A Feasibility Study of Bioremediation in a Highly Organic Contaminated Soil

Jami Beth Walsh
Worcester Polytechnic Institute

Follow this and additional works at: https://digitalcommons.wpi.edu/etd-theses

Repository Citation
https://digitalcommons.wpi.edu/etd-theses/838
A Feasibility Study of Bioremediation in a Highly Organic Soil

By

Jami B. Walsh

A Thesis

Submitted to the Faculty

of the

WORCESTER POLYTECHNIC INSTITUTE

in partial fulfillment of the requirements for the

Degree of Master of Science

in

Environmental Engineering

May 1999

APPROVED:

______________________________
Dr. James C. O'Shaughnessy, Major Advisor

______________________________
Dr. Frederick L. Hart, Head of Department
ABSTRACT

The focus of this study is on the use of bioremediation, as the primary method of decontamination for a soil contaminated with industrial waste oils. The area from which the samples were taken was used as a disposal site for oily wastewater for a period of more than 20 years. During this time the soil became severely contaminated.

The site is approximately 1 acre in area and consists of three distinct soil strata: a solidified petroleum layer, a peat layer and a layer of muck and mud. This soil is approximately 96% organic matter. The purpose of this study is to determine if, given these site characteristics, is bioremediation a feasible option.

Three phases were conducted to determine the usefulness of bioremediation in this situation. Phase one focused on the removal of total petroleum hydrocarbons (TPH) through nutrient addition and aeration. Phase two focused on quantifying and characterizing the reductions observed in phase one. Phase three again focused on quantifying and characterizing the reductions observed in phase one. The three phases of the study provided strong evidence that bioremediation was occurring in the soil and therefore, would be a viable means of remediation for a site with similar characteristics.
ACKNOWLEDGEMENTS

There are many people who without their help and guidance this project would not be possible.

I would like to extend my thanks to the Environmental Engineering Department of the Wyman-Gordon Company for funding this project. Brad Middlesworth for providing the project samples and answering any questions that I had. Mary Pilion for doing a lot of the little things that made this project possible and also for acting as advisor on the project when Brad was away on business. And thank you to Brian Postale for his ongoing support of WPI and the project system.

Thank you to Dr. James C. O'Shaughnessy for advising this project. His guidance and help throughout the study was indispensable.

Thank you to Jennifer Roberge for helping trek through the muck and mud to get the samples that I needed for my research.

A special thank you to my mom for always supporting me and believing in me through my six years at WPI. I Love you, Mom.

And finally, thank you to my roommates: Troy Thompson, Derek Shute, and Nick Conti. The three of you have managed to keep me smiling and laughing through everything, but still provided great emotional support and strength when I needed it.

Thank you again to everyone involved.
# Table of Contents

ABSTRACT ........................................................................................................................................... 2  

ACKNOWLEDGEMENTS ................................................................................................................... 3  

CHAPTER 1: INTRODUCTION ........................................................................................................ 9  

1.1 INTRODUCTION: ........................................................................................................................... 9  

1.2 REGULATIONS:............................................................................................................................ 10  

1.2.1 Resource Conservation and Recovery Act (RCRA) ............................................................. 10  

1.2.2 Comprehensive Environmental Response Compensation and Liability Act (CERCLA)....... 12  

1.2.3 Superfund Amendment and Reauthorization Act (SARA)..................................................... 13  

1.3. THE SITE ................................................................................................................................... 14  

CHAPTER 2: LITERATURE REVIEW ........................................................................................... 17  

2.1 INTRODUCTION: ........................................................................................................................... 17  

2.2 THE MICROORGANISMS: ........................................................................................................... 21  

2.2.1 Temperature ........................................................................................................................ 22  

2.2.2 pH ....................................................................................................................................... 22  

2.2.3 Moisture Content ................................................................................................................. 22  

2.2.4 Amount of Substrate Present .............................................................................................. 23  

2.2.5 Energy Sources ................................................................................................................... 24  

2.3 IN SITU BIOREMEDIATION METHODS: ................................................................................... 25  

2.4 PETROLEUM HYDROCARBONS: ............................................................................................. 28  

2.4.1 Aliphatic Hydrocarbons: ..................................................................................................... 28  

2.4.2 Alicyclic Hydrocarbons ....................................................................................................... 29
APPENDIX A LABORATORY DATA PRELIMINARY STUDY

APPENDIX B LABORATORY DATA PHASE ONE

APPENDIX C LABORATORY DATA PHASE TWO

APPENDIX D LABORATORY DATA PHASE THREE

APPENDIX E SAMPLE CALCULATION

BIBLIOGRAPHY
List of Figures

Figure 1: Schematic of Soil Present ................................................................. 15
Figure 2: Diagram of Sampling Locations ...................................................... 48
Figure 3: Setup for Phase One of the Study .................................................. 49
Figure 4: Schematic of the Setup for Phase Two .......................................... 51
Figure 5: Actual Setup for Phase Two ............................................................ 52
Figure 6: Schematic of Setup for Phase Three .............................................. 54
Figure 7: Actual Setup for Phase Three ........................................................ 54
Figure 8: Graphical Representation of TPH Concentrations for Phase One ....... 60
Figure 9: Phase Two TPH Concentrations ..................................................... 63
Figure 10: TPH Levels Phase Three ............................................................. 65
List of Tables

<table>
<thead>
<tr>
<th>Table</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>ORGANIC CARBON CONTENT</td>
<td>56</td>
</tr>
<tr>
<td>2</td>
<td>CONCENTRATION OF TPH</td>
<td>56</td>
</tr>
<tr>
<td>3</td>
<td>PERCENTAGE REMOVAL IN PHASE ONE</td>
<td>58</td>
</tr>
<tr>
<td>4</td>
<td>TPH CONCENTRATIONS FOR PHASE ONE</td>
<td>59</td>
</tr>
<tr>
<td>5</td>
<td>TPH CONCENTRATIONS PHASE TWO</td>
<td>62</td>
</tr>
<tr>
<td>6</td>
<td>TPH LEVELS PHASE THREE</td>
<td>64</td>
</tr>
<tr>
<td>7</td>
<td>VOCs OBSERVED IN PHASE THREE</td>
<td>67</td>
</tr>
</tbody>
</table>
1.1 Introduction:

In 1972, the nation's views on the environment and its protection were forever changed with the institution of the Safe Drinking Water Act (SDWA). The SDWA opened the door for regulations such as RCRA (the Reuse Conservation and Recovery Act), CERCLA (Comprehensive environmental Response Compensation and Liability Act; a.k.a. Superfund) and SARA (Superfund Amendments and Reauthorization Act). Actions that were legal once are no longer permissible.

The new regulations affected all aspects of industry. The federal and local governments were now regulating consumption and disposal of common chemical cleaners, such as trichloroethene (TCE). Prior to 1972, companies could obtain permit to discharge their wastes directly to rivers or streams or other bodies of water without any form of treatment. The current regulations allow for discharge to bodies of water via permitting. Unlike past permits, current permits dictate the type of and concentration of chemicals that may be released. The regulations relating to protection of the environment are becoming more stringent with the passing years.
1.2 Regulations:

As indicated in the preceding section, there are many regulations related to the environment that affect industry. The primary regulations that pertain to industry are RCRA, CERCLA and SARA. An overview of these regulations is provided in this section.

1.2.1 Resource Conservation and Recovery Act (RCRA)

The Resource Conservation and Recovery Act (RCRA) was enacted in 1976. RCRA amended the Solid Waste Disposal Act of 1965. The focus of RCRA is on active and future facilities. Abandoned and historic sites are not covered under this regulation, but rather are addressed under CERCLA.

The primary objective of RCRA is to protect the environment and human health. There are other objectives to RCRA including to conserve valuable material and energy resources by providing to local and state government for prohibiting open dumping; regulating the management of hazardous waste; encouraging recycling, reuse and treatment of hazardous waste; promoting beneficial solid waste management, resource recovery, and resource conservation systems; and providing guidelines for solid waste management. RCRA provides “cradle to grave” tracking and regulation of hazardous waste. This regulation targets not only the disposal of hazardous waste, but also the generation, transportation, storage and treatment of hazardous wastes.
RCRA requires that permits be issued to hazardous waste treatment, storage, and disposal facilities. RCRA enforces the “cradle to grave” policy through a record keeping and labeling system that requires the manifesting of hazardous waste shipments from point of generation to the point of disposal. If hazardous waste is allowed to accumulate for a period of greater than 90 days, then a permit is required. Generators must certify that they have a hazardous waste minimization program in place.

In 1984, the Hazardous and Solid Waste Amendments (HSWA) to RCRA took affect. The HSWA allowed for more stringent enforcement authority for the Environmental Protection Agency (EPA); more stringent hazardous waste management standards; and a comprehensive underground storage tank program. In 1986, amendments to RCRA allowed for the Environmental Protection Agency (EPA) to address environmental issues that could result from underground storage tanks (USTs) storing petroleum and other hazardous substances.

Hazardous waste is managed in accordance with the RCRA Subtitle C. A waste may be considered hazardous if it is ignitable, corrosive, or reactive. A waste may also be considered hazardous if it contains certain amounts of toxic chemicals. In addition to these characteristic wastes, the EPA has also developed a list of 500 specific wastes known as “listed wastes”.

Non-hazardous solid waste is managed in accordance with RCRA Subtitle D. Solid wastes covered under subtitle D are diverse including: municipal solid waste (MSW), some sludges, some semi-solid and liquid wastes, construction wastes, household
hazardous waste, and oil and gas waste. Solid waste is defined by EPA as an garbage, or refuse, sludge from a wastewater treatment plant, water supply treatment plant, or air pollution control facility and other discarded material including, solid, liquid, semi-solid, or contained gaseous material for industrial, commercial, mining and agricultural operations, and from community activities. (www.epa.gov)

1.2.2 Comprehensive Environmental Response Compensation and Liability Act (CERCLA)

Another regulation that affects industry is CERCLA. CERCLA is also known as Superfund. Superfund targets past contamination and mandates their clean up. CERCLA was enacted in December of 1980. CERCLA created a tax on the chemical and petroleum industries and provided broad Federal authority to respond directly to releases or threatened releases of hazardous substances that may endanger the environment or public health.

CERCLA accomplished three major things:

- Established prohibitions and requirements concerning closed and abandoned hazardous waste sites
- Provided for liability of persons responsible for releases of hazardous waste at these sites
- Established a trust fund to provide for cleanup when no responsible party could be identified.

The trust fund was created from the aforementioned tax which over the course of five years raise $1.6 billion dollars.
CERCLA also authorized two types of response action: Short term removal and Long term removal. Short term removal are where actions may b taken to address releases or threatened releases requiring prompt response. Long term remedial response actions permanently and significantly reduce the dangers associated with releases or threats of releases of hazardous substances that are serious, but not immediately life threatening. These actions can be conducted only at sites listed on EPA’s National Priorities List (NPL).

CERCLA also enabled the revision of the National Contingency Plan (NCP). The NCP provides guidelines and procedures needed to respond to releases and threatened releases of hazardous substances, pollutants, or contaminants, as well as, providing the NPL. The Superfund Amendments and Reauthorization Act (SARA) amended CERCLA in 1986. [www.epa.gov](http://www.epa.gov)

### 1.2.3 Superfund Amendment and Reauthorization Act (SARA)

The Superfund Amendment and Reauthorization Act (SARA) amended CERCLA. SARA is a reflection of the EPA’s experience in administering the complex Superfund program during the first six years. SARA also made several important changes and additions to the CERCLA program. These changes are:

- Stressed the importance of permanent remedies and innovative treatment technologies in cleaning up hazardous waste sites
- Required superfund actions to consider the standards and requirements found in other State and Federal environmental laws and regulations
- Provided new enforcement authorities and settlement tools
- Increased State involvement in every phase of the Superfund program
- Increased the focus on human health problems posed by hazardous waste sites
- Encouraged greater citizen participation in making decisions on how sites should be cleaned up
- Increased the size of the trust fund to $8.5 billion

SARA also required EPA to revise the Hazard Ranking System (HRS) to make sure that it accurately assessed the relative degree of risk to human health and the environment posed by uncontrolled hazardous waste sites that may be placed on the NPL. [www.epa.gov](http://www.epa.gov)

1.3. The Site

This paper focuses on the natural wetland that was used as a disposal site for oily wastewaters for over 20 years. Based upon the forging process, it is believed that this site contains various petroleum products and possibly BTEX (Benzene, Toluene, Ethyl Benzene, and Xylenes), as well as, the possibility of PCBs (Polychlorinated Benzenes).

The site is located on the east side of a metal forging plant and is approximately 1 acre in area. There are three distinct strata present in the site: a solid surface soil, an organic peat layer, and a saturated organic layer. (Figure 1) The solid surface soil appears to be a layer
of dried petroleum products. This was determined based upon the smell, feel and look of the top layer. Located below the top layer of solid soil is a layer of organic peat. The peat layer is 3 to 4 inches deep. In order to observe the peat layer the top layer must be penetrated. Upon the penetration of the top layer, the escape of off gases that were trapped beneath the surface was apparent audibly and olfactorily. Underlying the peat layer is a saturated organic soil. The water table in this area is located approximately 3 inches below the peat layer. The saturated soil appears to have a high organic content. Soils with high organic matter have limited remediation options. The options available for this type of soil are generally high in cost and/or extremely labor intensive. On site thermal desorption would be one option for the area, however, this process is extremely expensive and would have to be done at low temperatures do to the nature of the soil. Another option would be to excavate the soil and land farm it so that it may be

Figure 1: Schematic of Soil Present
transported off site. After treating the soil, the water present would also need to be treated. Once again this is a very expensive prospect. Another option for the soil is bioremediation. This is an in-situ process that requires a large amount of time, but relatively little capital. This appears to be a feasible means of remediation for the site because some vegetation has already begun to grow on the site. The focus of the remainder of this paper is on the prospect of bioremediation as a cleanup method for a highly organic soil.
Chapter 2: Literature Review

2.1 Introduction:

Beginning with the industrial revolution, a number of organic compounds have been synthesized. The use of these chemicals through direct and indirect applications generated chemical wastes in every economic sector. Unlike, naturally occurring organic compounds that are readily degraded, these synthetic chemicals are resistant to biodegradation. The environmental protection agency (EPA) estimates that only about 10% of all wastes are disposed of safely. (Chaudhry, 1994) There are many methods of treatment available to sites that have been contaminated. Some of these treatment methods are ex-situ while other are in-situ. Ex-situ processes require removing the soil or water from the site and transporting the waste to a treatment facility. In-situ processes take place with minimal disruption of the contaminated area.

Bioremediation is a process for the treatment of contaminated soil and groundwater. Biodegradation can be defined as the biologically catalyzed reduction in complexity of chemicals. Certain microorganisms have the capability to degrade contaminants in the environment. Not every contaminant can be remediated through the use of microorganisms. However, there are a number of contaminants that can be
degraded using this method. The contaminants that may be degraded via bioremediation can be subdivided into five main categories (Hickey and Smith, 1996):

- Organic solvents
- Polyaromatic hydrocarbon (PAH) (creosote oily wastes)
- Halogenated aromatic hydrocarbons
- Pesticides
- Munitions wastes

Contamination associated with pesticides and munitions wastes are not of concern in this study, therefore, the remainder of this paper will focus on organic solvents, polyaromatic hydrocarbon (PAH)-containing wastes (creosote a' oily wastes), and halogenated aromatic hydrocarbons. A brief discussion of the degradation of pesticides is also presented to provide a background in unique degradation pathways.

Bioremediation is generally viewed as a new technology, however, microorganisms have been used for the treatment and transformation of waste products for at least 100 years. Municipal waste water systems are based on the use of microorganisms in a controlled and engineered environment. Activated Sludge and fixed film treatment systems are examples of treatment methods dependent upon the metabolic activities of microorganisms that degrade wastes entering the facility. (King, Long and Sheldon, 1992) Making the transition to treating other forms of waste therefore should not be a surprise.

There are many advantages to the use of Bioremediation for treatment of hazardous waste sites (Alexander, 1994):

- Can be done in situ
- Keeps disruption to a minimum
- Eliminate transportation costs and liabilities
• Eliminates waste permanently
• Eliminates long-term liability
• Biological systems are often less expensive than conventional treatment
• Can be coupled with other treatment techniques to form a treatment train

One drawback of bioremediation is that only a select few bacteria and fungi act on a broad range of organic compounds. To date no organism is known that is sufficiently omnivorous that it will destroy a large percentage of the natural chemicals present. Another drawback to bioremediation is that it requires a large amount of time to obtain detectable results. However, this is only a draw back if time is a constraint on the cleanup of the site.

There are several bioremediation techniques that may be applied. Some examples are (Baker and Herson, 1994):

• Bioaugmentation
• Biofilters
• Biostimulation
• Bioreactors
• Bioventing
• Composting
• Landfarming

*Bioaugmentation* - The addition of bacterial cultures to a contaminated medium; frequently used as an ex-situ process.

*Biofilters* - The use of microbial stripping columns. Employed to treat air emissions.

*Biostimulation* - The stimulation of the indigenous microbial populations in soils and/or ground water. This process may be done in situ and ex situ.
Bioreactors - The use of biological processes in a contained area or reactor. This method is used to treat slurries or liquids.

Bioventing - The process of drawing oxygen through the soil in such a way to stimulate microbial growth and activity.

Composting - Aerobic, thermophillic process that mixes contaminated soil with a bulking agent. Done using static piles, aerated piles or continuously fed reactors.

Landfarming - Solid phase treatment system for contaminated soil. May be done as an in situ process or in a soil treatment cell.

Independent of the method of bioremediation there are several criteria that must be satisfied before biodegradation will take place in an environment. (Alexander, 1994)

- An organism must exist that has the necessary enzymes to bring about the biodegradation. The mere existence of an organism with the appropriate catabolic potential is necessary but not sufficient for biodegradation.

- That organism must be present in the environment containing the chemical. Although some microorganisms are present in essentially every environment near the earth's surface, particular environments may not contain an organism with the necessary enzymes.

- The chemical must be accessible to the organism having the requisite enzymes. Many chemicals persist even in environments containing the biodegrading species simply because the organism does not have access to the compound that it would otherwise metabolize. Inaccessibility may result from the substrate being in a different microenvironment from the
organism, in a solvent not miscible with water, or sorbed to solid surfaces.

- If the initial enzyme bringing about the degradation is extracellular, the bond acted upon by the enzyme must be exposed for the catalyst to function. This is not always the case because of sorption of many organic molecules.

- Should the enzymes catalyzing the initial degradation be intracellular, the molecule must penetrate the surface of the cell to the internal sites where the enzyme acts. Alternatively, the products of an extracellular reaction must penetrate the cell for the transformation to proceed further.

- Because the population or biomass of bacteria or fungi acting on many synthetic compounds is initially small, condition in the environment must be conducive to allow for proliferation of the potentially active microorganisms.

If the above conditions are not satisfied than biodegradation can not take place in the environment. The remainder of the paper will focus on the pathways for degradation of hazardous substances that the Environmental Protection Agency (EPA) has focused its research.

2.2 The Microorganisms:

As with any living creature, microorganisms need nutrients to survive. In particular the microorganisms require a carbon (C) source and an energy (E) source. There are also several environmental factors that affect the fate of the organism. These include:

- Temperature
• pH
• Moisture Content
• Amount of Substrate present

2.2.1 Temperature

Temperature affects the microorganism’s ability to survive let alone reproduce. If the temperature exceeds the allowable limit than enzyme denaturazation begins and leads to the inhibition of reproduction and eventually to death. If the temperature drops, reproduction again will come to a halt while the organism focuses its energy on sustaining life. If the temperature drops too low, then the organism will cease to exist. There are ideal temperatures for each microorganism and a small range in which these organisms may survive.

2.2.2 pH

The pH of the soil and the ground water affect the microorganism in a similar manner as temperature. There is generally a small range of pH in which the organism is capable of sustaining life. If the soil becomes extremely acidic or alkaline than the concentration of microorganism slowly diminishes.

2.2.3 Moisture Content

A source of water is a necessity for life. Fore each microorganism there is an optimal moisture content for it to grow. Microbes are limited to soluble materials that are transported across their cell membranes into the interior of the cell. The moisture
solubilizes the substrate and allows the substrate to enter the cell. "For hydrocarbon-contaminated soils, moisture level below 50% appear to inhibit degradation." (Cookson, 1995)

2.2.4 Amount of Substrate Present

A source of nutrition is needed. Growth of the microorganism will continue until the substrate is eliminated. Carbon has been said to be the building block of life. Therefore it is no surprise that the presence of carbon is a necessity for microscopic life to flourish. The carbon used to support life may be present in many forms. This is one of the reasons why microorganisms are an effective means of remediation. The determination of the amount of C used by microorganisms has long been a source of study.

The assimilation of C is an important issue. The percentage of C used by the microbial population reflects the biological efficiency of converting the substrate into biomass. Higher percentages indicate greater efficiency of the organism in the conversion of substrate. The greater the efficiency of conversion the quicker and more complete the remediation of the site by the microorganisms. The determination of the percent used is straightforward in liquid media, but becomes complicated in soils, wastewater, sediments or sewage. The complications exist due to the particulate matter as well as the water-insoluble products. An estimate of the assimilated C can be found using the following equation:

$$C_{\text{assimilated}} = C_{\text{substrate}} - C_{\text{mineralized}}$$
The assimilated C becomes mineralized further as the cells that are metabolizing the substrate are themselves decomposed or consumed by predators. (Alexander, 1994)

2.2.5 Energy Sources

Another key factor in the existence of the microbial population is an E source. Many environmental pollutants represent a novel C and energy source for a particular population still is transformed by the metabolic pathways that are characteristic of heterotrophic microorganisms. In order for the organism to grow on the compound, the compound must be converted to the intermediates that characterize the major metabolic pathways that are characteristic of the heterotrophic microorganisms. Compounds that cannot be modified enzymatically to provide the necessary intermediates, then it will not be able to serve a C and E source. This is due to the fact that the energy yielding and biosynthetic processes are not able to function. This indicates that the primary phases of the biodegradation process involves the modification of the novel substrate to yield a product that in itself is an intermediate, or a substance that can be further metabolized into an intermediate. This need to convert synthetic molecules to intermediates is common to both aerobes and anaerobes. (Alexander, 1994)

If sufficient organic nutrients are not present, then inorganic elements may be used as an energy source. The inorganic elements that may be used are oxygen (O₂), nitrogen (N), phosphorous (P) and sulfur (S). For heterotrophic organisms the limiting factor is generally the availability of C. Because C is the limiting element and because it is the
element of intense competition, a species that has the unique ability to grow on synthetic molecules has an advantage. As the organisms use these molecules as a C or energy source, the biodegradative process usually will still lead to the mineralization of the other elements in the chemical.

Typically microbial cultures produce extracellular surfactants that aid in solubilizing hydrocarbons. These surfactants consist of a complex mix of protein, lipids, and carbohydrates. Nonbiological surfactants have been used to disperse hydrocarbons.

### 2.3 In Situ Bioremediation Methods:

In situ implies that the remediation occurs on site. This will provide little if any disruption to the site. The aim in using bioremediation is to utilize the microbes to degrade hazardous constituents in the soil and water. This section will address the issues of remediating hazardous materials in situ. Prior to bioremediation being employed a biostudy must be performed. Typically there are two stages to a bioassessment: an initial bioassessment (screening study to determine if bioremediation is possible) and a more detailed treatability study (determination of the most likely kinetics and degradation pathways for the materials on site). If bioremediation is determined to be a viable method of remediation then treatment may begin: The following is an overview of a typical bioassessment.
• "A composite soil sample is prepared from samples obtained from the contaminated zones. The average hydrocarbon content is measured, and specific compounds (volatile or semivolatile compounds) may be determined. The bacterial population is estimated from plate counts of total heterotrophic bacteria, and often a specific hydrocarbon degrading population is estimated. The degrader population is estimated using target contaminant vapors as the sole carbon source for laboratory cultures. An alternative method utilizes the Most Probable Number (MPN) method. This method is more flexible and allows a better quantitative value to be obtained.

• The same characterization previously described is applied to ground water from the contaminated zone. The water pH and other wastewater parameters such as suspended solids, biochemical and chemical oxygen demand (BOD and COD), total organic carbon (TOC), and background nutrient concentrations are measured.

• Soil and water are combined in suitable flasks, usually at 10 to 25% solids, and treated with several ratios of nutrients and oxygen. A biotic poison is administered to provide at least one "killed" control sample to measure nonbiological effects of the treatment conditions (i.e. air stripping or chemical oxidation in the flasks). At periodic points during some predetermined treatment duration from 3 days to 2 weeks, the hydrocarbon content is measured to determine the approximate rate and degree of degradation. Other test parameters such as oxygen consumption, relative toxicity of the treatment conditions, nutrient content, pH, and BOD may also be measured to provide an indication of the effect of biodegradation under ideal conditions.

• Geochemical testing is strongly recommended to determine the soil/water system response to the addition of nutrients and oxygen. These tests are necessary to avoid geochemical reactions, which may cause site problems during the course of the remediation. One such problem is the precipitation of the orthophosphate in high calcium soils or hard water. The
objective of the test is to verify that the intended injection concentration of phosphate (especially orthophosphate as the most suitable form for biodegradation) does not result in the precipitation of calcium phosphate that can clog well screens and perhaps the geological formation. Chelating agents may be added to the nutrient blend to minimize this problem or complex phosphate forms may be used in higher concentrations to provide the essential nutrient.

• Another geochemical effect has to be considered when hydrogen peroxide is being considered as the oxygen source. Iron, copper, and manganese will catalyze the decomposition of hydrogen peroxide, which is possible without wasting the material and possibly causing outgassing in the formation. These metals can cause this effect at soil or water concentrations as low as 10 ppm. In the presence of these metals at or above these concentrations, it may be more practical to consider the use of oxygen to produce the optimum effect at minimum cost.

• Where the degradation pathway requires detailed documentation, either to support a health-based alternative remediation target value or to satisfy fate and transport concerns of regulatory agency, the frequency and type of analysis of the test flasks will be more involved and hence more expensive. These requirements may range from determination of the disappearance rate of specific compound (i.e. Polyaromatic hydrocarbons) using GC/MS techniques to a determination of the full degradation pathway including the daughter products rising from the biodegradation of a suit of fuel constituents. Radiolabeling of target compounds can be an effective technique for studying contaminant breakdown sequences. In addition, the types of bacteria active in the degradation may be identified using staining or other suitable technique, if it is a regulatory requirement.” (King, Long and Sheldon, 1992)

A bioassessment is the primary process for determining the feasibility of bioremediation on a site. The aforementioned typical procedure should be kept in mind throughout the
discussion of each type of contaminant. It should also be noted that the bioassessment outlined above is generalized and each study will be unique.

2.4 Petroleum Hydrocarbons:

Understanding the nature of the contaminant is important in any remediation work. Petroleum hydrocarbon plumes generally extends down to at least 2 ft. below the water table and may extend downgradient from the source for as far a one-mile. If the hydrocarbon source is above the water table, the vadose (or unsaturated) zone will also contain some hydrocarbons. Petroleum hydrocarbons include gasoline, oil, and organic solvents.

Petroleum hydrocarbons are highly insoluble and sorb to soil and sediment particles. Understanding the chemical structure of the compound is pertinent to degrading the contaminant. There are three types of hydrocarbons: aliphatic, alicyclic and aromatic.

2.4.1 Aliphatic Hydrocarbons:

Aliphatic Hydrocarbons have straight or branched chains of carbon atoms with sufficient hydrogen to satisfy the valency requirements of the carbon. Aliphatic hydrocarbons can be further broken down to: Alkanes (CnH2n+2); Alkenes (CnH2n); and Alkynes (CnH2n 2).
2.4.2 Alicyclic Hydrocarbons

Alicyclic Hydrocarbons are characterized by the presence of a carbon ring. Alicyclic hydrocarbons can be broken down into three subcategories: Cycloalkanes (CnH2N); Cycloalkenes (CnH2N2); and Cycloalkynes (CnH2N4).

2.4.3 Aromatic Hydrocarbons

Aromatic Hydrocarbons can be identified by the presence of one or more resonance-stabilized six-carbon rings. There are two types of aromatic hydrocarbons: unsubstituted and substituted. (See Appendix A for examples of chemical structures of each hydrocarbon)

There are several factors involved in the degradation of these compounds. These factors can be used as “rules of thumb" for petroleum hydrocarbons.

• "Aliphatic hydrocarbons are generally easier to degrade than aromatic compounds

• Straight-chain aliphatic hydrocarbons are easier to degrade than branched-chain hydrocarbons. The introduction of branching into the hydrocarbon molecule hinders biodegradation.

• Saturated hydrocarbons are more easily degraded than unsaturated-hydrocarbons. The presence of carbon-carbon double or triple bonds hinders degradation.

• Long-chain aliphatic hydrocarbons are more easily degraded than short-chain hydrocarbons. Hydrocarbons with chain lengths of less than 9 carbons are difficult to degrade because of their toxicity to microorganisms. Some specialized microorganisms (methanotrophs) can degrade these short-chain
hydrocarbons. The optimal chain length for biodegradation appears to be between 10 and 20 carbons." (Baker and Herson, 1994)

2.5 Degradation Pathways:

There are several degradation pathways for petroleum hydrocarbons. The purpose of this section is to provide an overview of the common pathways of degradation.

Straight chain alkanes are degraded primarily through the oxidation of the terminal methyl group, followed by cleavage of the molecules between the second and the third carbon in the chain. The initial reaction in the degradation of the straight chain alkanes involves the direct addition of oxygen to the terminal carbon. This forms an alcohol that can subsequently oxidize to a corresponding aldehyde and finally forms a fatty acid. From the fatty acid a two carbon long fragment is cleaved. This action provides the intermediate hydrocarbon of length Cn 2. This process is repeated until complete oxidation of the hydrocarbon molecule is achieved. The presence of branching in the molecule will prohibit the cleavage reaction and therefore significantly reduce the molecule's susceptibility to biodegradation.

Aromatic hydrocarbons are found mainly in light petroleum products, however, they may be present in small amounts in any petroleum product. Aromatic hydrocarbons are also widely used in industrial solvents. Aromatic hydrocarbons, in general, are very soluble in water and have low boiling points due to their small molecular size. These compounds are also very volatile. There are a large number of different pathways that are
used by bacteria to degrade aromatic compounds. To aid in the understanding of the degradation of aromatic compounds, the degradation of benzene follows.

Benzene is first converted to catechol or protocatechuate. The aromatic nucleus is subsequently opened by one of two pathways: the orthocleavage or the metacleavage. Orthocleavage: The aromatic ring of catechol or protocatechuate is opened as a result of the introduction of molecular oxygen into the hydroxyl groups. Acetyl-CoA and succinate are formed as a result of the cleavage. These products can than be further oxidized by the Krebs cycle and the electron transport system. Meta-cleavage: Once again the aromatic ring is opened by the introduction of molecular oxygen. In this case, the cleavage occurs between a hydroxylated carbon and the adjacent unsubstituted carbon. Acetaldehyde and pyruvate, which can be broken down by the Krebs Cycle and electron transport are the products of the ring cleavage. (Baker and Herson, 1994)

The above processes are aerobic in nature. Aerobic degradation is the most common, however, anaerobic degradation can occur. Anaerobic degradation will occur under denitrifying conditions, sulfate-reducing conditions, and methanogenic conditions. The initial step in anaerobic degradation is dissimilar to the aerobic degradation path. The first stage of degradation in an anaerobic system is the hydrogenation of the benzene ring, thus destabilizing the ring. Cleavage through hydration reaction yield aliphatic hydrocarbons that can be further metabolized to the Krebs cycle intermediates as described above. In anaerobic degradation, water acts as the oxygen source for metabolic reactions.
2.5.1 Polyaromatic Hydrocarbons (PAHs):

Polyaromatic hydrocarbons are generated and released from the incomplete combustion of organic material, including automobile exhaust. Petroleum related activities are reported to account for more than 70% of the artificially generated sources of PAHs. The degradation of these compounds are dependent upon the complexity of the PAH chemical structure. The ease of degradation is dependent upon the following (Cookson, 1995):

- Solubility of the PAH
- Number of Fused Rings
- Number of Substitutions
- Type of Substitutions
- Position of Substitutions
- Nature of Atoms in Heterocyclic Compounds

Polyaromatic hydrocarbons contain two or more fused aromatic rings. They are found in trace amounts in heavy petroleum products. PAHs are present as contamination in the form of naphthalene, phenanthrene, pyrene and benzopyrene. Degradation of two and three ring compounds, such as naphthalene and anthracene, has been shown to occur among aerobic bacteria. Biodegradation of the higher ring structures is dependent upon the molecule's solubility in water.

*Anaerobic Degradation:*
Degradation of PAHs by anaerobic organisms has not been very successful. However, some degradation has been achieved under denitrifying, sulfate reducing, and methanogenic conditions. Napthalene and anthracene was found to be slightly degraded anaerobically under denitrifying conditions. Under sulfate reducing or methanogenic conditions the degradation rate is independent of the nitrate concentrations, but is dependent upon the soil to water ration. However, Naphthol that contains a hydroxyl group substitution was found to be anaerobically degraded by denitrifying conditions, sulfate reducing conditions, and methanogenic conditions.

Aerobic Degradation:

As the number of fused rings increases the degree of degradation decreases. One methyl addition significantly decreases the degree of degradation. The influence of alkyl substitutes is less predictable. The effect of the methyl addition varies with the position in which it is substituted in the ring. Another way of reducing the degradation is to increase the degree of saturation through the addition of hydrogen atoms and the removal of double bonds between the carbons providing another valence bond. (Cookson, 1995)

For unsubstituted PAHs degradation occurs readily in the presence of soil bacteria. the rate of degradation of unsubstituted PAHs appear to be related to the solubility in
water of these compounds. There is little information on the degradation of PAHs with more than three rings. (Cookson, 1995)

Eukaryotic organisms have also shown an ability to degrade PAHs under aerobic conditions. The mechanism in this situation involves a reaction sequence called NIH shift in the initial stages of transformation. An example of an eukaryotic organism that is capable of degrading PAHs is the white-rot fungi. (Baker and Herson, 1994)

2.5.2 Halogenated Aliphatic and Aromatic Hydrocarbons:

Halogenated hydrocarbons are widely used as industrial solvents and degreasers. The definition of a halogenated hydrocarbon is a compound that has one or more of the hydrogen molecules have been replaced with a halogen. Common halogenated compounds are illustrated in Appendix B.

2.5.3 Halogenated Aliphatic Hydrocarbons:

Trichlorethene (TCE) is the most common halogenated aliphatic hydrocarbon contaminant in groundwater. These compounds can be degraded under both aerobic and anaerobic conditions. Many water and soil chemical properties will influence the stability of the halogenated aliphatic hydrocarbons. These compounds undergo abiotic transformations in the environment. These transformations include substitution and
dehydrohalogenation of a haloaliphatic compound in water. Dehydrohalogenation results in the removal of the halogen to form an alkene.

*Anaerobic Degradation Pathway:*

Anaerobically a process called reductive chlorination takes place. In reductive chlorination halogen atoms are removed sequentially from the molecule and replaced with hydrogen atoms. In reactions such as this, the halogenated hydrocarbon is not used as a C source but rather as an electron acceptor. This indicates that in order for reductive chlorination to occur an ample carbon source must be present to allow for microbial growth.

Dehalogenation is dependent upon the oxidation-reduction (redox) potential of the molecule. Redox potential is determined by the strength of the halogen-carbon bond. The higher the bond strength, the less likely the halogen will be removed. The bond strength in turn is dependent upon the type and the number of halogen atom present and the degree of saturation of the halogenated molecule. As the degree of saturation decreases the bond strength increases and the molecule becomes more difficult to degrade. This indicates that alkanes are more susceptible to reductive dehalogenation than alkenes and alkynes. (Baker and Herson, 1994)

*Aerobic Degradation Pathway:*
Until recently it was believed that aerobic degradation did not occur in the case of Halogenated Aliphatic Hydrocarbons. Degradation has been shown to occur in soils that have been exposed to methane or natural gas. It was therefore determined that a group of organisms known as methanotrophs. Methanotrophs have been isolated and in the presence of aromatic compounds can degrade TCE. The understanding of the degradation pathway for aerobic degradation is incomplete. The initial stage involves the oxidation of the molecule. It is believed that the initial oxidation is carried out in the same manner as in the degradation of aliphatic and aromatic hydrocarbons. (Baker and Herson, 1994)

2.5.4 Halogenated Aromatic Compounds:

Sources of Halogenated Aromatic Compounds are vast and varied. Many of these compounds are produced from commercial use and as chemical intermediates during the synthesis of chemicals. Potential releases are associated with the industrial operations dealing with pharmaceutical, pesticide formulation, dyes, rubber, solvents, cleaners, etc.. (Cookson, 1995)

Halogenated Aromatic Compounds include toluene and phenol. It is an immense group or chemical related to benzene. Due to the diversity of this group a thorough overview of the microorganisms and metabolic pathways involved is not possible.

Aerobic Degradation:
One requirement for microbial degradation of any compound is the need to induce the production of enzymes. However, not all halogenated aromatic compounds will generate enzymes. Most halogenated aromatic compounds are degraded by cometabolism. The relatively nonspecific nature of the enzyme that transform benzoate to catechol. In some cases, this degradation is not complete.

Degradation of Halogenated Aromatic Compounds proceeds through many of the same pathways as nonhalogenated compounds. For example, the first stage is to convert the compound to a chlorocatechol type substance. This is followed by the aromatic nucleus being broken down. The next stage is the dechlorination of the ring cleavage products. Halogenated polyaromatic compounds, such as chlorinated biphenyls, are generally degraded by the cleavage of a nonsubstituted ring, followed by the degradation of the resulting chlorobenzene. (Cookson, 1995)

The susceptibility of the Halogenated Aromatic Hydrocarbons is dependent on the nature of the halogen substitution, the number of substitutes and the placement of the substitutes. The susceptibility is decreased as the number of substitutions increases. However, some highly chlorinated compounds such as pentachlorophenol have been shown to be susceptible to aerobic degradation. (Baker and Herson, 1994) One method for the degradation of aromatic compounds is through ring cleavage.

*Anaerobic Degradation:*
Anaerobic degradation was found to occur in a variety of environments. Once again the process proceeds through reductive chlorination. Highly substituted compounds are more easily dehalogenated than monohalogenated compounds. Typically the degradation of these compounds is performed by a group of organisms rather than a single strain. Thus far, the majority of studies have focused on the use of methanogenic enrichment for degradative purposes. As for sulfur reducing agents, there is limited information. However, due to the lack of thermodynamic barriers to the degradation of Chlorinated Aromatic Hydrocarbons, the lack of information reflects a lack of research in this area, not a limitation to the organisms. (Baker and Herson, 1994)

2.5.5 Methanotrophic Treatment Technology:

Methanotrophic Treatment Technology (MTT) is based on the use of methanotrophs (a bacteria that derive energy from the oxidation of methane to methanol) to biodegrade chlorinated hydrocarbon. The organisms that are responsible for the degradation of compounds such a TCE do not derive energy from the transformation of the chemical, but instead the conversion is brought about by cometabolism with enzyme or cofactors produced by the microorganisms for other purposes. To do this, the methanotrophs use the enzyme methane monoxygenase to catalyze the oxidation of methane to methanol. This enzyme is not very specific. It will oxidize TCE to an unstable
epoxide that will undergo decomposition to yield a variety of products including carbon monoxide, glyoxylic acid and a range of chlorinated acids. (Hickey and Smith, 1996)

2.5.6 Pesticides:

Pesticides do not follow the general discussion of organic compounds discussed in previous sections of this report. This is a result of their uniqueness in chemical structure or from their use patterns by society and interaction with the environment. Pesticides can be divided into the following subcategories:

- Insecticides
- Herbicides
- Fungicides
- Polychlorinated Byphenyls
- Azo Dyes

The following contains discussions of the above subcategories. Pesticides have had a great impact on society and the environment. They have increased agricultural yields while detrimentally affecting the food chain. By design the chemicals in the pesticide are toxic to one form of life or another. This in turn makes these chemicals unsuitable for bioremediation, however they may be biologically degraded.

The microbial degradation of pesticides has been long recognized. The exact mechanism for adaptation to pesticides is not understood. Microorganisms may acquire genetic material to encode the biochemical mechanisms necessary to deal with a potential substrate. Another method is to transform a compound to remove the toxicity to their well being rather than as an energy source.
Aerobic Degradation:

Pesticides are subject to both biotic and abiotic transformation processes. In general, the abiotic transformations result in the partial degradation. The results of the partial degradation are a chemical that is more easily degraded by microorganisms. The most significant abiotic processes are the hydrolytic reactions. These transformations and occur through the interactions with reactive chemical groups on mineral surfaces, reactive organic compounds, and inorganic metals. Hydrolysis may be required for microbial degradation. Cometabolism by the organisms allows the microbe to catalyze the hydrolysis reaction. An important factor in the bioremediation of pesticide contaminated soil is the availability of the contaminant to the microorganisms. This availability is a function of the affinity of adsorption to the soil of the organic compound. Moisture content plays a role in the degradation of pesticides. The availability of the pesticide to the organisms is dependent upon the solubility of the compound in water. A moisture content of 50% will give good degradation rates. If the moisture content was dropped to 25% then the degradation rate would be decreased by approximately 90%. (Cookson, 1995)

The concentration of pesticide in the soil also contributes to the rate of degradation. When the concentration of pesticides present is less than 5 µg/l then the reaction rate can be described as a first order reaction rate. When concentrations exceed 5µg/l then the description becomes more complex, a biphasic breakdown occurs.

Often times when specialty compounds such as pesticides, are present in a soil, significant populations of microbes that degrade these compounds are not present.
Remediating these soils is generally done with the aid of bioaugmentation, the development of specific seed cultures and inoculation of the soil or bioreactor. For some pesticides such as DDT, lindane and heptachlor, anaerobic degradation works better than aerobic degradation. (Cookson, 1995)

*Anaerobic Degradation:*

There are many degradation reactions that characterize the breakdown of the pesticide family anaerobically. These include:

- Addition of a Hydroxyl Group
- Oxidation of an Amino Group
- Oxidation of a Sulfur Molecule
- Addition of an Oxygen to a Double Bond
- Addition of a Methyl Group
- Removal of a Methyl Group
- Removal of a Chlorine
- Chlorine Migration
- Reduction of a Nitro Group
- Replacement of a Sulfur with an Oxygen
- Cleavage of an Ether Linkage
- Metabolism of Side Chains
- Hydrolysis

*Addition of Hydroxyl Group:*

The hydroxyl group addition results in the replacement of one of the hydrogen-carbon bond with the hydroxy group.

*Oxidation of An Amino Group:*

The oxidation of the amino group has the result of converting the NH2 group to a NO2 group.
Oxidation of a Sulfur Molecule:

This reaction will result in the sulfur molecule (S) being replaced by an SO$_2$ molecule. This is a common reaction that results in the formation of an epoxide group. The epoxide group may be resistant to microbial degradation and toxic to the cell material of the microorganisms.

Addition/Removal of a Methyl Group:

Addition of Methyl group is common in the methylation of arsenic pesticides. Removal of a Methyl group is a result of the cleavage of the methyl from the nitrogen atoms of herbicides.

Removal of a Chlorine

The removal of a chlorine is common among halogenated compounds. In this process a hydrogen or hydroxide replaces the chlorine.

Chlorine Migration:

Migration is the movement of chlorine from one position to another position on the ring.

Reduction of a Nitro Group:

Another common reaction is the transformation of NO$_2$ to NH$_3$.

Replacement of Sulfur with a Oxygen:

Replacement of a sulfur with an oxygen may occur when an insecticide contains a sulfur-phosphorous double bond.
Cleavage of an Ether Linkage:

This method is self-explanatory it is simply the breakdown of the ether link.

Metabolism of Side Chains

Occurs through the cleavage of two carbon atoms by $\beta$ oxidation.

Hydrolysis:

The degradation of a compound through the addition of water.

Pesticides that contain a halogenated phenol or an aniline structure become complex because the compounds become mixed with the humic material of the soil. This tends to stabilize the compound and the soil. This is accomplished by removing the compound from the transport pathway. Thus the risk potential is lowered. The formation of humus in the natural environment involves the natural organic precursors as well as aromatic structures. This process is known as humification. The phenolic structure is important to the humic material. Hazardous chemical having this structure are therefore potential binding candidates for natural soil material. (Cookson, 1995)

2.6 Conclusions:

Bioremediation has numerous applications in terms of treatment of contaminated soils. There are millions of microorganisms and of those thousands are capable of degrading hazardous wastes. Degradation may occur with or without oxygen. The
environment will determine the method of degradation that will be used. Biodegradation is the only in-situ process that removes the contaminant 100%. Bioremediation reduces the future liabilities.

The regulations require that companies be responsible for their wastes from "cradle to grave". The liabilities are diminished because bioremediation is an in-situ process. Therefore, in 20 years there is no risk of being named as a Primary Responsible Party (PRP) at a superfund site.

The degradation pathways are complex and may occur in several manners. Understanding the pathways is essential in determining the nutrients and inoculants that should be used to increase the degradative potential. This section provides an overview of the most common contaminants and the common degradative pathways for those contaminants.
Chapter 3: Experimental Procedure

3.1 Introduction:

In an effort to determine the feasibility of bioremediation in-situ for a highly organic soil, samples were taken from the site and laboratory experiments were conducted. The purpose of these experiments was to simulate the in-situ environment under varying conditions. Each experimental situation simulates conditions under which bioremediation occurs. These experimental situations are discussed in the following sections. The experiments were conducted in three phases to aid in the determination of biodegradation feasibility. Phase one was conducted to assess the removal of contamination over a designated time period, phases two and three were conducted to determine if the removal observed in the reactors were due to volatilization or due to biological activity.

3.2 Preliminary Procedure:

Prior to beginning the first phase of the experimental portion of this study, a preliminary investigation was done to determine the type of contaminant present, as well as, the amount present. Several samples were taken from different locations in the area. The area in question has three distinct strata: an upper crust consisting of dried petroleum products, a peat layer that is 2 to 3 inches thick, and a saturated soil layer that is
underlying the peat layer. The water table in this area is approximately 8 to 12 inches below the surface. The saturated soil layer is comprised of a soil that was determined to be 98% organic. Due to the organic nature of the soil, other remediation options such as thermal desorption are not viable options for the site being investigated.

Samples were taken from 12 different locations around the area at different depths. The samples were then combined based on the depth and location from which they were taken to form a composite sample. Six composite samples were created and analyzed these include: a sample of the dried petroleum layer; a sample of the peat layer; and four samples of the saturated layer, one from samples taken near the inlet, one from samples taken in the middle of the area and one from samples taken at the outskirts of the site. (Figure 2)

The composite sample was then analyzed through the use of Gas Chromatography (GC). Utilizing the history of the area, the samples were tested for total petroleum hydrocarbons (TPH) utilizing 8100M test and for volatile organic compounds (VOCs) utilizing 8260 test. The preliminary results showed that TPHs are present, but VOCs were not detected in the samples that were analyzed. Utilizing the results of the GC, an experimental procedure for determining the viability of bioremediation in-situ was determined. The procedure is outlined in the following sections.
3.3 Phase One:

The first phase of the experimental procedure was to determine if through bioremediation or natural attenuation the levels of contamination could be significantly reduced. Four reactors were prepared to simulate different conditions for degradation. (Figure 3) The first reactor was a control reactor. The control group provides a basis for comparison throughout the experimental run. The control reactor did not have nutrient addition and it was not aerated on a regular basis. However, the control reactor had a constant moisture content that was monitored on a regular basis. Water was added as needed throughout the experimental run to maintain the moisture content at 25%. The setup for this phase can be seen in 18.

The second reactor contained nutrients and was aerated on a regular basis. The nutrients that were added were from a commercial fertilizer. The amount of nutrients added was determined based on a Carbon:Nitrogen:Phosphorous ratio (C:N:P) of 100:10:1 by eight. Again, the moisture content in this reactor was maintained at a constant level consistent to that of the natural environment from which it was taken (approximately 25%).
The reactor was aerated twice a week for 30 seconds at a time. This reactor will be referred to as the aerobic low mix reactor throughout the remainder of the paper.

The third reactor also contained nutrients and was aerated. The aeration occurred more frequently than in the second reactor. This reactor was aerated four times a week for 1 to 2 minutes at a time. Once again, the moisture content was maintained at 25% to simulate the in-situ condition.

The fourth and final reactor in this phase was an anoxic reactor. In the anoxic reactor, nutrients were added based on the C:N:P by weight. This reactor did not have an
aeration period. It should be noted that some oxygen was introduced into the reactor, during the sampling process. The moisture content was maintained at 25%.

![Figure 3: Setup for Phase One of the Study](image)

The results of the reactor tests are presented in the results portion of this paper. All reactors illustrated a decrease in TPH. This information lead to phase two of the study and an attempt to quantifiy the biological activity.

### 3.4 Phase Two:

The second phase of the study focused on determining the amount of biological activity that is occurring within the reactors. The four reactors described in phase one were again set up utilizing the same procedure described in the preceding section.
The reactors that were set up were then connected to a compressed air supply from one side and a series of traps designed to capture off gases from each reactor on the opposite side. The compressed air was filtered through a Cole-Parmer air filter to remove any moisture or residual oil that may be present in the line. The air was then fed through a Cole-Parmer flowmeter that allows one air source to be split between four samples. The flowmeter also allows for the regulation of the amount of air flowing through the system. The set up of one train of the experiment can be seen in Figures 4 and 5. Each reactor has an air source that enters through one side and is bubbled through a soil sample where the excess air and the off gases produced are released through the other side of the reactor. The flow then enters a series of four traps designed to capture the off gases produced in the soil. The first trap is a water trap followed by two methanol traps and another water trap. This setup was designed to capture any volatile compounds that may be given off through the aeration of the soil samples.

The purpose of phase two was to determine the fraction of removal that is associated with biological decomposition and the fraction that is associated with the chemical decomposition. Knowing the amount of volatilization that occurred and performing a mass balance, the amount of biodegradation occurring can be quantified.
Figure 4: Schematic of the Setup for Phase Two
3.5 Phase Three:

Phase three was once again a method for quantifying the amount of biodegradation occurring in the soil sample. The soil samples were prepared utilizing the same method as in phase one and phase two. Phase three, unlike phase two, utilized only one off gas trap. The reactors were attached to the compressed air line and air was introduced into the soil sample. The off gases produced would then exit the reactor and enter the methanol trap. Once again a mass balance would be used to determine the fraction of biodegradation occurring in the samples.
Another difference between the second phase of this study and the third phase is the addition of a fifth reactor. This additional reactor contained microbes that are believed to degrade petroleum products.

The microbes were obtained from a washwater tank at Wyman Gordon. It had been observed in the preceding months that the oil content of the washwater being held in these tanks was steadily decreasing. The reason for this was thought to be microbial degradation. A sample of this water was brought to the laboratory for inoculation into a soil sample. Prior to its addition, the washwater was tested for pH to determine if its addition would affect the balance of the system. With a pH of 8.0 it was within the acceptable range for biodegradation in soil to occur.

Due to the fact that it is known there are petroleum hydrocarbons present in the washwater, it was important to know the amount of TPH introduced into the system so an accurate analysis can be done. It is believed that these microbes will aid in the degradation of the TPH present and that the amount of TPH introduced via the inoculation will be less than the amount of the reduction in TPH observed.
Figure 6: Schematic of Setup for Phase Three

Figure 7: Actual Setup for Phase Three
Chapter 4: Results

4.1 *Introduction:*

The experiments outlined in the experimental procedure portion of this report yielded a number of results. The results are significant in the determination of feasibility of biodegradation in a highly organic soil. The results from all three phases are presented in this section of the report.

4.2 *Preliminary Results:*

The purpose of these results was to determine the amount of and the type of contamination present. The type of contaminant present will dictate the remediation method chosen. Also done in the preliminary portion of this study was the determination of the organic content of the soil. This is important because if the organic content of the soil is high, the options for remediation that are available are limited.

Samples were taken from various locations around the site (Figure 2) and then separated into six samples. Once the samples were segregated based on location and depth, the next step in the preliminary procedure was to determine the organic content of the soil. Each of the six samples that were sent for analysis was utilized to determine an
average organic content for the soil. Table 1 illustrates the results of the organic carbon testing.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Organic Content</th>
</tr>
</thead>
<tbody>
<tr>
<td>Top Soil</td>
<td>96%</td>
</tr>
<tr>
<td>SS</td>
<td>98%</td>
</tr>
<tr>
<td>Saturated Soil Location 1</td>
<td>95%</td>
</tr>
<tr>
<td>Saturated Soil Location 2</td>
<td>93%</td>
</tr>
<tr>
<td>Saturated Soil Location 3</td>
<td>98%</td>
</tr>
<tr>
<td>Saturated Soil Location 4</td>
<td>96%</td>
</tr>
<tr>
<td>Average</td>
<td>96%</td>
</tr>
</tbody>
</table>

Table 1  Organic Carbon Content

The high organic carbon content limits the remediation options. It indicates that biodegradation may be the best means of remediation for the site.

The six samples that were sent for analysis were analyzed utilizing the hydrocarbon fingerprint GC/FID or 8100M test. (Appendix A)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Concentration TPH (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Top Soil</td>
<td>302000</td>
</tr>
<tr>
<td>SS</td>
<td>717000</td>
</tr>
<tr>
<td>Saturated Soil Location 1</td>
<td>789000</td>
</tr>
<tr>
<td>Saturated Soil Location 2</td>
<td>6520</td>
</tr>
<tr>
<td>Saturated Soil Location 3</td>
<td>190000</td>
</tr>
<tr>
<td>Saturated Soil Location 4</td>
<td>19700</td>
</tr>
<tr>
<td>Average</td>
<td>114700</td>
</tr>
</tbody>
</table>

Table 2  Concentration of TPH

The high levels of TPH indicate that a massive clean-up effort is needed at the site. Any remediation technology that could be applied will have a long remediation time and may be relatively expensive. With these concentrations in mind, phase one of the study was designed.
4.3 Phase One Results:

Phase one was designed to determine if through agitation and the addition of nutrient, a significant reduction in contamination could be observed. As discussed in the Experimental Procedure portion of this report, phase one consisted of four reactors and three experimental conditions: a control reactor, a nutrient addition high mix reactor, a nutrient addition low mix reactor and an anoxic reactor. While gathering the samples in the field, it was apparent that there were gases trapped below the topsoil. When the topsoil was penetrated, odoriferous gases were emitted. This indicates that below the surface volatiles are being stripped from the soil and there is the possibility that biodegradation is occurring naturally.

Phase one was conducted over a 60-day period. Samples were taken to determine the TPH concentrations as well as if there were any VOCs present in the soil. The VOC sampling was done on days 1, 3, and 5. TPH sampling was conducted on days 1, 3, 5, 10, 20, 30 and 60.

VOC analysis was performed on all four reactors. The samples were analyzed using GC and EPA Method 8260. A complete list of the compounds that the samples were analyzed for can be located in Appendix A. Some of the chemicals tested for include: Ethyl Benzene, Vinyl Chloride, TCE and Naphthalene. The method reporting level (MRL) for the majority of the compounds tested for is 42 parts per billion (ppb). No VOCs were detected above the MRL. (Appendix B)
The samples were also analyzed for TPH. The Results can be seen in Table 3 and in Figure 8. The graphical representation illustrates the decline in contamination over the 60-day period. Figure 8 shows a decrease in contamination in all four reactors. The reduction of contamination can be seen in Table 4.

<table>
<thead>
<tr>
<th>Reactor</th>
<th>% Removal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>76.4</td>
</tr>
<tr>
<td>High Mix Frequency</td>
<td>88.9</td>
</tr>
<tr>
<td>Low Mix Frequency</td>
<td>88.8</td>
</tr>
<tr>
<td>Anoxic</td>
<td>81.1</td>
</tr>
</tbody>
</table>

Table 3 Percentage Removal in Phase One
<table>
<thead>
<tr>
<th>Sample</th>
<th>Concentration TPH (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial</td>
<td>79600</td>
</tr>
<tr>
<td>Day 1 Control</td>
<td>47800</td>
</tr>
<tr>
<td>Day 1 High Mixing Frequency</td>
<td>81800</td>
</tr>
<tr>
<td>Day 1 Low Mixing Frequency</td>
<td>51100</td>
</tr>
<tr>
<td>Day 1 Anoxic</td>
<td>36800</td>
</tr>
<tr>
<td>Day 3 Control</td>
<td>85500</td>
</tr>
<tr>
<td>Day 3 High Mixing Frequency</td>
<td>65700</td>
</tr>
<tr>
<td>Day 3 Low Mixing Frequency</td>
<td>70800</td>
</tr>
<tr>
<td>Day 3 Anoxic</td>
<td>53400</td>
</tr>
<tr>
<td>Day 5 Control</td>
<td>71000</td>
</tr>
<tr>
<td>Day 5 High Mixing Frequency</td>
<td>44200</td>
</tr>
<tr>
<td>Day 5 Low Mixing Frequency</td>
<td>53200</td>
</tr>
<tr>
<td>Day 5 Anoxic</td>
<td>50700</td>
</tr>
<tr>
<td>Day 10 Control</td>
<td>76900</td>
</tr>
<tr>
<td>Day 10 High Mixing Frequency</td>
<td>77500</td>
</tr>
<tr>
<td>Day 10 Low Mixing Frequency</td>
<td>66200</td>
</tr>
<tr>
<td>Day 10 Anoxic</td>
<td>44300</td>
</tr>
<tr>
<td>Day 20 Control</td>
<td>15800</td>
</tr>
<tr>
<td>Day 20 High Mixing Frequency</td>
<td>10700</td>
</tr>
<tr>
<td>Day 20 Low Mixing Frequency</td>
<td>11300</td>
</tr>
<tr>
<td>Day 20 Anoxic</td>
<td>13700</td>
</tr>
<tr>
<td>Day 30 Control</td>
<td>11300</td>
</tr>
<tr>
<td>Day 30 High Mixing Frequency</td>
<td>8240</td>
</tr>
<tr>
<td>Day 30 Low Mixing Frequency</td>
<td>8440</td>
</tr>
<tr>
<td>Day 30 Anoxic</td>
<td>10100</td>
</tr>
<tr>
<td>Day 60 Control</td>
<td>16300</td>
</tr>
<tr>
<td>Day 60 High Mixing Frequency</td>
<td>9050</td>
</tr>
<tr>
<td>Day 60 Low Mixing Frequency</td>
<td>7930</td>
</tr>
<tr>
<td>Day 60 Anoxic</td>
<td>12200</td>
</tr>
</tbody>
</table>

Table 4  TPH Concentrations for Phase One
Figure 8: Graphical Representation of TPH Concentrations for Phase One
the reductions observed in phase one of the study were encouraging. The reduction in TPH indicates that biodegradation may be occurring in the soil. However, the fact that a significant decrease was observed in the control reactor, indicates that the reduction may be due to other factors. Therefore, the second phase of the study will be focused on quantifying the biological reduction of contamination.

4.4 Phase Two Results:

Phase Two focused on determining the portion of remediation that can be attributed to biological activity. Because, phase one illustrated a uniform drop in concentration in all reactors, biological activity may not be the sole cause of degradation in the soil. To determine if biological activity was responsible for the reduction, an apparatus was designed to bubble air through the soil sample and collect any gases stripped from the soil. (Figure 4 and Figure 5) Using this information and the amount of degradation observed in the same time period, a mass balance can be performed for the soil. This will aid in determining the fraction of remediation associated with biological activity.

Phase two, did not provide the anticipated results. It is believed, based upon field observations, that there are VOCs present in the soil. However, neither phase one nor phase two detected volatiles in the samples. The volatile testing in phase two was on a smaller scale than phase one, BETX were the only volatiles tested for. If there was a detection of BTEX in the samples then a more comprehensive analysis would be done. BTEX were not observed in any of the samples. (Appendix C)
When dealing with an actual site, often times the data gathered is inconclusive. The soil in question is heterogeneous and anisotropic in nature. Due to these characteristics the data may not steadily decrease. Small jumps are observed in the phase one numbers, however, phase two does not illustrate any specific trends. (Figure 9) The lack of a specific trend makes it difficult to determine if there is an actual decrease in the contamination observed. Table 5 shows the actual data obtained. Note, that there as observed in Figure 9 there is no distinct downward trend in the reactors. However, about a 50% reduction occurred during the first 10 days when compared to the original samples.

<table>
<thead>
<tr>
<th>Sample</th>
<th>TPH Concentration (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial</td>
<td>345000</td>
</tr>
<tr>
<td>Day 1 Control</td>
<td>273000</td>
</tr>
<tr>
<td>Day 1 High Mixing Frequency</td>
<td>179000</td>
</tr>
<tr>
<td>Day 1 Low Mixing Frequency</td>
<td>151000</td>
</tr>
<tr>
<td>Day 1 Anoxic</td>
<td>131000</td>
</tr>
<tr>
<td>Day 3 Control</td>
<td>248000</td>
</tr>
<tr>
<td>Day 3 High Mixing Frequency</td>
<td>188000</td>
</tr>
<tr>
<td>Day 3 Low Mixing Frequency</td>
<td>198000</td>
</tr>
<tr>
<td>Day 3 Anoxic</td>
<td>141000</td>
</tr>
<tr>
<td>Day 5 Control</td>
<td>234000</td>
</tr>
<tr>
<td>Day 5 High Mixing Frequency</td>
<td>219000</td>
</tr>
<tr>
<td>Day 5 Low Mixing Frequency</td>
<td>169000</td>
</tr>
<tr>
<td>Day 5 Anoxic</td>
<td>192000</td>
</tr>
<tr>
<td>Day 10 Control</td>
<td>195000</td>
</tr>
<tr>
<td>Day 10 High Mixing Frequency</td>
<td>178000</td>
</tr>
<tr>
<td>Day 10 Low Mixing Frequency</td>
<td>221000</td>
</tr>
<tr>
<td>Day 10 Anoxic</td>
<td>147000</td>
</tr>
</tbody>
</table>

Table 5  TPH Concentrations Phase Two

VOC data is desirable to determine the amount of biological activity associated with reduction in contamination levels observed. This leads into phase three of the study.
Figure 9: Phase Two TPH Concentrations
4.5 Phase Three Results:

Phase three of this study again was aimed at determining the percentage of remediation associated with biological activity. Again the samples were analyzed for VOCs as well as TPH levels. The samples were collected at the same intervals as in phases one and two.

VOCs were collected via the procedure outlined in the Chapter 3. Methanol was used as a trap for the soil off gases. The samples were sent to the lab and analyzed using GC. The TPH analysis was similar to that observed in phase two. There was no distinct trend present in the data. The data obtained can be see in Appendix D. The results are presented in graphical form in Figure 10. The results can also be seen in Table 6.

<table>
<thead>
<tr>
<th>Sample</th>
<th>TPH Level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial</td>
<td>72500</td>
</tr>
<tr>
<td>Day 1 Control</td>
<td>80300</td>
</tr>
<tr>
<td>Day 1 High Mixing Frequency</td>
<td>148000</td>
</tr>
<tr>
<td>Day 1 Low Mixing Frequency</td>
<td>45500</td>
</tr>
<tr>
<td>Day 1 Anoxic</td>
<td>102000</td>
</tr>
<tr>
<td>Day 1 Microbe Addition</td>
<td>88800</td>
</tr>
<tr>
<td>Day 3 Control</td>
<td>144000</td>
</tr>
<tr>
<td>Day 3 High Mixing Frequency</td>
<td>239000</td>
</tr>
<tr>
<td>Day 3 Low Mixing Frequency</td>
<td>154000</td>
</tr>
<tr>
<td>Day 3 Anoxic</td>
<td>173000</td>
</tr>
<tr>
<td>Day 3 Microbe Addition</td>
<td>87000</td>
</tr>
<tr>
<td>Day 5 Control</td>
<td>132000</td>
</tr>
<tr>
<td>Day 5 High Mixing Frequency</td>
<td>152000</td>
</tr>
<tr>
<td>Day 5 Low Mixing Frequency</td>
<td>141000</td>
</tr>
<tr>
<td>Day 5 Anoxic</td>
<td>151000</td>
</tr>
<tr>
<td>Day 5 Microbe Addition</td>
<td>194000</td>
</tr>
</tbody>
</table>

Table 6 TPH Levels Phase Three
Figure 10: TPH Levels Phase Three
The VOCs were also tested at the same intervals as the TPH levels. The results can be seen in Table 7. Several VOCs were observed in phase three of the study. The VOCs observed were not byproducts of the breakdown of petroleum hydrocarbons, but were chlorinated compounds. This fact substantiates the assumption that the reduction of TPH observed in the soil is due to microbial degradation of the compounds.

The VOCs were not observed in the previous phases of the study. However, it is believed that these compounds were present and just not detected. The significance of these compounds will be discussed in the Discussion and Conclusion portion of this report. Table 7 contains the amount of some of the VOCs observed. The focus of the discussion will be on the chloromethane and the n-butanol (Methylethyl ketone), because the amount of the compounds observed were an order of magnitude higher for these compounds.

Based upon the concentrations observed, the amount of butanol present in the soil has been estimated to be 0.958 g. While the amount of chloromethane present can be estimated to be 17.69 g. Calculations for both concentrations can be found in Appendix E. These large concentrations are indicative of a larger problem in the site.
<table>
<thead>
<tr>
<th>Sample</th>
<th>1,2,4-Trimethylbenzene</th>
<th>1,3,5-Trimethylbenzene</th>
<th>2-Butanone</th>
<th>Chloro-methane</th>
<th>Isopropyl-benzene</th>
<th>n-butylbenzene</th>
<th>1,2-Dichlorethane</th>
<th>Toluene</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 1 Control</td>
<td>ND</td>
<td>ND</td>
<td>4750</td>
<td>1930</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Day 1 High</td>
<td>109</td>
<td>ND</td>
<td>8930</td>
<td>7100</td>
<td>ND</td>
<td>ND</td>
<td>104</td>
<td>ND</td>
</tr>
<tr>
<td>Day 1 Low</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Day 1 Anoxic</td>
<td>2360</td>
<td>754</td>
<td>10300</td>
<td>120000</td>
<td>207</td>
<td>794</td>
<td>ND</td>
<td>134</td>
</tr>
<tr>
<td>Day 1 Microbe</td>
<td>208</td>
<td>ND</td>
<td>10100</td>
<td>139000</td>
<td>ND</td>
<td>135</td>
<td>122</td>
<td>ND</td>
</tr>
<tr>
<td>Day 3 Control</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>548</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Day 3 High</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Day 3 Low</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Day 3 Anoxic</td>
<td>640</td>
<td>183</td>
<td>11300</td>
<td>5270</td>
<td>107</td>
<td>296</td>
<td>142</td>
<td>ND</td>
</tr>
<tr>
<td>Day 3 Microbe</td>
<td>264</td>
<td>ND</td>
<td>9610</td>
<td>36400</td>
<td>ND</td>
<td>140</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Day 5 Control</td>
<td>ND</td>
<td>187</td>
<td>7760</td>
<td>367</td>
<td>111</td>
<td>289</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Day 5 High</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Day 5 Low</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Day 5 Anoxic</td>
<td>868</td>
<td>254</td>
<td>9810</td>
<td>24600</td>
<td>121</td>
<td>346</td>
<td>126</td>
<td>ND</td>
</tr>
<tr>
<td>Day 5 Microbe</td>
<td>456</td>
<td>130</td>
<td>8980</td>
<td>9430</td>
<td>103</td>
<td>157</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

* Concentrations are in µg/L

Table 7  VOCs Observed in Phase Three
Chapter 5: Discussion and Conclusion

5.1 Introduction

The experiments conducted throughout the course of this study, provided many encouraging and interesting results. In general the results illustrated that microbial activity is responsible for the majority of the decrease in the TPH observed in all three phases. Through the following discussion of the results, the affect of biological activity in the soil in regards to the decrease in TPH levels observed will be illustrated.

5.2 TPH Reduction

A reduction in TPH can be observed in all phases of the experiment to some extent. The contamination observed in the site is present in high concentrations. The TPH levels observed in the preliminary stages of the study were in excess of 700,000 parts per million (ppm). This level of TPH indicates a large contamination problem on the site. However, the concentrations that were observed on the end of the site farthest from the inlet, were much lower than those concentrations near the inlet location. (Figure 2). These concentration levels indicate that the contamination is moving very slowly across the wetland. This distribution of contaminant indicates that the petroleum is virtually contained and therefore remediation of the site is possible.

The first phase of the experimentation had an initial concentration of approximately 80,000-ppm. The four reactors showed a decrease in TPH levels to
approximately 8,000 ppm. This concentration is still far above the state acceptable level, but shows a decrease of around 88% in TPH. These reductions were observed in all four reactors. The control reactor decreased in a similar manner to the three experimental conditions. It should be noted that the control reactor did experience slight agitation when moisture was added to maintain the in-situ moisture content. This agitation would introduce more oxygen to the system. An increase in oxygen allows for stimulation to the microbial population and allows for the population to grow.

Phases two and three of the study showed a slight decrease in levels of TPH followed by a marked increase in TPH levels, which was again followed by a decrease in TPH concentrations observed. The increase after the initial decrease implies biological activity. One possibility for the observed increases is that microbial populations are freeing sorbed contaminants from the soil. The organic nature of the soil makes it feasible that a large amount of contamination is sorbed tightly to the soil. The sorbed contamination would not be detected in the samples analyzed by the laboratory due to the fact that the extraction methods are not sufficient to break the bond between the contamination and the soil.

The decreases in TPH that were observed were significant. As previously stated approximately 88% of the TPH were removed in a 60 day period. This indicates that biodegradation appears to be a viable means of reduction for the contamination. The issue at hand after the observation of TPH reduction was quantifying the amount of the reduction that may be associated with biological activity within the soil. The quantification of microbial activity was down though the analysis of VOCs collected as off gas from the soil.
5.3 VOC Findings

During all three phases of the study analysis for VOCs were conducted. Phase one VOC analysis was conducted upon the soil itself. No VOCs were observed during this analysis. The lack of VOCs observed was contradictory to field observations made. Phase two of the study was designed to gather the off gases of the soil to aid in the determination of the biological impacts on the soil.

The set up of phase two, consisted of a series of four traps designed to capture the off gases produced in the soil. Air was fed through the system to strip the volatile compounds from the soil. The amounts of off gas produced that are petroleum in nature will account for the percent of remediation that would not be attributed to biological activities. The results of this portion of the study did not provide information on VOCs present in the soil. This could be due to several factors including: leaks in the system, too fast of airflow through the system or too large of air bubbles being introduced to the traps.

If a leak, or multiple leaks, was present in the system then the off gases may have escaped prior to their collection. High airflows through the system may not allow sufficient time for the off gases to be dissolved into the traps. The large air bubbles may also not have allowed for dissolution into the traps. The belief that VOCs existed in the system led to phase three and another attempt at quantifying the percent of removal that can be associated with biological activity.
Phase three of the study revealed several VOCs in the methanol traps. These VOCs were not byproducts of the breakdown of petroleum products, but rather chlorinated compounds. The majority of these compounds were present in quantities around 100 to 500 ppm. Two of the compounds, however, were present in much greater quantities. Butanone (MEK) and chloromethane were present in concentrations of one and two orders of magnitude greater respectively. Due to the fact that no petroleum byproducts were detected in the methanol traps, the reduction seen in all three phases may be attributed to biological activity.

The presence of chlorinated compounds in the soil, indicates a much larger problem than first thought. While biological processes may be able to degrade the petroleum product, the removal of the chlorinated compounds will be much more difficult. Work is currently being done on the use of microbes for the remediation of chlorinated contaminated soil, but they require different microbes from the microbes present to degrade the petroleum products. More investigation will need to be done to characterize the type and concentrations of the chlorinated compounds present on the site. Then a determination of remediation methods and time required for remediation may be made.

5.4 Conclusion

There is a distinct decrease in the concentration of TPH observed. Based upon the three phases of the experimentation, biological activity is the primary means of degradation for the system. All of the setups demonstrated a removal of TPH. It is believed, based upon the data collected, that the aeration of system aided in the remediation of the soil. Reductions were observed in the control situation as well as the
experimental conditions. The fact that there were large quantities of product still present after 30 years of discontinued use, yet a reduction occurred in the control reactor when air was introduced, implies that through the introduction of oxygen the microbial populations flourish.

Biodegradation appears to be a very viable solution to the problem of the TPH contamination in the soil. Other possible methods for remediation include excavating the soil and drying it thoroughly, then removing it from the site. To accomplish this a wall will need to be constructed around the wetland to prevent groundwater flow, and then the contaminated water may be pumped and treated. A newly constructed wetland may then be put into place.

More investigation into the chlorinated products present in the soil will need to be done to determine a remediation approach to this portion of the contamination problem. Biodegradation is an inexpensive and effective means for treating the TPH contamination in the soil.

Further study should be done to determine the concentrations of the chlorinated compounds present and means of remediation. The petroleum contamination should also be further investigated, including research into other means of remediation for the sight as well as cost and time estimates for bioremediation. Other possible areas of research would include cultivation of the bacteria present in the soil as well as further efforts to quantify the reduction in petroleum products that can be associated with biological activity.
Appendix A: LABORATORY DATA PRELIMINARY STUDY

Appendix B: LABORATORY DATA PHASE ONE

Appendix C: LABORATORY DATA PHASE TWO

Appendix D: LABORATORY DATA PHASE THREE

To be added at a later date…
Appendix E

Sample Calculation

Determination of amount of Chlorinated Compounds present in the wetland:
Area of wetland = 1 acre
Depth of wetland= 2 ft

Volume = 2 acre-ft Convert to metric = 2537.86 m³ or 2.54 L

High Concentration of Butanone = 11,300 µg/L
High Concentration of Chloromethane = 139,000 µg/L

Sample size sent for analysis = 10 ml

Amount of methanol present in Trap for Butanone = 20 ml
Amount of methanol present in Trap for Chloromethane = 30 ml

Concentration:

\[
\left[ \frac{observed \ Concentration}{Concentration} \right] \times \left[ \frac{Sample}{Size} \right] = Concentration
\]

\[
[11,300 \, \mu g/L] \times [20 \, ml] = 226 \, \mu g \, (butanone)
\]

\[
[139,000 \, \mu g/L] \times [30 \, ml] = 4170 \, \mu g \, (chloromethane)
\]

Wt. of Soil = 835 g

Concentration in Soil = 0.27 µg/g (butanone), 4.99 µg/g (chloromethane)

unit wt = wt/volume = 835 g/0.5 L = 1670 g/L

total wt = unit wt x volume = 4241.8 g in the site

Total in site = concentration * wt of soil = 226 * 4241.8 = 958646 µg = 0.958 g
Total in site = concentration * wt of soil = 4170 * 4241.8 = 17688306 µg = 17.69 g
Bibliography


Environmental Protection Agency [online], www.epa.gov


Wyman-Gordon Company [online], www.wymangordon.com