done with mesophilic bacteria at temperatures ranging between 15 - 45 °C, some evidence exists which suggests that psychrophilic (or at least psychrotolerant) (Kellems *et al.*, 1994) and thermophilic bacteria also possess pollutant biodegrading ability (Chen and Taylor, 1995).

Bacteria can also be classified according to the pH range within which they can grow. Our knowledge of biodegradation has been derived chiefly from studies conducted at "neutral" pH range, between approximately 6 - 8. Most groundwater also falls into this pH range as well. Acidophilic bacteria can tolerate a pH as low as 2, although most are lithoautotrophs which do not degrade organics. Alkaline conditions exist (pH 9-11) in certain areas where carbonate rocks predominate. Sodium concentrations in these alkaline environments are often more than 10X greater than seawater, thus indigenous bacteria are halophiles in addition to their adaptation to extreme pH. Knowledge of pollutant biodegradation in extreme pH environments is scarce.

Bacteria are sensitive to salinity. Halophilic bacteria have evolved a mechanism to counteract the tendency for cells to become desiccated by high salt concentrations. Numerous studies have demonstrated that marine bacteria are capable of hydrocarbon biodegradation, however, knowledge about pollutant biodegradation by extreme halophiles is lacking. Since a primary concern is the bioremediation of contaminated aquifers which are sources of potable water, acquiring knowledge about organisms adapted to extreme environments has not been a high priority.

All living things, including bacteria require inorganic nutrients for growth, in addition to a source of energy and carbon. Nitrogen and phosphorus are the nutrients which are most likely to be a limiting factor for biodegradation and, similar to agricultural applications, are often added to the environment to remove potential nutrient limitations.

Oxidation/reduction potential is by definition the electrical potential (in volts) of the oxidation/reduction reaction occurring between the electron donor and electron acceptor. In practice when considering conditions in the environment the term "redox" potential is commonly used. Redox potential typically refers to the reduction potential of the dominant electron acceptor in the environment. Electron acceptors tend to be the dominant selector of microbial activity in the subsurface and there are a number of factors to consider when calculating the assimilative capacity of a contaminated site (Table 3). Probes are available to measure environmental redox potential, however when assessing biodegradation, actual measurement of the electron acceptor of interest is more useful. For example, if aerobic biodegradation is desired oxygen concentrations should be monitored.

Table 3. Electron Acceptors

Electron Acceptors	Phase in Environment	Comments
O_2	gas	21% of atmosphere
NO_3^-	dissolved ion	From Nitrification - Limiting Nutrient
Fe^{3+} Mn^{4+}	solids	Regional Geology
SO_4^{2-}	ion, solid	Regional Geology
CO_2	gas, ion, solid	$CO_{2(g)} \leftrightarrow H_2CO_3 \leftrightarrow HCO_3^- \leftrightarrow CO_3^{2-} \leftrightarrow CaCO_{3(s)}$

Likewise, the simultaneous disappearance of nitrate and organics, with the concurrent appearance of nitrite is better circumstantial evidence of the existence of denitrifying bacteria than a redox measurement. More reducing, anaerobic conditions can be inferred from measurements of sulfate, sulfide, and the production of methane, as well as redox measurements.

Biotransformations

Bacteria biodegrade organic compounds by breaking large molecules apart with an array of biotransformation reactions such as hydrolysis, oxidation, reduction, dehalogenation, deamination, decarboxylation, and rearrangement reactions (Table 4). As mentioned previously, molecular oxygen participates directly in a number of biotransformation reactions (Table 5).

Anaerobic bacteria can perform many of the biotransformation reactions listed in Table 5 by different mechanisms without molecular oxygen (Schink, 1988). In addition, anaerobic bacteria can mediate reductive dehydroxylation, reductive deamination, and reductive dehalogenation reactions. Of these, reductive dehalogenations are of the greatest interest with respect to the biotransformation of hazardous pollutants. Anaerobic bacteria have only recently been shown to be able to hydroxylate alkanes and environmental observations reveal that saturated hydrocarbons generally persist under anaerobic conditions. While BTEX biodegradation by denitrifying and iron reducing bacteria has been well documented, and a few reports of aromatic hydrocarbon degradation under sulfate reducing and methanogenic conditions exist, detailed knowledge about the biochemistry of anaerobic aromatic hydrocarbon transformation is limited (Evans, 1988; Lovley et al., 1988; Lovley and Lonergan, 1990).

Table 4 Biotransformation Reactions

Hydrolysis:	esters, amides, nitrates, double bonds
Oxidations:	ß-oxidation, hydroxylation, dihydroxylation, decarboxylation, epoxidation, oxidative coupling, ring fission, ether cleavage, alcohols, aldehydes
Reductions:	double bonds, nitro groups, azo groups, aldehydes, ketones, alcohols, dehydroxylation
Dehalogentations:	oxidative, reductive, hydrolytic
Deaminations:	oxidative, reductive, hydrolytic
Alkylation / Dealkylations	N-dealkylations, O-dealkylations, C-dealkylations (includes methylation & demethylation)
Carboxylations and Rearrangements	

Table 5 Biotransformation Reactions Mediated by Oxygenase Enzymes

Biotransformation	Compound	Reference
Deamination	Aniline	Bachofer *et al.*, 1975
Dehalogenation	Pentachlorophenol	Xun *et al.*, 1992
Ether cleavage	2,4,-Dichlorophenoxyacetate	Tiedje and Alexander, 1969
Aromatic ring hydroxylation	Benzene	Gibson, 1984
Aromatic ring fission	Benzene	Gibson, 1984
Alkane hydroxylation	Octane	Watkinson and Morgan, 1990

In most cases bacteria biodegrade organic compounds to obtain energy for growth. Due to the relaxed specificity of some enzymes, bacteria perform certain biotransformation reactions on compounds which are not growth substrates. The term "cometabolism" can be broadly taken to mean the "gratuitous biotransformation of a compound from which the organism derives no benefit." Often these gratuitous reactions produce products which can be more easily biodegraded by other organisms (Figure 5).

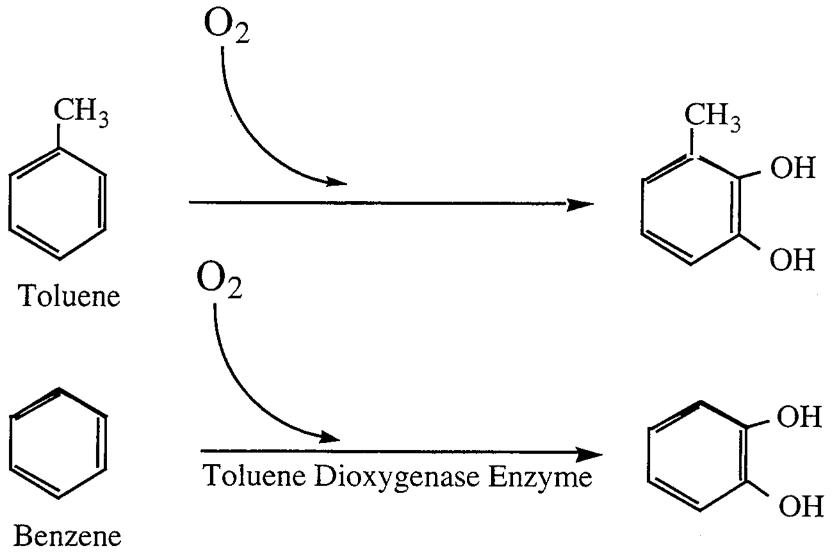
Hydrocarbons

Biodegradation of alkanes is usually initiated with terminal hydroxylation by a monooxygenase enzyme and subsequent oxidation of the alcohol to a carboxylic acid. The initial step requires oxygen, and thus far, no other anaerobic mechanism for the initiation of biodegradation of alkanes has been reported. The carboxylic acid can easily be further oxidized to CO_2 through two nearly universal biochemical pathways, ß-oxidation and the Krebs (tricarboxylic acid) cycle (Figure 3). Branched alkanes can be more difficult to degrade depending on the degree of branching. Branching interferes with ß-oxidation and significant branching can result in complete inhibition of biodegradation (Watkinson and Morgan, 1990). Alkenes can be aerobically biodegraded similarly to alkanes, however the double bond can also be hydrolyzed to initiate biodegradation under anaerobic conditions (Schink, 1988). Since alkanes have relatively low water solubilities they are less of a hazard to migrate as soluble constituents of groundwater. Biodegradation of alkanes often occurs at the hydrocarbon/water interface and is mediated by bacteria which produce biosurfactants for hydrocarbon uptake.

As described previously, aerobic biodegradation of aromatic compounds is initiated by the hydroxylation of the ring by oxygenase enzymes. Ring cleavage also requires oxygen. The aromatic compounds of most common concern are benzene, ethylbenzene, toluene, o-xylene, m-xylene, and p-xylene (BTEX).

Initiation of the biodegradation of the alkylbenzenes occurs either by dioxygenase attack on the aromatic ring or by monooxygenase attack on the alkyl group followed by a dioxygenase mediated ring cleavage (Smith, 1990).

Cometabolism



Without toluene the enzyme is not induced
and biodegradation may not occur

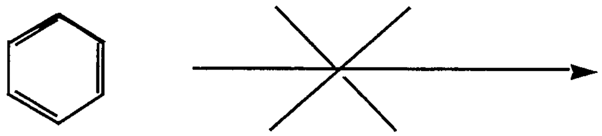


Figure 5 Conceptual Description of Cometabolism

Biodegradation rates are generally observed in the following order which may vary from site to site: toluene, ethylbenzene > benzene > m-xylene, p-xylene > o-xylene. BTEX are constituents of gasoline and therefore often appear together as a mixture in contaminated groundwater. Interactions between bacteria and BTEX mixtures may be complex and site specific (Alvarez and Vogel, 1991). Biodegradation of monoaromatic hydrocarbons (e.g. toluene, ethylbenzene) also occurs under anaerobic nitrate reducing conditions. Benzene appears to be more recalcitrant under anaerobic conditions than other monoaromatics. The biodegradation of aromatic rings has also been observed under iron reducing, sulfate reducing, and methanogenic conditions (Edwards *et al.*, 1992; Grbic-Galic and Vogel. 1987; Lovley et al., 1989).

Simple nonhalogenated hydrocarbon solvents such as ethanol, methanol, and acetone are easily biodegraded at dilute concentrations (e.g. 0.1 %). The cyclical ethers, and ether structures in general, are typically recalcitrant, however the biodegradation of furans and 1,4-dioxane has been reported, although biochemical mechanisms and the distribution of this ability in the environment remains unknown. Space limitations prohibit an exhaustive review of the biodegradation of all large production organics which might be found in groundwater, but several more complete references are available (Gibson, 1984; Howard, 1989; Leahy and Colwell, 1990; Young and Cerniglia, 1995).

Halogenated Compounds

A brief discussion of the biodegradation of halogenated compounds is included to serve as a contrast to petroleum hydrocarbon biodegradation. Halogenated organic compounds are among the most problematic environmental pollutants encountered (Chaudhry and Chapalamadugu, 1991). Halogenation typically increases environmental stability and toxicity, and significantly alters the kinds of biochemical and chemical reactions compounds undergo (Vogel *et al.*, 1987). Halogenation increases the oxidation state of a compound relative to analogous nonhalogenated compounds, and this significantly influences biodegradation as well. Several reviews of microbial transformation of halogenated compounds exist (Fetzner and Lingens, 1994; Mohn and Tiedje, 1992; Neilson, 1990). Biodegradation of halogenated compounds can be considered from two perspectives, bioenergetics and biochemical mechanisms. In general, very oxidized compounds are thermodynamically less favorable electron donors than reduced compounds, and therefore, as the degree of halogenation (and oxidation) increases compounds have fewer and fewer electrons to give up as electron donor and they potentially would yield correspondingly less energy when microorganisms oxidize them. Alternatively, polyhalogenated compounds are potentially good electron acceptors (Dolfing and Harrison, 1992). Halogenated compounds acting as electron acceptors can undergo a reaction called reductive dehalogenation, in which two electrons are transferred to the compound, the halogen leaves as a halide ion, and is replaced by a hydrogen atom. Depending on the degree of halogenation, and the type of environment and microorganisms present where it is found, halogenated compounds may be used as either electron donors or electron acceptors, with the more halogenated compounds making better electron acceptors and the less halogenated compounds making better electron donors. Consideration of thermodynamics suggests that sequential anaerobic dechlorination followed by aerobic biodegradation would be successful.

Halogenated organics influence biodegradation mechanistically because of the large atomic size of halogens relative to hydrogen (which halogens usually replace), halogen electronegativity, and the strength of the carbon-halogen bond. Halogen size may prevent biochemical reactions simply due to steric hindrance. Halogen electronegativity causes charge separation in bonds and may result in dipole moments in molecules, profoundly affecting chemical reactivity. For example, halogen substitution may result in compounds more susceptible to nucleophilic substitution reactions (e.g. hydrolysis) whereas many oxygenase enzymes are strong electrophiles which are better suited to attack saturated non-halogenated hydrocarbons. Strong bonds require large activation energies for cleavage and may prevent reactions from occurring. For example, the carbon-fluorine bond is exceptionally strong and is rarely broken during biological processes.

Halogenated Aliphatic Solvents

The halogenated aliphatic solvents which are most commonly found in groundwater are the chlorinated methanes (e.g. carbon tetrachloride (CCl4)) and chlorinated ethenes (tetrachloroethene or "perc" (PCE) and trichloroethene (TCE)). (Vogel *et al.*, 1987) Under anaerobic conditions, PCE can undergo stepwise reductive dechlorination to TCE, then dichloroethene (DCE), chloroethene (i.e. vinyl chloride (VC)), and finally ethene. The rates of dechlorination tend to be proportional to the number of chlorine, thus PCE dechlorinates faster than dichloroethene. In groundwater systems where PCE and/or TCE are undergoing reductive dechlorination, vinyl chloride often accumulates for reasons that are currently unknown, but possibilities include thermodynamic, kinetic, or toxic limitations. Since vinyl chloride is a known human carcinogen, the presence of this metabolite is extremely undesirable.

Aerobically, TCE, DCE, and VC, but not PCE, can be cometabolized by certain bacteria with monooxygenase enzymes. This phenomena can potentially be exploited for use in the restoration of contaminated aquifers. These bacteria require specific growth substrates (e.g. methane or phenol) which induces synthesis of the monooxygenase enzymes which act on the chlorinated compounds. The metabolites of (at least TCE) cometabolism are potentially toxic, and kill the cells mediating the reaction. Since the growth substrate and the cometabolized pollutant both compete for the same reactive enzyme site, a balance must be achieved between maintaining sufficient growth rates and acceptable degradation rates. Optimizing growth with excessive substrate could inhibit degradation of the pollutants, while low substrate concentrations may not induce the monooxygenase enzymes and biomass could be lost due to the production of suicide metabolites. For effective biodegradation, cell growth and pollutant biotransformation could potentially be separated in either space or time. This could be accomplished in groundwater by alternating periods of growth enhancement (adding growth substrate) with periods of starvation (Alvarez-Cohen, McCarty, 1991; Nelson *et al.*, 1987).

Halogenated Aromatic Compounds

The biodegradation of halogenated aromatic compounds is highly dependent on the position and number of halogen substituents (Reineke and Knackmuss, 1988). It is useful to subdivide halogenated aromatics into two groups, non-ionizable compounds and ionizable compounds. PCBs and chlorobenzenes are examples of non-ionizable halogenated aromatic compounds found in the environment. In general, increased halogen substitution results in greater hydrophobicity. Therefore, the more soluble, less chlorinated PCB and chlorobenzene congeners are a greater threat to migrate in groundwater relative to more highly chlorinated congeners. The biodegradation of PCBs and chlorobenzenes is highly dependent on the degree of chlorination as well (Bedard and Haberl, 1990; Furukawa *et al.*, 1978). PCBs and chlorobenzenes can be aerobically degraded similarly to non-halogenated aromatics, however, chlorine substitution often inhibits one or more enzymatic reactions. Thus, less chlorinated congeners are significantly more easily biodegraded by aerobic microorganisms than highly chlorinated compounds. Anaerobically, highly chlorinated PCBs and chlorobenzenes undergo microbially mediated reductive dechlorination to less chlorinated congeners which could potentially be biodegraded aerobically, but tend to persist under anaerobic conditions

(Abramowicz, 1990). A field demonstration of PCB bioremediation was recently described by Harkness *et al.*, (1993) during which it was found that bioavailability of PCBs severely limited biodegradation.

The behavior of ionizable aromatic compounds, such as phenols, anilines, and benzoates, is dependent on groundwater pH. For example, at a typical pH of 7, greater than 99% of the dissolved pentachlorophenol (PCP) (pKa = 4.75) will exist in the more soluble, less hydrophobic, ionized form. Unlike most PCB and chlorobenzene congeners, the complete biodegradation of PCP to CO_2 occurs under both aerobic and anaerobic conditions. In the better understood aerobic process, a single microorganism can catabolize PCP for energy and carbon. Anaerobically, PCP is sequentially dechlorinated to phenol, which can be further degraded to methane and CO_2. Relatively little is known about the microbiology and biochemistry of anaerobic PCP biodegradation (Haggblom and Valo, 1995; McAllister *et al.*, 1996).

Limits to Biodegradation

It should be mentioned that the environmental conditions which influence biodegradation and the limits to biodegradation are intrinsically related, and therefore, should not necessarily be viewed as separate topics. The most extreme limit to biodegradation is the absence of any known biochemical mechanism for the transformation of a specific compound (McCarty and Semprini, 1993). Discoveries of new transformations and microbial evolution of new enzymes will continue to challenge this limitation (Shannon and Unterman, 1993). Biodegradation potential can often be predicted from structure-activity models and a review of the biodegradation literature (Huesemann, 1995; Scow, 1990). However since microbial distribution and environmental conditions are extremely heterogeneous, actual site specific assays, such as laboratory treatability studies, provide the most reliable evidence that in situ biodegradation is possible.

Toxic Environmental Conditions

As discussed previously, microorganisms have adapted to life in many naturally occurring extreme environments, however, most of these organisms are autotrophs which will not significantly biodegrade organic pollutants. Extreme toxic conditions resulting from human activities and chemical releases to the environment are more difficult to ameliorate. The addition of oxygen and nutrients, as well as a moderate ability to influence pH, comprise the options currently available to influence environmental conditions. High concentrations of pollutants may cause toxic conditions and prevent biodegradation. Under these circumstances removal of source material might lower groundwater concentrations to non-toxic levels at which biodegradation could occur. Moderate environmental conditions at near neutral pH, with adequate moisture, nutrients, and electron acceptor are the most likely to promote biodegradation.

Bioavailability and Mass Transfer Limitations

Most bacteria take up dissolved nutrients and substrate from the surrounding water. Phenomena which lowers the dissolved concentrations or dissolution rate of compounds will limit biodegradation. Sorption of hydrophobic compounds to soils results in significant mass fractions of these compounds being unavailable to microorganisms. Biodegradation of the soluble phase results in desorption to reestablish the phase distribution equilibrium. In this situation, desorption rates may control biodegradation kinetics (Bosma et al., 1997). Long-term exposure of hydrophobic compounds to soils often results in a fraction of compound which remains unavailable for biodegradation due to mechanisms which are not yet fully understood (Hatzinger and Alexander, 1995). Therefore, it should be clear that in situ biodegradation kinetics may reflect processes other than microbial metabolism such as desorption of the pollutants, pollutant transport, and availability of electron acceptors.

Absence of Organisms

In some cases novel pollutant degrading microorganisms have been isolated and cultured in laboratories, while the widespread existence of these microorganisms in the environment has not been observed. The introduction of novel non indigenous pollutant degrading microorganisms to resolve this situation has several potential problems. The survival and effectiveness of non indigenous microorganisms in situ has rarely been carefully documented. Current research is attempting to assess the transport and survival of introduced organisms in contaminated zones, however the ability to introduce and effectively distribute pollutant degrading microorganisms into groundwater currently does not exist. The well known ecological problems caused by the introduction of invasive non indigenous macroscopic organisms (e.g. zebra mussels, invasive weeds, rabbits) has provoked considerable evaluation of the practice of introducing non-native organisms. The ecological effect of introducing invasive microorganisms is a relatively unexplored topic.

Mixed Wastes/Metals

Mixtures of different chemicals may influence biodegradation in several ways. Microorganisms have biochemical mechanisms for optimizing energy production by specifically utilizing preferred substrates while repressing catabolism of other substrates. Thus, compounds which are readily biodegraded when present individually, may persist when present in a mixture. Studies of BTEX biodegradation have revealed that substrate interactions are important, and are likely complex and diverse (Alvarez and Vogel, 1991). Compounds which require different redox conditions for biotransformation may further complicate the biodegradation of mixtures. For example, aerobic conditions are desirable for petroleum hydrocarbon biodegradation, but anaerobic conditions are necessary for reductive dechlorination of chlorinated solvents. Thus it may not be possible to have optimum biodegradation conditions for all compounds present in a mixture. Metals may undergo several microbially mediated processes such as oxidation, reduction, immobilization (as phosphates or sulfides), or alkylation. These biotransformations significantly affect the mobility and toxicity of metals, however, the metals are never destroyed.

Field Applications of Bioremediation

Bioremediation is the utilization of naturally occurring microbial biodegradation processes to restore a site to a non hazardous condition. A detailed discussion of bioremediation application and design is presented elsewhere (Baker and Herson, 1994; Cookson, 1995; Flathman *et al.*, 1994; King *et al.*, 1992; Riser-Roberts, 1992). There are many advantages to using bioremediation.

Why Bioremediation?

- **Final Solution**

Pollutants are degraded to harmless compounds such as CO2 and H2O, not merely transferred to another environmental compartment (e.g. landfill).

- **Less Hazardous**

Since the pollutants are left in place, bioremediation results in less exposure of workers and the local community to pollutants than conventional treatment processes.

- **Reduced Liability**

Reduced human exposure and no transportation of contaminated materials to off-site facilities results in less risk.

- **Effective**

Recent field experience has demonstrated that bioremediation of contaminated sites can reduce pollutant concentrations to acceptable levels.

- **Economics**

Bioremediation is usually less costly than conventional remediation processes, thus, bioremediation can save $.

Since pollutant destruction occurs in situ, the potential liability and environmental risk associated with the removal, handling, transport, and storage of hazardous contaminated materials is eliminated. An additional important advantage of bioremediation is that it is often the most economical solution available. Successful bioremediation requires a thorough site investigation and evaluation of treatment options. A site history is compiled which should include property uses, chemicals stored, and location of utilities and buildings, as well as the location of nearby wells. Available information about local hydrogeology, geology, and topography should also be included. Soil and groundwater samples are obtained to identify the contaminants present, and estimate their concentration and distribution. Soil borings are made to determine the local hydraulic gradient and sometimes in conjunction with pumping tests, the hydraulic conductivity is estimated. Whenever possible, an assessment of the local groundwater geochemistry should be made by measuring alternative electron acceptor concentrations (nitrate, sulfate), pH and aquifer buffering capacity. Data from samples taken outside the zone of contamination are useful for assessing background microbiological and geochemical conditions. Laboratory studies are currently the most reliable method for assessing the biodegradation potential of the indigenous organisms. From the assembled information, predictions of contaminant migration and biodegradation can be used to evaluate the current hazard posed and potential remediation options (Table 6).

Table 6 Proving In-Situ Biodegradation has Occurred

- Documented loss of contaminants
- Laboratory assays showing biotransformation potential at the site
- Evidence of biodegradation in the field

Monitored Natural Attenuation

Monitored natural attenuation is a type of bioremediation where the natural in situ biodegradation of pollutants occurs without the engineered manipulation of environmental conditions. Natural attenuation is appropriate when a site investigation shows that natural biodegradation processes are sufficiently attenuating the migration of contaminants, there is little imminent risk or liability associated with the site, and when sufficient evidence exists to demonstrate the likelihood that it will be safe and effective. An ongoing monitoring program is essential to regularly evaluate the progress of natural attenuation until site closure. Naturally occurring processes, including biodegradation, remove contaminants from the environment. The components required to obtain approval of natural attenuation include site characterization, evaluation of contaminant distribution and attenuation mechanisms, and implementation of a monitoring program. Soil conditions and hydrogeology have a significant impact on the environmental fate of pollutants as well as the type and numbers of microflora. Light distillates and components of middle distillates may migrate in the environment at rates faster than natural attenuation mechanisms can remove them. Under such conditions natural attenuation is not acceptable. The advantages of natural attenuation are a potentially lower cost for equivalent environmental protection so that financial resources are conserved for more hazardous contaminants. This depends significantly on the length of time it takes to achieve clean-up objectives. The length of time to remediate the site, as well as public and regulatory acceptance are potential disadvantages.

Engineered Bioremediation

Engineered systems are installed when it is necessary to overcome some limitation to biodegradation, or health and liability concerns make it desirable to accelerate naturally occurring processes. In current practice the addition of some form of oxygen to the aquifer is usually the primary objective. Nutrients (N&P) are often added as well, usually without site specific evidence of a nutrient deficiency, but because it is an easy and inexpensive option. Pumping wells, injection wells, infiltration galleries, vacuum pumps, and compressors may be installed in various combinations and configurations depending on the design objective. In addition to adding an electron acceptor or nutrients to an aquifer, it may be desirable to attempt to contain the contaminant plume, install a groundwater recirculation system, or combine bioremediation with other remediation processes.

In Situ Bioremediation (Cost: $20 - 200/ cubic meter soil)

In Situ bioremediation is not a defined unit operation, but rather a term used to describe a combination of unit operations for in situ soil and groundwater bioremediation. Bioventing, SVE, and/or air sparging can be combined with nutrient recirculation systems, infiltration galleries, above ground treatment systems, and hydrologic control to form an in situ treatment system. Interdiction wells and/or physical barriers may be used to contain contaminant plumes. Oxygen may be added to enhance biodegradation rates and, if their concentration is found to be rate-limiting, nutrients such as nitrogen (as ammonia or nitrate) and phosphorus may be added as well. The governing parameters which control biodegradation in-situ include soil hydraulic conductivity and the type of petroleum. All in-situ systems work better with more porous soils because aeration and environmental control are more effective. In situ bioremediation is more effective with light and middle petroleum distillates primarily because of mass transfer limitations (bioavailability) of the heavier constituents. In situ bioremediation is flexible, cost effective, and non-intrusive. Both soil and groundwater can be treated. There are a few potential disadvantages of in-situ bioremediation. Above ground treatment may be required for any extracted groundwater prior to discharge to a POTW or reintroduction to the subsurface and in-situ bioremediation may be less effective in low porosity soils. Site heterogeneities may result in inconsistent performance.

Bioventing (Cost: $10 - 70/ cubic meter soil)
Bioventing is a bioremediation unit operation during which air is pumped through a well inserted into the contaminated soil. The additional oxygen increases petroleum biodegradation rates. Air pumping flow rates are balanced to optimize biodegradation and minimize volatilization. If air is injected too rapidly the more volatile contaminants may be pushed into the atmosphere rather than biodegraded. The governing parameters which influence bioventing effectiveness are soil permeability, petroleum type, and soil moisture. Bioventing works best in more permeable sandy soils due to the ease with which oxygen can be distributed. Bioventing works best with middle to light distillates, however, volatile compounds tend to be removed by stripping rather than biodegradation if air flow rates are too fast. Too much soil pore water will impede air flow and too little moisture will inhibit biodegradation. The advantages of bioventing are that it can be used to treat large areas, it is relatively low-cost, non-intrusive, and flexible. One disadvantages is that bioventing does not work well in low permeability soils and high groundwater elevations (see air sparging) and saturated soils impede the effectiveness of bioventing.

Soil Vapor Extraction (Cost: $10 - 250/ cubic meter soil)
Soil Vapor Extraction (SVE) is a unit operation where air is extracted through a well inserted into the contaminated soil. This process is conceptually the opposite of bioventing, in other words the air is extracted rather than injected. SVE flow rates are typically about an order of magnitude higher than in bioventing and the objective is usually removal by volatilization rather than biodegradation. The contaminants partition out of the soil matrix into the air flowing through the pore spaces. However, reducing the flow rates will essentially allow the system to behave in a similar fashion as bioventing. The governing parameters which influence SVE are soil permeability, petroleum type, and site conditions. SVE works best in sandy soils which are more permeable, and since volatilization is usually the primary removal mechanism, SVE works best with light distillates. Utility trenches, foundations, and the soil heterogeneity of the site may affect air flow paths. The major advantage of SVE over bioventing is that the extracted vapor phase is easily collected and treated if required by regulations, however, this will increase costs. SVE does not work well in low permeability soils and cold temperatures affect chemical partitioning, and hence volatilization more significantly. In addition, groundwater (see air sparging) and saturated soils impede the effectiveness of SVE.

Air Sparging

Air sparging is a bioremediation operation where air is pumped through a well inserted into the contaminated groundwater. The primary distinction from bioventing is that air is pumped directly into groundwater. The contaminants partition out of the groundwater/soil matrix into air flowing through the soil pore spaces and oxygen in the air promotes biodegradation. Air sparging works best in highly porous sandy soils which are contaminated with lighter petroleum distillates. Air sparging removes light distillates by air stripping more efficiently than heavier distillates. Air sparging work best when contaminants are relatively shallow (<20 ft.). Air sparging provides a potentially very effective method for contaminant source control in groundwater. Air sparging can be combined with SVE for improved efficiency and vapor control. However, vapor recovery may be required which will increase costs. A significant risk is that impermeable or semi-impermeable layers above the injection point can cause significant contaminant plume spreading.

Composting / Landfarming

(Cost: Composting \$20 - 260/ cubic meter soil,
Landfarming \$15 - 250/ cubic meter soil).

Composting and landfarming are ex situ biological treatment processes for use after soil excavation. Composting is done by constructing mounds of contaminated soils which may contain aeration and leachate collection systems. Bulking material, such as wood chips or straw, and manure may be used to improve aeration and nutrient content. Alternatively, soil can be piled in windrows and aeration may be accomplished with commercially available equipment. Landfarming petroleum contaminated soil uses more land space than composting since soil is spread out 12-18 inches deep. Aeration is done by tilling. A significant removal mechanism is volatilization. If petroleum vapor release to the atmosphere is not allowed, composting offers a better system for vapor collection. The concentration and composition of the petroleum products affect biodegradation rates. Heavy distillates may degrade very slowly or not at all. Adding sufficient moisture and nutrients will ensure that these will not be limiting factors for biodegradation. Ex situ treatment offers an opportunity for better control of soil conditions and higher contaminant concentrations (up to 50,000 ppm total petroleum hydrocarbons (TPH)) can be treated with composting and landfarming. The major disadvantage is the amount of space required for ex-situ treatment. In addition, above ground treatment is more significantly affected by cold temperatures and leachate collection and vapor control systems may be required.

Bioaugmentation

The purchase, addition or utilization of commercially available petroleum biodegrading microorganisms should be considered very cautiously. Petroleum biodegrading microorganisms are ubiquitous in the environment, therefore, their alleged absence in petroleum contaminated soil should be viewed with concern and suspicion. Extreme environmental conditions (temperature, pH, salinity) may result in low or absent populations, however, the addition of exogenous organisms will not remedy this situation. Toxic concentrations of petroleum may also result in low microorganism populations. In this case, the utilization of bioaugmentation after the appropriate dilution in a bioslurry reactor may be appropriate. Other than the profit accumulated by the seller, there is little evidence of any advantage of bioaugmentation for petroleum bioremediation. One possible exception in the future may be for the bioremediation of added fuel oxygenates (e.g. MTBE), however, currently there is insufficient information available.

Alternate Ex Situ Remediation Technologies

Thermal desorption and landfilling are the most frequently used technologies for remediation of petroleum contaminated soils. Thermal desorption may be done on-site using truck-mounted units or soil can be trucked to permanent off-site facilities. Trucking distance is often the cost factor which determines economic feasibility. Landfilling costs are primarily influenced by geographic location. The primary advantage of these technologies is the reduced time to site remediation. A major drawback of landfilling is the continued liability of the waste generator for the material deposited.

Typical costs:
Thermal Desorption: $55 - 250/ cubic meter soil.
Landfilling: $40 - 220/ cubic meter soil.

Emerging Technologies

Increased utilization of bioremediation will depend on technological advances which will remove limitations to when bioremediation can be reliably and effectively applied (Atlas, 1995). Advances in understanding novel bioremediation technologies have been made in several areas, for example, bioavailability, molecular biology, microbiology, and anaerobic biodegradation.

Microbially produced biosurfactants and synthetic surfactants increase the apparent solubility of hydrophobic compounds and could potentially reduce bioavailability limitations. However, much remains to be learned about the biodegradation of surfactant solubilized compounds and potential surfactant toxicity, as well as cost and effectiveness. A combined treatment process consisting of surfactant/solvent soil washing followed by bioremediation to remove residual contamination has potential for the remediation of NAPL (Non-Aqueous Phase Liquids) contaminated sites. However, the microecological effect of the soil washing is unknown.

Advances in molecular biology have contributed much to our understanding of the biochemistry and microbiology of biodegradation, and will continue to do so. Use of molecular techniques is now commonplace in biodegradation research. The importance of understanding the biochemistry, genetics, and microbiology of biodegradation cannot be overestimated. In the coming years, molecular techniques for detecting and enumerating bacteria in situ will likely become routine. Genetic techniques are being used to construct novel biodegradation pathways, essentially creating organisms with new capabilities.

Additional examples of emerging technologies include water recirculation systems, oxygen releasing peroxides, the use of zero valent metals to enhance reductive dechlorination, as well as advances in situ measurement of physical, chemical, and biological parameters. Ongoing development of pollutant degrading thermophiles, alkaline tolerant bacteria, and bacteria with membranes resistant to high solvent concentrations will ensure that biodegradation will continue to grow as a primary groundwater remediation option.

CHAPTER 4 PROBLEM STATEMENT

Bioremediation is often the most cost-effective and successful technique available for the remediation of soils and groundwater contaminated with organic pollutants (e.g. petroleum). Leaking underground storage tanks have resulted in petroleum contaminated soils at many INDOT facilities and these sites now require remediation. Contractors implementing bioremediation must be certain that their design is justified by sound science and engineering principles. However, there are no currently existing "design codes" or "standard practices" for the design, implementation or monitoring of bioremediation projects. For example, some bioremediation strategies are only marginally justified by the research literature, and often not justified at all by the contractor's own data. In addition, many soils in Indiana have low hydraulic conductivities which increases the difficulty of implementing successful bioremediation projects. Low hydraulic conductivity of soil is one of the factors which has been identified by the National Research Council as limiting the potential applicability of bioremediation with our current state of knowledge (National Research Council, 1993).

Objectives

- Increase implementation of bioremediation by INDOT and other agencies.
- Improve the quality of engineering science utilized for bioremediation.
- Decrease costs associated with Leaking Underground Storage Tank (LUST) remediation.
- Improve environmental quality.
- Improve public and environmental health.
- Reduce uncertainty associated with the design and implementation of bioremediation systems.
- Reduce reliance on landfills for disposal of contaminated soils.
- Reduce long-term liability associated with hazardous waste.
- Develop a guidance manual for use by INDOT personnel and contractors.

These objectives will be achieved, in part, through the following activities:

- Implementation of a bioremediation field demonstration using Monitored Natural Attenuation.
- Implementation of a land farming field demonstration using excavated low hydraulic conductivity soils.
- Implementation of an engineered bioremediation field demonstration at a site with contamination in the saturated zone.
- Development of a Bioremediation Guidance Manual.

CHAPTER 5 WORK PLAN

The following tasks will enhance the deployment and utilization of bioremediation by INDOT. Overall, implementation of three field demonstrations is planned.

- **Develop a guidance manual for use by INDOT personnel and contractors.** The manual will describe procedures for determining when implementation of bioremediation is appropriate, selection of rational bioremediation strategies, and will provide standards for good practice.

- **Investigate methods for bioremediating soils with low hydraulic conductivities.** Many soils in Indiana have low hydraulic conductivities which increases the difficulty of implementing successful bioremediation projects. Low hydraulic conductivities of soil is one of the factors which has been identified by the National Research Council as limiting the potential applicability of bioremediation with our current state of knowledge. Several approaches will be investigated. Implementation of a land farming field demonstration is planned. Other possibilities which will be considered are soil fracturing and phytoremediation.

- **Implementation of an Engineered bioremediation field demonstration at a site with contamination in the saturated zone.** At sites with contaminated groundwater, remediation is necessary to prevent transport of contaminants off-site or into drinking water wells. Using bioremediation can significantly reduce the time and cost of groundwater remediation when compared to physical/chemical treatment methods. Evaluation of a demonstration project which follows standards of good practice for bioremediation will catalyze additional utilization of this technology.

- **Implementation of a bioremediation field demonstration using Monitored Natural Attenuation.** At sites with clayey soils and without potable groundwater receptors nearby, the risk of exposure or transport of the contaminants is greatly reduced. Therefore, less rapid and less costly forms of remediation are feasible. Intrinsic bioremediation, or natural attenuation, is one such remediation technique. In this field implementation we intend to use Monitored Natural Attenuation.

The following steps will be required at all of the field demonstrations:

1) site selection,
2) site characterization,
3) system design,
4) system installation,
5) site monitoring.

CHAPTER 6
IMPLEMENTATION OF BIOREMEDIATION - A GUIDANCE DOCUMENT

The purpose of a guidance manual is to provide Department of Transportation (DOT) decision makers with information for technology selection, management, and operation for bioremediation at petroleum contaminated sites.

Most DOT's utilize the services of consulting engineers and other environmental professionals for the remediation of petroleum releases to the environment. The bioremediation guidance document provides information about the basic fundamentals of bioremediation and bioremediation technologies. Remediation technology selection is often not a straightforward decision. In addition to considering the most effective technology and cost, desired clean-up end points and completion dates must be considered as well. To aid with this decision process a petroleum remediation decision tree was constructed. With this information, DOT decision makers will be better able to ensure that consulting engineers and environmental professionals follow sound practices and that effective and appropriate technologies are selected for remediation.

This document is not intended to serve as a manual for the design of bioremediation systems. The references listed at the end of this document provide additional information, including design procedures.

The document entitled "Guidance Manual for Managing Implementation of Bioremediation at Petroleum Contaminated Sites" is appended to this report (Appendix A).

CHAPTER 7
IMPLEMENTATION OF A LAND FARMING FIELD DEMONSTRATION

CHRISNEY, INDIANA

Introduction

Land farming of petroleum contaminated soils is an ex-situ biotreatment method. There are several advantages of land farming under the appropriate circumstances. Land farming offers the opportunity for greater control of biological conditions which influence biodegradation such as, aeration and moisture, and ease of nutrient addition if desired. Low permeability soils may be more effectively treated by land farming due to their easy accessibility for tilling which significantly improves aerobic biodegradation. In addition, due to greater control of soil conditions biodegradation kinetics may be significantly faster than in-situ degradation. However, excavation of the contaminated material is necessary and construction of holding cells (the land farm) are required to contain any petroleum contaminated run-off which might result from excessive rainfall. Therefore, land farming is most appropriate when small volumes of contaminated materials exist or in-situ treatment is not an option, such as when contamination is discovered during road construction activities or a property transfer necessitates nearly immediate closure.

The site selected for a land farming demonstration project was the Chrisney Unit located on US 231 just north of Chrisney, Indiana in Spencer County.

Hoosier Equipment Service, Inc. was contracted to remove one 10,000-gallon diesel fuel Underground Storage Tank (UST), one 2,000-gallon diesel fuel UST, and one 2,000-gallon gasoline UST from the Chrisney site in May 1995. Petroleum contamination was discovered during the tank removal which was reported to the Indiana Department of Environmental Management (IDEM) on May 26, 1995 and assigned incident number 9505539.

In late 1995 Environmental Management Consultants, Inc. conducted a Leaking Underground Storage Tank (LUST) site investigation. Soil borings from the site were analyzed for Total Petroleum Hydrocarbons (TPH). Petroleum contamination from the LUSTs which exceeded the IDEM action level of 100 TPH was present from 3.5 to 6 feet below ground in the area surrounding the former UST pump island and along the fuel product service lines.

Keramida Environmental, Inc. was contracted to construct a land treatment facility. During November 20 -26, 1997, the soil in the former pump island and product line trench areas were excavated. Approximately 300 cubic yards of soil were removed and placed in two containment areas "A" and "B" (Figure 6). Each containment area was constructed by pushing earth into a 24 inch tall berm around an area from which surface rocks and vegetation had been scraped. Over the entire area, including the berms, a 12-mil plastic bottom liner was put in place. Petroleum contaminated soil was placed in the containment area to a depth of 18 inches and covered with another layer of 12-mil plastic (Figure 7). Each area was divided into cells of approximately 260-330 square feet of surface for purposes of record keeping. Area "A" consisted of cells numbered 1 - 14, and area "B" consisted of cells numbered 15 - 20 (Figure 6)(Keramida Environmental, Inc., 1997). Periodically, the top cover was removed and the soil tilled with a hand tiller and soil samples were taken to monitor biodegradation progress. This type of simple landfarm design prevents infiltration of rainfall and eliminates the need for run-off and leachate collection systems.

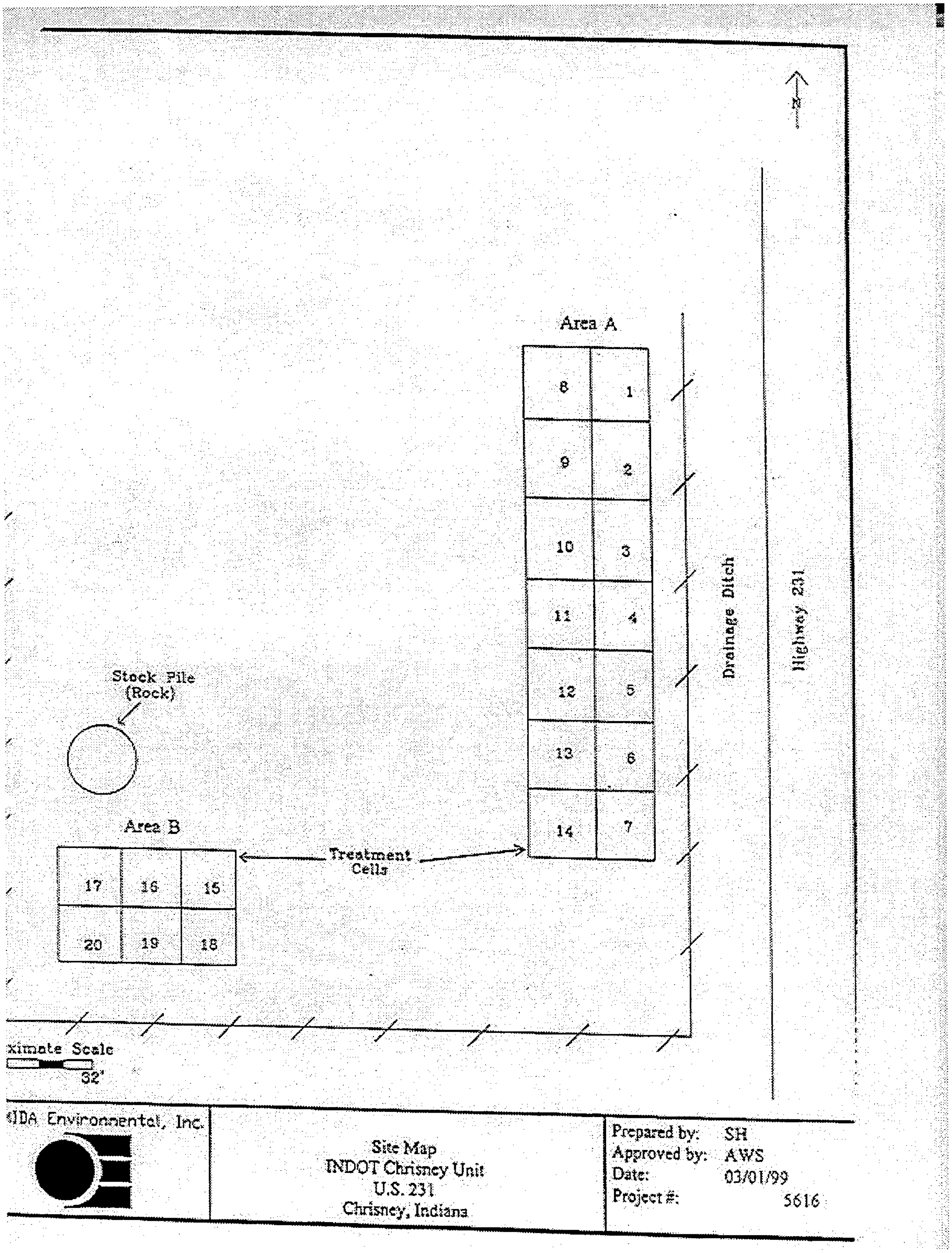


Figure 6 Layout of the Chrisney Land Farm

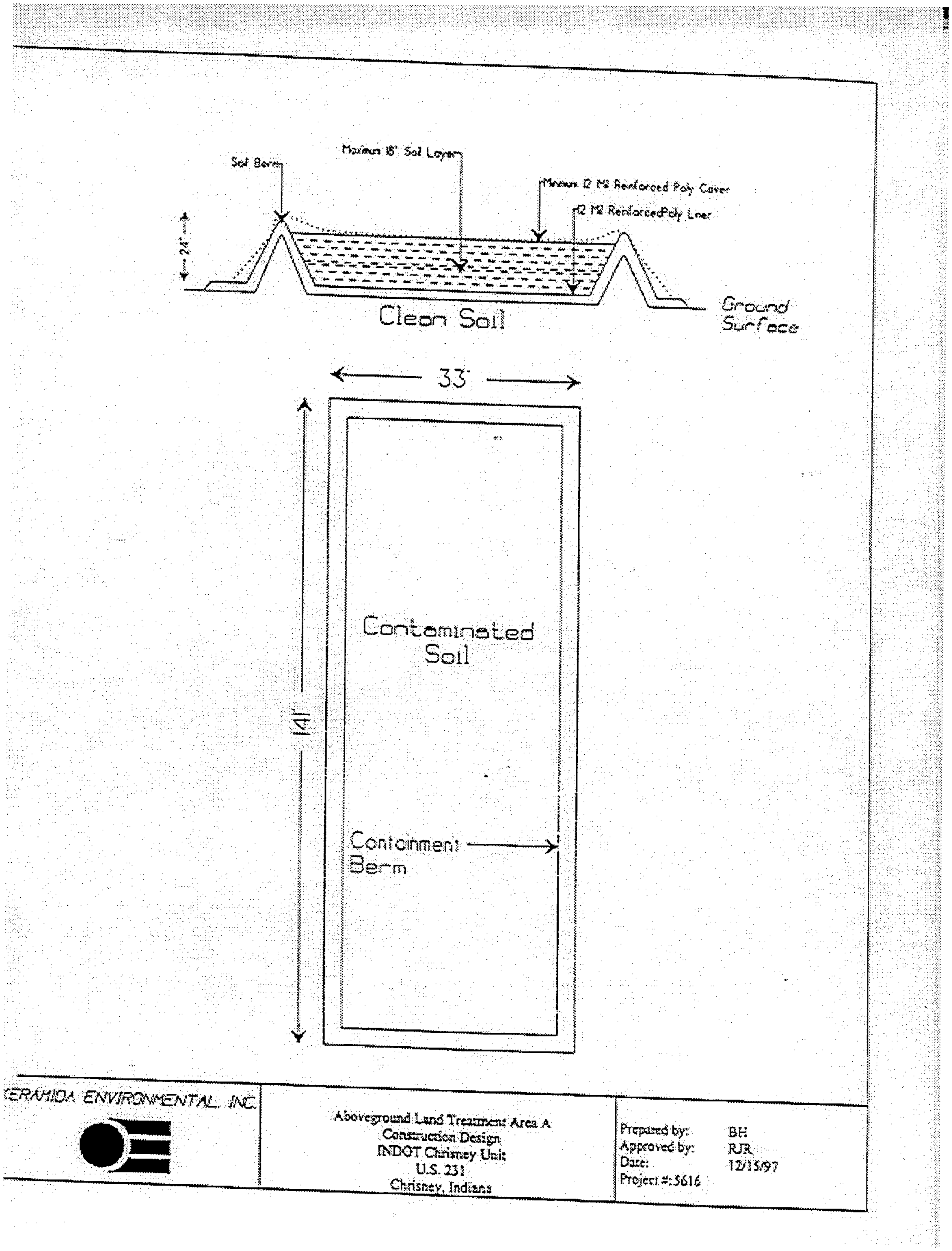


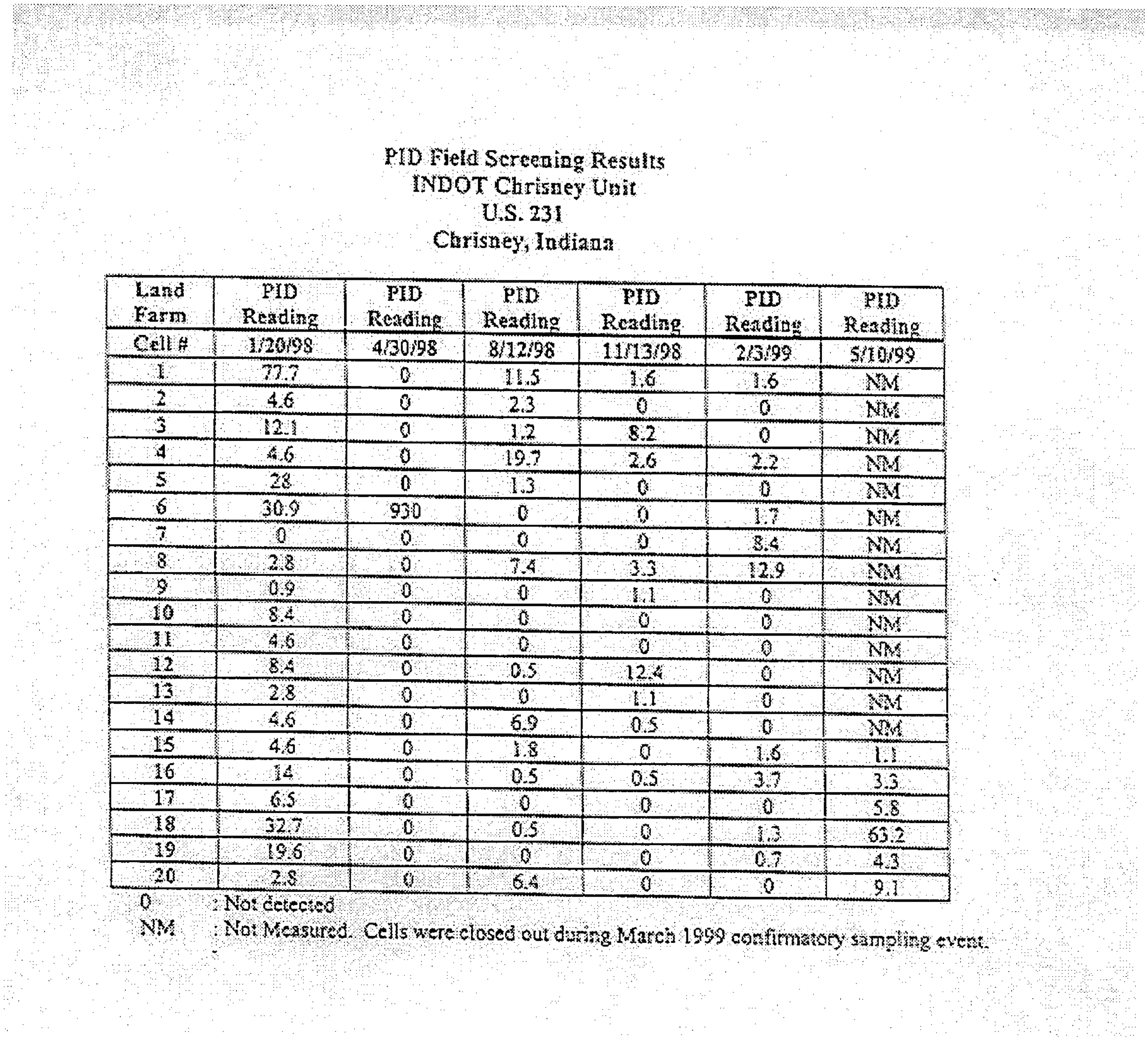
Figure 7 Construction Design of the Chrisney Land Farm

The clean-up criteria for petroleum contaminated soil is 100 mg/kg TPH if the soil will be used as fill material on-site and 20 mg/kg TPH if the soil is to be used off-site. On a quarterly basis each cell was field screened for TPH using a photoionization detector (PID) (Table 7). PID analysis will detect aromatic constituents of petroleum mixtures, however, PID analysis is not very quantitative and is not acceptable for achieving regulatory compliance. When field screening data showed that sufficient biodegradation may have occurred, confirmatory samples were taken and analyzed for volatile and semi-volatile TPH by gas chromatography according to EPA Method 8015.

Confirmatory sampling that was done on February 3, 1999 showed that TPH contamination in the soil contained in Area "A" was below the 100 mg/kg required for on-site soil use. Only cell 18 in Area "B" remained above this 100 mg/kg threshold (Table 8a). Cells 8-U and 18-U were also above 100 mg/kg TPH, however, it was determined that the TPH in these samples was actually from the underlying asphalt surface at the site. Area "B" was sampled and analyzed again on June 29, 1999 and found to be below the 100 mg/kg TPH clean-up objective (Table 8b). In August 1999 a closure report was submitted to IDEM and a letter of "No Further Action" requested (Keramida Environmental, Inc. 1999).

It is planned that the soil in Area "B" will be used as on-site fill material. One additional confirmatory sampling is planned for Area "A." If the TPH is found to be below the 20 mg/kg TPH threshold Area "A" soil will be used for off-site construction fill material.

Table 7

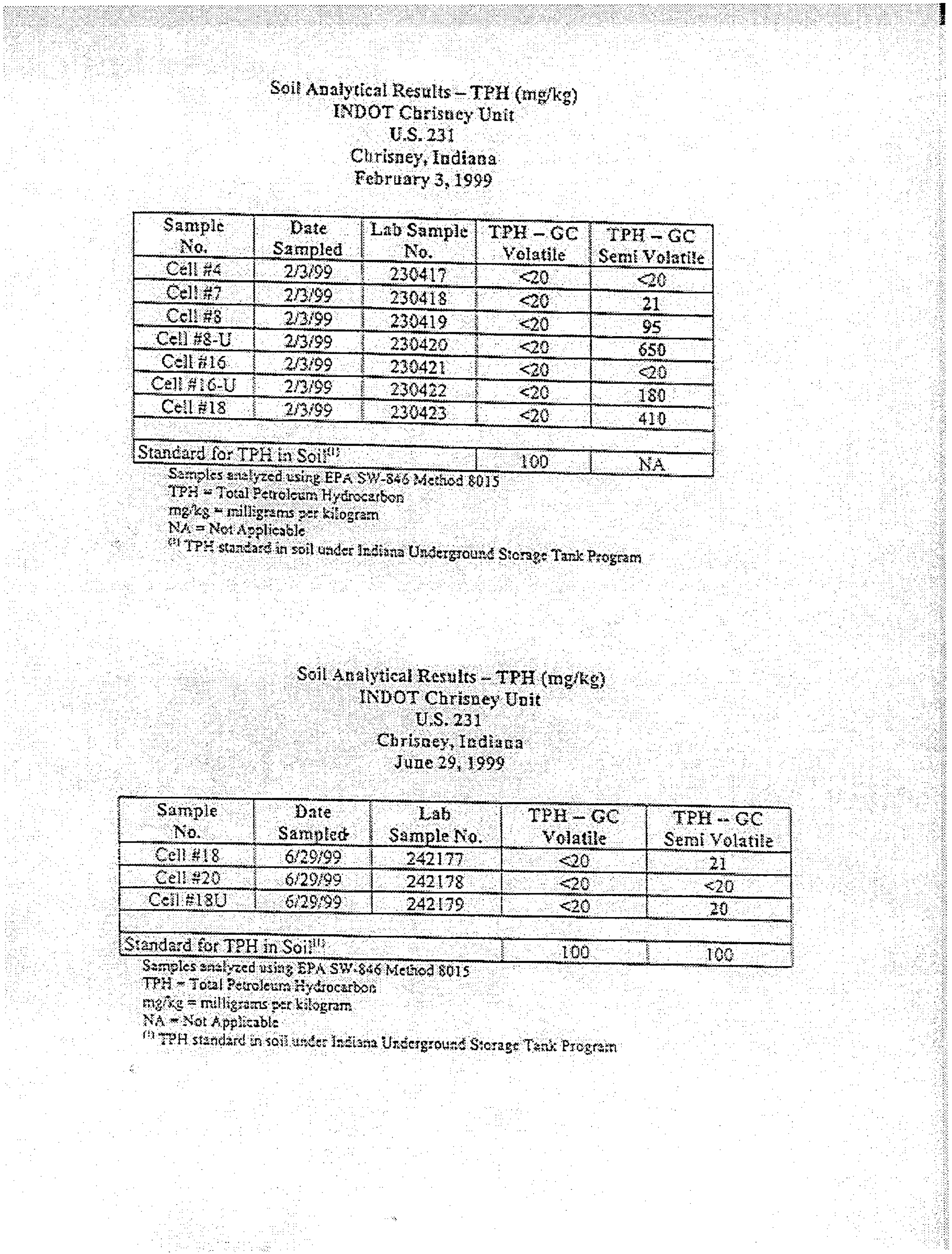


PID Field Screening Results
INDOT Chrisney Unit
U.S. 231
Chrisney, Indiana

Land Farm Cell #	PID Reading 1/20/98	PID Reading 4/30/98	PID Reading 8/12/98	PID Reading 11/13/98	PID Reading 2/3/99	PID Reading 5/10/99
1	77.7	0	11.5	1.6	1.6	NM
2	4.6	0	2.3	0	0	NM
3	12.1	0	1.2	8.2	0	NM
4	4.6	0	19.7	2.6	2.2	NM
5	28	0	1.3	0	0	NM
6	30.9	930	0	0	1.7	NM
7	0	0	0	0	8.4	NM
8	2.8	0	7.4	3.3	12.9	NM
9	0.9	0	0	1.1	0	NM
10	8.4	0	0	0	0	NM
11	4.6	0	0	0	0	NM
12	8.4	0	0.5	12.4	0	NM
13	2.8	0	0	1.1	0	NM
14	4.6	0	6.9	0.5	0	NM
15	4.6	0	1.8	0	1.6	1.1
16	14	0	0.5	0.5	3.7	3.3
17	6.5	0	0	0	0	5.8
18	32.7	0	0.5	0	1.3	63.2
19	19.6	0	0	0	0.7	4.3
20	2.8	0	6.4	0	0	9.1

0 : Not detected
NM : Not Measured. Cells were closed out during March 1999 confirmatory sampling event.

Table 8



Soil Analytical Results – TPH (mg/kg)
INDOT Chrisney Unit
U.S. 231
Chrisney, Indiana
February 3, 1999

Sample No.	Date Sampled	Lab Sample No.	TPH – GC Volatile	TPH – GC Semi Volatile
Cell #4	2/3/99	230417	<20	<20
Cell #7	2/3/99	230418	<20	21
Cell #8	2/3/99	230419	<20	95
Cell #8-U	2/3/99	230420	<20	650
Cell #16	2/3/99	230421	<20	<20
Cell #16-U	2/3/99	230422	<20	180
Cell #18	2/3/99	230423	<20	410
Standard for TPH in Soil[1]			100	NA

Samples analyzed using EPA SW-846 Method 8015
TPH = Total Petroleum Hydrocarbon
mg/kg = milligrams per kilogram
NA = Not Applicable
[1] TPH standard in soil under Indiana Underground Storage Tank Program

Soil Analytical Results – TPH (mg/kg)
INDOT Chrisney Unit
U.S. 231
Chrisney, Indiana
June 29, 1999

Sample No.	Date Sampled	Lab Sample No.	TPH – GC Volatile	TPH – GC Semi Volatile
Cell #18	6/29/99	242177	<20	21
Cell #20	6/29/99	242178	<20	<20
Cell #18U	6/29/99	242179	<20	20
Standard for TPH in Soil[1]			100	100

Samples analyzed using EPA SW-846 Method 8015
TPH = Total Petroleum Hydrocarbon
mg/kg = milligrams per kilogram
NA = Not Applicable
[1] TPH standard in soil under Indiana Underground Storage Tank Program

CHAPTER 8
IMPLEMENTATION OF A MONITORED NATURAL ATTENUATION FIELD DEMONSTRATION

LINTON, INDIANA

Introduction

When historical and field evidence indicates that naturally occurring removal mechanisms, such as biodegradation, are effectively degrading contaminants and sensitive environmental receptors, such as drinking water wells, are not at risk of contamination natural attenuation may be an appropriate remediation technology.

The site selected for a natural attenuation demonstration project was the Linton Subdistrict site, 89 Southwest D Street, Linton Indiana. The site originally had three USTs which were replaced in 1987 with one 10,000-gallon diesel fuel UST and one 2,000-gallon gasoline UST. In May of 1995 Hoosier Equipment Service, Inc. was contracted to remove the newer tanks. During excavation gasoline odors and soil staining indicative of petroleum leakage were noticed. Soil samples were collected from the excavation sidewalls and from groundwater. Soil samples contained between 110 - 870 mg/kg TPH and groundwater samples had 210 ug/L benzene which exceeded the maximum contaminant level (MCL) of 5 ug/L. The release was reported to IDEM and assigned an incident number of 9505512 (August Mack Environmental Inc., 1995).

The site encompasses approximately three acres which slopes slightly to the southwest. The adjacent nearby land is predominantly residential and light commercial, with agricultural land further to the southwest and southeast. The Town of Linton provides utilities to the site, including water and sewer service.

A shallow perched groundwater aquifer exists at the site. The water table is approximately eight feet below the surface with a thickness of approximately seven feet. The aquifer lies above an impermeable sandstone and shale layer at a depth of approximately 15 feet. The aquifer soil is a low permeability stiff brown and mottled gray clay. The groundwater elevations and hydraulic gradient are significantly influenced directly by local precipitation. The hydraulic gradient varies, depending on rainfall infiltration from approximately 0.01 to 0.02 ft/ft and the direction varies from northwest to northeast. For example, a groundwater potentiometric surface map from data taken in July 1999 shows a hydraulic gradient towards the northwest (Figure 8). However, data taken in November 1999 reveals a gradient towards the northeast (Figure 9). Groundwater flow velocities were estimated to be less than one foot/year.

The initial site investigation was performed by August Mack Environmental, Inc on August 30, 1995. Soil borings were taken and five groundwater monitoring wells were installed. One additional monitoring well was installed in January of 1998. Soil borings revealed that petroleum contamination existed from 3 - 14 feet below the surface surrounding the former tank pit areas. It was estimated that approximately 1,800 cubic yards of soil exceeded the 100 mg/kg action threshold. Two of the monitoring wells are located within the contaminated plume and four of the monitoring wells are located outside the contaminated area to serve as sentinels for migration of the contaminants.

A survey of wells within two miles of the Linton site identified four low capacity wells (< 70 gpm) within one mile and one high capacity surface water intake within two miles. The Linton municipal wellfield is located more than two miles from the site. Three of the low capacity wells are hydraulically upgradient from the Linton site. The remaining low capacity well is drilled to a depth of 73 feet and is not screened over the contaminated aquifer at the site. The high capacity water withdrawal is from a surface pond more than one mile from the site.

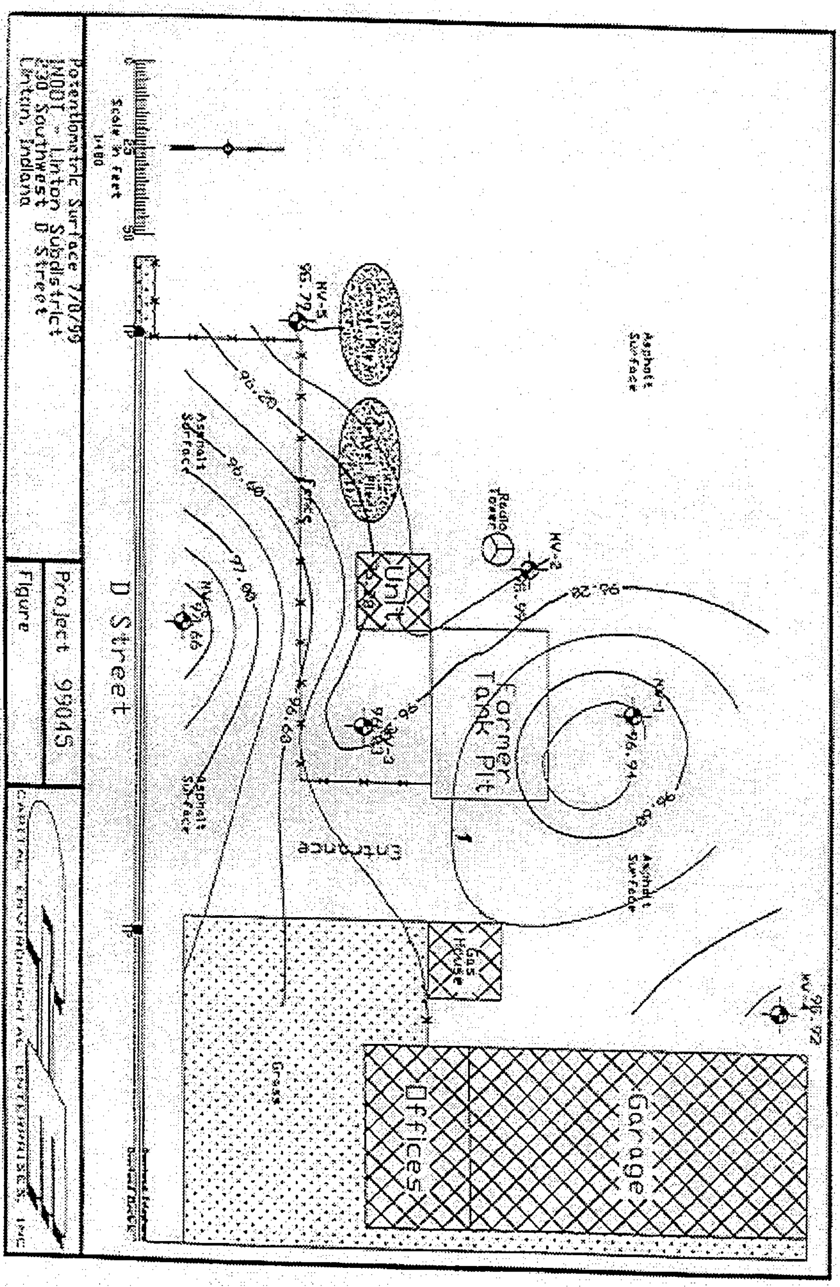


Figure 8 Linton Potentiometric Groundwater Surface 7-8-99

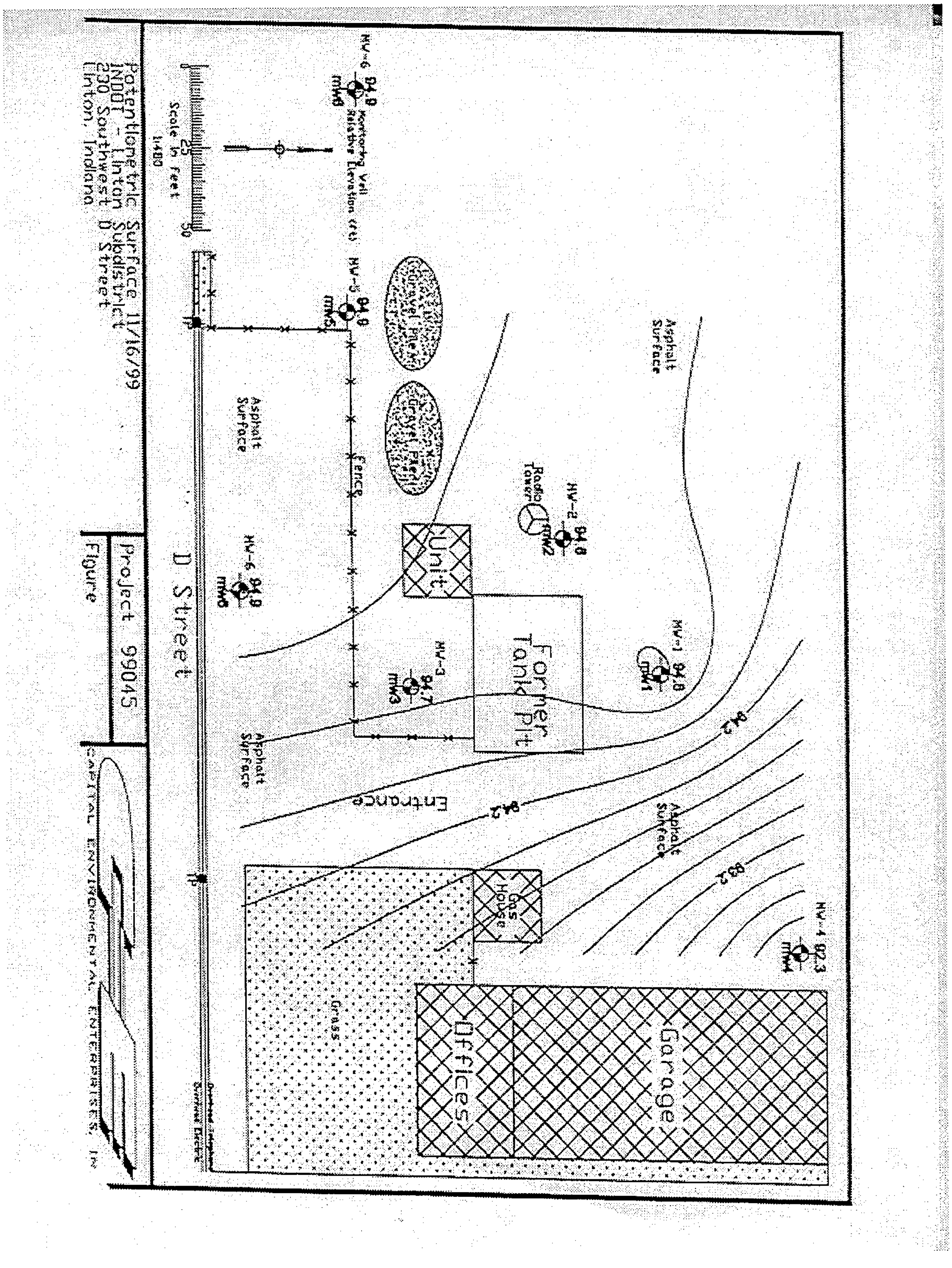


Figure 9 Linton Potentiometric Groundwater Surface 11-16-99

Considering the low groundwater flow rates and the on-site monitoring wells which are located to detect any contaminant migration, the risk of off-site migration is extremely low. Therefore, the petroleum contamination poses no serious or immediate hazard to human health or environmental receptors and a monitored natural attenuation program is appropriate for this site (Baldwin and Nies, 1998).

Since benzene is the most toxic and strictly regulated constituent of petroleum products it serves as a good example compound for evaluating natural attenuation. There have been some fluctuations in the concentrations of contaminants detected in the monitoring wells (Figure 10). This is very likely due to the variations in the direction of groundwater flow. As the groundwater flow changes directions in response to local rainwater infiltration it passes through contaminated soil near the former tank pit. However, the general trend is a decrease in contaminant concentrations. A summary of all groundwater monitoring data is shown in Table 9 (Capital Environmental Enterprises, Inc. 1999).

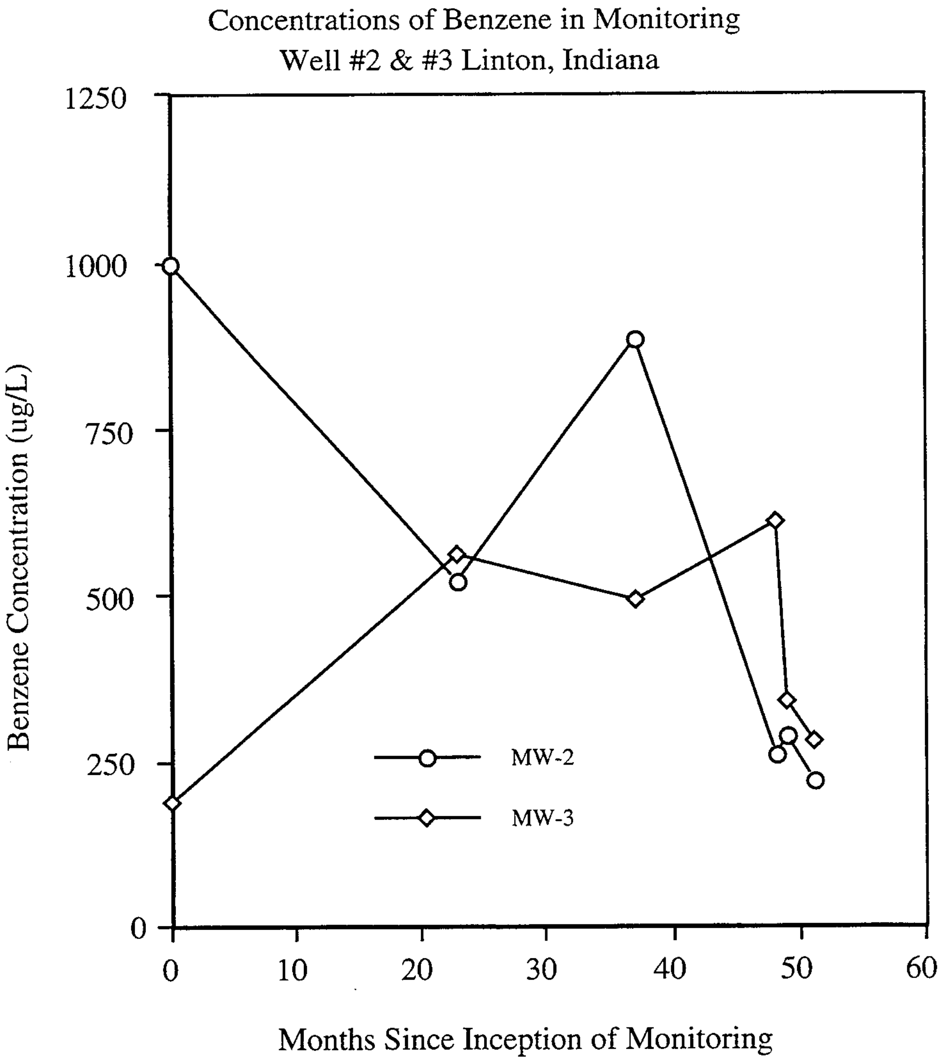
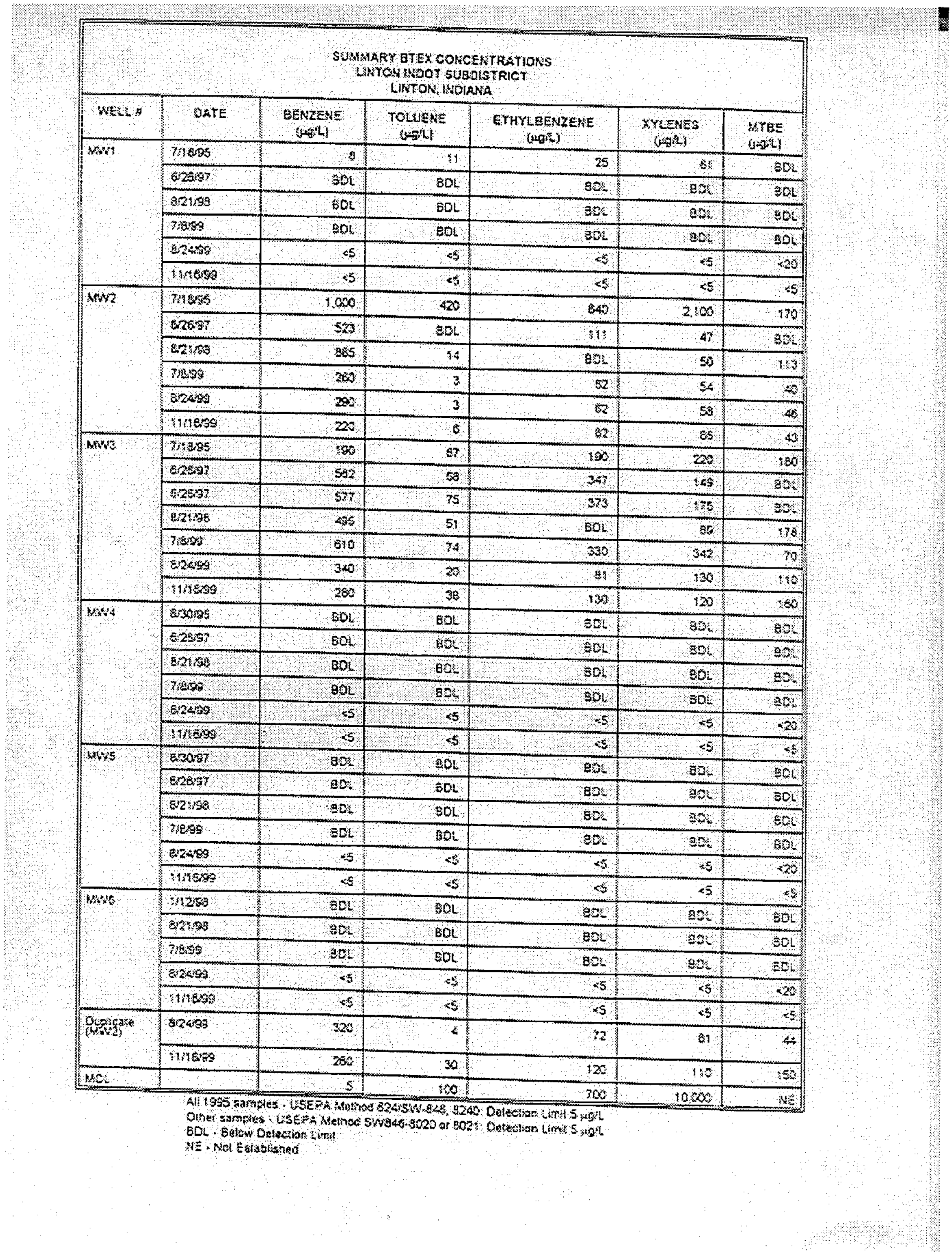

Concentrations of Benzene in Monitoring
Well #2 & #3 Linton, Indiana
Benzene Concentration (ug/L)
1250
1000
750
500
250
0
0
10
20
30
40
50
60
Months Since Inception of Monitoring
MW-2
MW-3

Figure 10.

Table 9 Summary of Groundwater Analysis at All Monitoring Wells at Linton, Indiana



SUMMARY BTEX CONCENTRATIONS
LINTON INDOT SUBDISTRICT
LINTON, INDIANA

WELL #	DATE	BENZENE (µg/L)	TOLUENE (µg/L)	ETHYLBENZENE (µg/L)	XYLENES (µg/L)	MTBE (µg/L)
MW1	7/18/95	8	11	25	61	BDL
	6/26/97	BDL	BDL	BDL	BDL	BDL
	8/21/98	BDL	BDL	BDL	BDL	BDL
	7/8/99	BDL	BDL	BDL	BDL	BDL
	8/24/99	<5	<5	<5	<5	<20
	11/18/99	<5	<5	<5	<5	<5
MW2	7/18/95	1,000	420	840	2,100	170
	6/26/97	523	BDL	111	47	BDL
	8/21/98	885	14	BDL	50	113
	7/8/99	260	3	52	54	40
	8/24/99	290	3	62	58	46
	11/18/99	220	6	82	86	43
MW3	7/18/95	190	67	190	220	180
	6/25/97	562	58	347	149	BDL
	6/25/97	577	75	373	175	BDL
	8/21/98	495	51	BDL	89	178
	7/8/99	610	74	330	342	70
	8/24/99	340	20	81	130	110
	11/18/99	280	38	130	120	160
MW4	8/30/95	BDL	BDL	BDL	BDL	BDL
	6/25/97	BDL	BDL	BDL	BDL	BDL
	8/21/98	BDL	BDL	BDL	BDL	BDL
	7/8/99	BDL	BDL	BDL	BDL	BDL
	8/24/99	<5	<5	<5	<5	<20
	11/18/99	<5	<5	<5	<5	<5
MW5	6/30/97	BDL	BDL	BDL	BDL	BDL
	6/28/97	BDL	BDL	BDL	BDL	BDL
	8/21/98	BDL	BDL	BDL	BDL	BDL
	7/8/99	BDL	BDL	BDL	BDL	BDL
	8/24/99	<5	<5	<5	<5	<20
	11/18/99	<5	<5	<5	<5	<5
MW6	1/12/98	BDL	BDL	BDL	BDL	BDL
	8/21/98	BDL	BDL	BDL	BDL	BDL
	7/8/99	BDL	BDL	BDL	BDL	BDL
	8/24/99	<5	<5	<5	<5	<20
	11/18/99	<5	<5	<5	<5	<5
Duplicate (MW2)	8/24/99	320	4	72	81	44
	11/18/99	262	30	120	110	150
MCL		5	100	700	10,000	NE

All 1995 samples - USEPA Method 624/SW-846, 8240: Detection Limit 5 µg/L
Other samples - USEPA Method SW846-8020 or 8021: Detection Limit 5 µg/L
BDL - Below Detection Limit
NE - Not Established

CHAPTER 9
IMPLEMENTATION OF AN ENGINEERED BIOREMEDIATION FIELD DEMONSTRATION

SHOALS, INDIANA

Introduction

Engineered bioremediation is appropriate in situations where the natural assimilative capacity of the site environment is exceeded and the contamination may be spreading via groundwater migration. In this situation clean-up objectives cannot be met without source removal and environmental manipulation to increase the in-situ microbial petroleum biodegradation rate, most often accomplished by increasing the availability of oxygen in the subsurface. An additional concern is the migration of the contaminants off-site.

The site chosen for an engineered bioremediation demonstration project was the Shoals Maintenance Unit located on US 50 West, Shoals, Indiana. The site contains two active fiberglass USTs, one a 10,000-gallon diesel fuel tank presently used for storage of calcium chloride and a 4,000-gallon gasoline tank, both of which were installed in June, 1990. A 2,000-gallon steel UST installed in 1966 and used for gasoline and diesel fuel was abandoned in 1990. Soil borings done in 1991 by Enviroscience, Inc. revealed soil TPH contamination up to 580 mg/kg in the vicinity of the 2,000-gallon UST. This UST was removed by Tank Technologies April 4, 1991. Additional sampling at that time showed that TPH concentrations ranged from 237 to 10,500 mg/kg.

The surface of the site slopes sharply to the northeast. A small creek lies at the bottom of the slope. A small local unconsolidated aquifer lies from 15 to 31 feet below grade at the site with an estimated flow northeasterly at a velocity of approximately 7 ft/yr. This small aquifer has an extremely steep hydraulic gradient of approximately 0.15 ft/ft (Figure 11). The regional aquifer in the area lies in water bearing sandstone, shale, and limestone at depths of 145 to 245 feet. Water is supplied to the Shoals Unit by the city of Shoals from a municipal well field located two miles east of the site. A water-supply well located 55 feet east of the 2,000-gallon tank was abandoned in 1993. To prevent petroleum contamination from migrating down the well casing, the pump was removed and the well closed in 1996.

From May 16, 1996 to September 30, 1997 three additional subsurface sampling events occurred at the Shoals site. Fifteen soil borings were taken for petroleum analysis (Table 10) and ten of these borings were converted into monitoring wells (Table 11 and 12). The highest TPH concentrations were found northeast of the former tank pit (Figure 12). Groundwater concentrations up to 44,200 ug/L BTEX were found including 20,000 ug/L benzene (Figure 13). Total BTEX found in monitoring wells sampled September 30, 1997 are shown in Figure 14. A notification of release was submitted to IDEM on August 8, 1996 and assigned an incident number 9608506 (ATC Associates, Inc., 1998).

The closest residential well is 115 feet deep and located approximately 1,500 feet west of the site. There are an additional 8 low-capacity wells within one mile of the site. A high capacity 200-gpm well is located 8,000 feet southeast of the site and four additional high capacity wells are within two miles of the site. The contaminated aquifer beneath the site is not currently used as a potable water source. The greatest risk of contaminant migration off-site is from the contaminated water exiting the steeply sloping hill to the northeast as a spring and running as a surface discharge into Nut Creek which flows south into the East Fork of the White River.

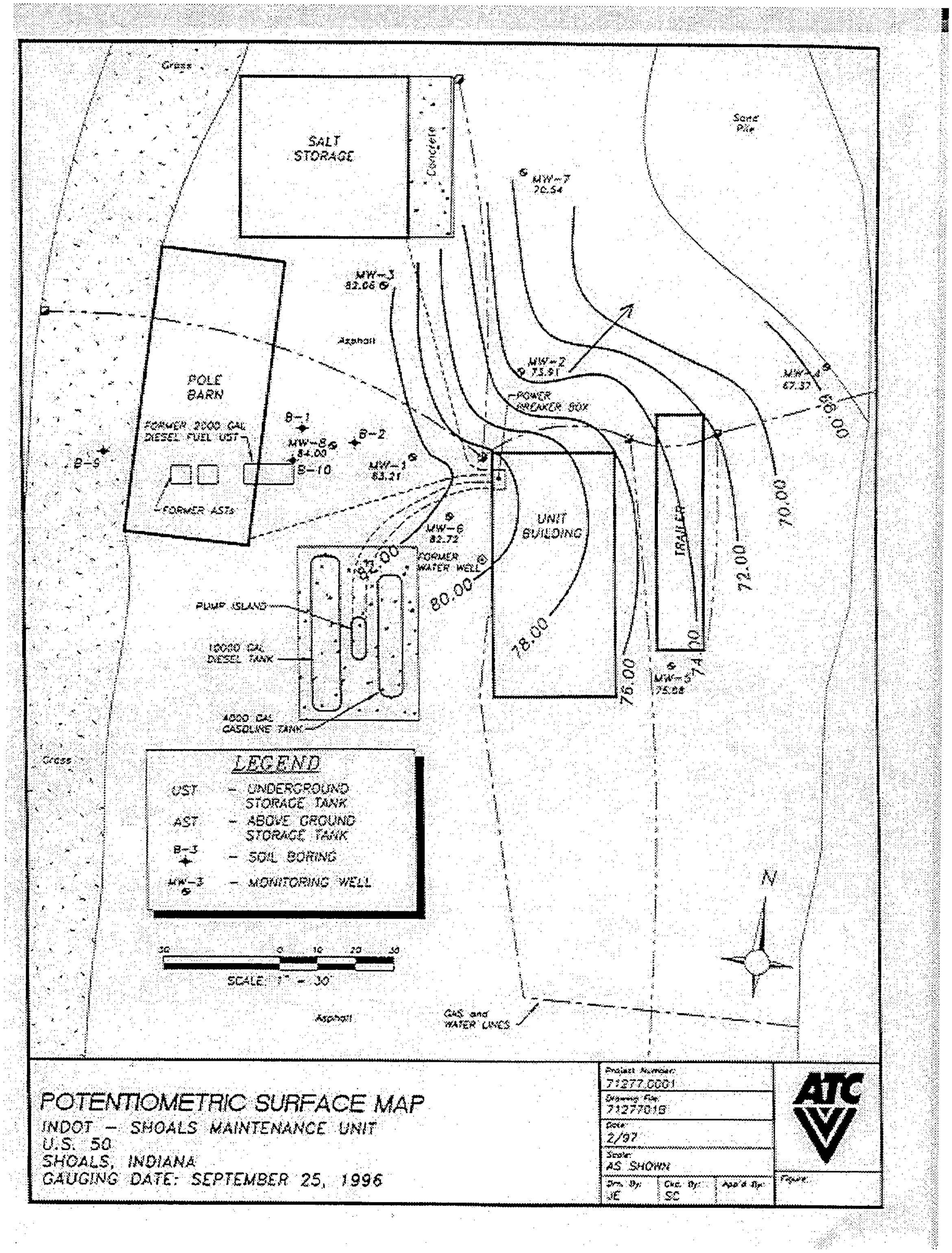


Figure 11 Shoals Potentiometric Groundwater Surface

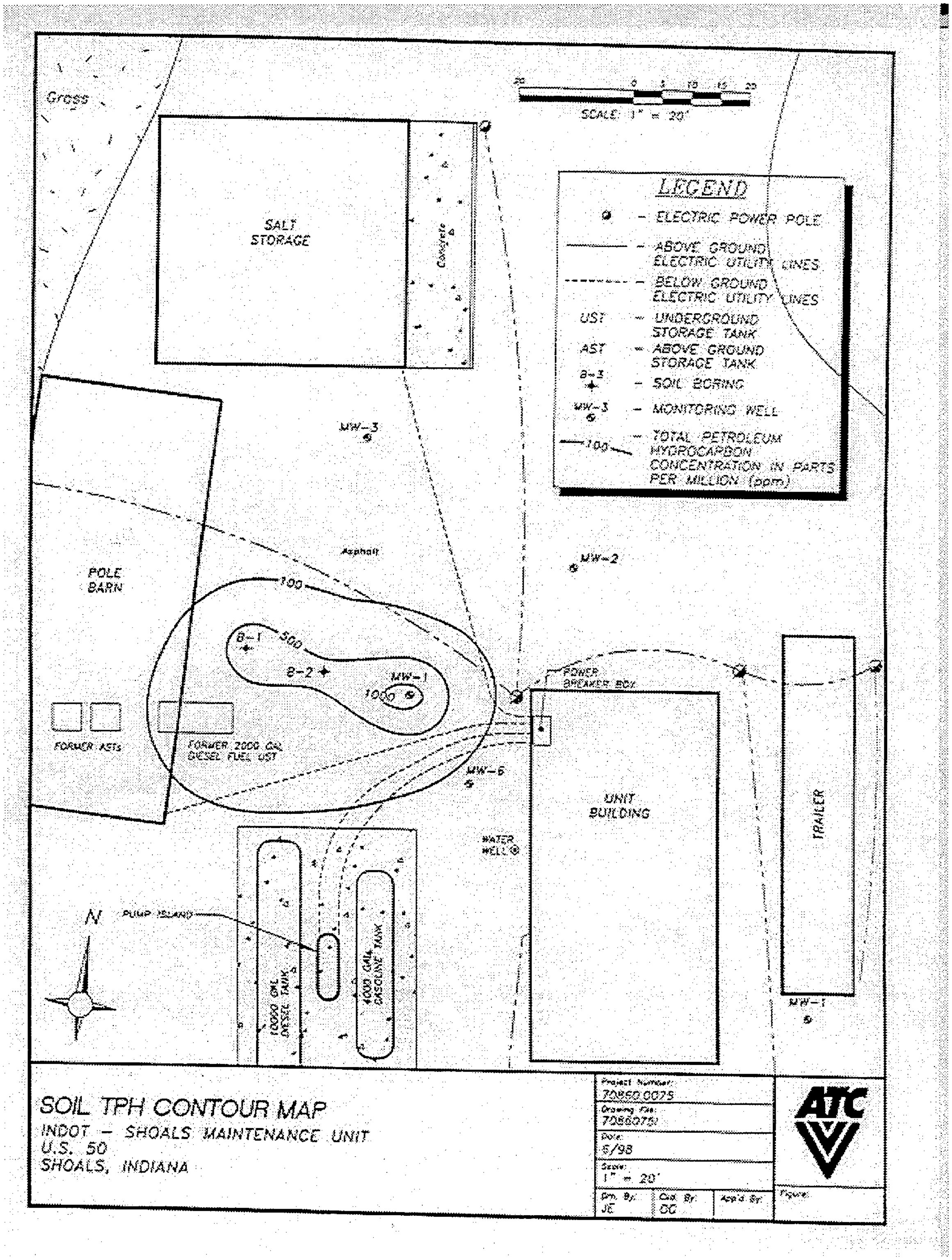


Figure 12 Total Petroleum Hydrocarbon Contours at Shoals

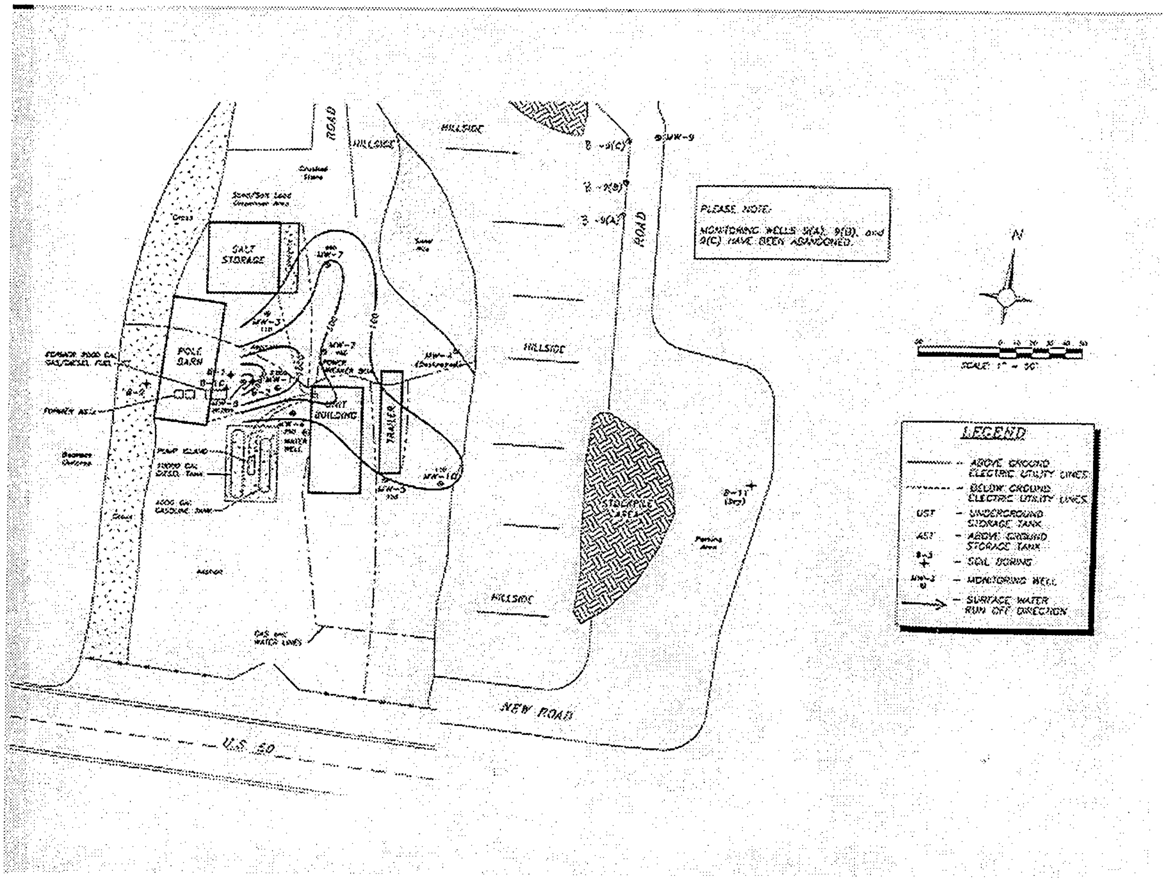


Figure 13 Benzene Groundwater Concentration Contours at Shoals

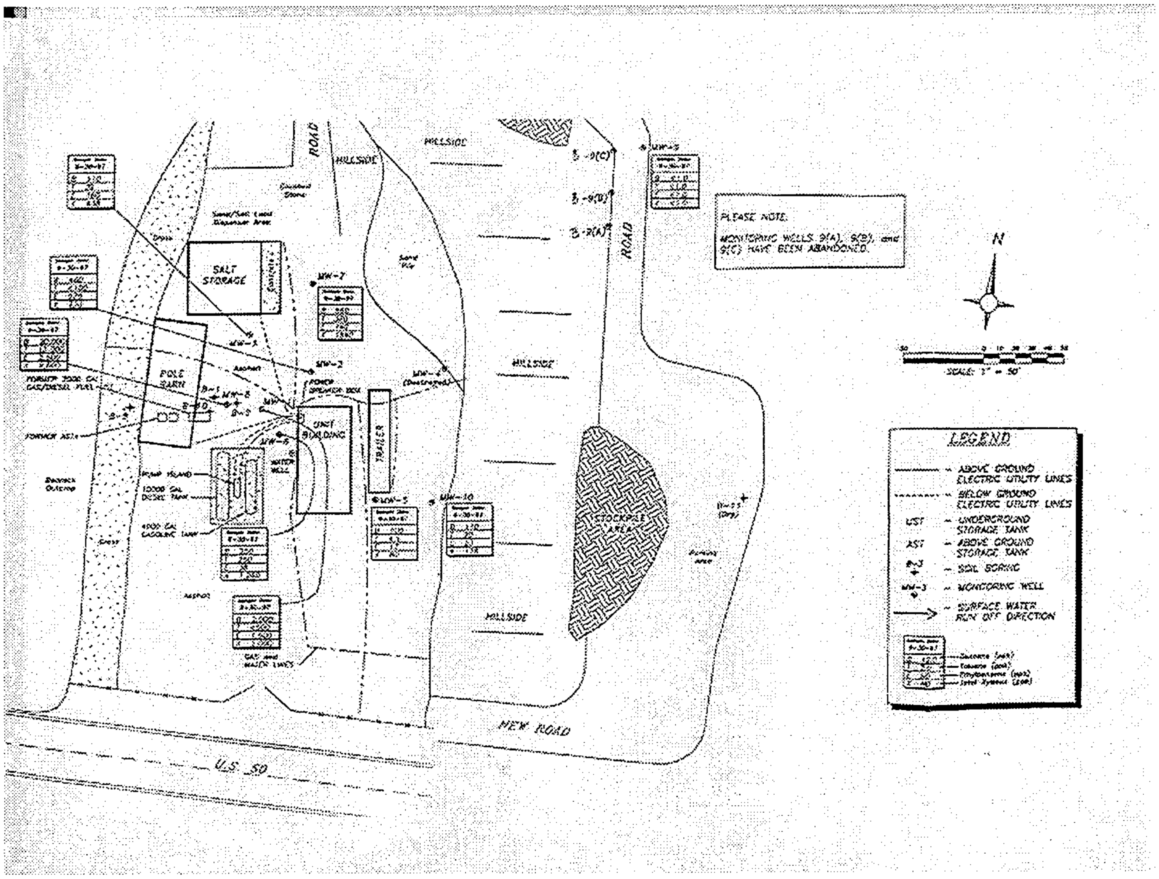
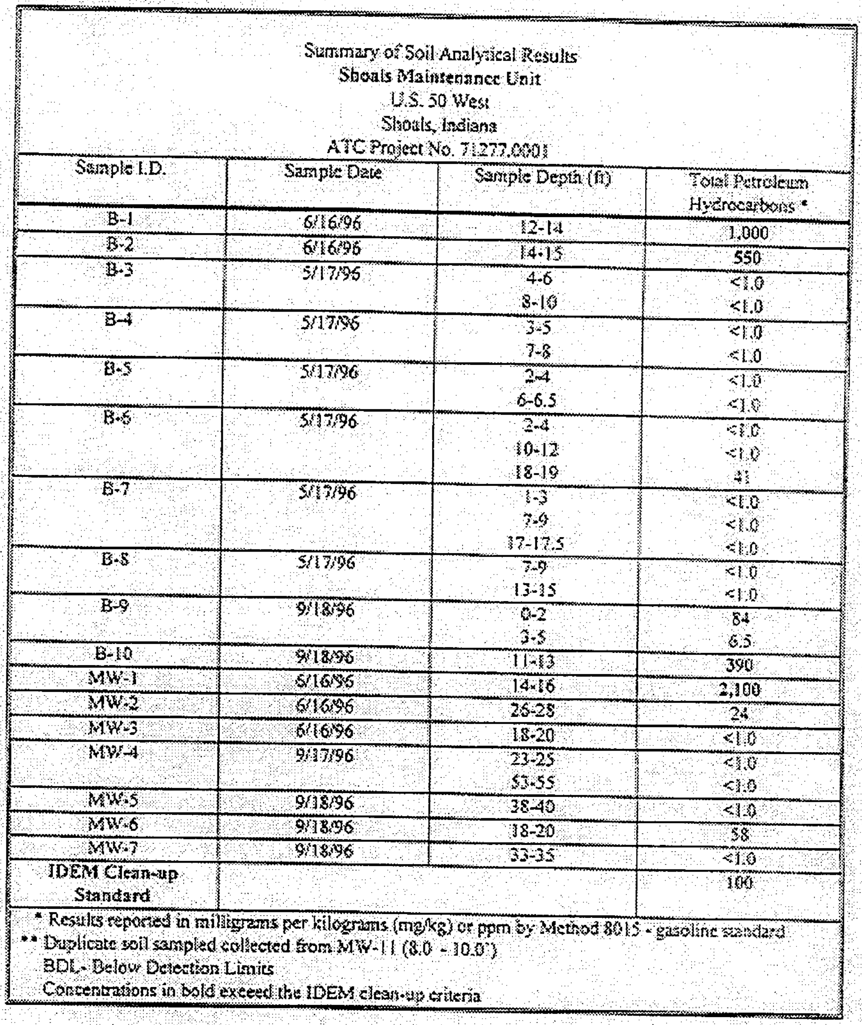


Figure 14 BTEX Groundwater Concentrations at Shoals

Table 10

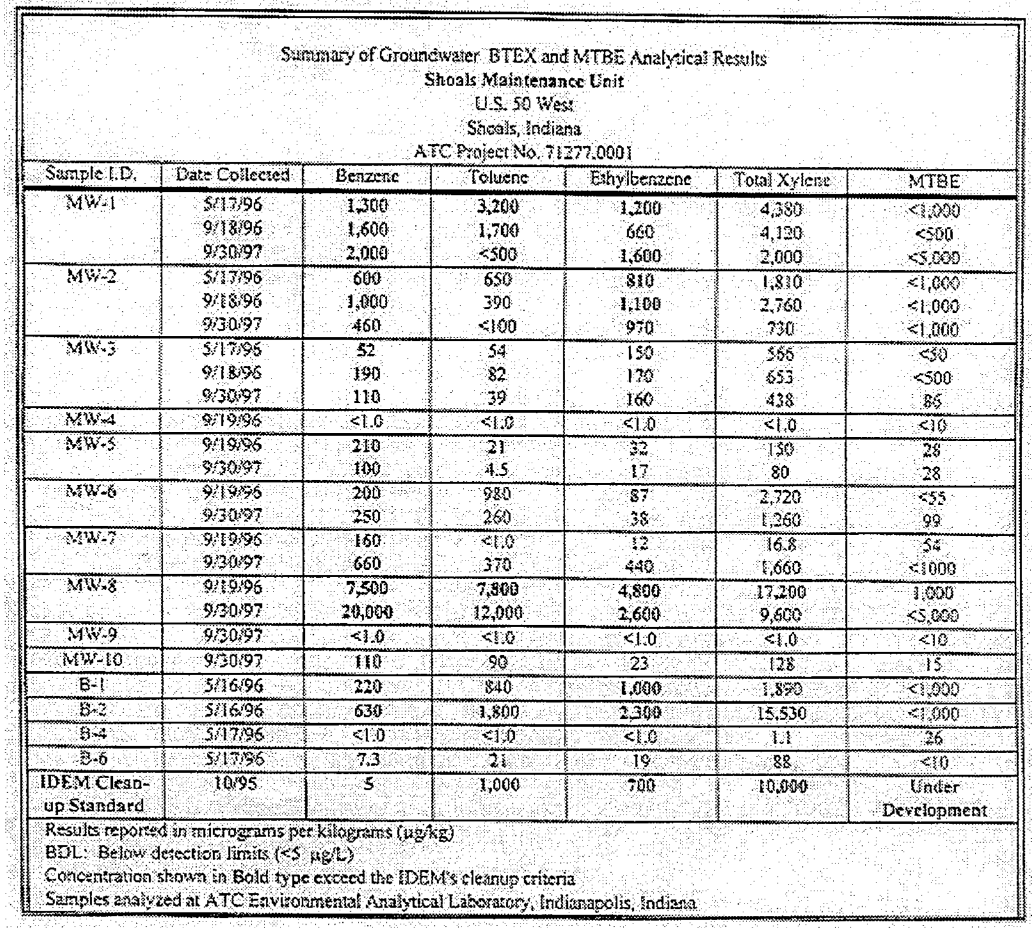


Summary of Soil Analytical Results
Shoals Maintenance Unit
U.S. 50 West
Shoals, Indiana
ATC Project No. 71277.0001

Sample I.D.	Sample Date	Sample Depth (ft)	Total Petroleum Hydrocarbons *
B-1	6/16/96	12-14	1,000
B-2	6/16/96	14-15	**550**
B-3	5/17/96	4-6	<1.0
		8-10	<1.0
B-4	5/17/96	3-5	<1.0
		7-8	<1.0
B-5	5/17/96	2-4	<1.0
		6-6.5	<1.0
B-6	5/17/96	2-4	<1.0
		10-12	<1.0
		18-19	41
B-7	5/17/96	1-3	<1.0
		7-9	<1.0
		17-17.5	<1.0
B-8	5/17/96	7-9	<1.0
		13-15	<1.0
B-9	9/18/96	0-2	84
		3-5	6.5
B-10	9/18/96	11-13	390
MW-1	6/16/96	14-16	**2,100**
MW-2	6/16/96	26-28	24
MW-3	6/16/96	18-20	<1.0
MW-4	9/17/96	23-25	<1.0
		53-55	<1.0
MW-5	9/18/96	38-40	<1.0
MW-6	9/18/96	18-20	58
MW-7	9/18/96	33-35	<1.0
IDEM Clean-up Standard			100

* Results reported in milligrams per kilograms (mg/kg) or ppm by Method 8015 - gasoline standard
** Duplicate soil sampled collected from MW-11 (8.0 - 10.0')
BDL- Below Detection Limits
Concentrations in bold exceed the IDEM clean-up criteria

Table 11

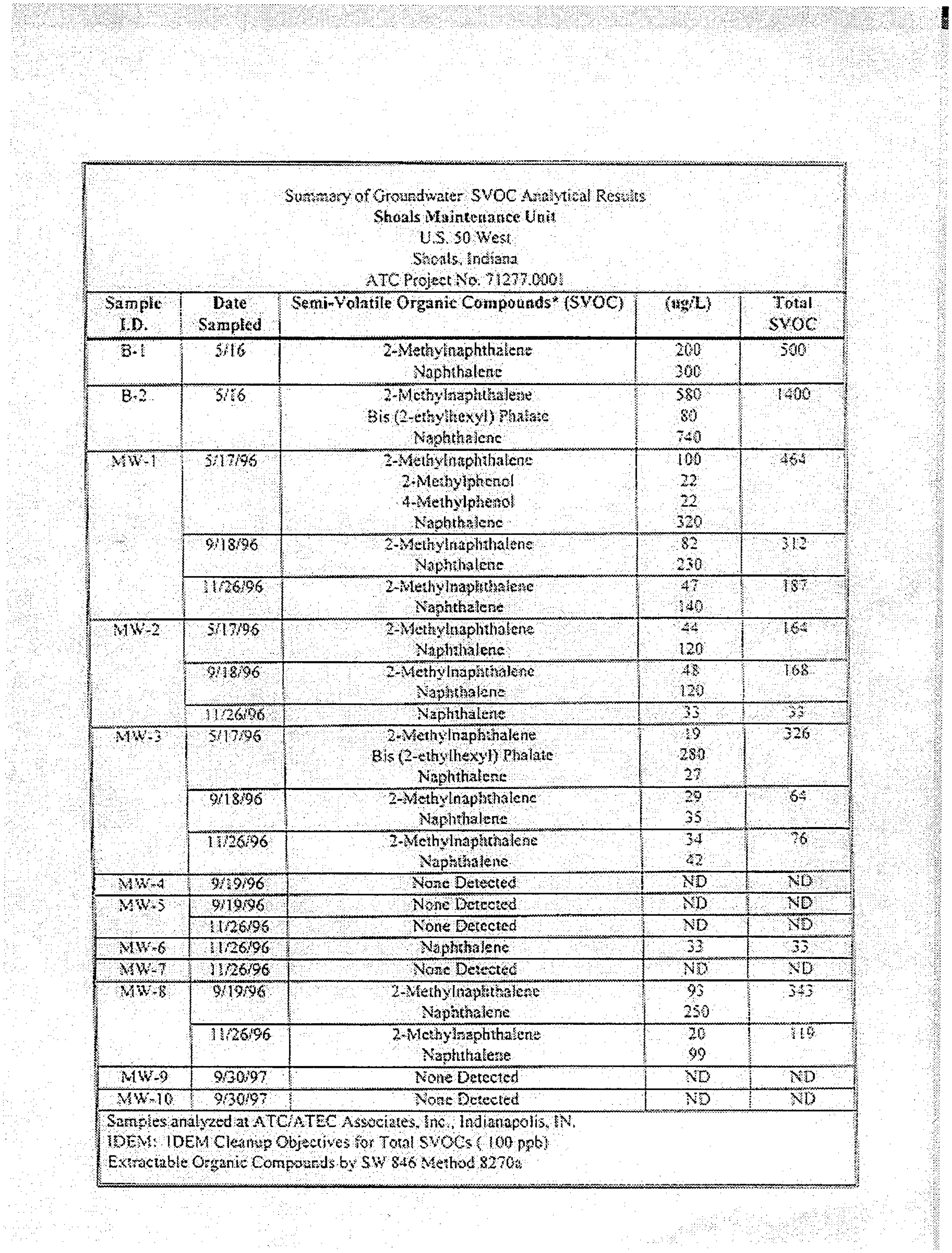


Summary of Groundwater BTEX and MTBE Analytical Results
Shoals Maintenance Unit
U.S. 50 West
Shoals, Indiana
ATC Project No. 71277.0001

Sample I.D.	Date Collected	Benzene	Toluene	Ethylbenzene	Total Xylene	MTBE
MW-1	5/17/96	**1,300**	**3,200**	**1,200**	4,380	<1,000
	9/18/96	**1,600**	**1,700**	660	4,120	<500
	9/30/97	**2,000**	<500	**1,600**	2,000	<5,000
MW-2	5/17/96	**600**	650	**810**	1,810	<1,000
	9/18/96	**1,000**	390	**1,100**	2,760	<1,000
	9/30/97	**460**	<100	**970**	730	<1,000
MW-3	5/17/96	**52**	54	150	566	<50
	9/18/96	**190**	82	170	653	<500
	9/30/97	**110**	39	160	438	86
MW-4	9/19/96	<1.0	<1.0	<1.0	<1.0	<10
MW-5	9/19/96	**210**	21	32	150	28
	9/30/97	**100**	4.5	17	80	28
MW-6	9/19/96	**200**	980	87	2,720	<55
	9/30/97	**250**	260	38	1,260	99
MW-7	9/19/96	**160**	<1.0	12	16.8	54
	9/30/97	**660**	370	440	1,660	<1000
MW-8	9/19/96	**7,500**	**7,800**	**4,800**	**17,200**	1,000
	9/30/97	**20,000**	**12,000**	**2,600**	9,600	<5,000
MW-9	9/30/97	<1.0	<1.0	<1.0	<1.0	<10
MW-10	9/30/97	**110**	90	23	128	15
B-1	5/16/96	**220**	840	**1,000**	1,890	<1,000
B-2	5/16/96	**630**	**1,800**	**2,300**	**15,530**	<1,000
B-4	5/17/96	<1.0	<1.0	<1.0	1.1	26
B-6	5/17/96	**7.3**	21	19	88	<10
IDEM Clean-up Standard	10/95	5	1,000	700	10,000	Under Development

Results reported in micrograms per kilograms (µg/kg)
BDL: Below detection limits (<5 µg/L)
Concentration shown in Bold type exceed the IDEM's cleanup criteria
Samples analyzed at ATC Environmental Analytical Laboratory, Indianapolis, Indiana

Table 12



Summary of Groundwater SVOC Analytical Results
Shoals Maintenance Unit
U.S. 50 West
Shoals, Indiana
ATC Project No. 71277.0001

Sample I.D.	Date Sampled	Semi-Volatile Organic Compounds* (SVOC)	(ug/L)	Total SVOC
B-1	5/16	2-Methylnaphthalene	200	500
		Naphthalene	300	
B-2	5/16	2-Methylnaphthalene	580	1400
		Bis (2-ethylhexyl) Phalate	80	
		Naphthalene	740	
MW-1	5/17/96	2-Methylnaphthalene	100	464
		2-Methylphenol	22	
		4-Methylphenol	22	
		Naphthalene	320	
	9/18/96	2-Methylnaphthalene	82	312
		Naphthalene	230	
	11/26/96	2-Methylnaphthalene	47	187
		Naphthalene	140	
MW-2	5/17/96	2-Methylnaphthalene	44	164
		Naphthalene	120	
	9/18/96	2-Methylnaphthalene	48	168
		Naphthalene	120	
	11/26/96	Naphthalene	33	33
MW-3	5/17/96	2-Methylnaphthalene	19	326
		Bis (2-ethylhexyl) Phalate	280	
		Naphthalene	27	
	9/18/96	2-Methylnaphthalene	29	64
		Naphthalene	35	
	11/26/96	2-Methylnaphthalene	34	76
		Naphthalene	42	
MW-4	9/19/96	None Detected	ND	ND
MW-5	9/19/96	None Detected	ND	ND
	11/26/96	None Detected	ND	ND
MW-6	11/26/96	Naphthalene	33	33
MW-7	11/26/96	None Detected	ND	ND
MW-8	9/19/96	2-Methylnaphthalene	93	343
		Naphthalene	250	
	11/26/96	2-Methylnaphthalene	20	119
		Naphthalene	99	
MW-9	9/30/97	None Detected	ND	ND
MW-10	9/30/97	None Detected	ND	ND

Samples analyzed at ATC/ATEC Associates, Inc., Indianapolis, IN.
IDEM: IDEM Cleanup Objectives for Total SVOCs (100 ppb)
Extractable Organic Compounds by SW 846 Method 8270a

Due to the relatively high contaminant concentrations and the risk of off-site migration source removal is required. A combination air sparging - soil vapor extraction system was designed by ATC Associates, Inc. Sparging wells and vapor extraction wells are to be placed throughout the contaminated zone northeast of the former tank pit area (Figure 15). The sparging wells and soil vapor extraction wells will be connected by piping placed in trenches (Figure 16). This will allow the bioremediation system to operate with minimal disturbance to INDOT activities at the site (ATC Associates, Inc., 1998).

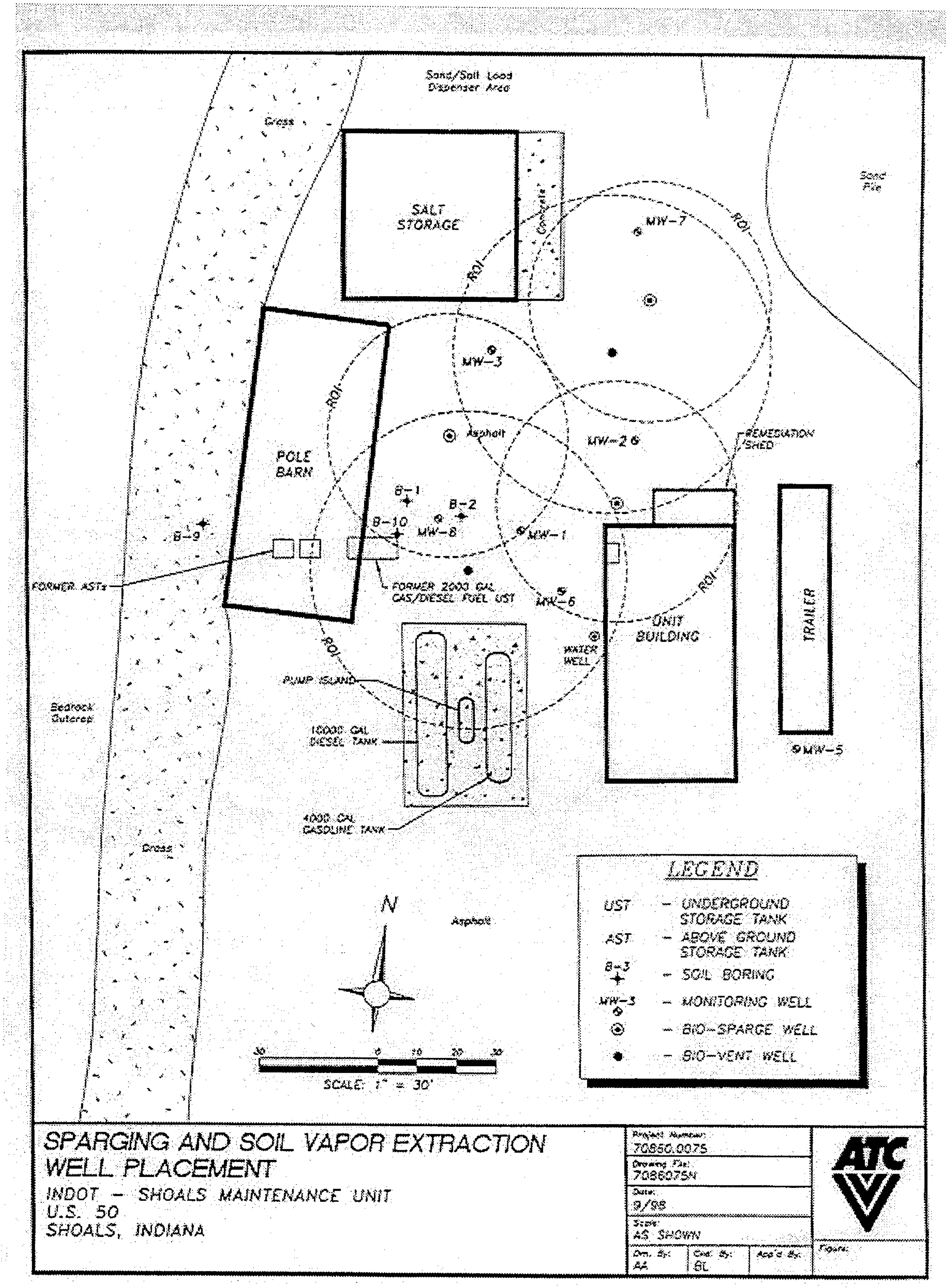

Sand/Salt Load Dispenser Area
Grass
SALT STORAGE
Concrete
Sand Pile
MW-7
ROI
ROI
MW-3
ROI
POLE BARN
Asphalt
MW-2
REMEDIATION SHED
B-1
B-2
B-10
MW-8
MW-1
B-9
FORMER ASTs
FORMER 2000 GAL GAS/DIESEL FUEL UST
MW-6
ROI
UNIT BUILDING
TRAILER
ROI
WATER WELL
PUMP ISLAND
Bedrock Outcrop
10000 GAL DIESEL TANK
MW-5
4000 GAL GASOLINE TANK
Grass
N
Asphalt
30
0
10
20
30
SCALE: 1" = 30'
LEGEND
UST - UNDERGROUND STORAGE TANK
AST - ABOVE GROUND STORAGE TANK
B-3 - SOIL BORING
MW-3 - MONITORING WELL
- BIO-SPARGE WELL
- BIO-VENT WELL
SPARGING AND SOIL VAPOR EXTRACTION WELL PLACEMENT
INDOT - SHOALS MAINTENANCE UNIT
U.S. 50
SHOALS, INDIANA
Project Number: 70850.0075
Drawing File: 7086075N
Date: 9/98
Scale: AS SHOWN
Drn. By: AA
Ckd. By: BL
App'd By:
Figure:
ATC

Figure 15

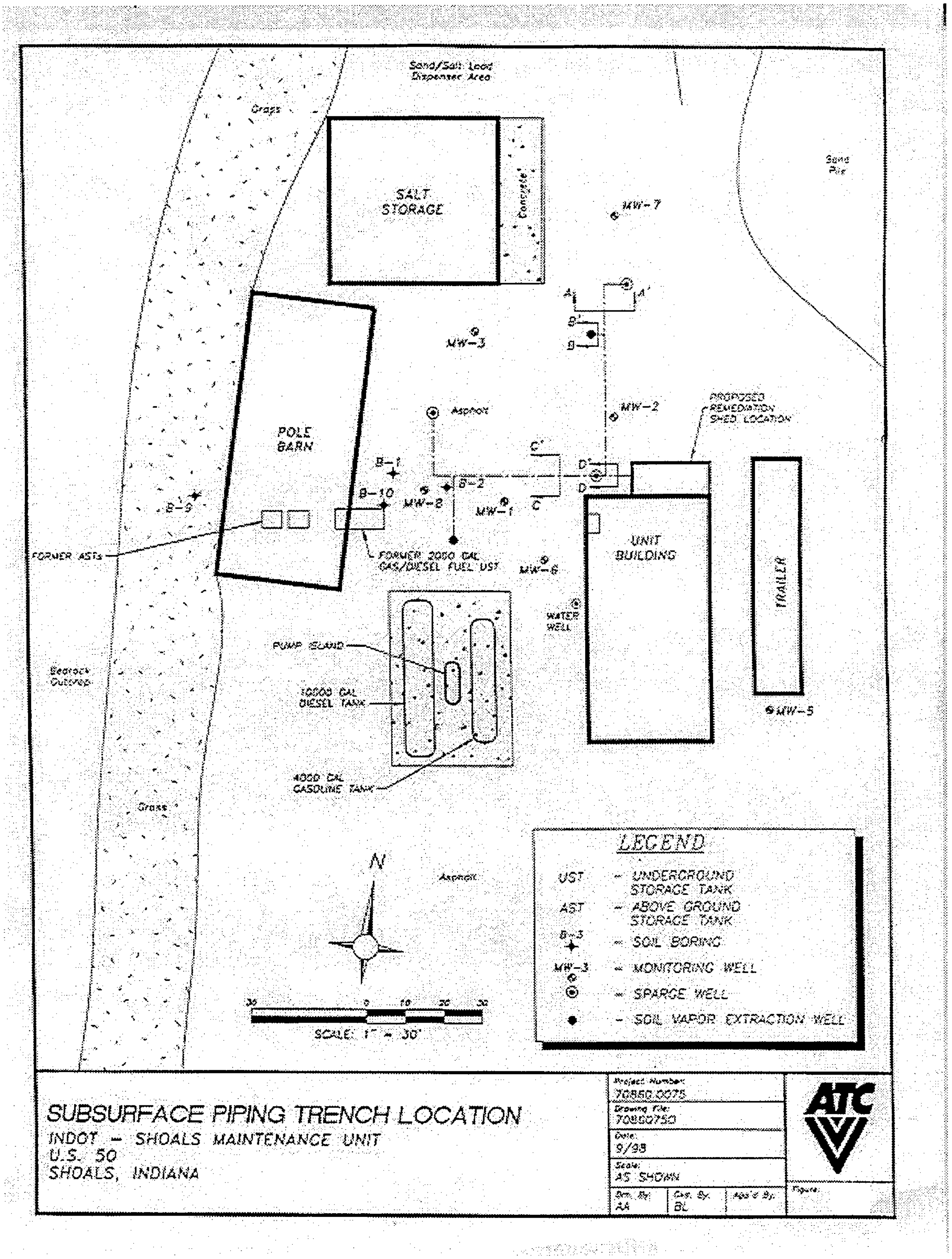

Sand/Salt Load
Dispenser Area
Grass
SALT
STORAGE
Concrete
Sand
Pile
MW-7
A
A'
B'
B
MW-3
POLE
BARN
Asphalt
MW-2
PROPOSED
REMEDIATION
SHED LOCATION
C'
C
D'
D
B-1
B-2
B-10
MW-8
MW-1
B-9
FORMER ASTs
FORMER 2050 GAL
GAS/DIESEL FUEL UST
MW-6
UNIT
BUILDING
TRAILER
WATER
WELL
PUMP ISLAND
10000 GAL
DIESEL TANK
4000 GAL
GASOLINE TANK
Bedrock
Outcrop
MW-5
Grass
N
Asphalt
30
0
10
20
30
SCALE: 1" = 30'
LEGEND
UST - UNDERGROUND STORAGE TANK
AST - ABOVE GROUND STORAGE TANK
B-3 - SOIL BORING
MW-3 - MONITORING WELL
- SPARGE WELL
- SOIL VAPOR EXTRACTION WELL
SUBSURFACE PIPING TRENCH LOCATION
INDOT - SHOALS MAINTENANCE UNIT
U.S. 50
SHOALS, INDIANA
Project Number:
70850.0075
Drawing File:
70850750
Date:
9/98
Scale:
AS SHOWN
Drn. By:
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Figure:
ATC

Figure 16

CHAPTER 10 RECOMMENDATIONS

Introduction

Bioremediation has become an important remediation option for addressing the clean-up of petroleum contaminated soils at INDOT facilities. This is beneficial to the State of Indiana because it improves environmental quality, reduces reliance on landfilling, and because bioremediation ultimately destroys the contaminants, it reduces Indiana's long-term liability associated with the existence of harmful substances either in the environment or stored in a landfill.

A number of improvements can be made to how bioremediation is implemented at INDOT facilities such as standardization of CAPs, acquisition of knowledge concerning more innovative bioremediation technology (specifically ORC), and development of a landfarming program for INDOT. These improvements are outlined in the implementation report accompanying this document. Implementation of one additional recommendation could improve the economics of bioremediation.

Few of the INDOT petroleum contaminated sites pose an immediate extreme hazard which requires an immediate clean-up and few of the sites pose a high risk of contamination of potable drinking water sources. Site investigations typically include data from which an initial risk assessment can be completed, however, these data do not seem to be used to select the most economical and effective remediation option within the context of acceptable risk. In addition, remediation decisions are often made without sufficient monitoring data to determine whether an active remediation is even required.

For example, two years of quarterly monitoring data are required to determine the stability of a contaminated groundwater plume. Soil borings from initial site investigations can easily be converted to monitoring wells, and therefore, the infrastructure for a site monitoring program can economically be put in place. However, if remediation decisions are made based only on a single set a data, then active remediation systems may unnecessarily be utilized. Passive option such as monitored natural attenuation can only be implemented with sufficient data. Therefore, a preliminary risk assessment to determine whether an imminent hazard exists, and if one does not, implementation of a monitoring program to acquire data to determine whether passive options will achieve site closure is recommended.

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APPENDIX A

Guidance Manual for Managing Implementation of Bioremediation at Petroleum Contaminated Sites

August 1999

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Introduction

The purpose of this manual is to provide Department of Transportation (DOT) decision makers with guidance for technology selection, management, and operation for bioremediation at petroleum contaminated sites.

Most DOT's will utilize the services of consulting engineers and other environmental professionals for the remediation of petroleum releases to the environment. This manual provides information about the basic fundamentals of bioremediation and bioremediation technologies. With this information, DOT decision makers will be better able to ensure that consulting engineers and environmental professionals follow sound practices and that effective and appropriate technologies are selected for remediation.

This document is not intended to serve as a manual for the design of bioremediation systems. The references listed at the end of this document provide additional information, including design procedures.

Bioremediation Basics

Bioremediation is the utilization of naturally occurring biodegradation processes.

Engineered bioremediation is the enhancement, acceleration and manipulation of naturally occurring biodegradation processes.

In Situ bioremediation is implemented on-site and in the ground.

Ex Situ bioremediation means the contaminated soil or groundwater is excavated or removed from the ground.

Natural attenuation is a remediation alternative that uses natural processes which include transport (dispersion, volatilization, and dilution), sorption, chemical transformation (photolysis, hydrolysis), and biodegradation. Intrinsic biodegradation processes are a very significant component of natural attenuation of petroleum products. Natural attenuation may be used as a remedial option only after evaluation of site characteristics, risk assessment, source control, and modeling indicate that remedial endpoints will be achieved by this method without undue hazards to human health and the environment.

What are the Advantages of Bioremediation?

- **Final Solution**

Pollutants are biodegraded to harmless compounds such as CO_2 and H_2O, not merely transferred to another environmental compartment (e.g. landfill).

- **Less Hazardous**

Since the pollutants are left in place, bioremediation results in less exposure of workers and the local community to pollutants than conventional treatment processes.

- **Reduced Liability**

Reduced human exposure and no transportation of contaminated materials to off-site facilities results in less risk. Destruction of pollutants, rather than long-term storage, eliminates liability.

- **Effective**

Recent field experience has demonstrated that bioremediation of contaminated sites can reduce pollutant concentrations to acceptable levels.

- **Economics**

Bioremediation is usually less costly than other remediation processes, thus, bioremediation can save $.

Petroleum Products Biodegradation Requirements

These requirements are based on a common sense approach to bioremediation.

1. An organism must exist that is capable of biodegrading the pollutants.
2. The organism must be present in the environment.
3. The pollutant must be accessible to the organisms.
4. Environmental conditions must exist that are conducive to growth of the desirable organisms (pH, temperature, nutrients (nitrogen & phosphorus), electron acceptor (e.g. oxygen)).

When these conditions can be met, bioremediation should be considered as an appropriate remediation technology.

Applications of Bioremediation

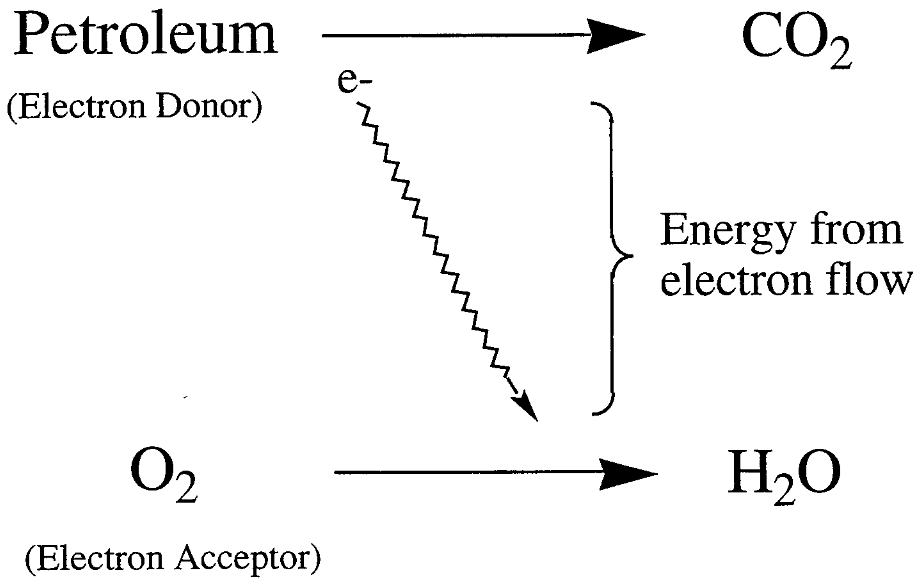
Pollutants	Bioremediation Technology
Petroleum products	Conventional
Non-halogenated Solvents	Conventional
Halogenated Solvents	Innovative

Optimum Soil Characteristics

Hydraulic Conductivity	$> 10^{-4}$ cm/s
Permeability	$> 10^{-9}$ cm^2
Temperature	10 - 45 °C (50 - 115 °F)
pH	6 - 8
Moisture	25 - 75% field capacity
Salinity	< 10 mmhos/cm
Concentration toxicity (in situ petroleum)	< 25,000 ppm TPH

Basic Microbial Metabolism

Aerobic microorganisms, like humans, obtain energy by mediating oxidation-reduction reactions in a process called respiration. During respiration the electron donor is oxidized and the electron acceptor is reduced. Living organisms obtain energy from the flow of electrons from the electron donor to the electron acceptor. During bioremediation, petroleum serves as the electron donor, and for reasons that will be explained later, oxygen (O_2) is the most desirable electron acceptor.



Humans are extremely limited in their utilization of electron acceptors, since we are able to use only oxygen. However, microbial communities have very diverse capabilities and are able to respire by utilizing a wide variety of different electron acceptors. One reason that oxygen is the best electron acceptor is it yields more energy during respiration than any other electron acceptor.

As the reduction potential of the electron acceptor becomes more positive, more energy is obtained from any given oxidation-reduction reaction. As we can see in the table below, oxygen has the most positive reduction potential.

Reduction Potentials of Important Biological Oxidants

(electron acceptors)

	E°'(Volts)
$\frac{1}{2}O_2(g) + H^+ + e^- = \frac{1}{2}H_2O$	0.820
$\frac{1}{2}MnO_2(s) + \frac{1}{2}HCO_3^-(10^{-3}) + \frac{3}{2}H^+ + e^- = \frac{1}{2}MnCO_3(s) + H_2O$	0.527
$\frac{1}{2}NO_3^- + H^+ + e^- = \frac{1}{2}NO_2^- + \frac{1}{2}H_2O$	0.423
$\frac{1}{6}NO_2^- + \frac{4}{3}H^+ + e^- = \frac{1}{6}NH_4^+ + \frac{1}{3}H_2O$	0.344
$FeOOH(s) + HCO_3^-(10^{-3}) + 2H^+ + e^- = FeCO_3(s) + 2H_2O$	-0.047
$\frac{1}{6}SO_4^{2-} + \frac{4}{3}H^+ + e^- = \frac{1}{6}S(s) + \frac{2}{3}H_2O$	-0.195
$\frac{1}{2}S(s) + H^+ + e^- = \frac{1}{2}H_2S(g)$	-0.243
$\frac{1}{8}CO_2(g) + H^+ + e^- = \frac{1}{8}CH_4(g) + \frac{1}{4}H_2O$	-0.244
$H^+ + e^- = \frac{1}{2}H_2$	-0.414

Values apply for unit activity (1M or 1 atm) in water at pH 7.0 and 25°C,
except $HCO_3^- = 10^{-3}M$ which more typically represents environmental conditions.

Importance of Oxygen

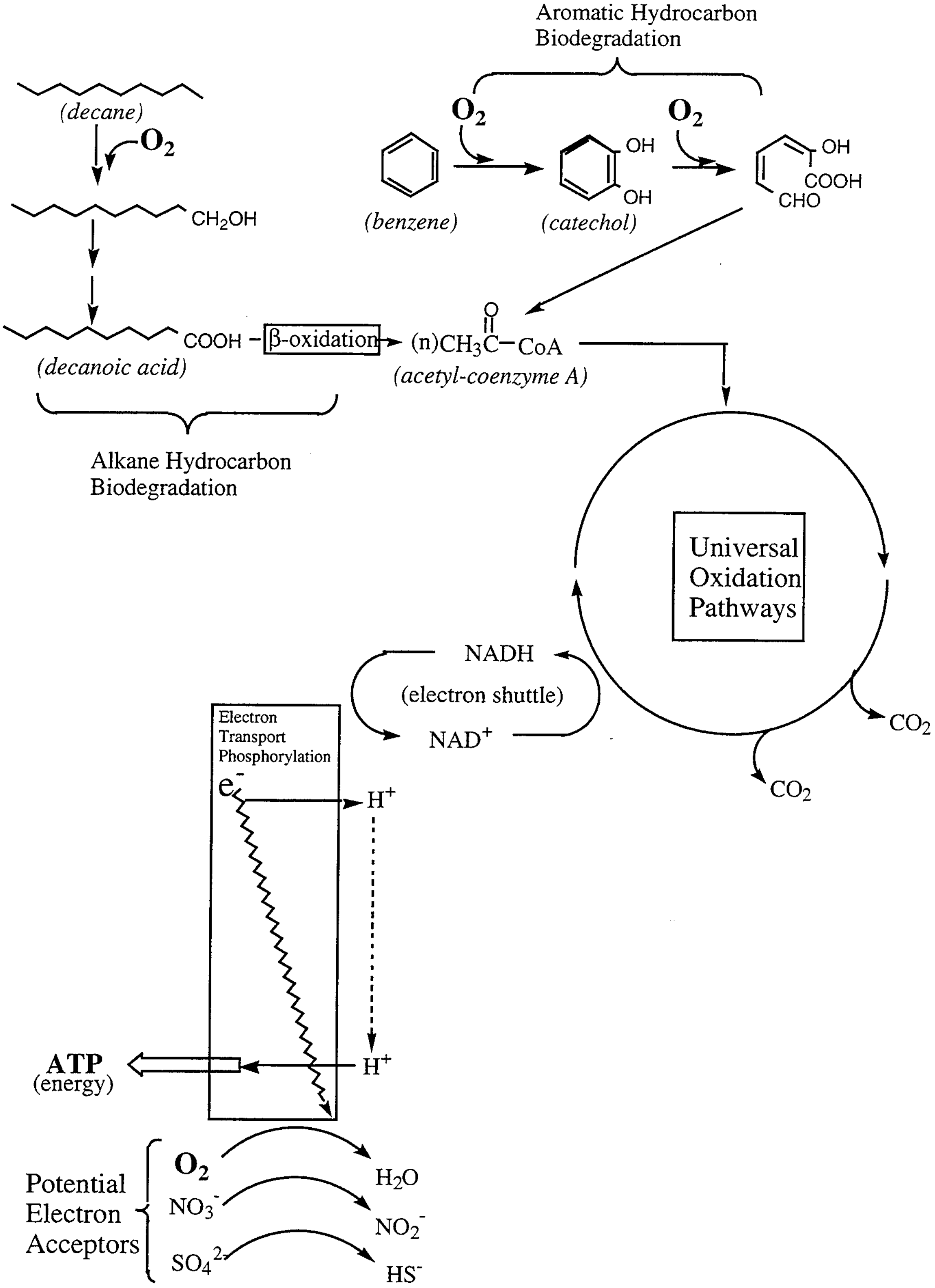
Molecular Oxygen (O_2) has 2 functions:

- ➨ electron acceptor (respiration)
- ➨ reactant (oxygenase enzymes)

As can be seen in the schematic on the following page, oxygen (shown in bold ➨ **O_2**) is involved in initial reactions of biodegradation and oxygen can also be used as an electron acceptor. There are many alternative electron acceptors which can be utilized by bacteria in place of oxygen, however, there are few alternative reactants to initiate biodegradation. This dual function of oxygen is the reason it is so important for successful petroleum bioremediation.

Petroleum hydrocarbons can biodegrade without oxygen, however, these anaerobic reactions are not as well understood and reaction rates tend to be slower than aerobic biodegradation rates. One good alternative to oxygen as an electron acceptor is nitrate (NO_3^-). Significant research has also been devoted to the feasibility of using nitrate as a supplemental electron acceptor. Nitrate is more soluble in water than oxygen, is a strong oxidant (not as strong as oxygen), and could potentially be more readily introduced into groundwater aquifers through injection wells. Many monoaromatic compounds are biodegraded under anaerobic nitrate reducing conditions, although the nitrate is used only as an electron acceptor and not as a reactant in biotransformation reactions. Nitrate does have known health hazards (methemoglobinemia) and is a regulated drinking water contaminant, and therefore, is used as a supplemental electron acceptor only under carefully controlled situations.

Schematic of Biochemistry of Petroleum Biodegradation



Basic Environmental and Petroleum Chemistry

Petroleum Composition

Product	Major Carbon Range	Boiling Range (°C)	Mono-aromatics (%)	Poly-aromatics (%)	Branched & Normal Alkanes(%)
Gasoline	$C_3 - C_{12}$	25 - 215	30 ± 10	~ 1	70 ± 10
Kerosene & Jet Fuel	$C_{11} - C_{13}$	150 - 250	15 ± 5	15 ± 5	70 ± 10
Diesel & Light Fuel Oil	$C_{10} - C_{20}$	160 - 400	~ 1	30 ± 5	70 ± 10
Heavy Fuel Oil	$C_{19} - C_{25}$	315 - 540	_	25 ± 10	75 ± 10
Motor Oils	$C_{20} - C_{45}$	425 - 540	_	20 ± 10	80 ± 10

Petroleum products are mixtures of hundreds of different compounds and their composition varies considerably due to different sources of crude oil and refining processes. Products can be classified according to their boiling point range into light distillates (gasoline, kerosene, jet fuel), middle distillates (diesel & light fuel oil), and heavy distillates (heavy fuel oil, & motor oils). The components of petroleum products can be divided into three categories, monoaromatics, polyaromatics, and alkanes. The monoaromatics are the compounds of greatest environmental concern due to their known human toxicity and environmental mobility, however, they are readily biodegradable. Light distillates contain significant amounts of monoaromatics. Polyaromatics are of some environmental concern because of their toxicity, although, they are fairly immobile in the environment. The biodegradation of polyaromatics is limited by their low solubility in water (slow mass transfer) which also contributes to their relative immobility. Alkanes are of lesser environmental concern due to their lower toxicity.

Alkane biodegradation is influenced by the degree of branching of the individual molecules, with straight chains (normal) being the most easily degraded. Unlike the heavy polyaromatics, bacteria have unique mechanisms to acquire and biodegrade very insoluble n-alkanes.

<u>Properties of Select Chemicals in Petroleum</u>

Compound	Formula	MW	BP °C	Solubility (mg/L)	Vapor (Pa) Pressure	Log K_{ow}	H (Pa m^3/mol)
Benzene	C_6H_6	78.1	80	1,750	12,700	2.13	557
Toluene	C_7H_8	92.1	111	535	3,800	2.65	680
p-xylene	C_8H_{10}	106.2	138	215	1,170	3.18	578
Naphthalene	$C_{10}H_8$	128.2	218	31	10.4	3.37	43
Methyl-Naphthalene	$C_{11}H_{10}$	142.2	241	27	5.5	3.9	29
Anthracene	$C_{14}H_{10}$	178.24	340	0.045	0.026	4.54	3.96
Phenanthrene	$C_{14}H_{10}$	178.24	339	1	0.091	4.57	3.24
Pyrene	$C_{16}H_{10}$	202.26	360	0.132	0.00009	4.88	1.895
Fluoranthene	$C_{16}H_{10}$	202.26	375	0.206	0.00067	4.90	1.037
Benzo[a]pyrene	$C_{20}H_{12}$	252.32	495	0.0012	0.0000007	5.98	0.046
Propane	C_3H_8	44.1	-42	62	861,263	1.9	71,535
Iso-butane	C_4H_{10}	58.1	-12	49	296,882	2.7	352,018
n-dodecane	$C_{12}H_{26}$	170.3	216	0.004	40.5	3.2	1,724,288

The environmental mobility of chemicals can be estimated from chemical and physical properties. Solubility in water and vapor pressure are the most important parameters because water and air are the fluids which move through soil and mobilize petroleum. Compounds with a relatively high vapor pressure are more readily volatilized and compounds with relatively high solubility are more readily transported with groundwater. Partition coefficients are used to estimate the relative distribution of a chemical between two phases. Log K_{ow} indicates the propensity of a chemical to partition between octanol (o) and water (w). A larger K_{ow} (e.g. benzo[a]pyrene) means the chemical "prefers" an organic phase, such as soil organic matter or petroleum free product, relative to water.

Henry's Constant (H) is a measure of the relative distribution of a chemical between air and water. Compounds with large H values may be removed from water by air-stripping (passing large flows of air through the water phase). Caution must be exercised when using the data for predictions about environmental mobility. For example, n-dodecane has a very large H due to its very low solubility in water, although, it would be poorly removed by air-stripping because insufficient mass of the compound would exist in the water phase. Also, the properties of the chemicals are determined from measurements of pure compounds, however, petroleum is a complex mixture which may exist as free product. Mixtures of compounds behave somewhat differently than individual compounds.

The monoaromatics benzene, toluene, and xylenes are relatively soluble in water and have relatively large vapor pressures. These compounds tend to adsorb less to soil organic matter (lower log K_{ow}) and can be removed from water by air-stripping (higher H). The polyaromatics (naphthalene - benzo[a]pyrene) are relatively insoluble in water and tend to adsorb strongly to soil. The properties of alkanes vary considerably (propane - n-dodecane) depending on the molecular weight of the compound.

Petroleum Bioremediation Technologies

The following pages contain conceptual descriptions and schematics of bioremediation technologies.

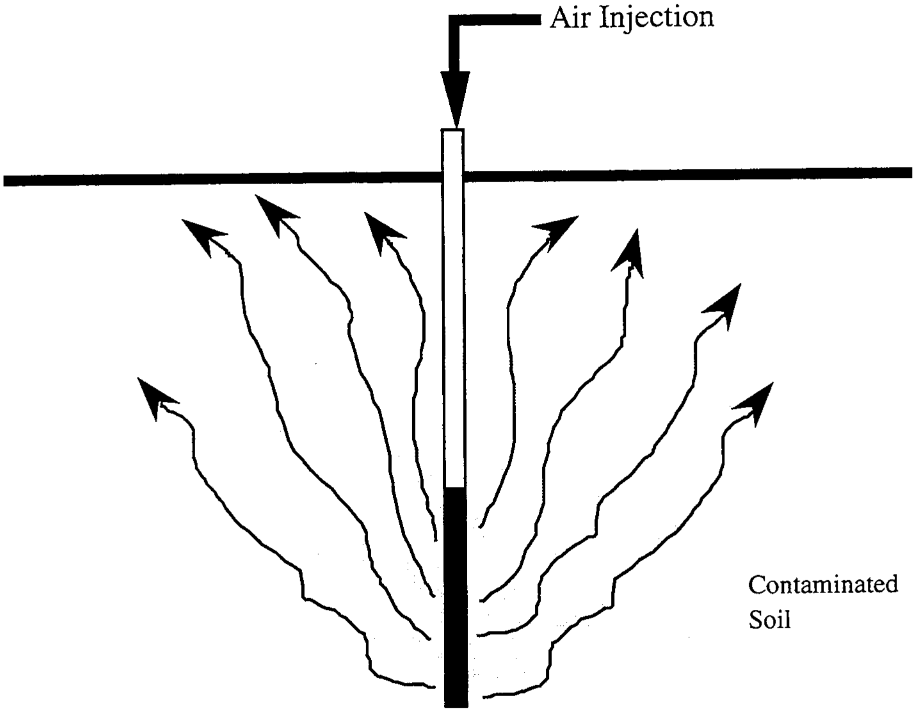
Each schematic is followed by a brief description of the process and the fundamental principles on which the technology is based.

The governing parameters which influence the feasibility and efficiency of each technology are included. Governing parameters are generally site specific and contaminant specific.

The relative advantages and disadvantages of each technology are listed.

A range of technology costs are listed. The range represents data which are available, including cases where the technology was under development or was marginally feasible. Actual costs which are likely to be realized will be in the mid to lower range of the costs listed.

Bioventing



Bioventing is a bioremediation operation where air is pumped through a well inserted into the contaminated soil.

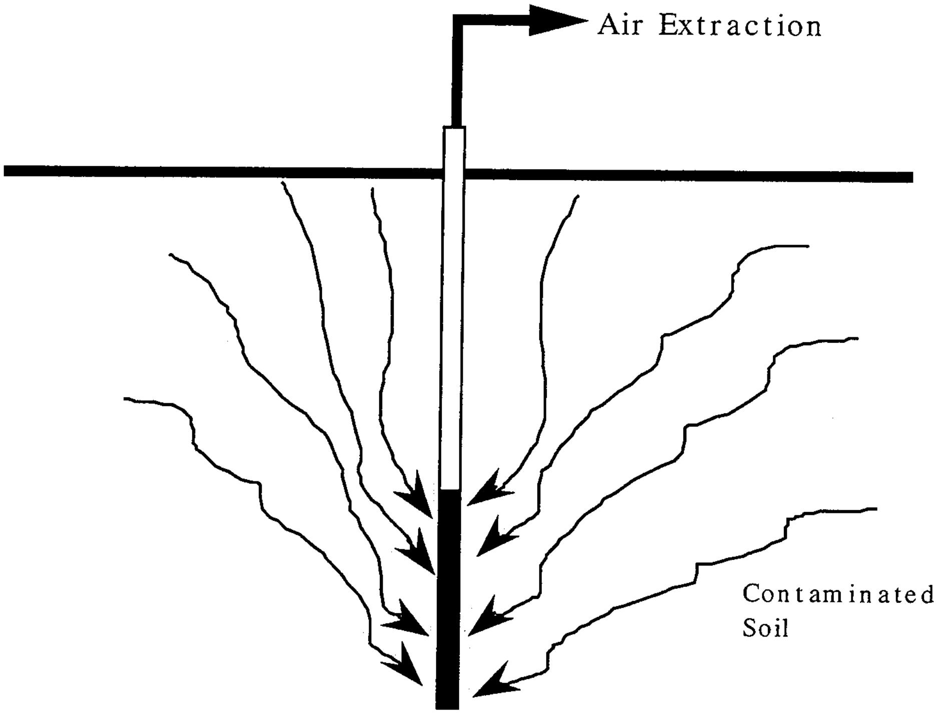
Principles
Oxygen increases petroleum biodegradation rates.
Air pumping flow rates are balanced to optimize biodegradation and minimize volatilization.

Governing Parameters
Soil permeability: Bioventing works best in sandy soils which are more permeable.
Petroleum type: Bioventing works best with middle to light distillates. Volatile compounds tend to be removed by stripping rather than biodegradation if air flow rates are too fast.
Soil moisture: Too much soil pore water will impede air flow and too little moisture will inhibit biodegradation.

Advantages
Bioventing can treat large areas.
Bioventing is relatively low-cost, non-intrusive, and flexible.
Disadvantages
Bioventing does not work well in low permeability soils.
High groundwater elevations (see air sparging) and saturated soils impede the effectiveness of bioventing.

Cost: \$10 - 70/ cubic meter soil.

Soil Vapor Extraction



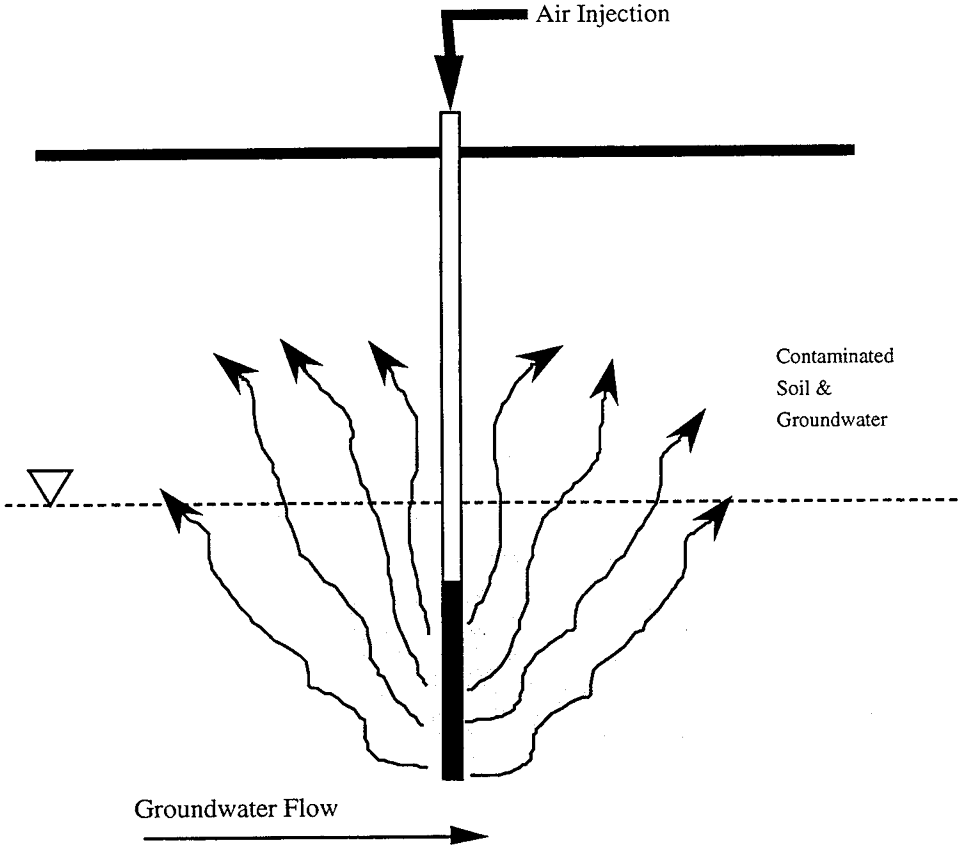
Soil Vapor Extraction (SVE) is a unit operation where air is extracted through a well inserted into the contaminated soil. This process is conceptually the opposite of bioventing, i.e. air is extracted rather than injected. SVE flow rates are typically about an order of magnitude higher than in bioventing and the objective is removal by volatilization rather than biodegradation. However, reducing the flow rates will essentially allow the system to behave in a similar fashion as bioventing.

Principles
Contaminants partition out of the soil matrix into the air flowing through the pore spaces.
Governing Parameters
Soil permeability: SVE works best in sandy soils which are more permeable.
Petroleum type: SVE works best with light distillates.
Site conditions: Utility trenches, foundations, and soil heterogeneity affect air flow.

Advantages
Air flow promotes biodegradation.
SVE and bioventing can be combined together.
Vapor phase can be collected and treated if necessary.
Disadvantages
SVE may require vapor control which increases cost.
Cold temperature affects chemical partitioning.
SVE does not work well in low permeability soils.
Groundwater (see air sparging) and saturated soils impede the effectiveness of SVE.

Cost: $10 - 250/ cubic meter soil.

Air Sparging



Air sparging is a bioremediation operation where air is pumped through a well inserted into the contaminated groundwater.

Principles

Contaminants partition out of the groundwater/soil matrix into air flowing through the pore spaces.

Oxygen in the air promotes biodegradation.

Governing Parameters

Soil permeability: Air sparging works best in porous sandy soils.

Contaminant type: Air sparging removes light distillates by air stripping more efficiently than heavier distillates.

Contaminant depth: Air sparging work best when contaminants are relatively shallow (<20 ft.).

Advantages

Air sparging enhances biodegradation.

Air sparging provides a method for contaminant source control in groundwater.

Air sparging can be combined with SVE for improved efficiency and vapor control.

Disadvantages

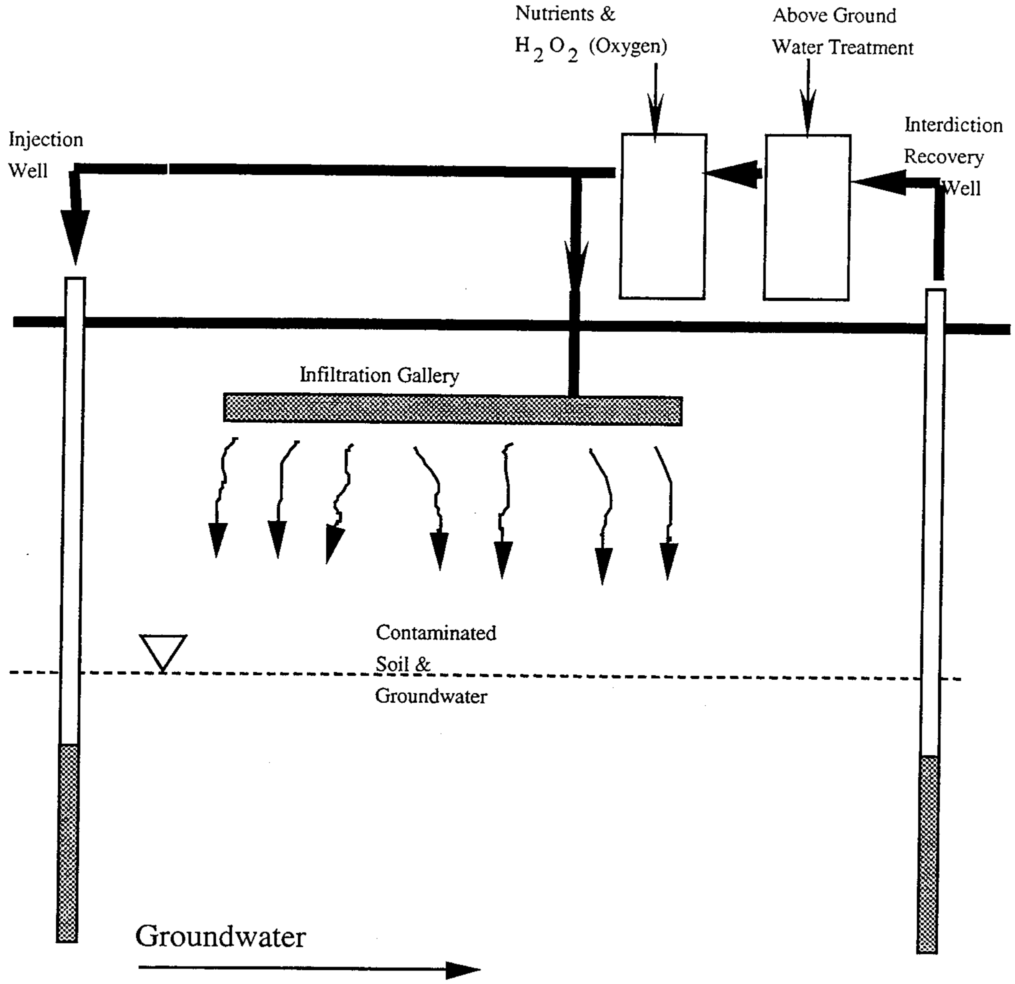
Vapor recovery may be required which will increase costs.

Limited applicability due to constraints of governing parameters.

Impermeable layers above injection point can cause plume spreading.

Cost: $40/ cubic meter soil (based on data from only one site).

In Situ Bioremediation



In Situ bioremediation is not a defined unit operation, but rather a term used to describe a combination of unit operations for in situ soil and groundwater bioremediation. Bioventing, SVE, or air sparging can be combined with nutrient recirculation systems, infiltration galleries, above ground treatment systems, and hydrologic control to form an in situ treatment system.

Principles

Nutrients and oxygen enhance biodegradation rates.

Interdiction wells and/or physical barriers may be used to contain contaminant plumes.

Governing Parameters

Soil hydraulic conductivity: All in situ systems work better with more porous soils.

Petroleum type: In situ bioremediation is more effective with light and middle distillates.

Advantages

Both soil and groundwater can be treated.

In situ bioremediation is flexible, cost effective, and non-intrusive.

Disadvantages

Above ground treatment may be required for extracted water.

In situ bioremediation is less effective in low porosity soils.

Site heterogeneities may result in inconsistent performance.

Cost: \$20 - 200/ cubic meter soil.

Natural Attenuation

Natural attenuation may be appropriate when sufficient evidence exists to demonstrate the likelihood that it will be safe and effective.

Principles

Naturally occurring processes, including biodegradation, remove contaminants from the environment. The components required to obtain approval of natural attenuation include site characterization, evaluation of contaminant distribution and attenuation mechanisms, and implementation of a monitoring program.

Governing Parameters

Site conditions: Soil conditions and hydrogeology have a significant impact on the environmental fate of pollutants as well as the type and numbers of microflora.
Contaminant characteristics: Light distillates and components of middle distillates may migrate in the environment at rates faster than natural attenuation mechanisms can remove them. Under such conditions natural attenuation is not acceptable.

Advantages

Cost
Conserves financial resources

Disadvantages

Time
Acceptance
Applicability

Cost: $7 - 20/ cubic meter soil. This cost is only for implementation of a monitoring program. The cost is highly dependent upon the duration of the remediation.

Composting / Landfarming

Composting and Landfarming are ex situ biological treatment processes for use after soil excavation.

Principles

Composting is done by constructing mounds of contaminated soils which may contain aeration and leachate collection systems. Bulking material, such as wood chips or straw, and manure may be used to improve aeration and nutrient content. Alternatively, soil can be piled in windrows and aeration may be accomplished with commercially available equipment.

Landfarming petroleum contaminated soil uses more land space than composting since soil is spread out 12-18 inches deep. Aeration is done by tilling. A significant removal mechanism is volatilization. If petroleum vapor release to the atmosphere is not allowed, composting offers a better system for vapor collection.

Governing Parameters

Aeration: Oxygen accelerates biodegradation.

Contaminants: Concentration and composition of the petroleum products affect biodegradation rates. Heavy distillates may degrade very slowly or not at all.

Moisture & Nutrients: Adding sufficient moisture and nutrients will ensure that these will not be limiting factors for biodegradation.

Advantages

Ex situ treatment offers an opportunity for better control of soil conditions.

Simple.

Soil reuse.

Higher contaminant concentrations (up to 50,000 ppm total petroleum hydrocarbons (TPH)) can be treated with composting and landfarming.

Disadvantages

Space required.

Above ground treatment is adversely affected by cold temperatures.

Leachate collection and vapor control systems may be required.

Cost: Composting $20 - 260/ cubic meter soil.
Landfarming $15 - 250/ cubic meter soil.

Bioslurry Reactors

Bioslurry reactors are another ex situ treatment option which have systems for stirring and aeration. Bioslurry reactors allow the best control over biodegradation conditions.

Principles
Contaminated soil is blended with water and nutrients into an aqueous slurry and continuously stirred and aerated.

Governing Parameters
Aeration: Oxygen accelerates biodegradation.
Contaminants: Concentration and composition of the petroleum products affect biodegradation rates. Heavy distillates may degrade very slowly or not at all.
Mixing: Improves mass transfer (bioavailability) of contaminants.
Nutrients: Adding sufficient nutrients will ensure that this will not be a limiting factor for biodegradation.

Advantages
Greater control.
Higher concentrations tolerated.
Lower permeability soils can be treated

Disadvantages
Soil volume limited by reactor size.
Wastewater generated from dewatering.
Cost.

Cost: $100 - 200/ cubic meter soil.

Alternate Ex Situ Technology Disposal Costs

Thermal desorption and landfilling are the most frequently used technologies for remediation of petroleum contaminated soils. Thermal desorption may be done on-site using truck-mounted units or soil can be trucked to permanent off-site facilities. Trucking distance is often the cost factor which determines economic feasibility. Landfilling costs are primarily influenced by geographic location.

Thermal Desorption: $55 - 250/ cubic meter soil.
Landfilling: $40 - 220/ cubic meter soil.

Bioaugmentation

The purchase, addition or utilization of commercially available petroleum biodegrading microorganisms should be considered very cautiously. Petroleum biodegrading microorganisms are ubiquitous in the environment, therefore, their alleged absence in petroleum contaminated soil should be viewed with concern and suspicion. Extreme environmental conditions (temperature, pH, salinity) may result in low or absent populations, however, the addition of exogenous organisms will not remedy this situation. Toxic concentrations of petroleum may also result in low microorganism populations. In this case, the utilization of bioaugmentation after the appropriate dilution in a bioslurry reactor may be appropriate. Other than the profit accumulated by the seller, there is little evidence of any advantage of bioaugmentation for petroleum bioremediation. One possible exception in the future may be for the bioremediation of added fuel oxygenates (e.g. MTBE), however, currently there is insufficient information available.

Site Assessment

An appropriate technology cannot be selected until a standard environmental site assessment has been completed. The objectives of a site assessment are to determine the source of the contaminant, determine the extent and distribution of the contaminant, estimate the impact of the contaminant on the environment, and obtain enough site information to choose a remediation technology. Listed are the data which an environmental professional should provide from Phase I and Phase II environmental site assessments. Additional data beyond Phase I and Phase II assessments may be necessary since many states have specific site characterization requirements for petroleum releases from underground storage tanks.

Phase I

- Site geology
- Site hydrogeology
- Topography
- Contaminant receptors
- Site history
- Well logs
- Depth to groundwater
- Subsurface structures
    - e.g. utilities
- Existing well survey
- Surrounding landuse
- Site map

Phase II

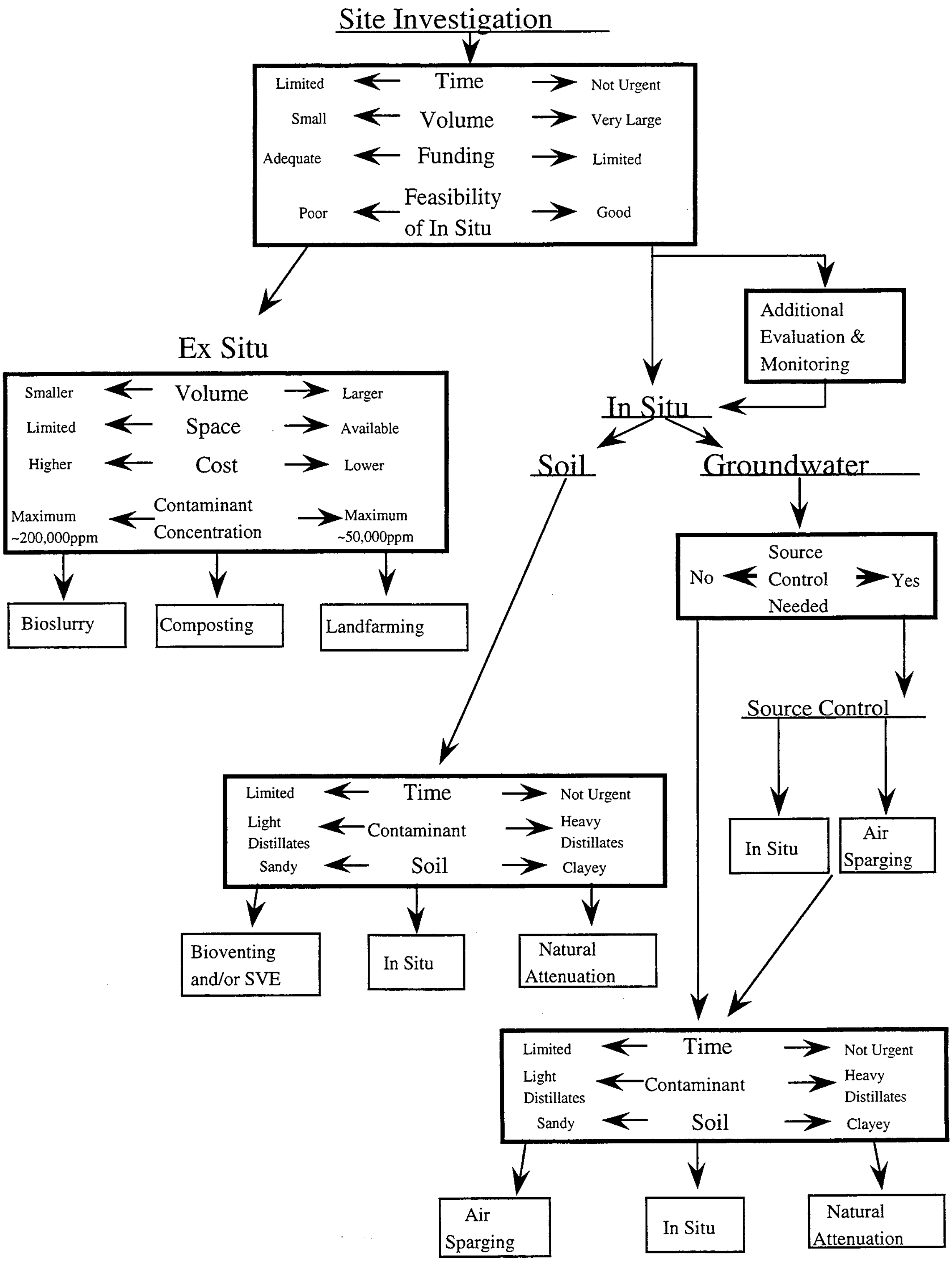
- Additional site history
- Sampling plan
- Soil characteristics
- Install monitoring wells
    - (if groundwater present)
    - water elevation, flow
    - water geochemistry
- Contaminant analysis
- Evaluation of data
- Site model
- Raw data
- Conclusions/recommendations

Technology Decision Tree

The following page contains a decision tree to aid DOT decision makers in determining whether the appropriate technology has been selected for petroleum remediation. Following a site investigation, a decision maker should consider the amount of time available for remediation (i.e. property sale pending?), the volume of contaminated material, and the availability of funding for remediation. Using the former information and data about the site and contaminants, the general feasibility of in situ remediation should be considered.

Once a decision regarding in situ or ex situ remediation has been made, then the appropriate technology can be selected. Technology selection is highly dependent on site characteristics and the type of petroleum products at the site. The decision tree should be used as a guide to help the decision maker communicate with their contractor, consultant, or other environmental professionals. In choosing a technology, it is not necessary that all governing factors are favorable, but rather that the information available suggests that the technology will best meet the remedial goals.

The most difficult challenge for technology selection is usually soil heterogeneity and clayey (low porosity) soils. Although the optimum hydraulic conductivity for bioremediation systems is $>10^{-4}$ cm/s (sandy soils), bioremediation may still be feasible at conductivities as low as 10^{-7} cm/s (clayey soils). Clayey soils limit the ability to introduce oxygen and nutrients and limit the efficiency of SVE and bioventing. Clayey soils also significantly retard contaminant transport. Consequently, clayey soils may reduce the rate of in situ bioremediation effectiveness, however, the acceptability of natural attenuation may be improved due to reduced contaminant transport.


Site Investigation
Limited
Time
Not Urgent
Small
Volume
Very Large
Adequate
Funding
Limited
Poor
Feasibility of In Situ
Good
Ex Situ
Smaller
Volume
Larger
Limited
Space
Available
Higher
Cost
Lower
Maximum ~200,000ppm
Contaminant Concentration
Maximum ~50,000ppm
Bioslurry
Composting
Landfarming
Additional Evaluation & Monitoring
In Situ
Soil
Groundwater
No
Source Control Needed
Yes
Source Control
In Situ
Air Sparging
Limited
Time
Not Urgent
Light Distillates
Contaminant
Heavy Distillates
Sandy
Soil
Clayey
Bioventing and/or SVE
In Situ
Natural Attenuation
Limited
Time
Not Urgent
Light Distillates
Contaminant
Heavy Distillates
Sandy
Soil
Clayey
Air Sparging
In Situ
Natural Attenuation

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