MISSISSIPPI STATE UNIVERSITY

USING CHEMICAL PRIMING AS A MEANS OF ENHANCING THE PERFORMANCE OF BIOCELLS FOR TREATING PETROLEUM PRODUCTS CONTAINING RECALCITRANT CHEMICAL SPECIES

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16. Abstract

Biocell technology is a soil remediation technology that utilizes commercial roll-off dumpsters as simple, yet effective bioreactors. Reported implementation costs for biocells range from $20 - $40 per cubic yard treated. Biodegradation of some petroleum hydrocarbons has been found to be difficult in terms of both the rate and extent of removal. Since petroluem products are literally made up of numerous organic chemcials, some of these chemicals are difficult to degrade due to bacterial associated limitations (i.e. enyzme reactivity toward targeted chemicals or cell permeability hinderances) and/or adsorption onto soil particles, which greatly limits the bioavailability of the pollutants to the cells. Chemical priming involves the addition of powerful chemical oxidizers, such as ozone and/or hydrogen peroxide, to chemically transform the targeted chemicals into more biodegradable compounds, plus the oxidizers attack the sorptive bonds between the soil and chemical(s) to increase bioavailability. Results from this study showed that the addition of chemical oxidizers, after a period when biodegradation rates approached zero, yet significant product remained in the soil, greatly enhanced the rate and extent of removal. The addition of the chemical oxidizer immediately reduced some of the petroleum in the soil via direct oxidation, plus restarting bioremediation after chemical priming also reduced a significant portion of the residual petroleum products in the soil samples. The major conclusion was that chemical priming indicated a high potential for enhancing the performance of bioremediation systems attempting to treat soils contaminated with petroleum products containing appreciable amounts of refractory chemicls.

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CHAPTER I

INTRODUCTION

Background

Petroleum Contamination

Man has dealt with the cleanup of petroleum products almost since the first day oil was discovered. Around the beginning of the 1990's, the spilling of more than 200,000 barrels of crude oil from the oil tanker Exxon Valdez in Prince William Sound, Alaska (Hagar, 1989), as well as smaller spills in Texas, Rhode Island, and the Delaware Bay (Anonymous, 1989), has recently refocused attention on the problem of hydrocarbon contamination in the environment.

In many countries, major oil spills come from storage tanks or from leaks in pipelines (Wardley, 1979). In the US, many private and government facilities are faced with the cleanup of petroleum spills. As of April 1995, over 287,000 underground storage tanks were confirmed to be leaking petroleum hydrocarbons into the surrounding environment (USEPA, 1995). The United States Environmental Protection Agency (USEPA) estimates that as many as 15 to 20% of the approximately 1.8 million regulated underground storage tank (UST) systems are either leaking or are expected to leak in the near future (Chen, 1995). Spills also occur during loading, discharging, and transporting of petroleum products by road and rail.

In most cases, the environmental impact of released wastes would be minimal if they continuously remained at the point of release; unfortunately, most wastes migrate from their release points to affect a wider area. Petroleum contamination most often is transported through groundwater along the local hydraulic gradient. This may raise more concern than the original spills, since over half of the potable water supply in the United States originates from groundwater (Brown, *et al*., 1985). In Mississippi, 90% of the public drinking water comes from groundwater sources (Burchell, 1996). Hence, prevention and treatment have to be carried out before further contamination of valuable groundwater resources takes place.

Another major contamination source is improper disposal. During the early stages of petroleum utilization, laws and regulations directed towards waste disposal were non-existent because of limited public concern over direct releases into the environment. Industries faced little pressure from the government or the general public to treat or dispose of the wastes they generated. Some petroleum industries simply excavated pits and used them as dumping grounds for petroleum wastes (Viraragpavan, *et al.,* 1998). Small businesses, even today, dump used oil onto the ground either because they can not afford sophisticated treatment or are ignorant of environmental regulations. Consequently, years of poor disposal practices have resulted in numerous contaminated sites.

Today, the effects of some industrial discharges and wastes, not only on people but also on the environment, have been realized and have become a cause for public concern. Since the late 1960's, Congress has enacted legislation which regulate many types of environmental

pollution. With this awareness of environmental impacts and promulgation of strict regulations, numerous sites are in need of cost-effective approaches to cleanup.

Environmental Health Aspects of Petroleum Contamination

The environmental impact of hydrocarbons varies considerably, because of their vastly different chemical structures. The toxicity of aromatic hydrocarbons is relatively high, while that of straight-chain paraffins is relatively low (Reis, 1996). In general, hydrocarbons stunt plant growth if the concentration in contaminated soil is above about 1% by weight (Wardley, 1979). Hydrocarbons can also impact higher organisms that may become exposed following an accidental release. Marine animals with hair or feathers that serve as insulation can die of hypothermia if coated with oil. Coated animals can also ingest fatal quantities of hydrocarbons during washing and grooming activities (Wardley, 1979).

One important group of petroleum hydrocarbons, which are particularly of concern by most parties, are polycyclic aromatic hydrocarbons (PAHs). PAHs, also known as polynuclear aromatic hydrocarbons or PNAs, are compounds of fused benzene rings in linear, angular, or cluster arrangements that contain only carbon and hydrogen. PAHs are formed during the pyrolysis of carbon- or petroleum-derived compounds at high temperatures (about 700EC) or other processes such as electrolysis with graphite electrodes, acetylene synthesis from natural gas, and refinery operations (Andelman and Snodgrass, 1974). As a result of this, PAHs have been detected in air, soil, and water matrices. Eleven of the forty PAHs listed by the National Academy of Sciences are classified as strongly carcinogenic or mutagenic, while another 10 are considered weakly carcinogenic and

mutagenic (NAS, 1972; Fouillet, *et al*., 1991). In 1980, EPA required that water quality be judged on the presence of 129 compounds. Sixteen of those were PAHs (Nowicki, *et al*, 1980). Also, some PAHs are known to be recalcitrant to biodegradation resulting in low removal efficiencies within traditional wastewater treatment processes (Herbes, *et al*., 1976). In general, the greater the number of rings, the more recalcitrant the PAH.

Current Treatment Alternatives for Petroleum Contamination

Commercial petroleum hydrocarbons are produced through distillation of crude oil. In general, the lighter fractions represent gasoline-range material. The intermediate or middle distilled fractions represent feedback for diesel, jet fuels, and "light" heating oils. The residuum in this process serves as heavy fuel oils or other non-fuel products (Nyer and Skladany, 1989; Potter, 1992).Table 1.1 presents some of the major commercial products associated with different distillation fractions. Because of their widespread usage, gasoline, diesel, and fuel oils are some of the most common petroleum products contaminating soils and groundwater (Nyer and Skladany, 1989).

The definitions of these three types of fuel products are (Hawley, 1981):

1. Gasoline is a mixture of volatile hydrocarbons suitable for use in internal combustion engines. The major chemical components of gasoline are branched chain paraffins (branches chain alkanes), cycloparaffins (cycloalkanes), and aromatics.
2. Diesel is Number 2 Fuel Oil, composed primarily of unbranched paraffins (straight chain alkanes) with a flash point between 110 and 190 EF (43 and 88EC).
3. Fuel oils are chemical mixtures having flash points greater than 100 EF (38EC). Fuel oils

can be distilled fractions of petroleum, residuum from refinery operations, crude petroleum, or a mixture of two or more of these materials.

Figure 1.1 shows gasoline, diesel, fuel oil, and other major petroleum hydrocarbon constituents as they would be separated using a gas chromatograph by increasing the boiling point.

Traditional Physicochemical Approaches

Traditional physicochemical techniques result in the abiotic destruction and/or separation of the chemicals from contaminated environmental media as their treatment mechanism. Most of the techniques that are well developed for use with petroleum hydrocarbons are either too costly or generate secondary waste streams which require further treatment. Typical treatment technologies used for remediation of petroleum hydrocarbon contaminated soil include thermal desorption, incineration, solvent extraction, and soil washing/soil flushing (Lighty, *et al.,* 1993).

Thermal desorption processes use transferred heat and pollutant volatility as the principal means to physically separate and transfer contaminants from soils, sediments, or other solids into the vapor phase. The contaminated material is excavated and delivered to the thermal desorber where water and the contaminants are volatilized due to the elevated heat. These processes typically operate at a temperature range of 350EF to 700EF (Hoeppel, *et al.*, 1991). Thermal desorbers are not used as stand-alone processes during remediation. Organics in the offgas have to be collected and recovered by condensation or adsorption or combusted in an afterburner. The selection of the gas treatment system depends on the

concentration of the contaminant, cleanup standards and regulations, and the economics of the offgas treatment system(s) employed (Lighty, *et al.*, 1993).

Incineration, also known as thermal destruction, is a well developed *ex situ* process that thermally destroys organic contaminants. Generally, incineration is a mature process which utilizes high temperatures, typically over 1800EF, in the presence of oxygen to oxidize organic compounds within contaminated solids or liquids (Long, 1993). Although, the destruction removal efficiency of a well designed incineration system can be in excess of 99.99%, the high cost (usually ranging from $300 to $1,500 per ton of soil treated) and possible secondary pollution caused by incomplete combustion or volatilization make the process difficult for acceptance by the general public (USEPA, 1996; Magee, *et al*., 1993; Long, 1993).

Solvent extraction is an *ex situ* separation and concentration process that uses non-aqueous liquid reagents to remove organic and/or inorganic contaminants from polluted soils, sediments, sludges, or water. As a separation process, solvent extraction does not destroy contaminants but produces a concentrated contaminated fraction, a treated solids fraction, and possibly, a wastewater stream. It may concentrate contaminants by a factor as high as 10,000:1 (Donnelly, *et al*.,1994). However, this concentrated portion is usually treated using incineration or other treatment methods depending on the type and concentration of contaminant(s). The treated solids fraction and wastewater may also contain residues of the contaminant and extraction fluid. Depending on the site cleanup requirements, further treatment of these fractions may have to be carried out. Contaminant removal efficiencies and levels of reduction vary depending on number of extraction stages,

type, concentration of contaminants present, and the nature of the medium to be treated. Costs for treating a ton of contaminated soil range from $95 to $700 (Donnelly, *et al.*, 1994).

Soil washing is an *ex situ* process that utilizes water-based solutions to extract and separate contaminants from soil. Contaminants are removed through abrasive scouring and scrubbing action using a washwater that is sometimes augmented by surfactants or other agents (i.e. alcohols). It is usually used as a pretreatment process followed by further treatment using thermal desorption, incineration, or bioremediation as required (Mann, *et al.*, 1994). Soil flushing is an *in situ* process which involves the injection of water, enhanced water, or gaseous mixtures to accelerate the mobilization of contaminants from subsurface soil (Mann, *et al*., 1994). Due to the slow rate of liquid phase diffusion into soil particles, this process may be time-consuming (Raws, 1996). When dealing with aged contaminaed soils, soil flushing will be less effective, because the chemicals can be relatively insoluble or tightly bounded to the soil particles (Mann, *et al*., 1994).

Bioremediation of Petroleum Hydrocarbons

Bioremediation employs microorganisms to biologically degrade organic contaminants present in various contaminated media. It is a natural process, where the end-products of contaminant oxidation are typically CO_2, H_2O, organic by-products, and additional biomass. Given this natural mechanism, bioremediation is easily accepted by the public as an environmentally friendly alternative.

It is well established that microorganisms are capable of degrading a wide variety of compounds, including aliphatic, aromatic, PAHs (Singer and Finnerty, 1984; Certiglia, 1984;

Zappi, *et al.*, 1996), chlorinated aliphatic hydrocarbons, such as TCE (Fogel, *et al*., 1986), explosives (Zappi, *et al.,* 1995), and chlorinated aromatics, such as PCP and PCBs (Valo, *et al.,* 1986; Focht and Brunner, 1985). Most field applications of bioremediation technologies have been directed toward soil that is contaminated with petroleum hydrocarbons (Autry and Ellis, 1992). The biodegradation potential of hydrocarbon compounds have been successfully tested in the laboratory using pure and mixed cultures (Singer and Finnerty, 1984; Watkinson and Morgan, 1990; Jing, 1998) and using full-scale field remediation units(Autry, *et al.*, 1991; Glaser, 1991). There are a number of engineered treatment systems available for bioremediation of petroleum products, such as, landfarming, biopiles, bioslurry, composting, and *in situ* bioremediation (Baker and Herson, 1994).

Bioremediation processes are typically more economical than traditional physical/chemical methods. It is estimated that bioremediation generally costs one-third to one-half that of incineration (Gabriel, 1991; Bollag and Bollag, 1995). Several factors contribute to the economical aspect of bioremediation (Block, *et al*., 1993):

1. Low capital and operating costs
2. Minimal specialized equipment requirements
3. Availability of trained contractors to implement the technology

Since the mid-1980s, bioremediation has been used at more than 100 locations to cost-effectively remediate hundreds of thousands of cubic yards of contaminated soil (Block, *et al.* 1993). The USEPA strongly believes in the future of bioremediation. To further the

utilization of innovative treatment technologies, the USEPA has created the Technology Innovation Office in 1990 to encourage studies on these technologies (King, et al., 1992). Bioremediation is considered by this group to be one of the most promising technologies for the remediation of contaminated environmental media.

Despite the positive performance of bioremediation, there are limitations to this process that prevent it from being used at some sites. First of all, bioremediation can be time-consuming. The speed of bioremediation is often measured in terms of half-lives of the hydrocarbon (i.e., the time for half of the hydrocarbon mass to be biologically degraded). Typical degradation half-lives range from a few days for low-molecular-weight compounds to a number of years for complex compounds, such as multi-ringed PAHs (American Petroleum Institute, 1984). Half lives for different kinds of fuel oils have been reported to range from less than 30 days to 57 weeks (Song, *et al.,* 1990; Loehr, *et al.,* 1992; Whiteside, 1993; McMillen, *et al.,* 1993; Martinson, *et al.,* 1993; Zappi, *et al.,*1996). Where time is an important criteria associated with cost, bioremediation may not be a preferable alternative at these sites.

Secondly, microorganisms typically utilize chemicals that are relatively water-soluble as food sources. Those chemicals that have low solubility in water, such as high boiling point petroleum products and PAHs, can be inaccessible to microorganisms. Also, in soil systems, the complex pore structure and fluid transport pathways of soil can make remediation difficult. Most hydrocarbons are trapped by capillary pressure and adsorption within the

pores of the soil and not available at soil-water interface (Reis, 1996). Adsorption of hydrocarbons onto natural organic matter (NOM) positioned on soil particle surfaces result in the formation of a bound fraction, also known as residue (Novak, *et al.,* 1995). This portion of the contamination may not be recoverable even with the exhaustive extraction methods (Kaufmam, *et al.,* 1976; Khan, 1982; 1991). Hence, microorganisms cannot utilize and degrade these adsorbed contaminants. Several studies support the concept that biological degradation is limited by the inaccessibility of adsorbed fractions of contaminants to microorganisms (Bailey and White, 1970; Meuler, *et al.,* 1992; Scow and Hutson, 1992; Novak, *et al.,* 1995). Because of these limitations, bioremediation may not be able to achieve the treatment goals for cleanup of the soil contamination. Enhancement strategies have to be carried out to increase the biodegradability and bioavailability of the contaminants.

Chemical Oxidation Enhanced Bioremediation

Advanced oxidation processes (AOPs) are defined as chemical oxidation processes which generate hydroxyl radicals in sufficient quantity to affect waste treatment (Glaze, 1987). These processes have the ability to rapidly oxidize recalcitrant compounds and convert them to potentially less toxic and more readily biodegradable intermediate products (Huang, *et al.,* 1993). AOPs have been successfully used for treating chlorinated solvents, PCBs, and other bio-recalcitrant chemicals. Recent research on advanced oxidation processes indicates that chemical constituents of fuels (i.e. PAHs and BTEX) are very

reactive to the hydroxyl radical and somewhat reactive to direct oxidation by ozone and other oxidants (Nelson and Brown, 1994). Oxidation products of most organic compounds are usually hydroxylated products, aldehydes, ketones, carboxylic acids, carbon dioxide, and water (Adams and Randke, 1992; Trapido, *et al.*, 1994; Zappi, *et al.*, 1995; Yao, *et al.*, 1996). These intermediates are usually more water-soluble (and biodegradable) than the parent compounds. Plus, oxidants can react with the sorption bond between the contaminant and soil NOM, thus increasing the bioavailability of the contaminants.

Figure 1.2 illustrates how oxidants attack both soil sorption sites and the recalcitrant compounds themselves. This figure serves as a summary of the proposed process which is referred to herein as chemical priming. The by-products of the reaction are likely to have higher biodegradation potential and bioavailability. Chemical oxidation usually excels in the oxidation and net cleavage of aromatic and cyclic chemical rings, while it typically becomes rate limiting for the subsequent oxidation of the straight chained by-products of ring cleavage (Hoigne and Bader, 1983). Bioremediation, on the other hand, often is effective at the mineralization of straight-chained chemical (Dragun 1988), but rate-limiting with the degradation of high molecular weight branched hydrocarbons and polycyclic aromatic structures. These two processes seem to be very complimentary of each other. Therefore, the combination of chemical oxidation processes and bioremediation may provide an optimistic result toward achieving a cost-effective alternative of soil decontamination.

Research Objectives

The primary objective of this investigation is to examine the feasibility of using chemical priming as an enhancement to the bioremediation of petroleum contaminated soils. The specific objectives were to:

1. Demonstrate the feasibility of using chemical oxidation processes to enhance the biotreatment potential of two soils having different types and levels of TPH contamination.
2. Evaluate various candidate oxidation strategies for use as chemical priming steps.
3. Compare the affects of chemical and/or biological oxidations on treating the two soil specimens.
4. Formulate implementation strategies to successfully integrate bioremediation and chemical priming.

Table 1.1

Petroleum Distillation Products*

Fraction	Distillation Temperature, EC	Carbon Number
Gas	Below 20	C-1 to C-4
Petroleum ether	20 to 60	C-5 to C-6
Ligroin (light Naphtha)	60 to 100	C-6 and C-7
Natural Gasoline	40 to 205	C-5 to C-10 and cycloalkanes
Kerosene	175 to 325	C-12 to C-18 and aromatics
Gas Oil	Above 275	C-12 and higher
Lubricating Oil	Non-volatile liquids	Probably long chains attached to cyclic compounds
Asphalt or Petroleum Coke	Non-volatile solids	Polycyclic structures

*Adapted from Morrison and Boyd, 1973.

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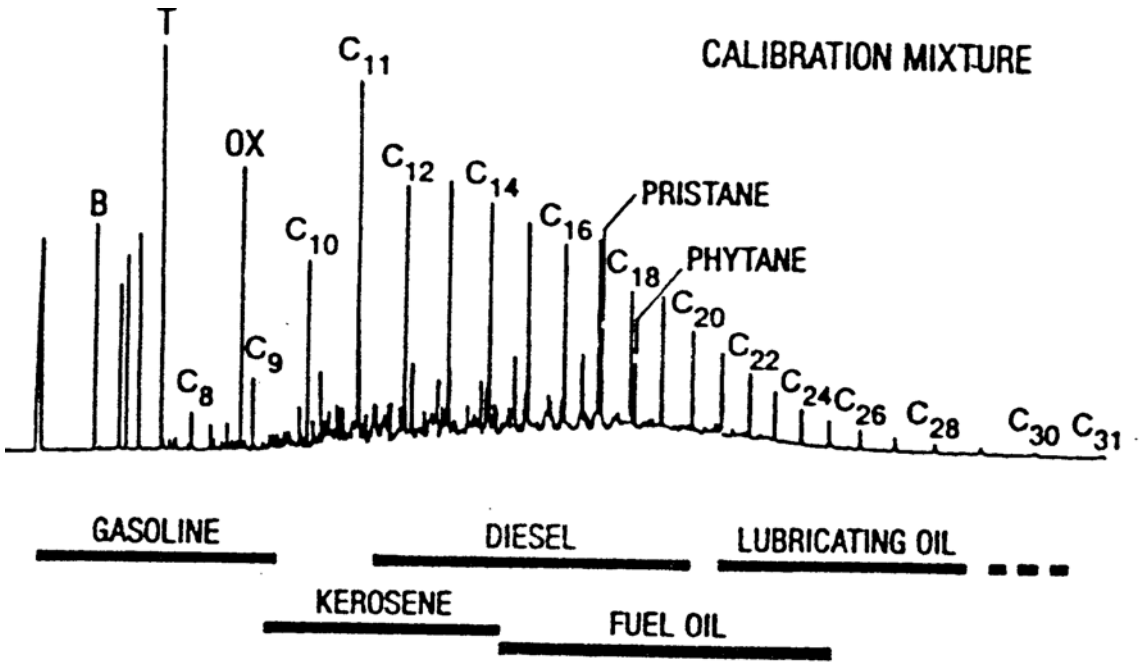
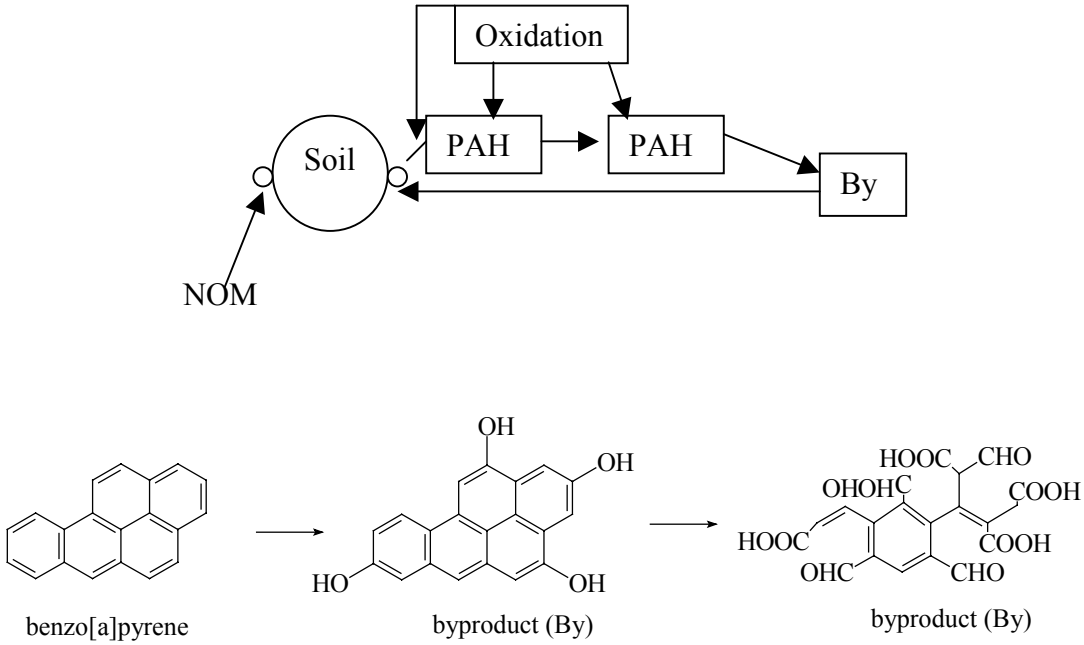


Figure 1.1. Approximate Boiling Ranges for Individual Hydrocarbon Products

Benzene (B) has a boiling point of 80.1°C and n-Hentriacontane (C-31) has a boiling point of 302°C (from Senn and Johnson 1985) (Reprinted with the permission of Ground Water Publishing Company. Copyright 1989).



Proposed decomposition reaction pathway of benzo[a]pyrene.

Figure 1.2. Illustration of the Mechanism for the Effects of Chemical Oxidation in Soil System

Note: By represents oxidation by-products

CHAPTER II

LITERATURE REVIEW

Treatment of petroleum hydrocarbon contaminated soil has been the subject of several studies throughout the years. A number of published studies and reviews discuss in detail the biodegradation of petroleum products in relation to chemical structure. Many different engineered treatment systems are available for bioremediation of petroleum products in soil. Besides biological methods, studies on chemical oxidation of petroleum products, especially PAHs, with or without bioremediation have been investigated. Yet, there are a limited number of studies conducted on soil decontamination. Presented in this chapter are the general aspects of biodegradation of contaminated soils, followed by a discussion on past studies that evaluated chemical oxidation enhanced bioremediation.

Bioremediation of Petroleum Hydrocarbons

Bioremediation processes utilize microorganisms to biologically degrade contaminants present within environmental media. The processes utilize natural biochemical reactions to remove contaminants, but the natural biodegradation rate must be accelerated and optimized to reduce the contaminant concentration within a reasonable time period. To increase the rate of biodegradation, three important considerations must be determined: the kind of pollutants that must be treated; the matrix that contains the pollutants; and, the type of microorganisms that will be used to degrade the target pollutants. Reviewed in this section are the

fundamentals of bioremediation and various factors that impact the biodegradation of petroleum hydrocarbons. Understanding the fundamentals, as well as key operational factors, is paramount to the optimization and success of biodegradation processes.

Fundamentals of Biodegradation

Bioremediation processes utilize microorganisms to degrade or decompose contaminants for the treatment of soil and/or water. Heterotrophic bacteria and fungi are the primary organisms used for this function (Ward, *et al.*, 1995). Organic materials are degraded by microorganisms for the generation of energy and synthesis of new cell tissue. In the absence of organic matter, the cell tissue is endogenously respired to obtain energy for maintenance. In most biological treatment systems, these three activities occur spontaneously, and they may be represented as follows:

- Oxidation (dissimilatory process):

  Organics + Electron Acceptor → CO_2 + H_2O + By-products + Energy + Cells

- Synthesis (assimilatory process):

  Organics + Electron Acceptor + Bacteria + Energy → new cells

- Overall pathway:

  Orgaincs + Electron Acceptors + Bacteria → CO_2 + $2H_2O$ + Cells + By-products

Factors Affecting Biodegradation

Several environmental factors must be fulfilled before a contaminant can be degraded or transformed by microorganisms. Failures of bioremediation that have been documented are mostly due to not attaining treatment goals or excessively long treatment times. (Block, *et*

al., 1993). Hence, understanding the environmental factors influencing biodegradation is very critical to the success of site remediation.

Biodegradation Potential of Petroleum Products: The biodegradation potential of a chemical refers to the ease and degree of utilization exhibited by target organisms when using the chemical as a carbon source for metabolism. The biodegradation potential of petroleum products is well correlated to their chemical structures. Petroleum hydrocarbons can be divided into four classes: saturates (aliphatics), simple aromatics (benzene, toluene, ethylene, and xylenes), asphaltenes (phenols, fatty acids, ketones, esters, and prophyrins), PAHs (polycyclic aromatic hydrocarbons), and resins (pyridines, quinolines, carbazoles, sulfoxides, and amides). Biodegradation rates have been shown to be highest for the saturates, followed by the light aromatics, with high-molecular-weight aromatics and polar compounds exhibiting extremely low rates of degradation (Block, *et al*., 1993, Long, 1993).

PAHs containing two or three aromatic rings are readily degradable, whereas PAHs containing four or more aromatic rings are much more difficult to degrade (Baker and Herson, 1994; Hoeppel and Hinchee, 1994; Zappi, *et al.,* 1995). Microbiological transformations of large PAH compounds have been documented (Keck, *et al.,* 1989). The transformation mechanisms appear to be primarily cometabolic (Mahaffey, *et al.,* 1988) and under aerobic conditions (Bauer and Capone, 1985; Delfino and Miles, 1985; Park, *et al.,* 1990; Wang, *et al.,* 1990).

Typical degradation half-lives range from a few days for low-molecular-weight compounds to a number of years for complex, high-molecular-weight compounds (American Petroleum Institute, 1984). Specific bioremediation half-lives have been reported as over 48 weeks for bunker C fuel (Song, *et al*., 1990), 37-57 weeks for crude oil sludge (Loehr, *et al*., 1992), less than 30 days for some normal alkanes and aromatics (Loehr, *et al*., 1992), six weeks for a mixed waste of heavy and light petroleum fractions (Banerjee, *et al.,* 1995), five weeks for a Saudi Arabian crude oil (Whiteside, 1993), eight weeks for crude oil under optimum conditions (McMillen, *et al*., 1993), and more than two years for crude oil under nonoptimized conditions (McMillen, *et al*., 1993). Bioremediation with composting has been successfully applied with remediation times of five weeks for sludges and diesel-contaminated soils (Martinson, *et al*., 1993).

Bioavailability of Petroleum Contaminants in Soil Environments: Bioavailability is defined as the ability of a compound to be freely transported across the cell membrane for intracellular metabolism and/or available for extracellular metabolism (Verschueren and Visschers, 1988). It is another important limiting factor controlling the bioremediation of contaminants in soil because it controls the physical availability of contaminants to microorganisms. In order to be metabolized by bacteria at an appreciable rate, it is generally believed that a compound must be in the dissolved state (Wodzinski and Coyle, 1974; Guerin and Boyd, 1992; Mihelcic and Luthy, 1991). Unfortunately, high molecular weight hydrocarbons and PAHs have low water solubilities. The low solubility and high hydrophobicity of these compounds makes them less susceptible to bacterial attack. The

solubility of selected petroleum products are listed in Table 2.1.

Besides the effects of low contaminant solubility on soil remediation, the complex pore structure of soils and strong adsorption bonds also make remediation difficult. It is well recognized during soil content analysis, after exhaustive solvent extraction, a portion of organics may not be recoverable (Kaufman, *et al.*, 1976; Khan, 1982; 1991). The adsorption sites occur within the internal sites of small soil pores and inside the organic matrix of soil humic substances (Novak, *et al.* 1995). For hydrophobic organic compounds, several researchers have shown that the dominant sorption mechanism is the affinity of such chemicals for natural organic material in the soil (USEPA, 1989; LaGrega, *et al.,* 1994). Chemical and biological reactions are known to influence the formation of bound residue. Aged residues are generally considered to be more protected from microbial degradation than freshly contaminated soils. As the length of time the chemical remains in the soil increases, the chemical binding or diffusional barriers increase; thereby, limiting the desorption process, which in turn minimize microbial degradation rate (Pignatello, 1989). Sorption processes also hinder the ability of the bacterial cells to attach to, absorb, and/or enzymatically degradate larger organic molecules (Cheng, *et al.,* 1983; Voice and Weber, 1983). Hence, degradation rate is limited by the inaccessibility of adsorbed molecules to microorganisms.

Microbial Numbers and Activity in Soil: Rapid biodegradation of petroleum hydrocarbons requires an appropriate level of active bacteria to be presented in the soil. Many of the heterotrophic microorganisms found in most soils possess the ability to degrade petroleum products (Perry and Scheld, 1968; Odu, 1978; Pinholt, *et al.,* 1979; Zappi, *et al.,*

1992; 1993). The quantification of the number of microorganisms present in soils is often based on indirect information, such as the population of bacteria growing on a particular solid growth medium. Unfortunately, no single growth medium supports all microorganisms of interest. Also, most growth medium used for total bacterial counts isolate only aerobic heterotrophic populations not anaerobic (Hoeppel and Hinchee, 1994).

It has been suggested that bacterial population density should be greater than 10^6 colony forming units (CFUs) per gram dry soil for sustaining appreciable degradation of petroleum hydrocarbons (Morgan and Watkinson, 1989). Contaminants in soil may have either a positive or a negative effect on the numbers and diversity of microorganisms. Many bacteria occurring in soils are in a dormant state and are stimulated by the addition of suitable food sources (Johnston and Robinson, 1982; Hoeppel and Hinchee, 1994). Johnston and Robinson (1982) found that application of oily sludges and fertilizer to soil resulted in a significant increase in bacterial colonies. Bacterial counts have been found to be 100 to 1,000 times higher in soils contaminated with jet fuel than in adjacent uncontaminated soils (Ehrlich, *et al.,* 1985). However, if the contaminant is not chemically similar to a natural material found in the ecosystem, it can pose a toxicity impact on bacterial consortia (Blum and Speece, 1991; Donnelly, *et al.,* 1991). High concentrations of many poorly soluble hydrocarbon contaminants in soils seem to inhibit biodegradation rates but not the numbers or metabolic activity of degradative microorganisms (Watts, *et al.,* 1989).

Electron Acceptors: Petroleum compounds are best biodegraded under aerobic conditions (Hoeppel and Hinchee, 1994). To support aerobic respiration, molecular oxygen is used as the terminal electron acceptor. Organisms that are dependent on aerobic

respiration to meet their energetic needs can exist only when there is an adequate supply of molecular oxygen. If the molecular oxygen is not available, NO3-, SO42+, CO2, and some organics can be used as electron acceptors (Metcalf & Eddy, 1991). Recent research has shown that nitrate, serving as a terminal electron acceptor in the absence of oxygen, can promote the degradation of many monoaromatic compounds common to most fuels (Major, *et al.,* 1988; Evans, *et al.,* 1991; Hutchins, 1991). However, oxygen carries the highest electron energy potential allowing the organisms derive more energy through organic oxidation using oxygen. Most studies evaluating anaerobic degradation have indicated appreciably lower degradation rates compared to those achieved with aerobic conditions (Major, *et al.,* 1988). These studies also demonstrate that when treating petroleum products in soil, the provision of oxygen is one of the key factors effecting the success of biodegradation (Dibble and Bartha, 1979; Lee, *et al.,* 1988; Ritter and Scarborough, 1995; Jing, 1998). Hence, the fastest contamination reduction rate is achieved by supplying sufficient molecular oxygen within soil masses undergoing aerobic bioremediation.

Nutrients: Microorganisms are composed of combinations of elements that are the components of their genetic material, structural molecules, enzymes, and intracellular plasma. The major elements that make up microorganisms are hydrogen, carbon, oxygen, nitrogen, and phosphorus (Metcalf & Eddy, 1991; Hoeppel and Hinchee, 1994). An approximate formula for the organic fraction of bacteria cell tissue is $C_{60}H_{87}O_{23}N_{12}P$ (Hoover and Porges, 1952; Metcalf & Eddy, 1991). In the case of attempting to establish an active biomass within petroleum hydrocarbon contaminated soils, carbon and hydrogen would not be limiting since they are the major components of hydrocarbons (i.e. TPH).

Phosphorus is often plentiful, but unavailable because of its poorly soluble natural forms. Nitrogen is usually present as dinitrogen gas within the soil pores, but is seldom in bioavailable forms such as amino acids, ammonium, or nitrate (Hoeppel and Hinchee, 1994). Other micronutrients are also needed, but they are almost always naturally present in soils to support bioremediation (Baker and Herson, 1994). Thus, the major nutrients that limit microbial growth in soils are nitrogen and phosphorus. A lot of researchers have investigated the effects of adding nitrogen and phosphorus as inorganic salts to enhance the bioremediation of crude oil, gasoline, and diesel fuel contaminated soils (Pritchard, *et al.,* 1992, Prince, *et al.,* 1993; Widrig, 1995; Chang, *et al.,* 1995; Venosa, *et al.,* 1996; Jing, 1998). Theoretically, the optimum amount of nitrogen and phosphorus present in soil should be based on a carbon : nitrogen : phosphorous (C:N:P) ratio similar to that stoichiometrically composing a typical bacteria cell. Some studies have indicated that adding nitrogen and phosphorus salts have negligible effect on biodegradation rate enhancement (Bossert and Bartha, 1984). One explanation for this discrepancy is the variability in soil composition, such as the nitrogen reserve level and the presence of nitrogen-fixing bacteria (Leahy and Colwell, 1990; Toccalino, *et al.,* 1993). In general, the addition of N and P is required for establishment of an optimized biotreatment system.

Soil pH: The pH of the environmental media is also a key factor in the growth of microorganisms. Most microorganisms can not tolerate pH levels above 9.5 or below 4.0. For soil, generally, the optimum pH for growth lies between 6.5 to 7.5 (Metcalf & Eddy, 1991; Englert, *et al.*,1993).

Soil Moisture: Water is an essential compositional material for microorganisms.

Biodegradation requires water for microbial growth, diffusion of nutrients, and removal transport of waste by-products (Hoeppel and Hinchee, 1994). However, too much water can limit air flow through soil, thus reducing the availability of oxygen. Dibble and Bartha (1980) demonstrated the feasibility of biodegradation of an oily sludge within a sandy loam with water saturation ranging from 20 to 80. Stegmenn (1994) found that the optimal biodegradation of diesel fuel occurred at a moisture content of 60% of field capacity.

Temperature: Temperature plays a vital role on activity of bacteria. It has been observed that the rate of biodegradation increases with increasing temperature, doubling with every 10°C of rise in temperature until a limiting temperature is reached (Kimball, 1966; Stanier, *et al.,* 1976; Metcalf & Eddy, 1991). Temperature can also indirectly influence biodegradation of a compound or mixture by changing its physical properties, bioavailability, or toxicity to microorganisms (Atlas and Bartha, 1972). Research has shown that soil microorganisms are capable of degradation at most ambient soil temperatures (Atlas, 1981; Leahy and Colwell, 1990). Optimum petroleum degradation rates by aerobic bacteria occur at temperatures between 15°C and 30°C (Fan and Tafuri, 1994). Jing (1998) observed a decrease in oxygen

uptake rate with temperatures below 20°C during the degradation of heavy motor oil contaminated soil.

Introduction to Chemical Oxidation Processes

Advanced Oxidation Processes (AOPs) are defined as those oxidation processes which generate hydroxyl radicals in sufficient quantity to affect waste treatment (Glaze, 1987). Many systems qualify under this broad definition of strong oxidants, for example: O_3 and H_2O_2 (also known as peroxone process), H_2O_2 and catalysts (which are transition metal ions), and oxidizer irradiation (*e.g.* ultraviolet [UV], ultrasound [US] and oxidizers, or electron beam [eb]) (Huang et al., 1993).

Ozonation Process

Although not an AOP, ozone is a strong oxidizer that has found significant usage in waste treatment (Rice, 1980; Hoigne, 1982). Ozone is an unstable gas with a boiling point at -112°C at atmospheric pressure. Ozone has a characteristic penetrating odor that can be detected at concentrations as low as 0.01 to 0.05 ppm (Rice, 1980). It is a powerful oxidant, having an oxidation potential (E°) of 2.07 volts at 25 EC. Table 2.2 lists the oxidation potentials of some oxidants (CRC, 1975). Hoigne, *et al.* (1982) proposed that O_3 can have two reaction modes: direct ozonation reactions and free radical (such as hydroxyl radical) indirect reactions. The primary reactions initiated by ozone in water can be described by a sequence of reactions as shown in Figure 2.1 (Hoigne, 1982). During ozonation, a portion of the dissolved ozone reacts directly with the dissolved organic, this is usually a rather slow reaction (compared to hydroxyl radical reactions) and is highly selective being the most

important for processes like disinfection and decoloration. Rate constants ranging from 1 to 10^6 M^{-1} sec^{-1} has been reported for ozone-organic pollutant reactions (Huang, *et al*., 1993; Kuo, 1995; Qui, 1999). Typically, a portion of the ozone decomposes before reacting with the chemicals or being stripped off. As suggested by Weiss (1935), this decomposition reaction occurs in aqueous solutions and is initiated by formation of hydroperoxide ions from the reaction of ozone with the hydroxyl ion.

$$O_3 + OH^- \rightarrow HO_2^- + O_2 , \qquad (2\text{-}1)$$

Hydroperoxide then reacts with ozone molecules generating the hydroxyl radical and oxygen as shown below (Langlains, *et al.,* 1991):

$$O_3 + HO_2^- \rightarrow OH\cdot + O_2^- + O_2 , \qquad (2\text{-}2)$$

The hydroxyl radical formed during the ozone decomposition reacts much more rapidly with organic compounds than molecular ozone due to its higher oxidation potential (E° = 2.33 v). The rate constants for hydroxyl radical-organic compound reactions are usually on the order of 10^8 to 10^{10} M^{-1} sec^{-1} (Huang, *et al*., 1993). Chemically, hydroxyl radicals are much less selective than molecular ozone.

The reaction of OH A with organic compounds can be classified into three mechanisms: hydroxyl addition, hydrogen abstraction, and electron transfer. Organic compounds containing aromatic systems or carbon-carbon multiple bonds easily react with OH A due to the rich π-electron cloud of the aromatic ring usually via a hydroxylation reaction (Huang, *et al*., 1993; Qui, 1999):

$$OH\ A + C_6H_6 \rightarrow A\ C_6H_6OH, \qquad (2\text{-}3)$$

Several hydroxyl radical reactions are illustrated in Figure 2.2. It illustrates that: (a) free or complex metal ions can be oxidized, possibly by one-electron transfer processes, to yield the ozonide radical anion which can in turn initiates the chain decomposition of ozone (Staehelin and Hoihne, 1983); (b) the hydroxyl radical can react with aromatic groups within humic molecules yielding hydroxylated forms which are more susceptible to further attack by ozone (Gurol and Singer, 1983); (c) the hydroxyl radical can react with aliphatic side chains or fatty acids usually by hydrogen atom abstraction reactions. The organic radicals thus formed will generally add dioxygen to form organic peroxides which decompose by eliminating the superoxide ion reentering into the chain causing the decomposition of ozone and increased formation of hydroxyl radicals (Staehelin and Hoigne, 1983;1985); and, (d) ozone can react with carbon-carbon double bonds within the humic molecule to first yield peroxidic intermediates and then produce hydrogen peroxide and carbonyl products (Bailey, 1978).

The mechanisms and kinetics of ozone reactions with saturated, unsaturated, and aromatic hydrocarbons have been investigated. The main reaction products of ozone with saturated hydrocarbons are ketones, acids, alcohols, peroxides, and water (Asinger, 1959; Razumovskii and Zaikov, 1984; Qui, 1999).

The reaction of ozone with the carbon-carbon double bond in different unsaturated compounds has been a subject of attention for almost a century. The primary product of the reaction of ozone with a double bond is a molozonide, or primary ozonide, which is not stable and rapidly decomposes into a bipolar ion and a carbonyl compound , such as aldehyde or ketone (Razumovskii and Zaikov, 1984). Examples of the principle reaction

products of ozone with multiple-bond compounds are summarized in Table 2.3.

Ozone seems to be the most appropriate oxidant to degrade PAHs (Bailey, 1982; Cornell and Kuo, 1984; Beltran, *et al.,* 1995). Beltran studied ozonation of fluorene, phenanthrene, acenaphthene, and naphthalene. He suggested that the ozonation of fluorene developed through hydroxyl radical and direct ozonations while radical reactions seemed to be negligible during the ozonation of the other PAHs investigated. Ozonation by-products of PAHs are polar aliphatic compounds, mainly carboxylic acids and aldehydes (Helleur, *et al.,* 1979; Legube *et al.,* 1986).

O_3/H_2O_2 System (Peroxone)

The O_3/H_2O_2 process is commonly referred to as the peroxone process. In the presence of hydrogen peroxide in an aqueous solution, the hydroperoxide ion HO_2- is formed via the disassociation of hydrogen peroxide as illustrated below:

$$H_2O_2 + H_2O \leftrightarrow HO_2^- + H^+ , \quad (2\text{-}4)$$

This additional generation of hydroperoxide ion favors the production of hydroxyl radicals as illustrated in Reaction (2-2). The following chain mechanism generates hydroxyl radicals during peroxone treatment:

$$HO_2 \leftrightarrow H^+ + O_2^- , \quad (2\text{-}5)$$

$$O_2^- + O_3 \rightarrow O_3^- + O_2 , \quad (2\text{-}6)$$

$$O_3^- + H^+ \rightarrow HO_3 \quad (2\text{-}7)$$

$$HO_3 \rightarrow OH^{\bullet} + O_2 , \quad (2\text{-}8)$$

Peroxone is a comparably new treatment technology. Studies on peroxone oxidation of

pesticides, tetrachloroethylene, explosives, and oxalic acid (a compound often formed during ozonation of aromatic molecules in water) have been conducted (Paillard, *et al.,* 1988; Glaze and Kang, 1989; Allemane, 1994; Zappi, *et al.,* 1994; 1995). Very little research is available on this type of oxidation with non-halogenated unsaturated and saturated hydrocarbons in non-aqueous systems . It is noteworthy to mention that Beltran *et al.* (1995) concluded that since direct reactions of ozone with many hydrocarbons are so dominant that the contribution of hydroxyl radical toward oxidation (formed due to the presence of hydrogen peroxide) is minimal.

Fenton's Reagent

The oxidizing properties of the mixture of H_2O_2 and ferrous iron (Fenton's Reagent) was first observed by Fenton at the end of the 19th century (Fenton, 1894). This reaction generates hydroxyl radicals according to the reaction below:

$$H_2O_2 + Fe^{2+} \rightarrow OH^{\bullet} + OH^- + Fe^{3+}, \quad (2\text{-}9)$$

In the absence of the added substrate, the hydroxyl radical will oxidize another molecule of Fe^{2+} as shown below:

$$Fe^{2+} + OH^{\bullet} \rightarrow Fe^{3+} + OH^- , \quad (2\text{-}10)$$

Thus, the overall stoichiometry of the reaction without additional reactants (i.e. a pollutant) is:

$$2Fe^{2+} + H_2O_2 + 2H^+ = 2Fe^{3+} + 2H_2O \quad (2\text{-}11)$$

The above equation indicates that Fenton's reaction is strongly dependent on reaction pH. Watts, *et al*. (1990) found that H_2O_2 decomposed rapidly at pH > 5; however, at pH 3,

the H_2O_2 consumption rates were low, while the rate of contaminant degradation was high due to increased hydroxyl radical production.

In the presence of organic substrates, the additional reaction below is included:

$$RH + OH\bullet \rightarrow R\bullet + H_2O, \quad (2\text{-}12)$$

where $R\bullet$ is an organic radical.

Similar reactions also occur when H_2O_2 is catalyzed by other metal ions, such as manganese (Watts and Dilly, 1996). Because iron compounds are comparably less toxic and cheaper, it has been successfully used for chemical oxidation of numerous organic compounds in water treatment.

Chemical Oxidation Enhanced Bioremediation

Various oxidation techniques have been successfully employed to transform organic compounds to improve their biodegradation potential (William, *et al*., 1994). The specific goal of the oxidation step is to convert the hazardous constituent into a form that is more amenable to subsequent biotreatment.

Numerous studies have been performed on the treatment of wastewater and groundwater using AOPs prior to or after biological treatment. The efficacy of chemical oxidation as a biodegradation enhancement step has been confirmed repeatedly and was the subject of a recent review (Scott and Ollis, 1995). Much of this work was done with aqueous wastes, unfortunately, little work has been performed with soil decontamination. The following

discussions detail studies that indicate promise for using AOPs and ozone for soil decontamination.

Ozonation and Peroxone Processes

Ozonation technology is useful in the oxidative degradation or transformation of a wide range of pollutants for the treatment of drinking water, ground water, and wastewater. Due to its high oxidant capacity and electrophilic character, ozone seems to be the most appropriate oxidant to degrade recalcitrant compounds (Beltran *et al*.,1995).

Zappi, *et al.* (1994) investigated the combined use of ozone and hydrogen peroxide (peroxone) to treat soils contaminated with 2,4,6- trinitrotoluene (TNT) using 5 liter bench scale slurry oxidation units. Results indicated that hydrogen peroxide dosing into an ozonated slurry reactor enhanced the rate of TNT disappearance by converting the TNT to trinitrobenzene (TNB), a known TNT oxidation by-product. Approximately 96% of the initial TNT was reduced within 90 minutes.

H_2O_2 Applications

The application of hydrogen peroxide for treatment of aqueous phase inorganic and organic pollutants is well established. It has been employed mainly for odor abatement at municipal wastewater treatment plants (Elizardo, 1991).

A two-step oxidation process was investigated for the treatment of phenanthrene contaminated soil fines generated from a soil washing process (Kemenade *et al.* 1995). Oxone ($2KHSO_5$A$KHSO_4$AK_2SO_4) and hydrogen peroxide were used as oxidants for the chemical pre-oxidation step, and unacclimatized municipal activated sludge was employed in

the subsequent biodegradation step. Air-dried and sieved soil was spiked with 10,000 mg/kg phenanthrene by adding a solution of phenanthrene in acetone to the soil. They reported that integrating a 24 hour chemical pre-oxidation step with 5 g/l oxone followed by 5 days of biological treatment was the most effective combination for the remediation of the soil.

Fenton's Reagent

To date, the most commonly practiced and well documented oxidant used for chemical priming as a pretreatment step is Fenton's Reagent. It has also been proposed as a soil decontamination technology. Research in this area includes Fenton's Reagent remediation of the explosives TNT (2,4,6-trinitrotoluene) and RDX (1,3,5-trinitrohexahydro-*s*-triazine) in a soil slurry (Kubarewicz, *et al*., 1985), petachlorophenol (Watts, *et al*., 1990), and pesticides contaminated soils (Tyre, *et al*., 1991).

Brown, *et al.* (1995) conducted a study on a site contaminated with PAHs and CPAHs (carcinogenic PAHs) using three reactors in sequence. Reactor 1 was a biological pretreatment step for the contaminated slurry, Reactor 2 was a Fenton's Reagent step used for oxidation, and Reactor 3 was used for biological polishing of Reactor 2 effluent. During operation, the reactor system demonstrated average total PAH and CPAH removals of 85 and 66%, respectively (Brown, *et al*., 1995).

Watts (1992) demonstrated that TPH levels in an oil and fuel contaminated soil from a spill site could be reduced from 2,000 mg/kg to below the regulatory limit of 100 mg/kg in 7 days using 12% hydrogen peroxide with and without iron addition during laboratory scale studies. The optimum pH values was reported to be less than 3. These conditions were

subsequently used for pilot testing of the process using 1.25 yd^3 of soil loaded into 55 gallon drums over a reaction period of one to three days. No degradation products were found; however, the possibility of polymerization and bound residue formation was noted (Watts, 1992).

Kawahara, *et al.* (1995) used Fenton's Reagent as a pretreatment technique to treat a PAHs contaminated soil. Soil slurries, consisting of 10 g of contaminated soil and 30 ml water were treated with 40 ml of Fenton's reagent (30% H_2O_2:8.84 *m*M $FeSO_4$) at a neutral pH (pH of this soil was 7). Results showed that the removal of PAHs after oxidation was in the range of 72% (naphthylene) to 93% (acenaphthylene). A signficant increase in the extractibility of the PAHs after 1 hour of treatment was observed. The concentrations of 12 of the 14 PAHs studied appeared to increase in a range from 13 to 56% relative to the initial analysis after one hour of treatment by Fenton's reagent. The authors suggested that the large increase in extractability was probably due to the iron ions complexing with the PAHs and weakening the adsorptive bonds of the PAH-humic complex formed on soil surfaces (Kawahara, *et al.,* 1995).

Table 2.1

Solubility of Selected Hydrocarbons at 25°C

Chemical	Molecular Weight (g/mol)*	Solubility (g/m^3)**
Benzene	78.10	1780
Toluene	92.10	515
Ethylbenzene	106.20	152
p-Xylene	106.20	185
n-Pentane	72.15	38.5
n-Octane	114.20	0.66
n-Decane	148.28	0.052
n-Dodecane	170.33	0.0034
n-Tetradecane	198.38	0.000655
Naphthalene	128.20	31.7
Fluorene	166.20	1.84
Phenanthrene	178.20	1.29
Anthracene	178.20	0.041
Pyrene	202.30	0.135
Chrysene	228.30	0.002
Benzo[a]pyrene	252.30	0.0038

*: CRC Handbook of Chemistry and Physics (64^{th} ed.)
**: Mackay and Shui (1981)

Table 2.2

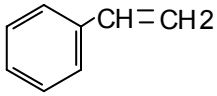
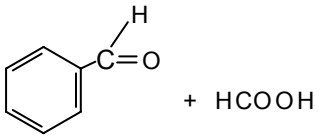
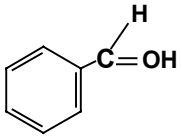
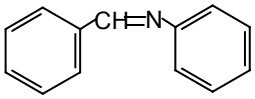
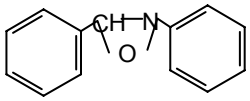
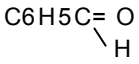
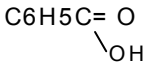
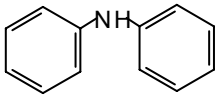
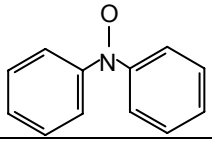
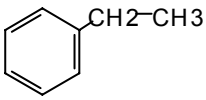
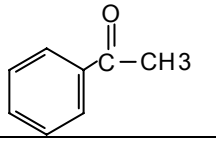
Oxidation Potential of Some Common Oxidants*

Reactions	Oxidation Potential (E° in Volts), 25°C
$F_2 + 2e = 2F^-$	2.87
$OH + H^+ + e^- = H_2O$	2.33
$O_3 + 2H^+ + 2e = O_2 + H_2O$	2.07
$O_3 + H_2O + 2e = O_2 + 2OH^-$	1.24
$H_2O_2 + 2H^+ + 2e = 2H_2O$	1.76
$H_2O_2 + H_3O^+ + 2e = 4H^2O$ (basic)	0.87
$Cl_2 + 2e = 2Cl^-$	1.36
$MnO^{4-} + 8H^+ + 5e = Mn^{2+} + 4H_2O$	1.49
$O_2 + 2H_2O + 4e = 4OH^-$	0.40

* Handbook of Chemistry and Physics, 56th Edition, 1975-1976. CRC Press inc., Cleveland, Ohio, pp. D-141-143.

Table 2.3

Products of Reactions of Ozone with Multiple-Bond Compounds

Compound	Principle Products	Ref.
	 	1
	  	2
		3
		4

References:
1. Razumovskii,1971
2. Criegee,1968
3. Reiber, *et al.,* 1960
4. Alfey, 1964

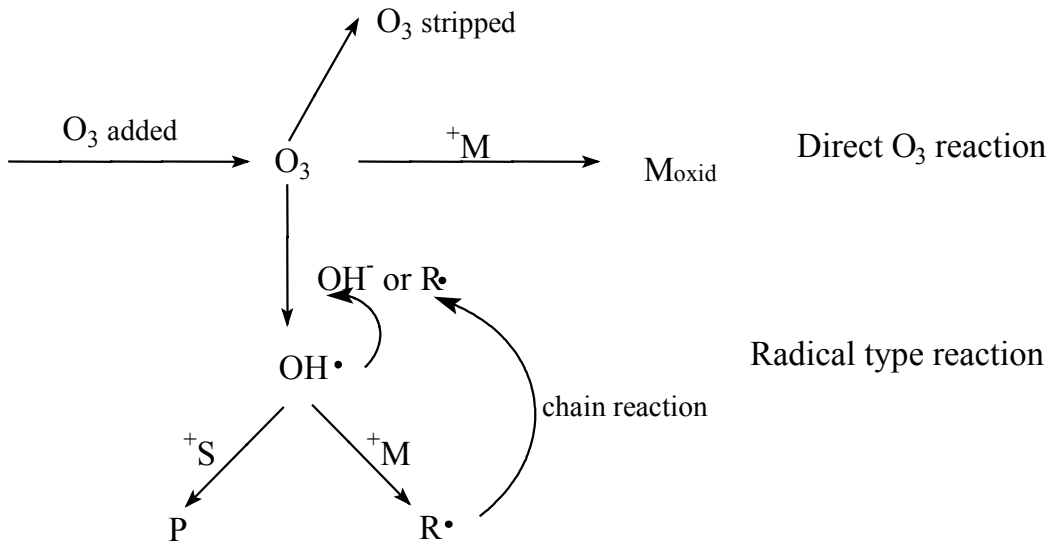


Figure 2.1. Reaction Scheme for Ozone Added to an Aqueous Solution (Hoigne, 1982)

M = Organic(s)
M_{oxid} = Oxidized organic(s)
S = Free radical scavenger
P = Products which do not catalyse the ozone decomposition
R = Free radicals which catalyze the ozone decomposition

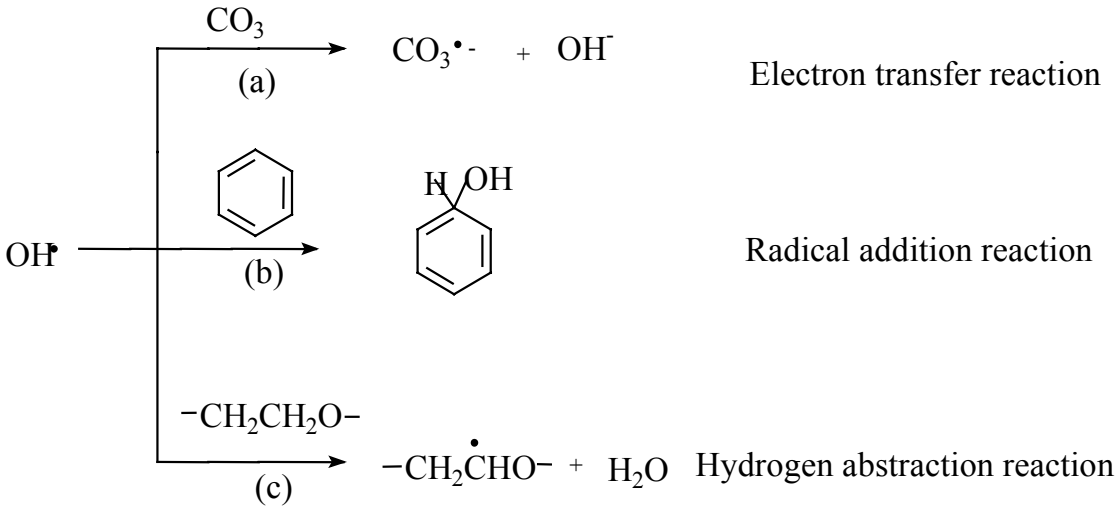


Figure 2.2. Hydroxyl Radical Reaction with Organic Compounds

CHAPTER III

METHODS AND MATERIALS

Materials

Soils

Two types of soil were examined during this study: a soil contaminated with high levels of used motor oil, known herein as the high molecular weight hydrocarbons contaminated soil (HMW-Soil), and a soil contaminated with lesser levels of diesel fuel, referred to as the lighter molecular weight hydrocarbons contaminated soil (LMW-Soil). The HMW-Soil was collected from an abandoned gas station located in Silver City, Mississippi. This site is a Mississippi Department of Transportation (MDOT) site obtained during Right-of-Way activities. For years, the owner of the store dumped waste crankcase oil onto the ground without any treatment. Initial characteristics of this soil are listed in Table 3.1. A treatability study on bioremediation of the HMW-Soil was previously performed within the MSU Environmental Technology Research and Application Laboratory (E-TECH Laboratory) by Jing (1998). Collection of the HMW-Soil for this study was performed on January 22, 1998. The contaminated soil was excavated by hand and sieved using a 6.3mm screen (1/4 in.) to remove rocks, root material, and other debris from the soil. At MSU, soil was homogenized thoroughly using a shovel within a container constructed of plastic sheets and bricks. Soil

was then sieved through a No. 4 (4.75mm) US standard sieve and stored in 5-gallon buckets until used.

The LMW-Soil was collected from a leaking tank farm area in Port Hueneme, California (a Navy fuel storage site). It was primarily contaminated with diesel fuel, but also contained lesser amounts of gasoline, fuel oil, and motor oil. Before used in the experiments, the LMW-Soil was sieved using a No. 10 (2.00 mm) US standard sieve and mixed completely using a hand-held shovel to ensure homogeneity. From visual observations, the LMW-Soil has an overall smaller particle size and higher sand content than the HMW-Soil. Table 3.1 also lists the characteristics of the LMW-Soil.

Nutrients

Ammonium nitrate (NH_4NO_3) and ammonium hydrogen phosphate ($(NH_4)_2HPO_4$) (both obtained from Fisher Scientific) were utilized as nitrogen and phosphorus sources, respectively. These are readily assimilated nutrient substrates for microorganisms (Dibble and Bartha, 1979; Harris and Arnold, 1995). They have been previously proven to have a positive effect on enhancing the bioremediation rate of the TPH in the HMW-Soil (Jing, 1998).

Surfactant

A nonionic, nontoxic, and readily biodegradable surfactant, Tween 80 (polyoxyethlene sorbitan ester), was purchased from Fisher Scientific and evaluated for its ability to enhance the bioavailability of the petroleum products in the soil system to the bacteria.

Hydrogen Peroxide

Hydrogen peroxide solutions of 3%(w/w) and 30%(w/w) were obtained from Fisher Scientific. Hydrogen peroxide solutions were formulated by diluting the original solution with distilled, deionized (DDI) water based on the required concentrations of the experiment. The hydrogen peroxide solutions were made on an as-needed basis during this study to prevent decomposition; thereby, preserving chemical integrity.

Iron Salt (Fe^{2+})

Fenton's Reagent is the combination of ferrous ion salt and hydrogen peroxide. The selection of iron salt used in this study was ferrous sulfate ($FeSO_4 \bullet 7H_2O$) purchased from Fisher Scientific.

Oxygen

Oxygen was generated using a laboratory ozone generator (Model LC-1234) manufactured by Ozonology Inc. (Evanston, IL). An AirSep Corporation Model AS-12 oxygen generator is incorporated into this system for feed gas preparation. The oxygen generator supplies oxygen at 90% ± 5% purity at flow rates below 12 scfh (standard cubic feet per hour) at a maximum of 9 psi.

Ozone

Ozone was generated using the ozone generator mentioned above. The ozone generator is a corona discharge unit that utilizes four stainless steel electrodes inside borosilicate glass dielectrics with copper jacketing. Ozone is produced within four cells and individually regulated by a single primary voltage autotransformer (supplies up to 10.5 KV). The multiple

ozone sources are used to conduct multiple experiments at the same time. Gas flow from each cell is controlled by individual rotameters within a range of 1-6 scfh. Figures 3.1 and 3.2 present the ozone content in the production gas of generator versus turndown voltage setting and flowrate, respectively. The volumetric flow rate of gas into the reactor for this study was maintained at approximately 2 scfh and was continuously supplied into the reactors during all experiments involving ozone or oxygen addition. Various turn-down settings were used to control ozone content based on experimental demands.

Experimental Methods

This study is composed of two experimental phases. Phase I involved slurry phase experiments, while Phase II utilized packed columns. For both phases, bioremediation was conducted first followed by chemical priming, then a reestablished bioremediation stage (i.e. BIO/OX/BIO).

Phase I: Shake Flask Experiments

The objective of this set of experiments was to screen several candidate biological and Fenton's Reagent treatment strategies in order to select a workable number of test conditions to be performed during the Phase II Experiments.

The soil-water slurries were formed by combining 100 grams of contaminated soil (dry soil weight) with 300 ml distilled water to form a 25% (w/w) slurry which was then added to a 500 ml Erlenmeyer flask. An orbital agitation table (Model M49235, Barnstead/Thermalyne, Bubuque, IA), set at 250 rpm, provided aeration via agitation. All incubations were performed in duplicate at room temperature.

The experiments performed using the slurry systems (Phase I) are listed below:

1. Determination of the optimum biotreatment conditions for both soils (BIO). The best bioremediation condition for the HMW-Soil was previously determined by Jing (1998). This optimal condition was periodic addition of nutrient sources at a TPH: N: P ratio of 100:25:10 and activated sludge augmentation at the initiation of incubation. This condition was used to generate a previously biotreated soil for input into the chemical priming experiments of this study. However, optimal bioremediation conditions were not known for the LMW-Soil. Therefore, the following candidate bioremediation conditions were tested using the LMW-Soil:

a. Control (aeration only),
b. Nutrients dosed at a TPH: N: P ratio of 100: 25: 10 batch added at the beginning of treatment,
c. Nutrients dosed at a TPH: N: P ratio of 100: 10: 5 batch added at the beginning of treatment and on Day 21,
d. Nutrients dosed at a TPH: N: P ratio of 100: 25: 10 and activated sludge seeding at the beginning of treatment. Activated sludge was obtained from the local wastewater treatment plant and 30 ml of settled sludge was used,

e. Nutrients dosed at a TPH: N: P ratio of 100: 10: 5 batch added at the beginning of treatment and on Day 21. Plus, the addition of 30 ml settled activated sludge also dosed at the beginning of the test,

f. Same as Condition d with the addition of Tween 80 at a concentration of 0.5% (w/w) of the dry soil on Day 21,

g. Same as Condition e with the addition of Tween 80 at a concentration of 0.5% (w/w) of the dry soil on Day 21.

2. Evaluation of chemical priming on the bio-treated soil in slurry phase (BIO/OX). The intent was to evaluate the various chemical oxidation processes (ozonation, peroxone process, and Fenton's Reagent) using bio-treated slurry in shake flasks once the observed biodegradation rate of the TPH had reached a point of diminishing return. The purpose of these experiments was to evaluate the effects of the candidate chemical oxidation processes on subsequent biotreatment (reestablished bioremediation).

During attempts to treat the slurries using ozonation and peroxone, excessive foaming of the flask contents was encountered. The foaming was so severe that the experiments could not be performed without excessive soil flotation out of the reactors. This condition is unacceptable. Descriptions of this problem and methods attempted to solve the problem are detailed as Appendix A. Since control of the foaming could not be resolved, only Fenton's Reagent tests were studied in this series of experiments for both soils. It was thought that Fenton's Reagent at least would be considered representative of chemical priming during the shake flask experiments.

Fenton's Reagent Reaction: The Fenton's Reagent experimental conditions used for

both soils were set up as:

1. 20,000 mg/l H_2O_2, 2,000 mg/l Fe^{2+} , and pH = 2.5
2. 20,000 mg/l H_2O_2, 2,000 mg/l Fe^{2+} , and pH = 5.5
3. 5,000 mg/l H_2O_2, 500 mg/l Fe^{2+} , and pH = 2.5
4. 5,000 mg/l H_2O_2, 2,000 mg/l Fe^{2+} , and pH = 2.5

Biologically treated slurry to be chemical primed was removed from the shaker table and transferred into a stirred flask. Hydrochloric acid was added to lower the slurry pH to about 2.5, for those experiments evaluating low pH. The amount of hydrogen peroxide used in this set of experiments was obtained by calculating the volume of 3% H_2O_2 solution needed to bring the concentration in the slurry solution to the desired value. Before the stock hydrogen peroxide solution was added, ferrous sulfate was added and mixed into the slurry. The H_2O_2 solution was then slowly added to the slurry to avoid severe foaming caused by the generation of oxygen from H_2O_2 decomposition. To monitor the fate of hydrogen peroxide, 5 ml of slurry samples were taken at various time intervals, filtered through filter paper, and tested using test strips to indicate the approximate concentration of hydrogen peroxide. When the concentration of hydrogen peroxide in the slurry phase had decreased to zero, fresh stock H_2O_2 solution was added to increase the H_2O_2 levels back to the initial concentrations. All experiments were carried over a five hour period. At the end of the test, catalase (Sigma Aldrich) was added to remove the residual H_2O_2 to stop any further oxidation from occurring. Samples for total heterotroph plate counts and pH readings were taken at the beginning and the end of the test. Plate counts were used to evaluate the influence of Fenton's Reaction on the soil microorganisms. The TPH content of both the

soil and water phases were analyzed at the beginning and the end of each test. Water samples were collected by pipetting liquid from settled slurry above the water-soil interface.

One additional rate test was conducted over an eight hour period using the best condition obtained from the above set of tests. TPH-GC samples were taken at the initiation of the reaction and at 1, 3, 5, and 8 hours. This rate test was designed to evaluate the TPH degradation rate achieved by Fenton's Reaction.

3. Reestablishment of bioremediation after chemical priming (BIO/OX/BIO). After chemical priming, results from total heterotroph plate counts, pH, and TPH analyses were carefully examined. A sodium hydroxide (1N) solution was used to increase the pH in the slurry for the pH adjusted systems. Activated sludge obtained from local sewage plant was augmented to increase the bacteria population within the soil. Nutrient sources were added based on the remaining TPH level in the slurry to achieve a TPH: N: P ratio of 100:25:10. During the first week of restarted bioremediation, pH values of all flasks were checked constantly, because continuous agitation of the slurry resulted in the desorption of organic acids, which are common by products of chemical oxidation reactions, from the soil particles. Reestablished bioremediation efficiency was monitored using the parameters of pH, total heterotrophs, and TPH concentration analyzed every two weeks.

Phase II: Column Experiments

Eighteen column reactors were assembled as illustrated in Figure 3.4. Eight of them were loaded with HMW-Soil and the other ten were loaded with the LMW-Soil. The candidate chemical priming conditions tested for both soils are listed in Table 3. 3. All test conditions were studied using duplicate columns. More details on this test phase is provided below:

Column Reactor Design: The main body of the column was constructed using a 12 in. long by 2 in. ID clear PVC pipe (see Figure 3.3). Each end of the column was capped with a 2 in. PVC union. The bottom of the column was reduced to ¼ in. ID and Swagelok® Quick-Connect inserted. A ¼ in. PVC pipe line was connected to the other end of the Quick-Connect and used as either a gas sparging line, solution pumping line, or drainage line depending on the requirement of the experiment at the time. A ½ in. Swagelok® female connector was inserted at the top of the column. The top of the column was capped with a ½ in. Swagelok® cap during bioremediation stage of the experiments. The ½ in. female connector was plumbed to a ¼ in. PVC pipe for off-gas treatment during the chemical priming stage. Before loading the soil, a piece of stainless-steel screen was inserted at the bottom of the column to support the contents. Washed pea gravel was loaded on top of the stainless-steel screen in order to provide distribution of injected gases. This layer was 1” of height and overlaid with a non-woven geotextile fabric which served as a filter to prevent soil fines from washing into the pea gravel layer and eventually draining out of the columns. After all the parts were assembled and glued together, leakage tests were conducted using

water and air to assure proper sealing of the systems.

Soil Loading: Eight of the columns were loaded with HMW-Soil obtained directly from Ms. Jing's biocells (Jing, 1998). From her experiments, it was shown that after 56 days of biotreatment, about 70% of TPH was removed. Remediation trends indicated that biological degradation was not capable of appreciably further degrading the residual TPH. Soil taken out of the biocells was re-mixed to increase homogeneity. Before loading into the columns, soil samples were collected and analyzed for pH, moisture content, total heterotrophic bacteria population, and GC-TPH level. Each column was charged with 350 ± 2 grams (wet) of HMW-Soil. Special care was taken when loading the soil into the columns to eliminate channeling and wall effects by packing the soil in lifts and gently compacting across the cross-section of the lift faces to ensure homogeneity. Since the initial biotreatment stage was already completed by Ms. Jing, the HMW-Soil loaded into the columns were immediately chemically primed without any further biotreament.

Each column containing the LMW-Soil was filled with 375 ± 2 grams soil (wet), 30 ml settled activated sludge, and nutrients at a TPH:N:P ratio of 100:25:10 (dissolved in the activated sludge). Before loading, all the ingredients were added to the soil and mixed to ensure homogeneity. This stage of experimental effort was performed to start the initial biotreatment stage (including the Control Set). Soil samples for testing of initial parameters were collected from the mixed soil. Every 5 days, pure oxygen (90% O_2 [v/v]) was sparged into the columns using the oxygen generated from the ozone generator at a rate of 2 scfh for 20 minutes per column. The oxygen was saturated with water before application into the

reactors in order to reduce volatilization of soil moisture. Biological treatment performance was monitored using several parameters which included soil TPH (GC-TPH), oxygen, oxygen uptake rate (OUR), carbon dioxide production rate, volatile hydrocarbon levels in the column headspace, total heterotrophic bacteria population, moisture content, and pH level. GC-TPH analyses were performed weekly. Total heterotrophic bacterial populations were enumerated every 2 weeks. Oxygen, carbon dioxide, OURs, and volatile organic concentrations in the columns headspace were measured every 5 days. Because the upper limit for the oxygen monitor is 30% (v/v) oxygen and since 90% (v/v) oxygen was sparged into the reactors, gas monitoring activity was carried on every 5 days prior to each sparging event to allow levels to reduce into the measurable range of the gas monitor.

Oxygen uptake and carbon dioxide production rates were calculated using the following equations, respectively:

$$\text{OUR} = (y^0{}_{O_2} - y_{O_2})/t, \tag{3-1}$$

$$\text{CO}_2\text{ prod} = y_{CO_2}/t, \tag{3-2}$$

where:

OUR = Oxygen uptake rate, % O_2 /day

CO_2 prod = CO_2 production rate, % CO_2 /day

$y^0{}_{O_2}$ = 90% (oxygen concentration from oxygen generator)

y_{O2} = Measured headspace oxygen concentration, %,

y_{CO2} = Measured headspace CO_2 concentration, %,

t = Time interval between aeration and measurement, day

Total incubation time for biological treatment of the LMW-Soil was 14 days (selected based on the Shake-Flask slurry phase bioremediation results). After the initial biotreatment stage was completed in the column, chemical priming was applied to the soil, followed by the second biotreatment stage.

Chemical Priming Experiments: The two gas phase ozone concentrations evaluated during the chemical priming stage were 1.09×10^{-3} mol/l (100% voltage setting and 2 scfh flow rate, 4.5% [v/v]) and 0.49×10^{-3} mol/l (80% voltage setting and 2 scfh flow rate, 2.5% [v/v]). Both concentrations were tested with the LMW-Soil, while only the 1.09×10^{-3} mol/l level was tested with the HMW-Soil. Off-gases exiting the columns were bubbled into two gas absorbers containing a 2% (wt%) potassium iodide (KI) solution. The gas-washing bottles were plumbed in series to ensure full capture of ozone. The reason why the ozone monitor was not used was because the monitor requires a minimum of 1ml/min inlet gas flow rate to measure the ozone concentration, which was too high for the column experiments. After completion of the priming stage, the KI solution was titrated with sodium thiosulfate to indicate the amount of ozone exiting the column via the off-gases over the entire reaction period. After the priming experiment, oxygen was sparged into the columns for 20 minutes to strip out the residual ozone to prepare for the subsequent second biotreatment stage and fully quantify reacted ozone. Samples for pH, moisture content, total heterotrophic bacterial enumeration, and GC-TPH analysis were taken before and after each test.

Peroxone was applied using a similar application as used with ozonation, except that 100 ml of a 500 ppm hydrogen peroxide solution was first slowly pumped into the columns using a Masterflex brand peristaltic pump (Cole-Parmer Instrument Company) before sparging ozone. For the HMW-Soil, the ozone generator was set at 100% voltage and a 2 scfh flowrate, yielding an ozone gas phase concentration of 1.09×10^{-3} mol/l (4.5% [v/v]). For the LMW-Soil, an 80% voltage setting was also evaluated resulting in a gas phase ozone concentration of 0.49×10^{-3}mol/l (2.5% [v/v]). Because of the presence of water and the generation of surfactant-like compounds from the first biotreatment stage, foaming could be a problem during the ozone sparging process. Soil particles carried by the foam could get into gas absorbers and clog the gas distribution stone. Therefore, to alleviate this event, an empty 100 ml flask was placed in the off-gas treatment line before the first gas absorber in order to trap any foam that may be produced. After reaction, oxygen was sparged into the system and water samples collected and analyzed for hydrogen peroxide levels. All of these procedures were performed to make sure that there was no residual oxidants remained in the system which may hinder the reestablishment of bioactivity. Excess water was drained out of the system after chemical priming testing was completed.

Soil loaded into the columns for the Fenton's Reagent experiment was initially dosed with the $FeSO_4$ solutions. The solution was let sit for 24 hours to allow the salt solution to soak into the soil. The amount of iron salt added was formulated to yield 2,000 mg Fe/kg dry soil dose. Hydrochloric acid was mixed with the soil to reduce the pH value to less than 3.

Then, 100 ml of a 20,000 mg/l hydrogen peroxide solution was slowly pumped into the columns. The hydrogen peroxide solution was drained every 4 hours of the first 12 hours and replaced with fresh hydrogen peroxide to ensure an adequate supply of hydrogen peroxide was present.

The Control Set was allowed to continue biological activity using the same biological method described earlier while other sets being treated chemically. For both soils, oxygen was sparged into the Control columns for 20 minutes when the oxidation processes were started on the other sets and same sample event was performed with other sets when the oxidation stage was completed.

Post-Chemical Priming Biotreatment Stage: After chemical priming, the soil in each column was mixed with 30 ml settled activated sludge and enough of a 2M sodium hydroxide solution to increase the soil pH up to about 7 (where needed), while nutrients were dosed to achieve a TPH:N:P ratio of 100:25:10 (based on actual residual soil TPH levels). These additions were also applied to the Control Sets for both soils. Biological treatment was then conducted using the same methods used in the first biological treatment stage until a distinctive decrease in biodegradation rate was observed. GC-TPH analyses were performed weekly. Total heterotrophic bacterial population was enumerated every 2 weeks. Oxygen, carbon dioxide, OUR, and volatile organic concentrations in the column headspace were measured every 5 days.

Analytical Methods

Moisture Level

The moisture content of the soil was measured gravimetrically after the soil was dried at 105°C for 12 hours using a laboratory oven. Moisture content was determined by:

$$MoistureContent(\%) = \frac{W_{total} - W_{dry}}{W_{total}} \times 100, \quad (3\text{-}3)$$

where,

W_{total} = Total weight of wet soil, g

W_{dry} = Dry weight of the soil, g

pH

pH measurement was performed using Accumet pH Model 15 meter (Fisher Scientific). The pH meter was calibrated with standard buffer solutions of pH-4, pH-7, and pH-10. Between sample analysis, the electrode was rinsed with distilled water and gently blot-dried with tissue. Constant stirring of sample was performed to quickly reach a steady potential.

Soil pH was measured using EPA Standard Method No. 9045C (USEPA, 1987). The method involves adding 20±0.1g of soil to 20 ml of distilled water and continuously stirring for 5 minutes. After allowing the soil suspension to stand for about 1 hour, most of the suspended clay is settled out from the suspension then pH measurement is taken on the aqueous phase. The glass electrode is immersed just deep enough into the clear supernatant

solution to establish good electrical contact. All results are reported as "soil pH measured in water at 20EC", which is the temperature at which pH meter is calibrated.

Microbial Enumeration

Total heterotrophic microbial counts were used to indicate active bacterial populations in the soil samples. Counts were accomplished using a pour plate technique amended with nutrient agar (Hach Company, Loveland, CO.). To enumerate total heterotrophs in soil, 10 grams of soil sample is mixed with 30 ml distilled water and shaken with a wrist-shaker for 2 hours to allow microorganisms to enter the liquid phase. 11 ml of sample is transferred into a dilution bottle (Fisher Scientific) with 99 ml of sterile phosphate buffered water resulting in 1:10 dilution. After several dilutions were made, 1 ml of each dilution and moderate amount of agar was added onto pour plates and incubated for 48 hours at 35°C within an incubator (Fisher Scientific Isotemp Standard 600 Series). Counts were expressed as colony forming units per gram of dry soil (CFUs/g soil). Countable plates are those that have 20 to 300 visible colonies. Counting was accomplished using a lighted colony counter (Leica Model 3325, Buffalo, NY).

Results were determined by:

$$Microbial\ Counts(CFUs/g\ dry\ soil) = \frac{N \times DF}{C_{slurry} \times (1 - MoistureContent)}, \qquad (3\text{-}4)$$

where:

N = Counts

DF = Dilution Factor

C_{slurry} = Slurry concentration, mg/l

Liquid Phase H_2O_2 Concentrations

H_2O_2 concentrations in the aqueous phase were analyzed using hydrogen peroxide colorimetric test strips (Merckoquant, Germany). When dipped into the test solution, the reaction zone on the strip transfers oxygen from the hydrogen peroxide to an organic redox indicator, which is then converted into a blue oxidation product. Hydrogen peroxide concentration was then determined by comparing the reaction zone with a color scale provided by manufacturer. The color scale has 6 concentrations (namely 0, 1, 3, 10, 30, and 100 mg/l). Hence, hydrogen peroxide liquid concentrations are within a close range rather than an exact concentration. Since the purpose of measuring hydrogen peroxide concentration is to maintain a target hydrogen peroxide concentration level in the liquid phase, this test is suitable and convenient for use during experimentation. Before the strips were used, standard hydrogen peroxide solutions of 0, 1, 3, 10, 30, and 100 ppm were made and a more refined calibration of the color scale was conducted.

To eliminate color interference during testing with soil slurries, about 5 ml of slurry sample was first filtered through 110 mm pore size filter paper and the filtrates used for testing following the same procedure used with the aqueous samples.

Ozone Concentrations

The method used for determination of ozone concentration is based on the oxidation of iodide in an aqueous solution to iodine and then its subsequent reduction with sodium thiosulfate. The reaction of ozone with iodide follows the reaction scheme presented below:

$O_3 + I^- \rightarrow O_2 = IO^-$ (fast), (3-5)

$IO^- + H_2O \rightarrow HIO + OH^-$ (fast), (3-6)

$HIO + 2I^- \rightarrow I_3^- + OH^-$ (fast), (3-7)

$3HIO + 3OH^- \rightarrow IO_3^- = 2I^- + 3H_2O$ (very slow), (3-8)

Upon acidification, the overall analytical mechanism is:

$O_3 + 3I^- + H_2O \rightarrow I_3^- + O_2 + 2OH^-$, (3-9)

One molecule of ozone liberates 2 equivalents of titrable iodine. The reaction between iodine and sodium thiosulfate is:

$I_2 + 2S_2O_3^{2-} \rightarrow 2I^- + S_4O_6^{2-}$, (3-10)

To measure ozone concentrations in the gas phase, an accurate measured volume of gas is bubbled through a 2% solution of KI. The solution is then transferred to a beaker and acidified with 10 ml 1N sulfuric acid. While stirring, the KI solution is titrated with the 0.1 N thiosulfate solution with 2 ml of starch indicator added just before the clear endpoint. The concentration of ozone (C) as mol/l at standard conditions is given by:

$$C = \frac{V_{th}(ml) \times N_{th} \times 2(mol/l)}{2 \times T_b(\min) \times V_{sc}(scfh) \times \frac{28.316 l/ft^3}{60 \min/h}}, \quad (3\text{-}11)$$

where:

V_{th} = Volume of thiosulfate used, ml

N_{th} = Normality of thiosulfate, mol/l

T_b = Bubbling time , hr

V_{sc} = Standard volume of ozone measured by ozone generator in standard cubic feet per hour, scfh

Headspace Gas Analysis

A portable multi-gas analyzer (Gas Tech GT Series Monitor, Newark, CA) was used during the column phase experiments to measure oxygen, carbon dioxide, and volatile hydrocarbon concentrations in the gas phase (column headspace). The monitor has an electrochemical oxygen sensor, an infrared carbon dioxide sensor, and a catalytic compensated sensor for total hydrocarbons. The unit also measures lower explosive levels (LEL) which were not measured during this study.

Petroleum Hydrocarbon Analysis

There are two methods available for analyzing petroleum hydrocarbons in the soil phase: the "Total Petroleum Hydrocarbon" Method (EPA Method 418.1) and "Target Compound(s), GC (Gas Chromatograph)-TPH" Method (EPA Method 8015B). In Method 418.1, the measurement of the "total hydrocarbon" content is analyzed using an infrared spectrophotometer. *N*-paraffins, the major composition of petroleum hydrocarbons, exhibit a strong adsorption band at this wavelength due to the presence of CH_2 groups in the molecules. Total hydrocarbon concentration is expressed relative to the detector response to a standard mixture containing a fixed ratio of aromatic and paraffinic hydrocarbons, or to a petroleum product reference sample (Potter, 1993). The advantages of IR method are that it

is easy, quick to perform the analysis, and comparably cheaper (Nyer and Skladany, 1989). The major drawback of EPA 418.1 when used to analyze complex petroleum products is that since infrared measure CH_2 bond, all materials, contaminants and benign materials, that are soluble in the solvent will be extracted and possibly show up as TPHs. They can create positive or negative interference. Hence, "Total Petroleum Hydrocarbons" method is valuable for use as a screening step in determining the presence of hydrocarbon contamination. Total Petroleum Hydrocarbons (TPH) Infrared Method EPA 418.1 was used during the first bioremediation stage of the LMW-Soil and to indicate the initial TPH levels in both soils.

Most current GC methods for the analysis of gasoline and diesel TPH are flame ionization detector (FID) based, primarily because of the FID's universal and sensitive response to all hydrocarbons (Xiang and Morgan, 1995). Method 8015B is responsible for the analysis of nonhalogenated organics using GC/FID. It may also be used to the analysis of petroleum hydrocarbons, including GRO (gasoline range organics) and DRO (diesel range organics). This method is used in the chemical priming and reestablished second stage bioremediation.

TPH-IR: In this method, an organic solvent (dichlorotrifluoroethene) is used to extract the hydrocarbons from the soil or slurry samples. Jing (1998) determined that 2 minutes of sonication using an ultrasonic processor was optimum to effectively extract TPH from soil

samples, while 30 minutes of agitation using a rotary shaker was sufficient for extraction of TPH from slurry samples.

To prepare a soil sample for sonication, 5 grams of wet soil was mixed with an equal amount of anhydous sodium sulfate (Fisher Scientific) to absorb the moisture and 25 ml of solvent (Horiba, Irvine, CA). Hydrochloric acid was also added to the soil samples to lower the pH to less than 2 which will assist with the dissociation of hydrocarbons from the soil. After sonication, the extract was filtered though silica gel to remove interfering polar compounds, such as humic acids and other detritus-based compounds.

Slurry sample extraction was performed using a wrist shaker. 5 ml of acidified slurry was mixed to 25 ml of solvent and shaken for 30 minutes. The bottom extract was then pipetted out and cleaned with silica gel. Measurements of TPH was performed using the infrared spectrophotometer (Horiba OCMA-350,Irvine, CA). The standard used for instrument calibration was a solution composed of 2,2,4-isooctane, cetane, and monochlorobenzene at a volume ratio of 3:3:2 (Horiba, Irvine, CA). TPH concentration in soil was determined by the following formula:

$$TPH(mg/kgsoil) = \frac{TPH_{reading}(mg/l) \times V_s(l)}{W_s(mg) or C_s(mg/l)}, \tag{3-12}$$

where:

V_s = Volume of solvent, l

W_s = Weight of soil sample, mg

C_s = Soil concentration in slurry, mg/l

TPH-GC/FID: In order to perform GC analysis, more stringent sample preparation, sample extraction, and sample clean-up procedures are required. In this research, an ASE 200 Accelerated Solvent Extractor (Dionex Corporation, USA) was used to extract the TPH from soil or slurry samples. This method, referred as Pressurized Fluid Extraction (PFE), was recently approved by EPA and added as Method 3545 in U. S. EPA SW 846 (USEPA, 1996). The Dionex ASE 200 Accelerated Solvent Extractor was the only device used to develop all of the equivalency and validation data that provided the basis for approval of Method 3545 for RCRA compliance monitoring. The ASE 200 accelerates the traditional extraction process using solvent at elevated temperatures. Pressure is applied to the sample extraction cell to maintain the heated solvent in a liquid state during the extraction. ASE 200 is able to perform extractions that are highly reproducible. This method also eliminates interferences caused by variations in temperature and sample quantity often encountered using traditional soxhlet extraction or ultrasonic extraction methods. Additionally, compared with soxhlet extraction, the PFE Method uses less solvent (<15 ml per 10 g) over a significantly less time span (minutes vs. hours). The programmed method that was used for extraction is summarized as follows:

Solvent:	Acetone (pesticide quality or equivalent)/ Hexane (pesticide quality equivalent) (1:1, v/v)
Oven Temperature:	100°C
Pressure:	10 MPa (1500 psi)
Oven Heat-up Time:	5 min

Static time: 5 min

Flush Volume: 60% of extraction cell volume

Upon initiation, the method proceeds as follows (DIONEX, 1997, ASE 200 Accelerated Solvent Extractor Operator's Manual, Dionex Corporation):

1. The oven begins heating to 100°C.
2. When the oven has reached the set point, the pumps fill the cell with solvent. The static valve closes and the pump continues pumping until the pressure reaches 1,500 psi.
3. An initial 5-minute heat step occurs, followed by the first 5 minute static step.
4. After the static step, the static valve opens and the pump flushes 20% of the cell volume of fresh solvent through the cell.
5. The static and flush steps are repeated. The method then continues to the 120 seconds purge step to remove the residue solvent from the system into the collection vials.

Dichloromethane (DCM) and the mixture of hexane and acetone (50:50, volume ratio) are widely used as organic solvents (USEPA, 1987). A comparison study on the effects of using these two solvents was accomplished with the assistance of the Mississippi State Analytical Laboratory located at Mississippi State University. The results, attached as Appendix C, showed that hexane and acetone system was a more efficient solvent than DCM when used as solvent for the extraction of petroleum compounds.

Soil samples were mixed with a drying or dispersing agent before loading into the ASE cells. Two drying and dispersing agents are most often used: sodium sulfate and diatomaceous earth (hydromatrix). Hydromatrix is easier to work with because it dries out

samples more quickly, provides a cleaner transfer of the mixtures to the cell, and extracts well. Although, sodium sulfate has a higher availability, it tends to clump the samples, making transfer more difficult. Also, temperature changes during extraction may cause gel formation in the purging line if sodium sulfate is used.

Since the existing water in the original sample will also be extracted by ASE and water content influences subsequent GC analysis, the extracted water layer has to be discarded. The majority of the water/acetone layer is pippetted out using a disposable glass pipette. Anhydous sodium sulfate is then added to absorb the remaining water from the extract. After separating the solid and extract using paper filter (110 mm pore size), the extract was concentrated to 4 ml using an isothermal water bath (temperature set at 40°C). After the concentration step, the extract is ready for GC analysis.

During the chemical priming process of slurry experiments, TPH concentrations in the water phase were also measured. Liquid-liquid extraction based on the USEPA Standard Methods was used. This technique is summarized as follows:

1. Allow the slurry to settle and quantitatively transfer a 20 ml sample from the water layer to a 50 ml separatory funnel. Add 10 ml of hexane to the funnel. Seal and shake the separatory funnel vigorously for 1-2 minutes with periodic venting to release excess pressure.
2. Allow the organic layer to separate from the water phase for a minimum of 10 minutes.
3. Repeat the extraction two more times, using 5ml of fresh hexane each time. Combine the three solvent extracts. This gives 1:1 solvent to sample ratio with the extract then being ready for GC analysis.

GC analysis

A Hewlett Packard 6890 Series capillary column gas chromatograph equipped with an FID detector and a HP automatic liquid sampler (HP G1512A controller, HP G1513A injector, and HP 18596C tray) was used for TPH analysis. Detailed GC parameters are provided in Table 3.2. A 30m × 0.53mm ID column having a film thickness of 1.5 μm was used as the analysis column. HP 6890 Series ChemStation (based on Microsoft Windows® 95) was used to collect and analyze the data.

A No. 2 Fuel Oil standard was purchased from Sigma-Aldrich company as the calibration standard for GC-TPH analysis. The No. 2 Fuel Oil, also known as Diesel Range Organic (DRO), was diluted to four different concentrations in methanol and calibration was conducted by corresponding summed total peak areas for all compounds to the known concentrations (Appendix B). Unfortunately, not all of the GC-TPH analytical results fall nicely into the DRO range. As seen in the initial chromatograph of the HMW and LMW-Soil (Appendix E), a peak covering a large area was presented after retention time of approximately 30 minutes. This type of unseparated peaks, referred as humpograms by Potter *et al.* (1993), are the indication of motor lubricating oils and residual fuel product mixtures having high boiling points. Additionally, the standard of this high boiling point mixture is not available in the market, hence quantification of this portion of TPH was not achieved. Instead, a peak summing method described as following was used:

1. Peak areas, instead of individual peaks, were of interest
2. All petroleum hydrocarbons presented in the chromatograph were grouped into three

portions. Compounds having retention time of 7 minutes to 11.3 minutes indicated light petroleum hydrocarbons. Peaks present at retention time between 11.3 to 26 minutes were deemed medium petroleum hydrocarbons. The unseparated peak covering 26 to 46 minutes were defined as heavy petroleum hydrocarbons.

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Table 3.1

Initial Characteristics of the Soil Samples

	HMW-Soil	LMW-Soil	Units
TPH (GC)*	1,980	254	ppm
TPH (IR)	43,000	3,600	ppm
pH	7.1	8.1	
Microbial Count**	2.8×10^6	1.4×10^6	CFUs/g soil

* TPH was measured as diesel range organics by GC
** Microbial counts were total heterotrophic counts. The unit used was colony forming units (CFUs) per gram soil.

Table 3.2

GC Parameters

GC	HP 6890
Column type	SPB™-1, 30m × 0.53mm ID, 1.5μm film
Data station	HP 6890 ChemStation
Oven temperature program	35°C (2min) to 300°C at 8°C/min, hold 15 min
Run time	50.13 min
Detector	FID (flame ionization detector)
Detector temperature	280°C
Injector temperature	320°C
Helium flow rate	10 ml/min
Injection size	0.5 μl

Table 3.3

Summary of Candidate Column Operating Conditions used with the HMW and LMW-Soils

Set Number	HMW-Soil	LMW-Soil
1	Control	Control
2	Ozonation ($Q = 2$ scfh, $V_{set} = 100\%$, $t=4$ hr)	Ozonation ($Q = 2$ scfh, $V_{set} = 80\%$, $t=2$ hr)
3	Peroxone ($Q = 2$ scfh, $V_{set} = 100\%$, $C_{H2O2} = 500$ mg/l, $t=4$ hr)	Peroxone ($Q = 2$ scfh, $V_{set} = 100\%$, $C_{H2O2} = 500$ mg/l, $t=2$ hr)
4	Fenton's Reagent: ($C_{H2O2} = 20{,}000$ mg/l, $C_{Fe2+} = 2{,}000$ mg/l, pH = 3, $t = 24$ hr)	Peroxone: ($Q = 2$ SCFH, $V_{set} = 80\%$, $C_{H2O2} = 500$ mg/l, $t=2$ hr)
5	NA	Fenton's Reagent ($C_{H2O2} = 20{,}000$mg/l, $C_{Fe2+} = 2{,}000$mg/l, pH = 3, $t = 24$ hr)

Note: NA: Not Applicable
V_{set} : Voltage Setting for the ozone generator
C_{H2O2} : Hydrogen peroxide concentration
C_{Fe2+} : Ferrous sulfate concentration

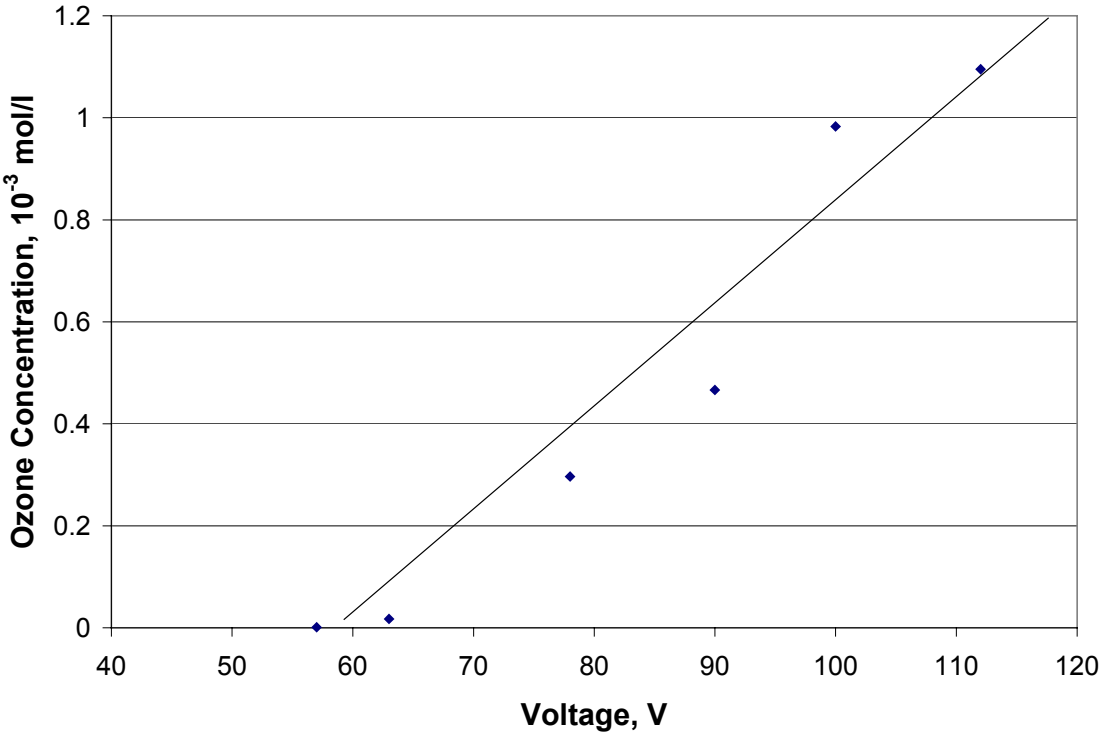

Figure 3.1. Ozone Concentration vs. Voltage (Flow Rate = 2 scfh)

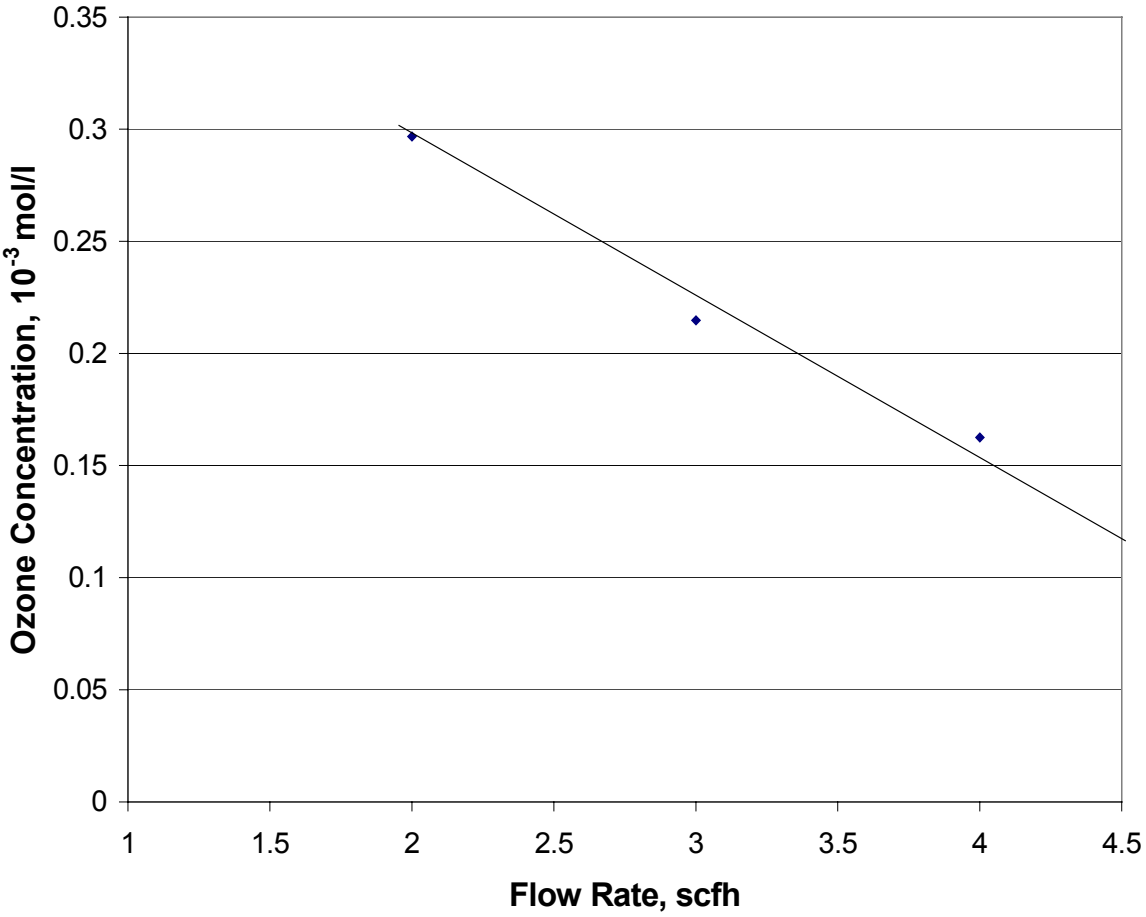

Figure 3.2. Ozone Concentration vs. Flow Rate (Voltage = 112 V)

Off-gas treatment line

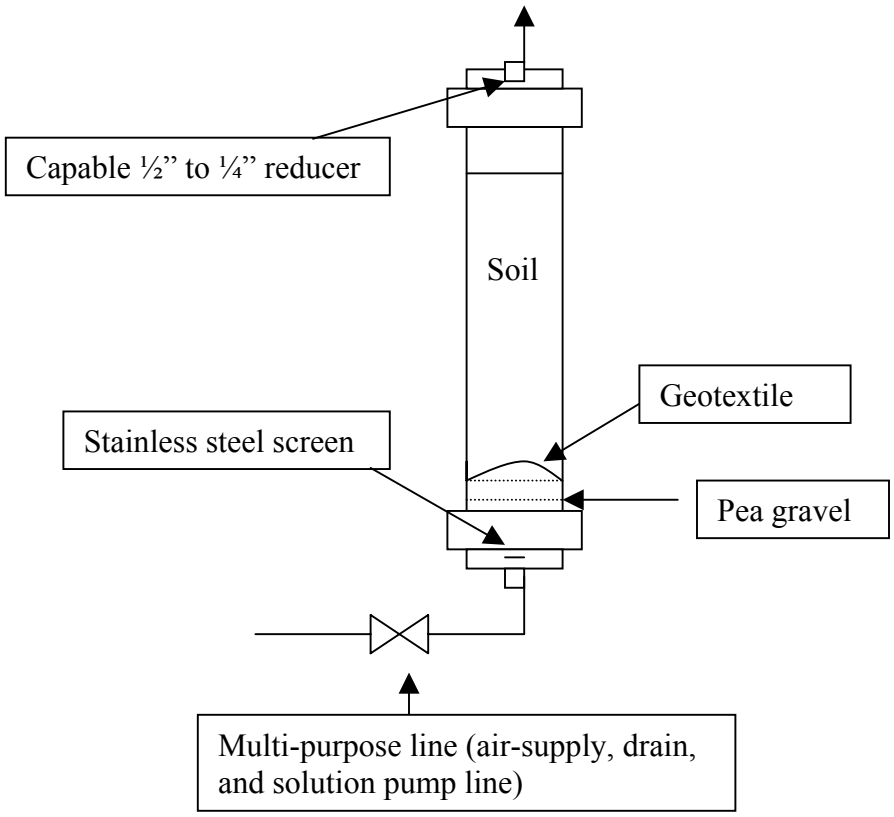

Figure 3.3. Schematic of a Bench Scale Column Reactor

CHAPTER IV

SHAKE FLASK EXPERIMENTAL (PHASE I) RESULTS

This series of shake flask experiments was performed to screen candidate biological and chemical conditions most conducive to TPH removal as both stand-alone and combined processes. They were designed to firstly determine if chemical oxidation could obtain further removal of bio-refractory compounds that are not degraded during the first stage biotreatment performed prior to oxidation. The contaminated soil-water formed slurry was initially treated using an optimized biological method. Chemical oxidizers were added once removal of the residual petroleum hydrocarbons within the bioreactors had reached a distinct point of diminishing returns. After contacting with oxidizers (i.e. chemical priming), the ecological conditions in the chemical primed slurries were adjusted to re-establish bacterial activity and further biological treatment conducted to remove the remaining TPH and oxidation by-products.

Biological Pretreatment Experiments

The purpose of these experiments was to test several candidate treatment strategies in order to determine the optimal operational parameters to be used in the first biological treatment stage. This effort was done to ensure biotreatment as a stand-alone process was given sufficient opportunity to perform. The information on enhanced bioremediation conditions for the HMW-Soil was adopted from Ms. Jing's experiments (Jing, 1998). She concluded that the biological rate of the soil TPH contamination appears to be best enhanced by periodic nutrient amendment and initial activated sludge augmentation. (Note: Ms. Jing

was a graduate student in the E-TECH Laboratory evaluating biotreatment strategies as a stand-alone process for treating the HMW-Soil at the same time this study was being initiated.)

Since no prior information was known concerning the bioremediation potential of the LMW-Soil, a series of shake flask experiments was conducted to determine the best biotreatment conditions for this soil. The intent being that the best biotreatment offers for treating this soil was to be attempted before chemical priming was evaluated. Figure 4.1 presents soil TPH concentrations (analyzed using IR Method) versus incubation time for this series of experiments. The highest and most complete biodegradation rate is observed when amending with activated sludge and nutrients in comparison with the control or nutrient addition alone. This indicates that the natural biodegradation of hydrocarbon contaminants in the system was hindered by the limited number of TPH active bacteria early during incubation and by the lack of nutrients. Little difference in performance was observed between the two levels of nutrient dosing. Tween 80 addition after 21 days of incubation time did not appear to enhance the rate or extent of TPH degradation. On the contrary, a slight increase in TPH is observed after the addition of Tween 80, which could be due to the increased extractability of the TPH from the soil afforded by the surfactant. However, in general, the overall performance of the conditions did not vary dramatically. None of the conditions removed the TPH to levels below 1,000 mg/kg within 40 days of incubation.

Table 4.2 lists the results of the heterotroph plate counts on the biotreated soil. It indicates a large increase of the amount of bacteria in all sets after 2 weeks of incubation. Those flasks augmented with activated sludge had much higher microbial counts than the non-augmented flasks. Enumeration of total heterotrophs in activated sludge showed that activated sludge had about 3.2×10^6 CFUs/ml resulting in a total of 6.4×10^5 CFUs spiked into each flask (20 ml per flask was added). After 2 weeks of incubation time, the bacterial populations in the bioaugemented sets exceeded 8.0×10^7 CFUs/g. Bioaugmentation likely provided a higher degradation rate because of the existence of increased bacteria diversity and population. By Week 4, the data indicated a decrease in bacterial populations in all sets. This population decrease corresponds nicely with the observed decreased TPH removal rate (see Figure 4.1) indicating that the remaining TPH was more recalcitrant than the degraded TPH.

In summary, biotreatment as a first treatment stage removed approximately 71% of the original TPH. The biotreatment condition to be used prior to chemical priming of the LMW-Soil was activated sludge addition at the beginning of the incubation and nutrients batch amended at a TPH:N:P ratio of 100:25:10.

Chemical Priming Experimental Results

HMW-Soil

Figure 4.2 presents the slurry phase TPH concentrations for the HMW-Soil after treatment with various Fenton's Reagent conditions tested. After five hours of treatment, these data show that all three acidic conditions yielded higher TPH removals than the neutral pH condition (especially with the heavy petroleum hydrocarbons). This agrees with the findings of Watts, *et al.* (1990) and Huang, *et al.* (1993) who observed that Fenton's Reaction favors acidic conditions over neutral or basic conditions. Higher dosages of hydrogen peroxide and ion salt showed only slightly improved results. Also, higher ratios of $FeSO_4$ to H_2O_2 did not provide an increase in hydrocarbon removal. Research has confirmed that excessive additions of ion salt does not guarantee higher treatment efficiency (Watts, 1992). Depending on the properties of the soil, ion salt addition may not be necessary for those soils having a rich mineral content already within the soil matrix (Watts, 1992).

The aqueous phase TPH concentrations from the shake flask experiments are presented in Figure 4.3. These data show that initially there was little dissolved hydrocarbons in the water phase. This occurred because the water soluble contaminants were likely degraded by the bacteria during the first stage of biological pretreatment. After chemical priming, significant increases in aqueous phase TPH levels are observed. It is speculated that Fenton's Reagent likely reacted with the organic sorption sites on the soil and/or degraded insoluble TPH compounds into more soluble by-products resulting in an increase in hydrocarbon levels within the aqueous phase. When evaluating the data presented in Figure 4.3, the solid phase results must also be taken into account to explain the results. It appears that higher

hydrogen peroxide and ion salt dosages not only yielded more TPH removal in the soil phase, but also provided a decrease in soluble TPH within the aqueous phase. It can be concluded from

these two charts that higher Fenton's Reagent dosages applied at acidic pH provided an increase in TPH removal efficiency.

Figure 4.4 shows the rate test experimental results for the previously biotreated HMW-Soil treated with Fenton's Reagent. The tested conditions used for this experiment were 20,000 ppm hydrogen peroxide and 2,000 ppm $FeSO_4$ (H_2O_2:Fe ratio of 27:1 [w/w]) at a pH of 2.5. As expected, petroleum hydrocarbons were removed as reaction time progressed. After the first hour of Fenton's Reagent treatment, the heavy TPH levels clearly decreased. No obvious difference in medium and light TPH levels was observed through about the reaction period. All totaled, approximately 53% removal of the heavy TPH was achieved after 8 hours of treatment.

LMW-Soil

The results of the investigations on the effects of Fenton's Reagent dosage on TPH removal conducted on the LMW-Soil are presented in Figures 4.5 and 4.6. Although these data are not as dramatic as the HMW-Soil data, these two charts do present comparable results to the HWM-Soil data. Clearly, the acidic pH conditions yielded better TPH removal efficiencies than the neutral pH experiments. Also, higher dosages of hydrogen peroxide and ion salt (at similar ratios) achieved higher TPH percent removals. The best TPH removal was achieved by the 20,000 ppm H_2O_2 and 2,000 ppm $FeSO_4$ at pH of approximately 2.5

condition.

Figure 4.7 presents the rate testing experimental results for the application of Fenton's Reagent on the previously biotreated LMW-Soil. The condition used for this experiment was 20,000 ppm hydrogen peroxide and 2,000 ppm $FeSO_4$ (H_2O_2:Fe ratio of 27:1 [w/w]) and a pH of 2.5. An increase in TPH concentration is observed early during testing. It is believed that Fenton's Reagent likely increased TPH extraction efficiency via oxidation of the sorption bonds. By oxidizing the sorption bonds, Fenton's Reaction breaks down the petroleum hydrocarbon-organic matter complex formed at the soil surfaces. After three hours, the reaction rate of heavy petroleum hydrocarbons decreased considerably. There was an increase in light petroleum levels after three hours which could be due to degradation of the heavy petroleum portion into lighter fractions. After eight hours of testing, approximately 91% of the heavy petroleum hydrocarbons were removed.

Table 4.2 and 4.3 summarize the pH measurements and heterotrophic bacteria enumerations before and after chemical priming, respectively. A slight decrease in pH was observed in almost all of the sets, except for Set 3 of the HMW-Soil. It is speculated that Fenton's Reaction acidifies the reaction system due to the formation of organic acids as by-products. As discussed in Chapter II, one major group of by-products of petroleum hydrocarbon oxidation is organic acids (carboxylics). The presence of these organic acids is the likely reason for the decrease in pH within the test slurry systems.

Table 4.3 presents the results of bacterial enumerations for total heterotrophs in the HMW and LMW-Soils. These data indicate that the bacterial populations were not affected by the addition of chemical oxidizers. In fact, an increase was observed in all of the sets. The exact reason for this phenomena is unknown. One possible explanation is that the stronger agitation provided by the magnetic stir bar and Fenton's Reaction increased the extractability of the microorganisms from the soil which might have been previously adsorped on the soil particles and/or heavy petroleum hydrocarbons. Recent studies have shown that plating techniques when applied to soils only account for approximately 10% of the total bacterial population due to the strong affinity of the microbes for the soil surface (Glaser, 1997).

Summary

It can be concluded from this series of experiments that chemical priming in the form of Fenton's Reagent is capable of increasing the rate and extent of TPH degradation in both the HMW and LMW-Soils. Dosing with 20,000 ppm hydrogen peroxide and 2,000 ppm ferrous sulfate at a pH of 2.5 appears to be the optimum condition among all conditions tested during chemical priming experiments. Fenton's Reagent appears to further decrease the pH within the soil slurry systems, hence, slurry pH will have to be adjusted to neutral to reestablish biological activity.

Reestablished Bioremediation Experimental Results

HMW-Soil

Figure 4.8 presents the results of the reestablished biological treatments within the shake flasks for the HMW-Soils. Figure 4.8 plots only the heavy portion of the GC analysis, while the results of the light and medium portions are presented in Tables 4.4 and 4.5. After Fenton's Reagent treatment, the reestablished bioremediation achieved further removal of the heavy TPH. All of the four sets that were previously treated with Fenton's Reagent yielding an additional 20% heavy TPH removal during the second stage of bioremediation. Compared with the Control Set, the chemical primed sets achieved more than 32% removal of the heavy TPH. Although 20,000 ppm hydrogen peroxide and 2,000 ppm ferrous sulfate at pH of 2.5 achieved the greatest extent of TPH removal during Fenton's Reagent treatment, it does not appear to have exhibited better enhancement than the other sets using different Fenton's Reagent conditions. But, from an overall TPH removal standpoint, this condition yielded the most TPH removal of all the conditions tested. Results of the light and medium fractions listed in Tables 4.4 and 4.5 do not show a clear pattern for the removal of these fractions of hydrocarbons. Higher levels of the light and medium fractions were observed after the first stage of bioremediation and chemical priming, which is likely due to increased presence of extractable hydrocarbons and heavy hydrocarbon degradation by-products. After reestablishing bioremediation, these fractions of hydrocarbons decreased dramatically.

LMW-Soil

Figure 4.9 presents the results of heavy TPH analysis in slurry phase for the LMW-Soil using BIO/OX/BIO. The light and medium TPH analytical results are presented in Tables 4.7 and 4.8. It is observed in Figure 4.9 that after Fenton's Reagent treatment, the bioremediation rate for the two sets that were previously treated by Fenton's Reagent under pH acidic conditions appeared to be very small. On the other hand, the set that was treated under neutral pH conditions had a much higher TPH removal within the same time period. The exact reason for this is unknown. However, from the pH measurement results listed in Table 4.9, it seems that there was a decrease in slurry pH in all of the three sets after two weeks of reestablished bioremediation. After proper adjustment was made, the biodegradation rate is observed to increase within Sets 1 and 2, which were previously treated under acidic conditions. Over 90% TPH removal was achieved within 68 days for the primed sets. The first stage of bioremediation yielded approximately 44% removal, the chemical priming stage achieved about 20% more TPH removal, and reestablished bioremediation yielded an additional 26% TPH removal. Slurry that was treated with biological methods alone only yielded 42.2% total removal of the initial heavy petroleum hydrocarbons. The light and medium TPH fractions followed the similar results to that of the HMW-Soil. An increase of the amount of light and medium TPH was first observed, then a decrease by the end of the reestablished bioremediation stage.

Summary

It can be summarized from these series of experiments that chemical oxidation as evaluated using Fenton's Reagent is capable of increasing the rate and extent of TPH removal once biotreatment is reestablished. Although the 20,000 ppm hydrogen peroxide and 2,000 ppm ferrous sulfate solution at a pH of 2.5 condition yielded the overall best TPH removal during the BIO/OX/BIO experiments for both soils, the lower dosage of 5,000 ppm hydrogen peroxide and 500 ppm ferrous sulfate solution yielded similar enhancement results during the reestablished bioremediation stage. Obviously, lower dosages are more economical making this a significant finding. The optimal BIO/OX/BIO system based on the results of these experiments is:

1. Initial biological treatment with bacteria augmentation and nutrient amendment using a TPH: N: P ratio of 100:25:10 (4 weeks of incubation).
2. Oxidize the soil with 5,000 ppm hydrogen peroxide and 500 ppm $FeSO_4$ at a pH of 2.5 for 2 hours.
3. Recondition the soil to a neutral pH and add activated sludge and nutrients at a TPH: N: P ratio of 100:25:10.

Although ozonation and peroxone are not conducted in slurry experiments because of the foaming problem, optimistic results are expected because ozone and the combination of ozone and hydrogen peroxide are more powerful oxidation systems than Fenton's Reagent.

The effects of ozonation and peroxone process on the treatment of TPH for HMW and LMW-Soils were tested in packed soil column experiments (next section).

Table 4.1

LMW-Soil Slurry Phase Bioremediation Bacterial Enumeration Results

Sets	Microbial Counts ($\times 10^{-6}$CFUs/g dry soil)		
	Initial	2 weeks	4 weeks
Control	1.35	28	26
Nutrient (100:25:10)	1.35	40	35
Nutrient (100:10:5)	1.35	32	44
Nutrient (100:25:10) + A.S.	1.35	89	76
Nutrient (100:10:5) + A.S.	1.35	101	96
Nutrient (100:25:10) + A.S.+ Tween 80	1.35	104	81
Nutrient (100:10:5) + A.S.+ Tween 80	1.35	120	73

Note: Nutrient doses are presented as TPH: N: P ratio

A. S.: Activated Sludge seeded microcosms

Table 4.2

Soil pH during Slurry Phase Fenton's Reaction

Conditions*	HMW-Soil		LMW-Soil	
	Before**	After***	Before	After
Set 1	2.40	2.37	2.53	2.28
Set 2	2.55	2.23	-	-
Set 3	2.45	2.64	2.41	1.82
Set 4	5.54	2.17	7.79	4.53

Table 4.3

Summary of Heterotroph Bacteria Enumeration in Slurry Phase Fenton's Reaction****

Conditions*	HMW-Soil		LMW-Soil	
	Before**	After	Before	After
Set 1	236	604	4.44	64
Set 2	260	1556	-	-
Set 3	224	260	5	76
Set 4	176	236	7.6	492

---

* Set 1: 5000ppm H_2O_2/ 500ppm $FeSO_4$, pH=2.5,
Set 2: 5000ppm H_2O_2/ 2000ppm $FeSO_4$, pH=2.5,
Set 3: 20000ppm H_2O_2/ 2000ppm $FeSO_4$, pH=2.5,
Set 4: 20000ppm H_2O_2/ 2000ppm $FeSO_4$, pH=5.5 for Silver City soil and pH=7.8 for Navy soil.

** Before: Before Fenton's Reagent treatment

*** After: After Fenton's Reagent treatment

****Unit: $\times 10^{-6}$ CFUs/ g dry soil.

Table 4.4

Summary of TPH Analytical Results for the HWM-Soil – Light Fraction

Time, day	Control	Set 1	Set 2	Set 3	Set 4
0	42.2	42.2	42.2	42.2	42.2
28	3180	2648	3246	2105	2563
30	3180	777	677	704	740
54	7291.1	1064.3	888.2	1227.3	2473.6
68	1395.7	204	95.5	201.4	69.9

Set 1: 5,000 ppm H_2O_2/ 500 ppm $FeSO_4$, pH=2.5
Set 2: 5,000 ppm H_2O_2/ 2,000 ppm $FeSO_4$, pH=2.5
Set 3: 20,000ppm H_2O_2/ 2,000 ppm $FeSO_4$, pH=2.5
Set 4: 20,000 ppm H_2O_2/ 2,000 ppm $FeSO_4$, pH=5.5

Table 4.5

Summary of TPH Analytical Results for the HWM-Soil – Medium Fraction

Time, day	Control	Set 1	Set 2	Set 3	Set 4
0	1750.4	1750.4	1750.4	1750.4	1750.4
28	7223	4869	6546	4012	3956
30	7223	5295	4686	4902	5009
54	3353.8	265.9	56.3	282.9	460.3
68	730.0	226.7	171.6	121.5	330.4

Set 1: 5,000 ppm H_2O_2/ 500 ppm $FeSO_4$, pH=2.5
Set 2: 5,000 ppm H_2O_2/ 2,000 ppm $FeSO_4$, pH=2.5
Set 3: 20,000ppm H_2O_2/ 2,000 ppm $FeSO_4$, pH=2.5
Set 4: 20,000 ppm H_2O_2/ 2,000 ppm $FeSO_4$, pH=5.5

Table 4.6

Summary of pH Data Collected during Slurry Phase Reestablished Bioremediation Tests for the HMW-Soil

	After Oxidation	After adjustment	2 weeks restarted bioremediation	4 weeks restarted bioremediation
Control	-	5.55	5.55	5.55
Set 1	2.37	7.42	7.08	7.06
Set 2	2.23	6.44	6.80	6.76
Set 3	2.64	6.71	6.70	6.45
Set 4	2.17	6.68	6.64	6.87

Set 1: 5,000 ppm H_2O_2/ 500 ppm $FeSO_4$, pH=2.5
Set 2: 5,000 ppm H_2O_2/ 2,000 ppm $FeSO_4$, pH=2.5
Set 3: 20,000ppm H_2O_2/ 2,000 ppm $FeSO_4$, pH=2.5
Set 4: 20,000 ppm H_2O_2/ 2,000 ppm $FeSO_4$, pH=5.5

Table 4.7

Summary of TPH Analytical Results for the LWM-Soil – Light Fraction

Time, day	Control	Set 1	Set 2	Set 3
0	246.3	246.3	246.3	246.3
28	1895.0	632.5	1156.5	1352.1
30	1895.0	2391.0	2094.0	1902.0
44	954.8	2040.0	880.9	868.7
58	3219.0	1328.1	8646.1	9456.5
68	2654.2	649.8	2130.5	4268.2

Set 1: 5,000 ppm H_2O_2/ 500 ppm $FeSO_4$, pH=2.5
Set 2: 20,000 ppm H_2O_2/ 2,000 ppm $FeSO_4$, pH=2.5
Set 3: 20,000 ppm H_2O_2/ 2,000 ppm $FeSO_4$, pH=7.8

Table 4.8

Summary of TPH Analytical Results for the LWM-Soil – Medium Fraction

Time, day	Control	Set 1	Set 2	Set 3
0	214.6	214.6	214.6	214.6
28	6326.0	5236.1	4653.1	5923.4
30	6326.0	3296.0	4125.0	4584.0
44	1716.7	2267.8	134.6	158.1
58	1109.6	338.1	0	47.5
68	1230.5	56.5	113.1	82.4

Set 1: 5,000 ppm H_2O_2/ 500 ppm $FeSO_4$, pH=2.5
Set 2: 20,000 ppm H_2O_2/ 2,000 ppm $FeSO_4$, pH=2.5
Set 3: 20,000 ppm H_2O_2/ 2,000 ppm $FeSO_4$, pH=7.8

Table 4.9

Summary of pH Values for the Slurry Phase Reestablished Bioremediation Stage for the LMW-Soil

	After Oxidation	After adjustment	2 weeks restarted bioremediation	4 weeks restarted bioremediation
Control	-	7.8	7.8	7.8
Set 1	2.28	7.49	5.14	6.47
Set 2	1.82	7.26	5.34	6.73
Set 3	4.53	7.12	4.98	7.01

Set 1: 5,000 ppm H_2O_2/ 500 ppm $FeSO_4$, pH=2.5
Set 2: 20,000 ppm H_2O_2/ 2,000 ppm $FeSO_4$, pH=2.5
Set 3: 20,000 ppm H_2O_2/ 2,000 ppm $FeSO_4$, pH=7.8

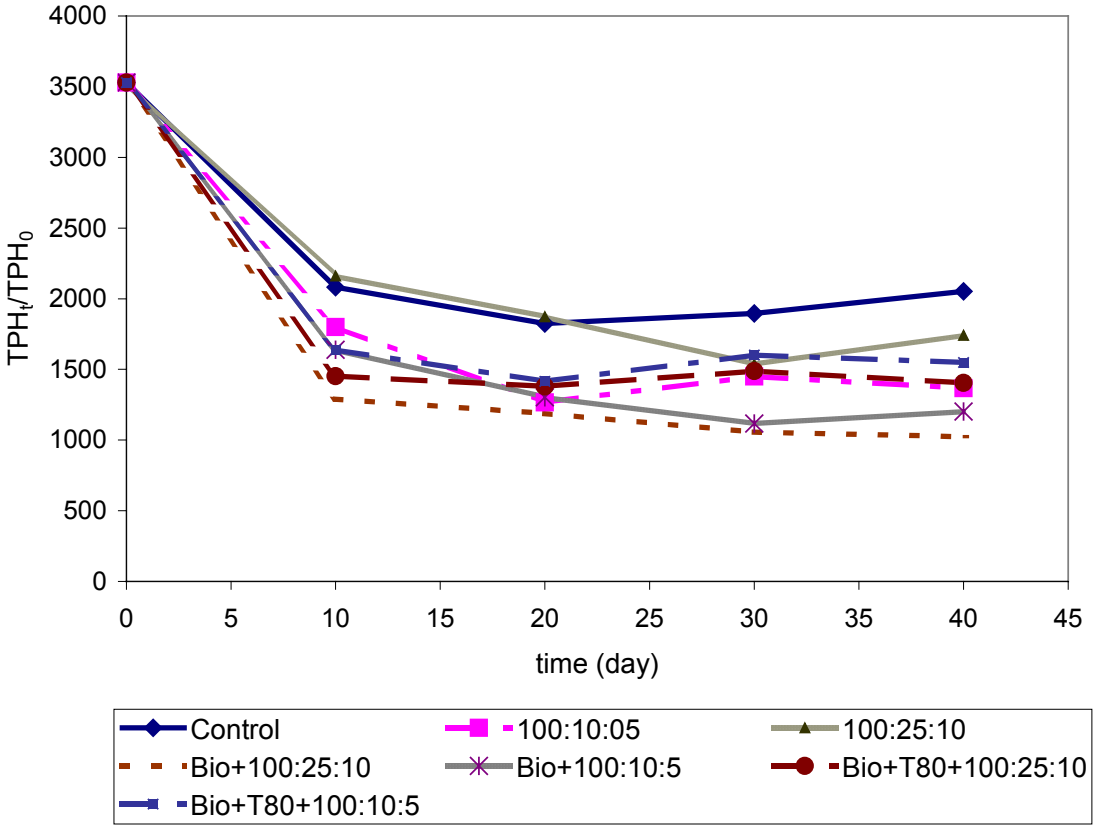

Figure 4.1. LMW-Soil Slurry Phase Initial Bioremediation Results

Bio: Bioaugmentation with activated sludge
100:25:10 or 100:10:05: Indicates TPH: N: P ratios that were dosed to slurry
T80: Addition of Tween 80 on Incubation Day 21

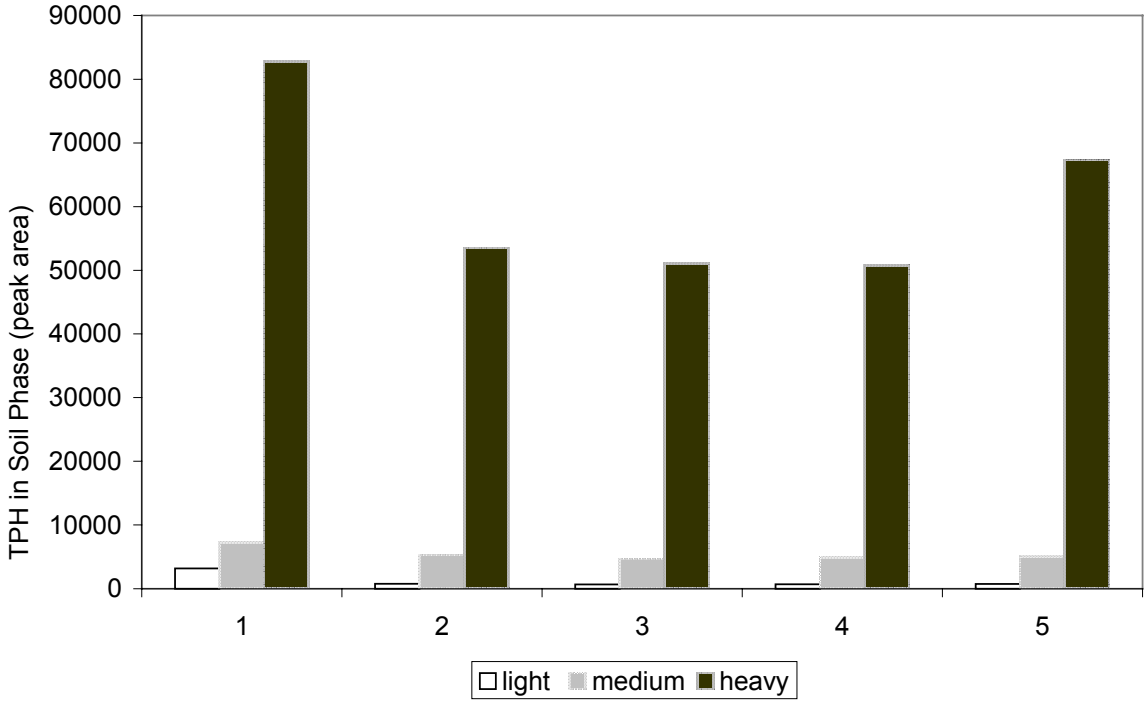

Figure 4.2. Soil Phase TPH Results of Slurry Phase Chemical Priming for the HMW-Soil

1. Post Biotreatment
2. 5,000 ppm HP/500 ppm Fe^{2+}, pH=2.5
3. 5,000 ppm HP/2,000 ppm Fe^{2+}, pH=2.5
4. 20,000 ppm HP/2,000 ppm Fe^{2+}, pH=2.5
5. 20,000 ppm HP/2,000 ppm Fe^{2+}, pH=5.5

Note: HP: Hydrogen Peroxide and Fe^{2+}: $FeSO_4$

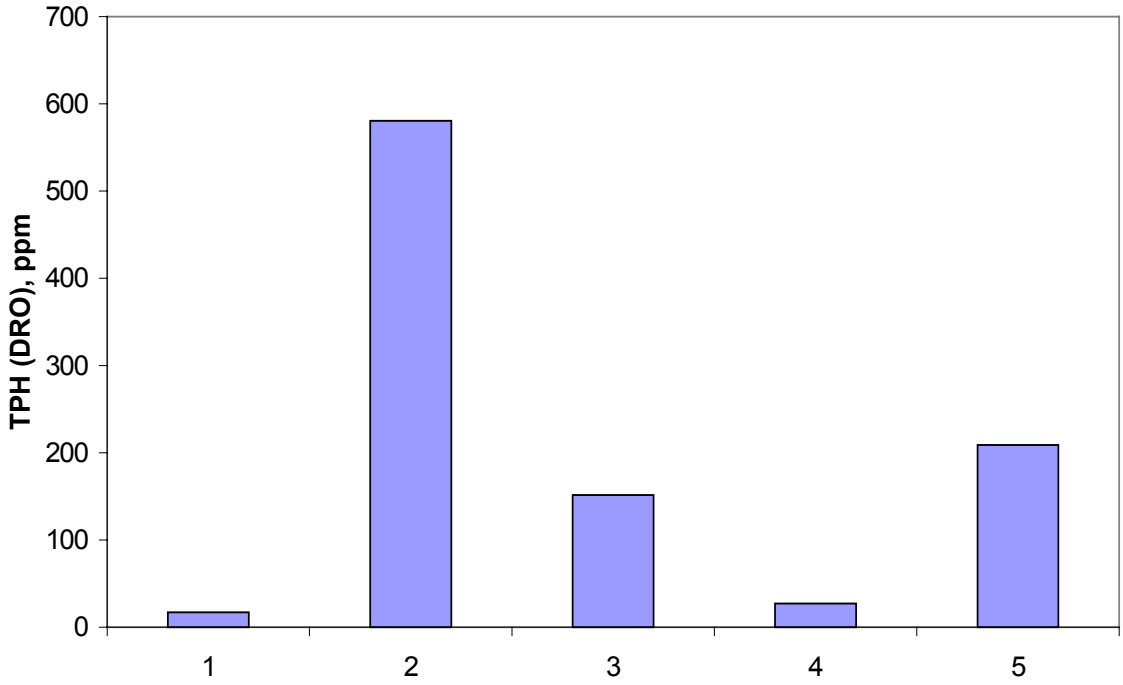

Figure 4.3. Aqueous Phase TPH Results of Slurry Phase Chemical Priming for the HMW-Soil

1. Post Biotreatment
2. 5,000 ppm HP/500 ppm Fe^{2+}, pH=2.5
3. 5,000 ppm HP/2,000 ppm Fe^{2+}, pH=2.5
4. 20,000 ppm HP/2,000 ppm Fe^{2+}, pH=2.5
5. 20,000 ppm HP/2,000 ppm Fe^{2+}, pH=5.5

Note: HP: Hydrogen Peroxide and Fe^{2+}: $FeSO_4$

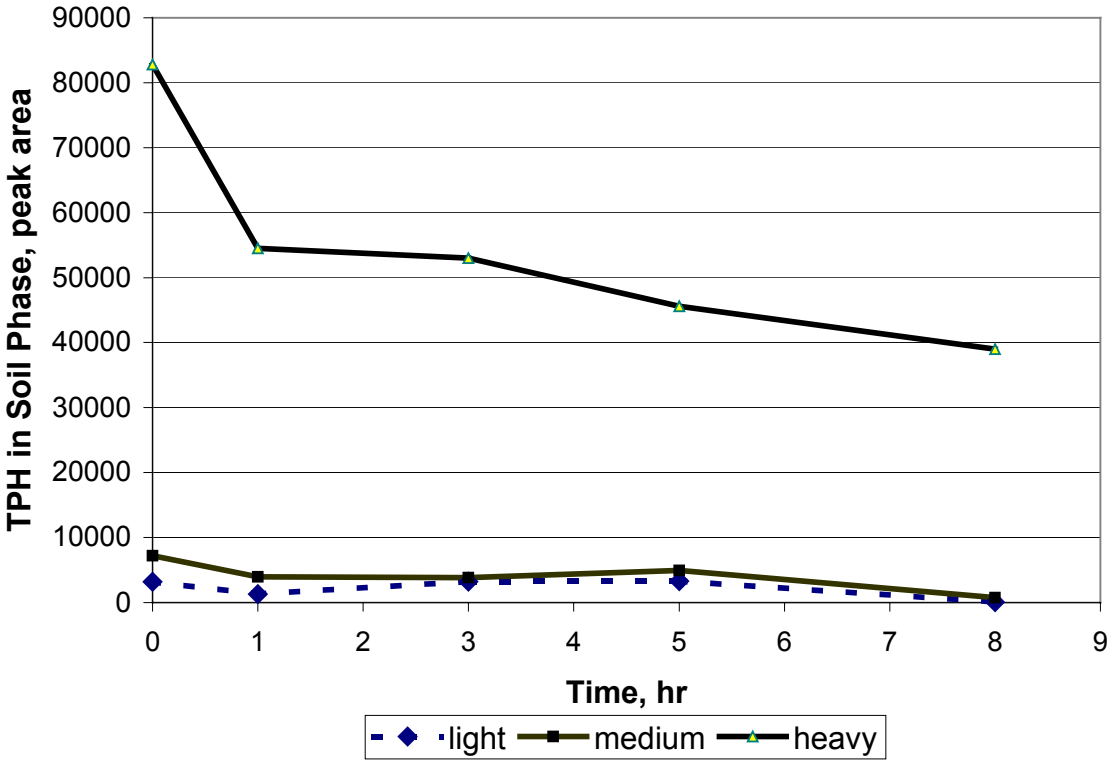

Figure 4.4. Results of Slurry Phase Fenton's Reagent Rate Test for the HMW-Soil

Conditions: 20,000 ppm HP/2,000 ppm Fe^{2+}, pH=2.5
Note: HP: Hydrogen Peroxide and Fe^{2+}: $FeSO_4$

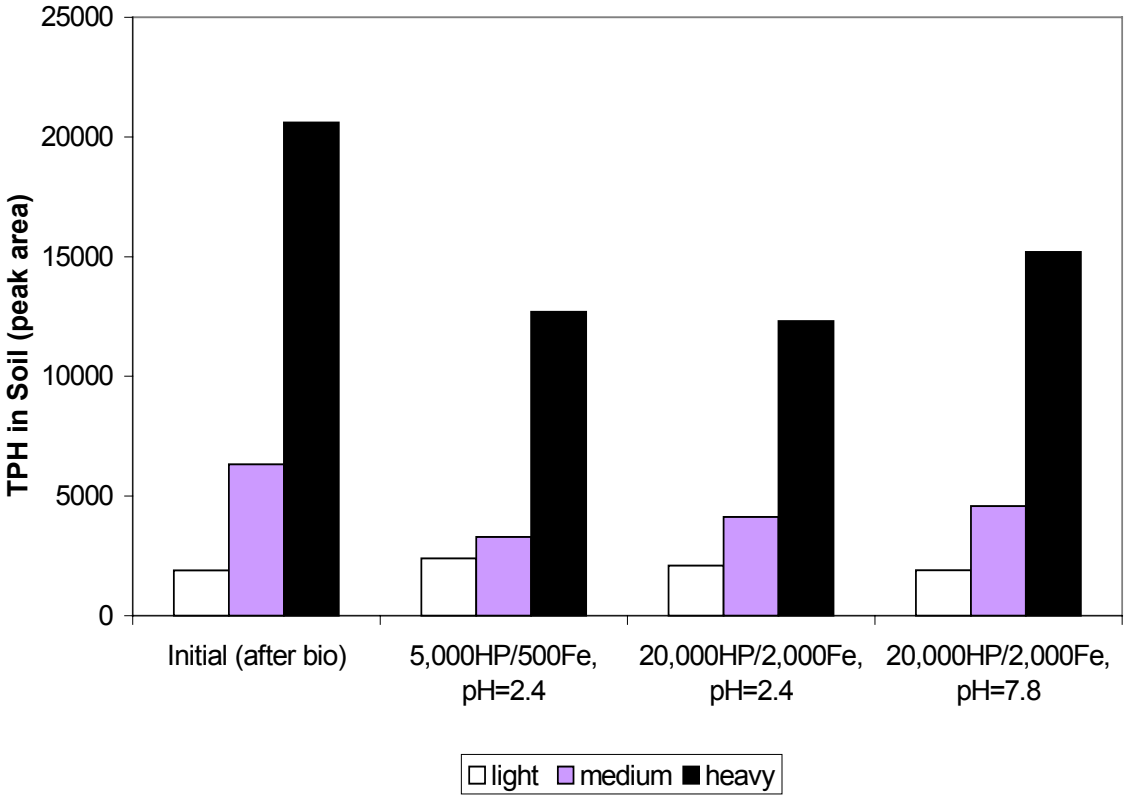

Figure 4.5. Soil Phase TPH Results of Slurry Phase Chemical Priming for the LMW-Soil

Note: HP: H_2O_2, mg/l and Fe: $FeSO_4$, mg/l

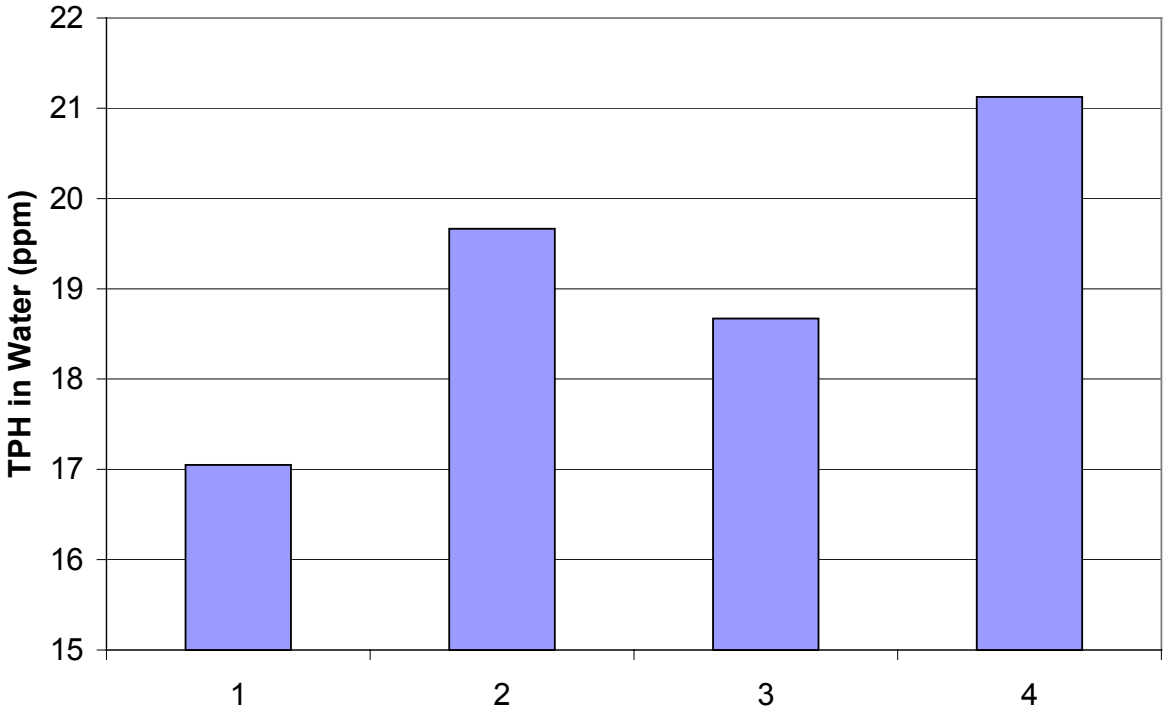

Figure 4.6. Aqueous Phase TPH Results of Slurry Phase Chemical Priming for the LMW-Soil

1. Post Biotreatment
2. 5,000 ppm HP/500 ppm Fe^{2+}, pH=2.4
3. 20,000 ppm HP/2,000 ppm Fe^{2+}, pH=2.4
4. 20,000 ppm HP/2,000 ppm Fe^{2+}, pH=7.8

Note: HP: H_2O_2, mg/l; ppm and Fe^{2+}: $FeSO_4$, mg/l

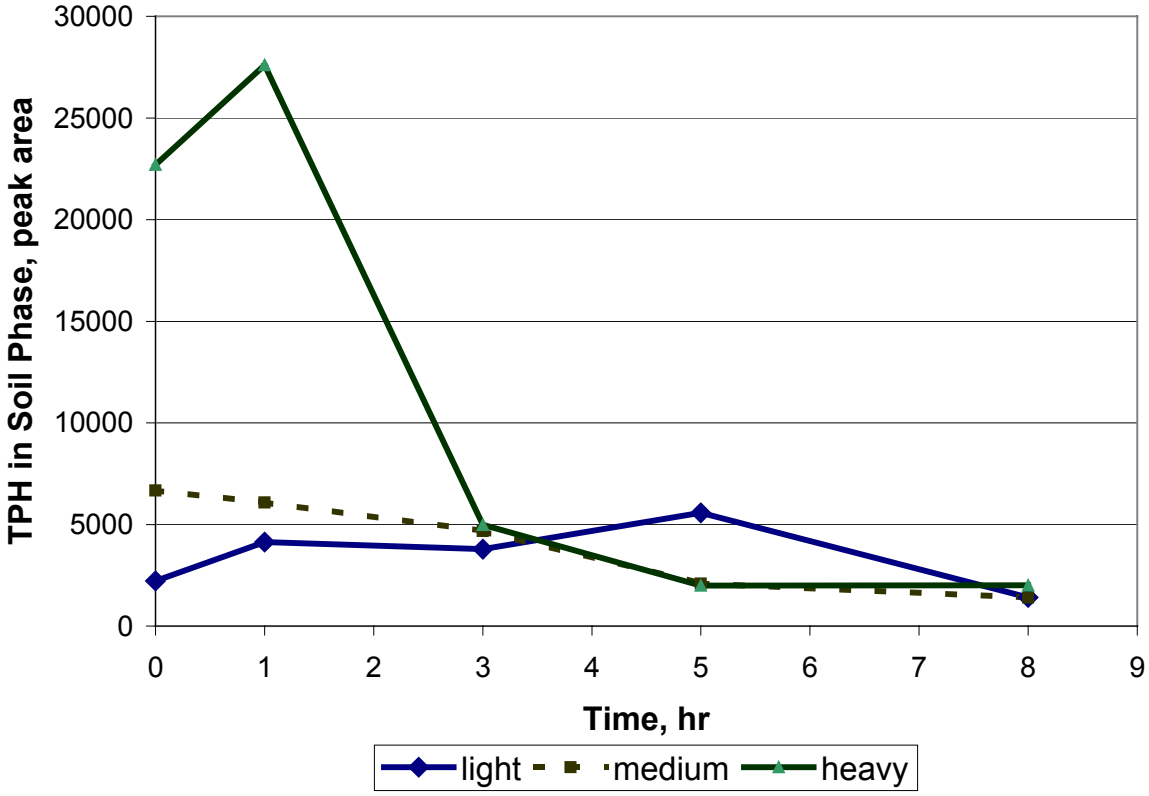

Figure 4.7. Results of Slurry Phase Fenton's Reagent Rate Test for the LMW-Soil

Conditions: 20,000 ppm HP/2,000 ppm Fe^{2+}, pH=2.5
Note: HP: Hydrogen Peroxide and Fe^{2+}: $FeSO_4$

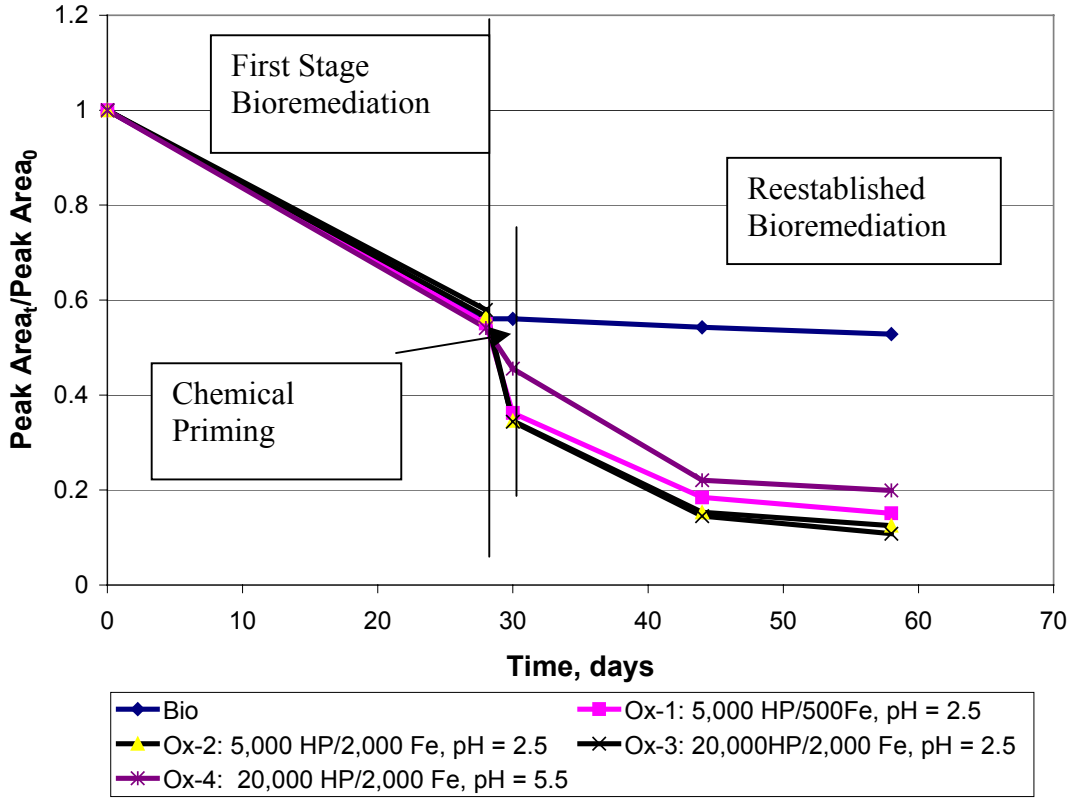

Figure 4.8. Heavy TPH Results of Fenton's Reagent Enhanced Bioremediation for the HMW-Soil

Note: Initial bioremediation stage occurred from Day 0 to Day 28, Fenton's Reagent treatment was conducted on Day 29, and post chemical priming bioremediation was applied from Day 30 to Day 58.
HP: H_2O_2, ppm
Fe: $FeSO_4$, ppm

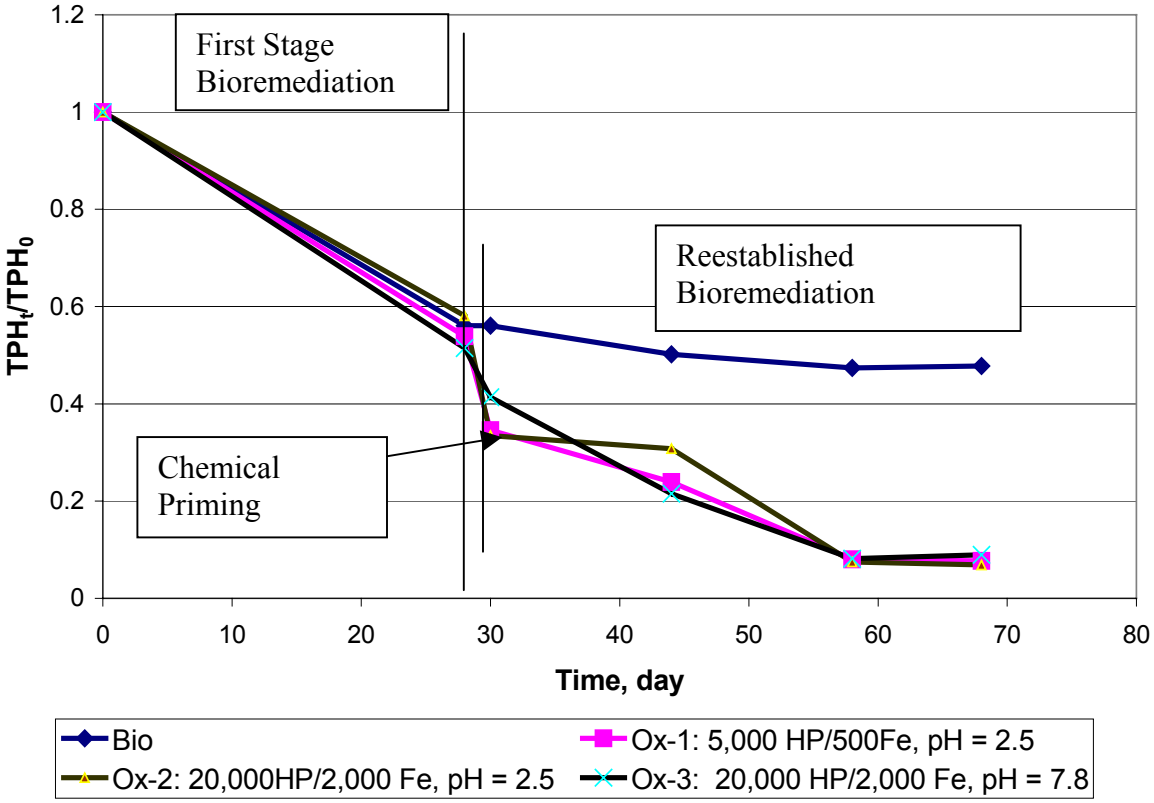

Figure 4.9. Heavy TPH Results of Fenton's Reagent Enhanced Bioremediation for the LMW-Soil

Note: Initial bioremediation stage occurred from Day 0 to Day 28, Fenton's Reagent treatment was conducted on Day 29, and post chemical priming bioremediation was applied from Day 30 to Day 68.
HP: H_2O_2, ppm
Fe: $FeSO_4$, ppm

CHAPTER V

SOIL COLUMN EXPERIMENTAL (PHASE II) RESULTS

The column experiments were designed to demonstrate the feasibility of chemical oxidation enhanced bioremediation for the HMW and LMW-Soils under more realistic soil conditions (i.e. packed soil columns). Experimental phase objectives were to determine the optimum chemical priming conditions for achieving the best enhancement results and evaluate the net results on the reestablished bioremediation stage in terms of TPH removal.

HMW-Soil Column Results

Chemical Priming Results

Figure 5.1 presents the results of the chemical priming experiments for the HMW-Soil. The HMW-Soil used in these experiments was previously treated biologically during Ms. Jing's experiments for approximately 5 months (Jing, 1998). Initial TPH concentrations shown in Figure 5.1 were those measured after Ms. Jing's experiments and was obtained by averaging soil TPH analytical results from all of the columns. The Control Set (oxygen sparged column [no oxidation]) heavy TPH levels show a slight difference compared to the initial level after 24 hours of bioremediation. This is likely due to the heterogeneous property of the soil contamination. From Figure 5.1, it is observed that all of the chemical oxidation

experiments yielded more TPH removal than the Control Set indicating that chemical priming has a positive effect on TPH removal. Comparing the extent of heavy TPH removal achieved by both ozone and peroxone systems at the 2 hour and 4 hour sampling time indicates continuous removal over the total 4 hour reaction period. This indicates that further treatment is likely with longer reaction times. The same observation can be made to the Fenton's Reagent Set in that over the 24 hour reaction period continuous heavy TPH removal is observed. The peroxone process had the highest extent of heavy TPH removal. Four hours of peroxone treatment yielded about 90% removal of the residual heavy TPH remaining from the previously applied biotreatment step. Ozonation and Fenton's Reagent achieved over 30% and 49% additional removal of the heavy TPH, respectively. Ozonation had the lowest heavy TPH removal of all sets. The likely reason for these results is that both Fenton's Reagent and peroxone generate hydroxyl radicals which are likely more reactive with the heavy TPH than ozone. Ozonation and peroxone sets exhibited an increase in the light TPH fractions. This may be due to the degradation of heavy petroleum hydrocarbons into lighter hydrocarbon fractions. Note that the medium fraction was not detected during any of the analyses.

Table 5.1 summarizes the experimental conditions used in the chemical priming experiments. The applied ozone mass listed in Table 5.1 represents the total dose of ozone sparged into the columns throughout the entire reaction period. These data were analyzed using the methods described in Chapter 3. The un-reacted ozone mass was determined via capturing all ozone exiting the column within the KI traps, then analyzing for ozone content. Reacted ozone mass represents the total mass of ozone reacted within the column (includes

ozone reacted with soil constituents, contaminants, and ozone that was self-decomposed over the reaction period). These data were calculated by the following mass balance around the column:

$$m_r = m_{in} - m_{out} - m_h$$

where:

m_r = mass of ozone reacted, g O_3,

m_{in} = mass of ozone sparged into the column reactor, g O_3,

m_{out} = mass of ozone captured in the offgas absorber, g O_3,

m_h = mass of ozone left in the headspace, g O_3.

After application of either ozonation or peroxone processes, oxygen was sparged for an additional 20 minutes to remove residual ozone from the column headspace and soil pores, hence the mass of the headspace ozone in the equation is zero. It is then assumed that all other ozone sparged into the column was reacted. The above equation is then simplified as:

$$m_r = m_{in} - m_{out}$$

The data in Table 5.1 indicate the same level of ozone was reacted in the ozonation and peroxone process sets. However, with the input of hydrogen peroxide, more than 50% additional heavy TPH was removed in the peroxone set than in the ozonation set. Again, it is very likely that the formation of hydroxyl radicals during the peroxone process resulted in this significantly higher extent of TPH removal.

Comparing the hydrogen peroxide usage within the peroxone process and Fenton's Reagent, it is observed that Fenton's Reagent required a larger input of hydrogen peroxide,

approximately 120 times greater than the peroxone process, and longer oxidation time. Yet, peroxone process yielded more heavy TPH removal than the Fenton's Reagent treatment. On the other hand, Fenton's Reagent process is easy to implement and requires less capital cost than peroxone process (the generation of ozone in the field is costly). Hence, a thorough cost analysis of these oxidation processes should be evaluated prior to field application to determine which is economically and technically superior.

From Table 5.1, a decrease in soil pH can be observed after all of the chemical priming experiments. This is likely the result of organic acid formation during hydrocarbon degradation. This pH decrease was also observed with the slurry phase Fenton's Reaction experiments. As mentioned, several researchers have identified organic acids as a by-product of hydrocarbon oxidation (Asinger, 1959; Razumovskii and Zaikov, 1984; Kuo *et al.,* 1995).

Problems with foaming were also encountered with the peroxone and Fenton's Reagent columns. This was likely caused by the fact that these experiments used columns that were fully saturated with water. Foaming was not observed during the ozonation column experiments, this was likely the case because the ozone tests were performed under unsaturated conditions. However, because foaming was not as severe as with the slurry phase oxidation tests, it was successfully controlled through the addition of an empty gas absorper bottle installed in front of KI solution bottles. This bottle served as a foam trap. It is anticipated that if BIO/OX processes are applied in slurry systems, some foaming control technique should be incorporated. If applied in unsaturated systems, foaming should be minimal and of limited concern.

Results of Reestablished Bioremediation

Overall TPH Removal: Figures 5.2 and 5.3 present the heavy and light TPH analysis of the reestablished bioremediation, respectively. It is observed from Figure 5.2 that among the four treatment sets, the peroxone set exhibited no apparent removal of heavy TPH during the reestablished bioremediation stage. Only about 3% additional TPH removal was achieved during this stage. On the other hand, ozonation achieved the highest enhancement. An additional 39% of the heavy TPH was removed in the ozonation set. No apparent additional heavy TPH removal was observed for the Fenton's Reagent Set during the first two weeks of reestablished bioremediation; however, an additional 10% removal was achieved over the second two weeks. The Control Set achieved an additional 13% of heavy TPH removal. This removal is likely due to oxygen sparging into the column that provided significantly more oxygen to support biotreatment over just headspace oxygen turnover.

It can be concluded from Figure 5.2 that ozonation provided the best enhancement with regard to reestablishing bioremediation. Although, the peroxone process yielded the highest heavy TPH removal during the chemical priming step, it did not provide any additional removal of the heavy TPH fractions during the reestablished bioremediation stage. Although, Fenton's Reagent yielded a dramatic enhancement of heavy TPH removal in the slurry phase experiments, similar results were not observed with the column experiments. This is likely due to the fact that after chemical priming, soil

environment conditions were not as well adjusted as those with the slurry system. Hence, soil pH condition, bacterial populations, and nutrient levels might not have ideally suited for bacterial activity. Additionally, it is possible that the by-products of chemical oxidation might have built up within the peroxone and Fenton's Reagent systems to such an extent that they exhibited a toxic or inhibitory effect on the microorganisms. Consequently, little or no biodegradation was observed during this stage of the experiments.

The light TPH results presented in Figure 5.3 show an increase in light TPH levels after chemical oxidation prior to biotreatment indicating the likely generation of light by-products. Within the four tested sets, peroxone has the highest light TPH generation followed by Fenton's Reagent and ozonation. This order agrees well with the oxidation removal efficiencies for the heavy TPH. After the first two weeks of reestablished bioremediation, the presence of this build-up light fraction of hydrocarbons decreased for all systems. The peroxone set had the highest light TPH removal rate, while ozonation and Fenton's sets had similar removal rates. This suggests that during the first two weeks of reestablished bioremediation, lighter TPH fractions were degraded followed by the degradation of some of the heavy TPH. However, the overall light TPH removal obtained compared to the levels at the end of the first stage bioremediation is limited. The bulk of TPH removal is certainly with the heavy TPH fraction. It is likely that prolonged biotreatment may have yielded bettered light TPH removal.

Headspace Gas Analysis Results: Figure 5.4 through 5.6 present the results from off gas monitoring activities during the reestablished biodegradation stage. These figures present the averaged (duplicate columns) oxygen uptake rates (OUR) and CO_2 production rates for each column set, respectively. These data show that microbial activity was much higher between Day 5 and Day 10 based on the increased OURs and CO_2 production rates. An increase in VOCs indicated the production of lighter TPH by-products and/or degradation of sorption bonds (see Figure 5.3). This reflects an evidence of direct enhancement provided by chemical priming. The OUR data eventually decreased toward the end of the incubation indicating reducing quality ecological conditions over time. The initial increase was also observed with VOC data indicating an increase in the biodegradation rate, contaminant mobility, and biodegradation potential. This agrees with the fact that microorganisms, after acclimated to the environment, exhibited growth under the increased food source. Hence, higher OUR, CO_2 production rates, and TPH reduction were observed. Once the carbon source is exhausted or the residual TPH was biorecalcitrant in nature, bioactivity was reduced as witnessed by the reduced OUR and CO_2 production rates seen in Figures 5.4 and 5.5. In Figure 5.4, no distinct difference in OUR was observed with any of the test sets. This is likely due to the detection limit of the multi-gas analyzer. The multi-gas analyzer was originally designed to alarm oxygen deficient (less than 19% [v/v]) and oxygen rich and potentially explosive (greater than 26%[v/v]) environments (Multi-gas user menu). When oxygen levels are greater than 26%, with the alarm going off, actual readings from the analyzer are usually suspicious from a quantitative stand point. This oxygen rich situation was encountered during the

experiments because of the use of a high oxygen (90%) supply source, regardless of the processes under evaluation. Hence, the OUR data should be reviewed alone with the consideration of other parameters (i.e. CO_2 production and VOC generation rates) to analyze biological activity. Still, given the OUR analysis technique used, these data, albeit, the accuracy not perfect, are considered within range for oxygen levels less than 26% (v/v).

Figures 5.5 and 5.6 show that ozonation and peroxone had higher CO_2 production and VOC generation rates than the Fenton's Reagent and the Control Set. This agrees with the heavy TPH analytical results in that the ozonation set had the highest removal during the reestablished bioremediation experiment, while the peroxone set had the highest light TPH removal and greatest oxidation based reduction of the heavy TPH. The Control Set has the least CO_2 production and VOC generation of all the sets. This agrees with the lowest heavy TPH removal efficiency observed with the Control Set (witnessed by the least CO_2 production). The lower generation of VOC in the Control Set compared to the chemical primed sets confirms the suggestion that the high VOC levels observed in the primed sets were the result of the chemical oxidation stage. However, the presence of pure oxygen within the Control Set did increase bacterial activity, hence, an additional TPH removal was observed over the previous biotreatment stage conducted by Ms. Jing (Jing, 1998).

LMW-Soil Column Experiments

Ozonation of the LMW-Soil

Before chemical enhanced bioremediation was studied on the LMW-Soil, an ozonation-only treatment was first evaluated using the column reactors to test the feasibility of using chemical oxidation as a stand-alone process for the LMW-Soil. It was thought that given this soil is composed of primarily light, simple aromatics, that ozonation alone may be a feasible treatment process. Table 5.2 lists the experiment conditions and results of this experiment. From visual observations made on the soil after oxidation, the LMW-Soil was bleached by the large amount of ozone added. The soil color was lighter at the bottom than that at the top of the column. A layering soil color was observed with the top being the darkest and bottom being the lightest. This phenomena is explained by review of the likely fate of ozone in a soil column. As ozone enters the column, it immediately begins at react, as the sparged ozone reaches the top portion of the column, ozone levels in the gas phase has been reduced; hence, the bottom portion of the soil in the column had the lightest color while the top soil was the darkest. About 53% TPH was removed using ozonation alone. It seems that ozone is capable of treating the contaminated soil to some extent. It was observed during the 4 hours of ozonation, that the color of the KI solution almost remained unchanged during the reaction indicating little ozone exited the columns. The ozone concentration analytical results as listed in Table 5.2 show that a large portion of applied ozone was reacted during the experiment. The amount of reacted ozone per TPH mass treated is also listed in Table 5.2.

Table 5.2 also shows that ozonation did not impact soil pH. It is likely due to the higher buffering capacity of the LMW-Soil. However, oxidation did decrease the bacterial populations within the soil system. This was expected because ozone is one of the mostly commonly used disinfection agents in wastewater treatment.

It is concluded from this experiment, that ozonation can be used as a stand-alone treatment process for the contaminated LMW-Soil. However, it seems that a large amount of ozone has to be supplied into the system, which will possibly makes the process expensive when compared to the BIO/OX/BIO system discussed in the following section.

BIO/OX/BIO Treatment of the LMW-Soil

For the LMW-Soil, bioremediation was conducted in the columns first followed by chemical priming, then reestablished bioremediation (i.e. BIO/OX/BIO).

Overall TPH Removal: Figure 5.8 presents the TPH (as DRO) results collected throughout the total BIO/OX/BIO treatment. The LMW-Soil experiment results are presented as DRO (ppms) because all of the compounds appearing on the chromatographs fell within the DRO range (selected sample chromatographs are presented in Appendix E). After two weeks of the first stage bioremediation, the TPH levels in all columns were dramatically decreased indicating that the TPH in the LMW-Soil was very biodegradable. Within the first two weeks of the first biotreatment stage, it can be seen that the TPH degradation rate observed in the second week is higher than

that of the first week. This is believed to have occurred because during the first few days of incubation, the bacteria, especially the seeded portion, were exotic to the environment, and hence, experienced a lag phase. After acclimation, under the optimally provided ecological conditions, the bacteria rapidly metabolized the TPH as witnessed by the rapid TPH biodegradation rate shown in Figure 5.8.

Chemical priming evaluated after two weeks of bioremediation removed a large additional portion of the residual TPH. Figure 5.9 presents more detailed results for the chemical priming experiments. All of the four oxidation conditions tested yielded distinct TPH removals over those achieved with the Control Set. The ozonation process had slightly better results than the other processes; although, the rational for this very slight difference is not known. It could be that the unsaturated conditions used with ozone set (and not with the others) provided better oxidant distribution.

Table 5.3 lists the test conditions used for all of the chemical priming experiments. It shows that at the same applied ozone dose (comparing ozone versus the Peroxone 2 Set), the peroxone process consumed similar amounts of ozone as the ozonation process. However, from Figure 5.9, peroxone did not improve TPH removal over ozonation, yet it used 140 mg/kg soil hydrogen peroxide, where ozone used none. This contradicts the results achieved the HMW-Soil. The reacted ozone mass per TPH treated values also show that with the addition of hydrogen peroxide, the expectedly better results were not achieved. The possible explanation for this could be that the natural occurring soil organics and other constituents of this soil system acted as hydroxyl radical scavengers, hence, the generated hydroxyl radical may not have reacted with the target contaminants

as efficiently with the LMW-Soil. Another possible reason for the little performance difference between ozone and peroxone could be that the TPH in the LMW-Soil was very reactive with ozone. Hence, the radicals produced offer little additional removal over ozone alone. Similar results were observed in other research using peroxone process for treating PAHs (Beltran, *et al.,* 1996), where was suggested that the direct reaction of ozone with the contaminants were the primary removal mechanism and that the contribution of hydroxyl radical oxidation appeared minimal. With the Peroxone 1 Set, ozone at a higher concentration was sparged into the columns, but the results, as seen in Figure 5.9, indicated that the extra input of ozone yielded only slightly better TPH removal than the Peroxone 2 Set. The exact reason of this result is unknown. Clearly, in the case of the LMW-Soil, ozonation seems to be a more cost effective and technically superior process than peroxone process.

Fenton's Reagent treatment yielded the lowest TPH removal in all the oxidation sets. Yet over 75% additional removal of the residual TPH from the first stage bioremediation was achieved. This value was in the same general range observed the other oxidation sets. Comparing hydrogen peroxide usage in Fenton's Reagent to peroxone processes, Fenton's Reagent used 120 times more hydrogen peroxide than the peroxone processes, yet no better results were achieved. Again, an economic evaluation should differentiate an optimal process.

Table 5.2 shows that after oxidation, the soil pH did not decrease in any of the systems tested. This indicates that the LMW-Soil has a stronger buffering capacity compared to the HMW-Soil and/or a lesser amount of organic acids were produced with

this soil over the HMW-Soil. In either case, the soil pH was optimum for the reestablishing bioremediation.

Table 5.4 lists the percent TPH removal data of the reestablished bioremediation for the LMW-Soil. During the reestablished bioremediation stage, no appreciable additional TPH removal was observed with any of the chemical primed sets. These data show that reestablished bioremediation only achieved approximately 2% additional TPH removal. This likely occurred because of the limited bacterial food reserve remaining in the columns after chemical priming. The final TPH level in the oxidation sets was approximately 20 ppm in all the cells. Within the Control Set, an additional 14.6% TPH was removed during the continuous bioremediation stage yielding an total of 83.6% removal with just bioremediation alone. The final TPH in the Control Set was 59.6 ppm; which is below the regulated level of 100 ppm for most states. These data suggest that with proper bacterial seeding, nutrient addition, and oxygen sparging, the treatment goal can be reached within 4 weeks of bioremediation alone for the LMW-Soil. However, chemical oxidation can effectively treat this soil within a shorter period of time. It can be effectively used in the situation where time is an important criteria. Also, if even lower TPH levels are required, then chemical priming can be a useful technique to achieve this goal. Since no additional removal was achieved during the second stage bioremediation, a BIO/OX approach may be a good option for this soil.

Headspace Monitoring Results: Figures 5.10 through 5.12 present the headspace monitoring results throughout the entire 30 days of BIO/OX/BIO treatment. A dramatic increase in oxygen uptake rate, CO_2 production rate, and VOC production after Day 15

(i.e. after chemical priming) is observed. This is likely the result of the production of easier to biodegrade compounds produced from the chemical oxidation of the TPH. When the food sources in the columns were exhausted, biological activity slowed down as witness by the reduced OUR and CO_2 production rates (Days 15 to 25). Within the last 2 weeks of reestablished bioremediation incubation, the Fenton's Reagent Set had the highest carbon dioxide production rate and comparably higher VOC production among all the oxidation sets. This is due to the higher residual TPH level in Fenton's Reagent set than the other oxidation sets. It is noteworthy that the Fenton's Reagent Set yielded a slight removal of TPH (1.82%) during the 2 weeks of reestablished bioremediation period. This is the greatest removal observed with any of the oxidation sets. This further confirms the postulation that the limited additional TPH degradation rate achieved during the reestablished bioremediation stage was likely due to exhausted food supply.

Other Parameters: Table 5.5 through 5.7 present the pH, moisture content, and total heterotroph enumeration results. Bacteria enumeration results listed in Table 5.7 show that during the two bioremediation periods (Day 0-14 and Day 16-30), the bacterial populations were increased over time due to improved environmental conditions and a sufficient availability of degradable substrate which simulated the growth of soil bacteria. The oxidation step did decrease bacterial populations; however, oxidants dosages used in this test are not considered highly disinfective in nature, and as such after oxidation the bacterial populations were sufficiently high for reestablishing biotreatment. Additionally, soil pH and moisture levels within the soils system, as listed

in Tables 5.6 and 5.7, were in the optimum range for supporting bacterial growth and metabolic activity. Hence, the total heterotrophic bacterial populations were still within the 10^7 range. Although no further TPH removal was observed after 2 weeks of reestablished bioremediation, there were likely other organics presented to support the bacterial activity. This organic carbon was likely not lost during the concentration step during sample clean-up procedure and/or not measured with the analytical procedures used (i.e., not detectable by GC/FID) .

Comparison of the Experimental Results of the Two Soils

For the two soils evaluated, chemical oxidation seems to be capable of degrading TPH more rapidly and completely than using biological treatment alone. For the HMW-Soil, ozonation achieved a clear biotreatment enhancement in terms of heavy TPH removal during the reestablished bioremediation stage. However, for the LMW-Soil, reestablishing bioremediation did not yield additional TPH removal beyond the removal achieved by chemical priming.

In terms of the HMW-Soil, peroxone had a higher heavy TPH removal efficiency than did the ozonation and Fenton's Reagent sets. However, with regard to the LMW-Soil, peroxone did not yield improved TPH removal over ozonation. For both soils, peroxone did not result in additional TPH removal during the reestablished bioremediation stage. This suggests that the soil environmental conditions and contaminant-types play an important role in terms of treatment performance. It also

suggests that the use of peroxone process is a very powerful process that can be applied as a BIO/OX treatment.

The Fenton's Reagent column data contradicted the slurry phase results by not achieving the same level of enhancement during the reestablished bioremediation stage for both soils. This may be due to the heterogeneous property of the soil contamination and fabric and the better mixing conditions provided in a slurry system. The pH and total heterotroph enumeration data showed that oxidation produced slightly less than desirable environmental conditions for the targeted microorganisms. Given this, readjustment of environmental conditions becomes very crucial with the restarting of biological treatment. It is expected that pH adjustment, bacteria seeding, and nutrient amendment will be needed.

In the case of the LMW-Soil system, the contaminants were present at lower levels and their composition seemed to be biodegradable. Introduction of oxidants to this soil resulted in the rapid degradation of TPH within a short period of time. For this soil, biotreatment alone achieved appreciable TPH reduction. Hence, BIO or BIO/OX treatment is sufficient for the remediation of the LMW-Soil.

Table 5.1

Summary of the HMW-Soil Column Oxidation Conditions and Selected Results

		Control	Ozone	Peroxone	Fenton
O_2 Flow Rate (l/min)		0.944	-	-	-
O_3 Flow Rate (l/min)		-	0.944	0.944	-
Applied O_3 Mass (g O_3)		-	10.80	10.80	-
Un-reacted O_3 Mass (g O_3)		-	1.78	1.34	-
Reacted O_3 Mass (g O_3)		-	9.02	8.46	-
Normalized Applied Ozone Mass (g O_3/kg soil)			30.85	30.85	
Normalized Reacted Ozone Mass (g O_3/kg soil)			25.75	27.02	
H_2O_2 Dose (mg/kg soil)		-	-	142.86	17,142.86
$FeSO_4$ Dose (mg/kg soil)		-	-	-	2,000
H_2O_2: Fe^{2+} ratio (w/w)					23:1
Total Hetero-troph*	Before Oxidation	1.25×10^8	1.25×10^8	1.25×10^8	1.25×10^8
	After Oxidation	1.25×10^8	5.60×10^6	7.60×10^5	2.23×10^6
pH	Before oxidation	6.8	6.8	6.8	3.5 (adjusted)
	After Oxidation	6.8	5.8	5.6	3.8
Experiment Time (hours)		24	4	4	24

Notes for Table 5.1

Control: Oxygen sparging (90% [v/v]purity) @ 2 scfh

Ozonation : 4.5%(v/v) ozone @ 2 scfh

Fenton: 20,000mg/kg H_2O_2/2,000mg/kg $FeSO_4$

Peroxone : 4.5% ozone @2 scfh/500mg/kg H_2O_2.

* Total Heterotrophs: CFUs/g dry soil

Table 5.2

Ozonation Treatment Results for the LMW-Soil

O_2 Flow Rate (l/min)		0.944
Applied O_3 Mass (g O_3)		10.80
Un-reacted O_3 Mass (g O_3)		0.023
Reacted O_3 Mass (g O_3)		10.78
Normalized Applied Ozone Mass (g O_3/kg soil)		28.80
Normalized Reacted O_3 Mass (g O_3/kg soil)		28.75
Total Heterotroph (CFUs/g)	Before	1.35×10^6
	After	3.89×10^5
pH	Before	7.9
	After	7.9
Reaction Time (hr)		4
TPH (ppm)	Before	362.93
	After	193.68
Reacted O_3 Mass per TPH treated (g O_3/mg TPH)		0.170

Note: Ozonation was performed solely to the LMW-Soil without any biological pretreatment or post treatment during this experiment

Table 5.3

Summary of the LMW-Soil Column Oxidation Conditions and Selected Results

		Control	Ozone	Peroxone 1	Peroxone 2	Fenton
O_2 Flow Rate (l/min)		0.944	-	-	-	-
O_3 Flow Rate (l/min)		-	0.944	0.944	0.944	-
Applied O_3 Mass (g O_3)		-	2.37	5.04	2.37	-
Un-reacted O_3 Mass (g O_3)		-	0.98	1.55	0.80	-
Reacted O_3 Mass (g O_3)		-	1.39	3.49	1.57	-
Normalized Applied O_3 Mass (g O_3/kg soil)		-	6.76	14.40	6.76	-
Normalized Reacted O_3 Mass (g O_3/kg soil)		-	3.95	9.98	4.47	-
Reacted O_3 Mass per TPH treated (g O_3/mg TPH)			0.047	0.121	0.053	
H_2O_2 Dose (mg/kg soil)		-	-	142.86	142.86	17,142.86
H_2O_2: Fe^{2+} (w/w)		-	-	-	-	23:1
pH	Before oxidation	7.8	7.8	7.7	7.7	7.2 (adjusted)
	After Oxidation	7.7	7.7	7.7	7.7	7.2
Experiment Time (hours)		24	2	2	2	24

Notes for Table 5.3

Control: Bioremediation, oxygen (90% [v/v] purity) @ 2 scfh
Ozonation: 2.5%(v/v) ozone @ 2 scfh, 2 hours
Peroxone 1: 4.5% (v/v) ozone @2 scfh/500 mg/kg H_2O_2, 2 hours
Peroxone 2: 2.5% (v/v) ozone @2 scfh/500 mg/kg H_2O_2, 2 hours
Fenton: 20,000 mg/kg H_2O_2/2,000 mg/kg $FeSO_4$, 24 hours.

Table 5.4

Percent TPH Removal Achieved with the LMW-Soil during the Reestablished Bioremediation Stage

Time (day)	TPH Removal Percentage (%)				
	Control	Ozonation	Peroxone1	Peroxone 2	Fenton
After oxidation	68.92	94.79	93.67	93.48	92.04
14	80.64	94.61	94.69	94.30	92.41
28	83.58	94.94	94.99	94.37	93.86

TPH removal Percentage (%) = $(1- TPH_t/TPH_0) \times 100$
Control: Bioremediation, oxygen (90% (v/v) @ 1.5 scfh
Ozonation : 2.5%(v/v) ozone @ 1.5 scfh, 2 hours
Peroxone 1: 4.5% ozone @1.5 scfh/500 mg/kg H_2O_2, 2 hours
Peroxone 2: 2.5% ozone @1.5 scfh/500 mg/kg H_2O_2, 2 hours
Fenton: 20,000 mg/kg H_2O_2/2,000 mg/kg $FeSO_4$, 24 hours

Table 5.7

Summary of LMW-Soil Bacterial Enumeration (CFUs/g)

Time, day	Control	Ozonation	Peroxone 1	Peroxone 2	Fenton
0	1.35×10^6	1.35×10^6	1.35×10^6	1.35×10^6	1.35×10^6
14	2.45×10^7	4.95×10^7	5.34×10^7	1.91×10^7	1.45×10^7
16	1.98×10^7	9.57×10^6	1.60×10^7	1.67×10^7	3.35×10^6
30	2.68×10^8	3.54×10^8	1.82×10^8	1.54×10^8	2.00×10^8

Control: Bioremediation, oxygen (90% (v/v) @ 1.5 scfh
Ozonation : 2.5%(v/v) ozone @ 1.5 scfh, 2 hours
Peroxone 1: 4.5% ozone @1.5 scfh/500 mg/kg H_2O_2, 2 hours
Peroxone 2: 2.5% ozone @1.5 scfh/500 mg/kg H_2O_2, 2 hours
Fenton: 20,000 mg/kg H_2O_2/2,000 mg/kg $FeSO_4$, 24 hours

Table 5.6

Summary of LMW-Soil pH

Time, day	Control	Ozonation	Peroxone 1	Peroxone 2	Fenton
0	8.1	8.1	8.1	8.1	8.1
14	7.81	7.84	7.66	7.69	7.20
16	7.76	7.53	7.76	7.74	7.22
30	7.74	7.56	7.37	7.62	7.22

Control: Bioremediation, oxygen (90% (v/v) @ 1.5 scfh
Ozonation : 2.5%(v/v) ozone @ 1.5 scfh, 2 hours
Peroxone 1: 4.5% ozone @1.5 scfh/500 mg/kg H_2O_2, 2 hours
Peroxone 2: 2.5% ozone @1.5 scfh/500 mg/kg H_2O_2, 2 hours
Fenton: 20,000 mg/kg H_2O_2/2,000 mg/kg $FeSO_4$, 24 hours

Table 5.7

Summary of LMW-Soil Moisture Content (%)

Time, day	Control	Ozonation	Peroxone 1	Peroxone 2	Fenton
0	21.3	21.3	21.3	21.3	21.3
14	22.3	22.3	22.3	22.3	22.3
16	22.3	20.5	34.6	35.5	31.1
30	25.4	28.9	32.0	32.0	31.8

Control: Bioremediation, oxygen (90% (v/v) @ 1.5 scfh;
Ozonation : 2.5%(v/v) ozone @ 1.5 scfh, 2 hours;
Peroxone 1 : 4.5% ozone @1.5 scfh/500 mg/kg H_2O_2, 2 hours;
Peroxone 2 : 2.5% ozone @1.5 scfh/500 mg/kg H_2O_2, 2 hours;
Fenton: 20,000 mg/kg H_2O_2/2,000 mg/kg $FeSO_4$, 24 hours.

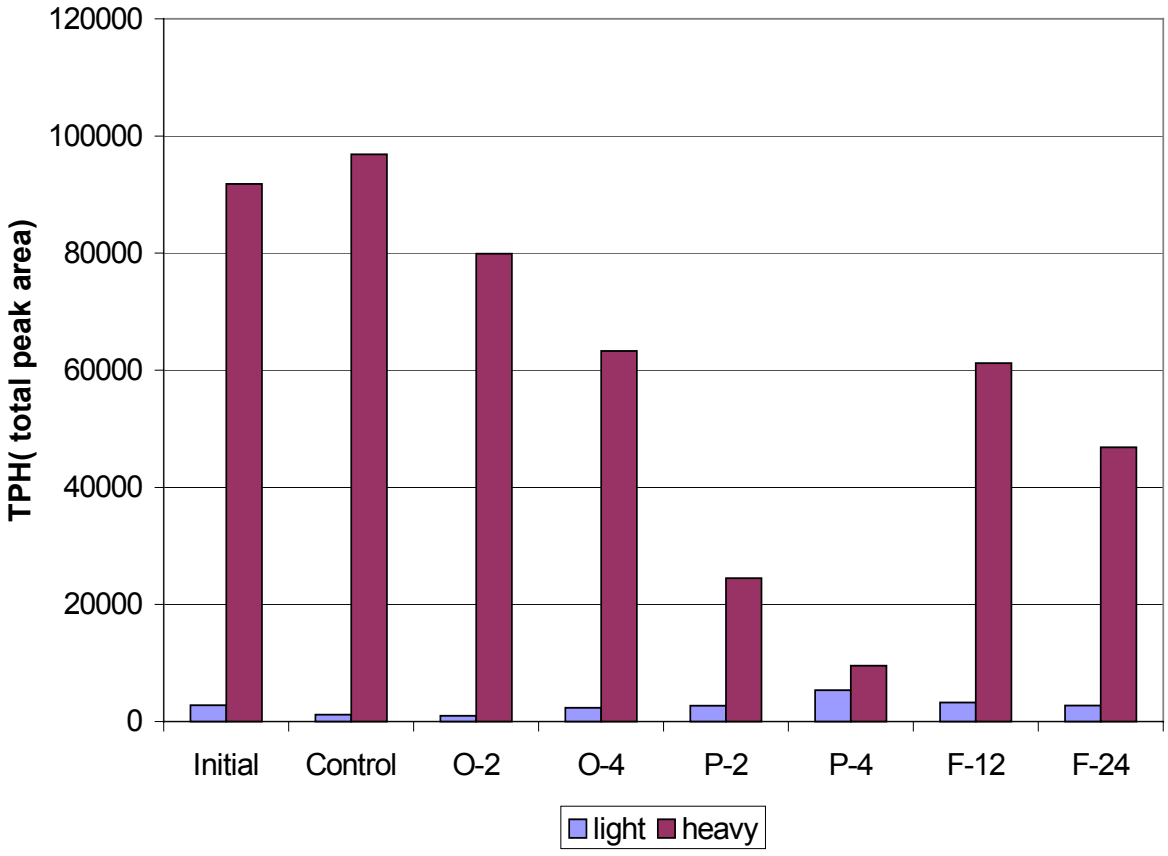

Figure 5.1. HMW- Soil TPH Results of Chemical Priming Experiments in Soil Columns

Initial: TPH analysis after biological treatment (initial data for chemical oxidation)
Control: Continuous biological treatment (after 24 hours incubation time)
O-2: 2 hours ozonation result O-4: 4 hours ozonation result
P-2: 2 hours peroxone reaction result P-4: 4 hours peroxone reaction result
F-12: 12 hours Fenton's reaction result F-24: 24 hours Fenton's reaction result
Ozonation : 4.5%(v/v) ozone @ 1.5 scfh
Fenton: 20,000 mg/kg H_2O_2/2,000 mg/kg $FeSO_4$
Peroxone : 4.5% ozone @1.5 scfh/500 mg/kg H_2O_2

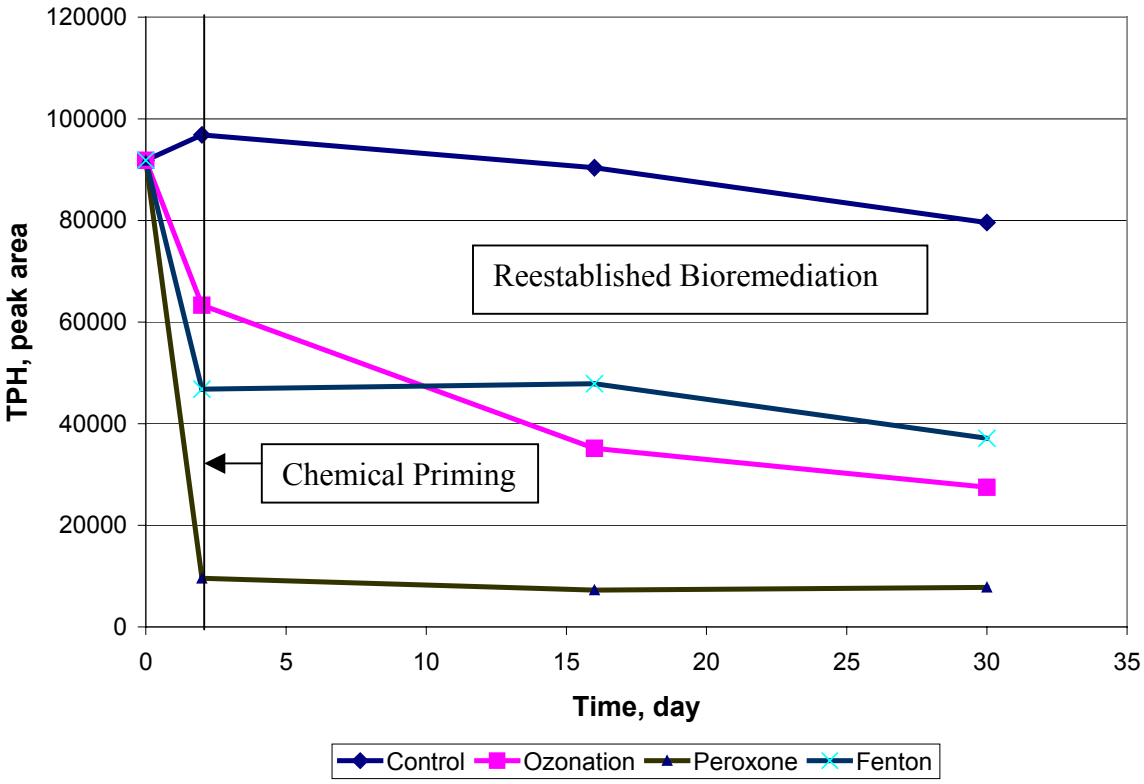

Figure 5.2. HMW-Soil Heavy TPH Results of Chemical Priming and Reestablished Bioremediation

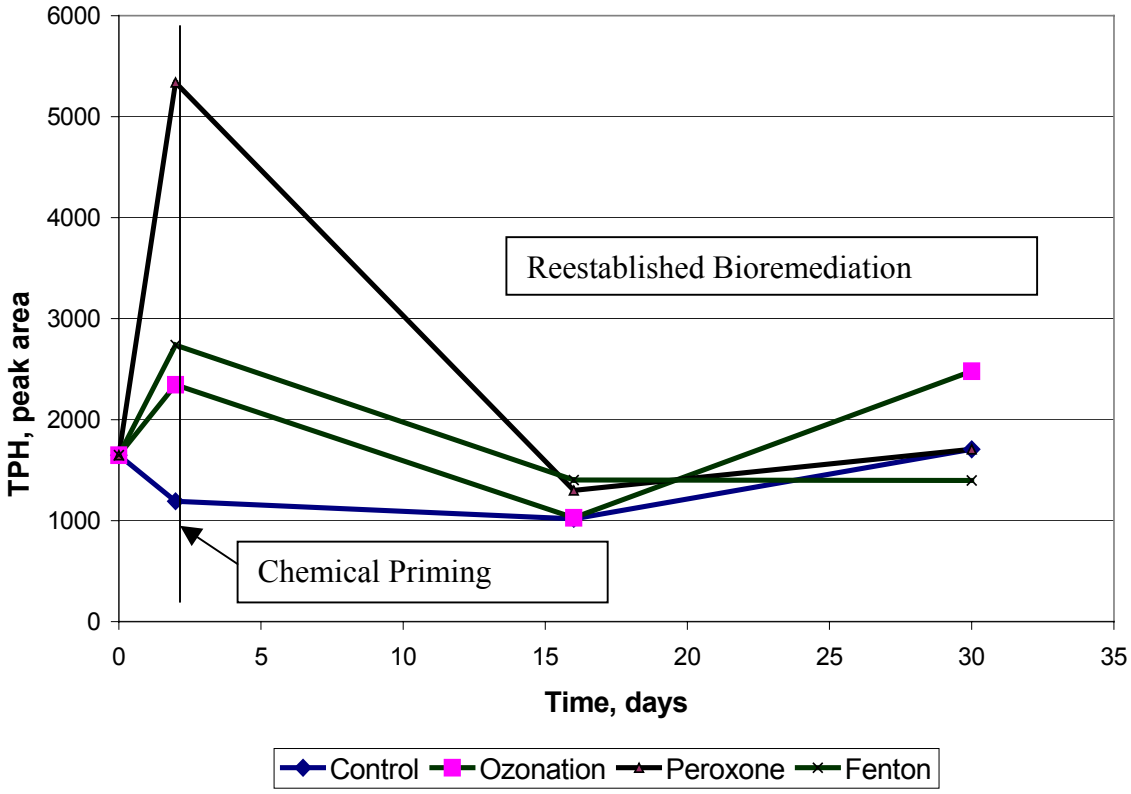

Figure 5.3. HMW-Soil Light TPH Results of Chemical Priming and Reestablished Bioremediation

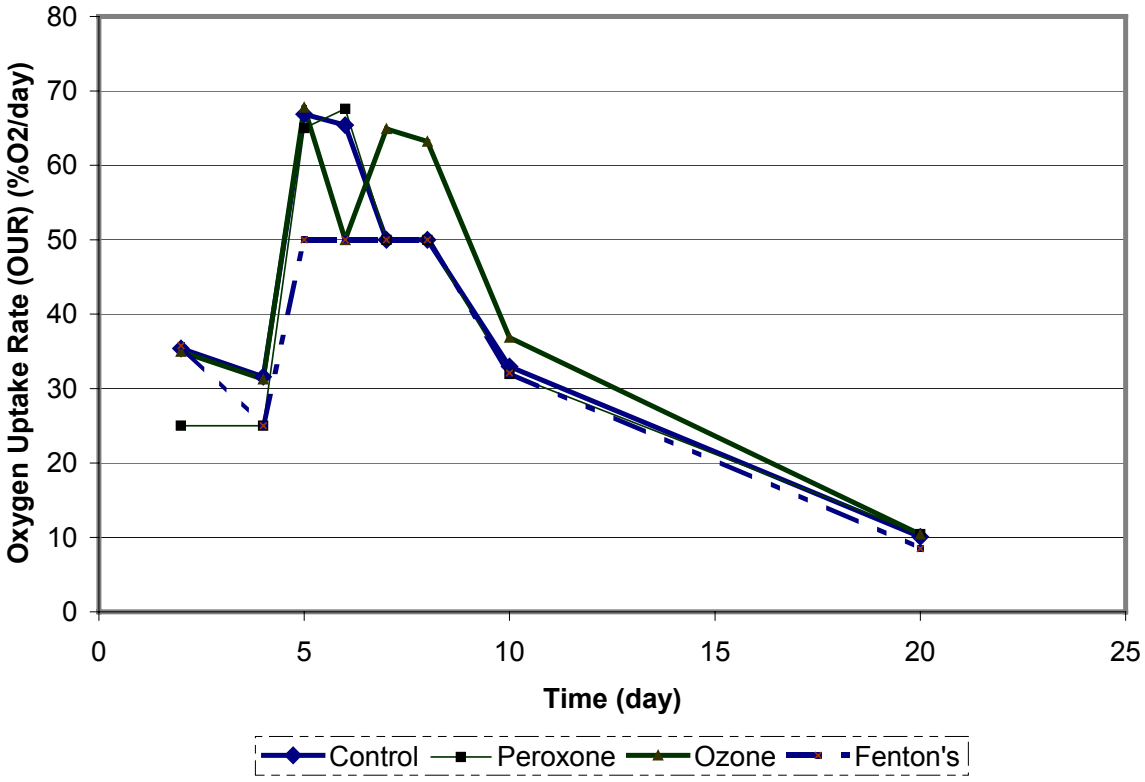

Figure 5.4. O_2 Evolution from Reestablished Bioremediation – HMW-Soil

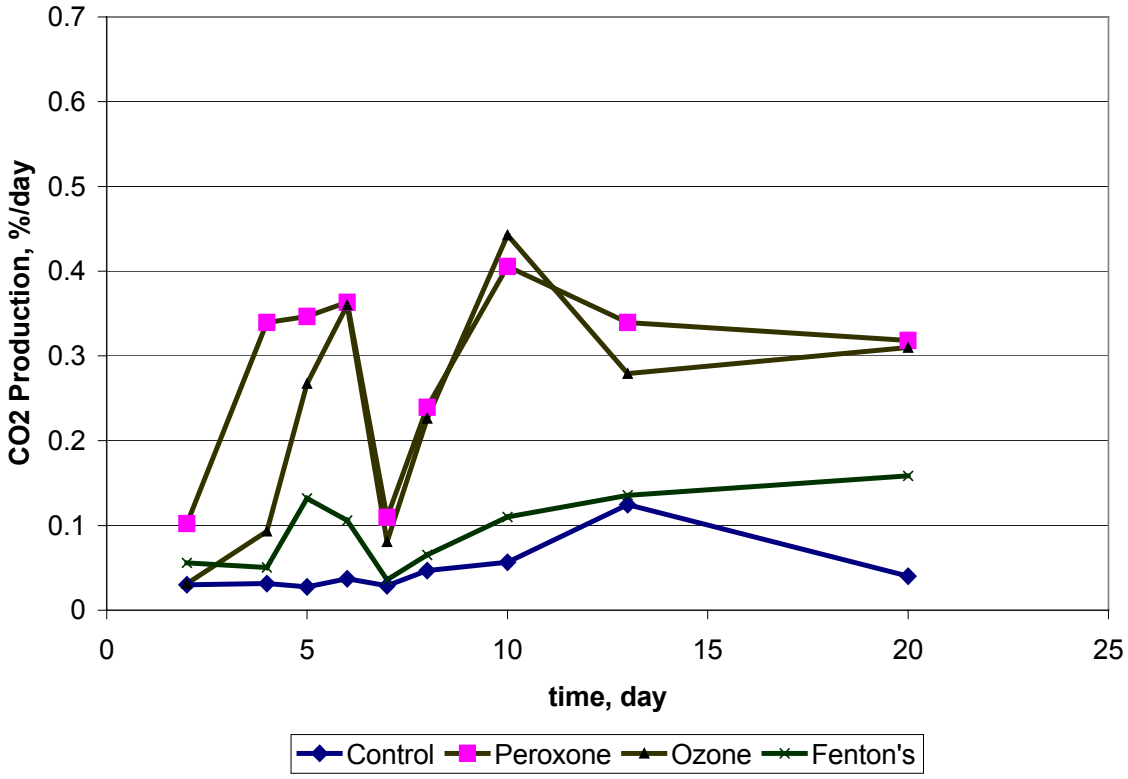

Figure 5.5. CO_2 Evolution from the Reestablished Bioremediation Stage – HMW-Soil

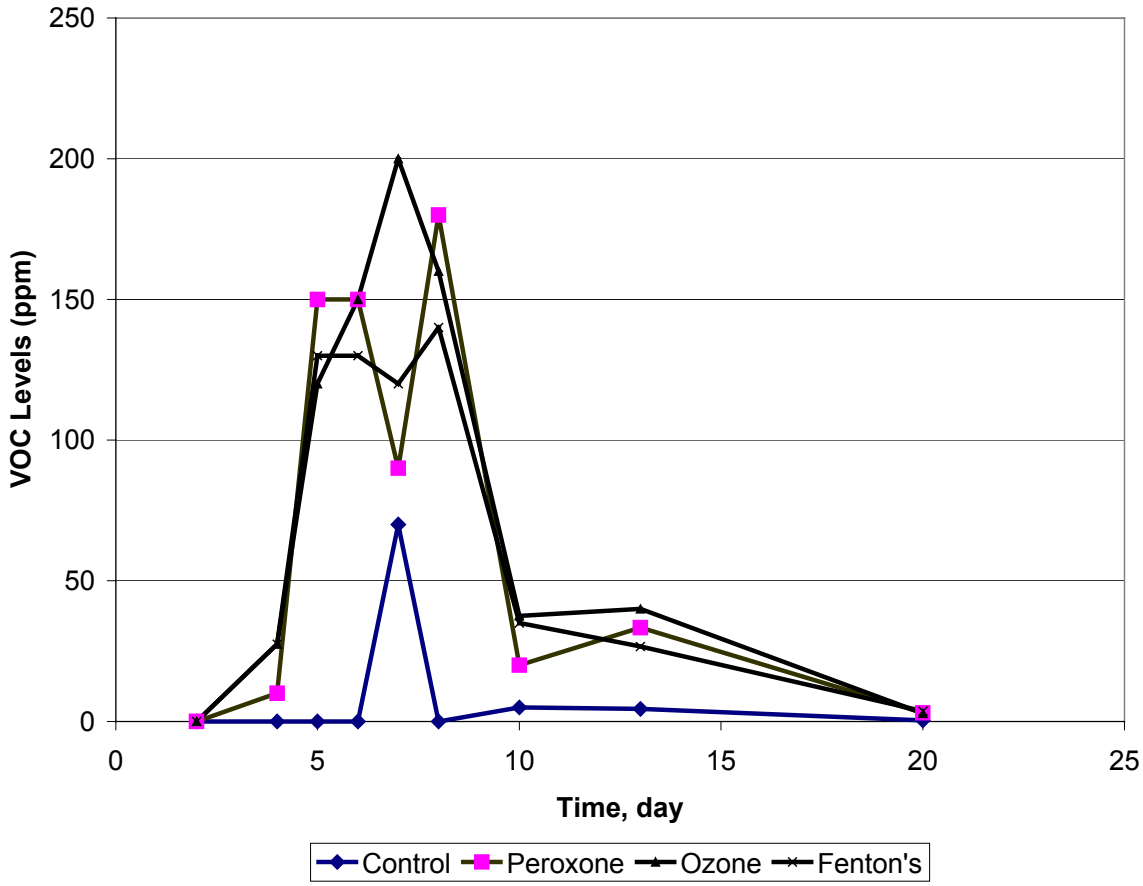

Figure 5.6. Headspace VOC Levels during the Reestablished Bioremediation Stage - HMW-Soil

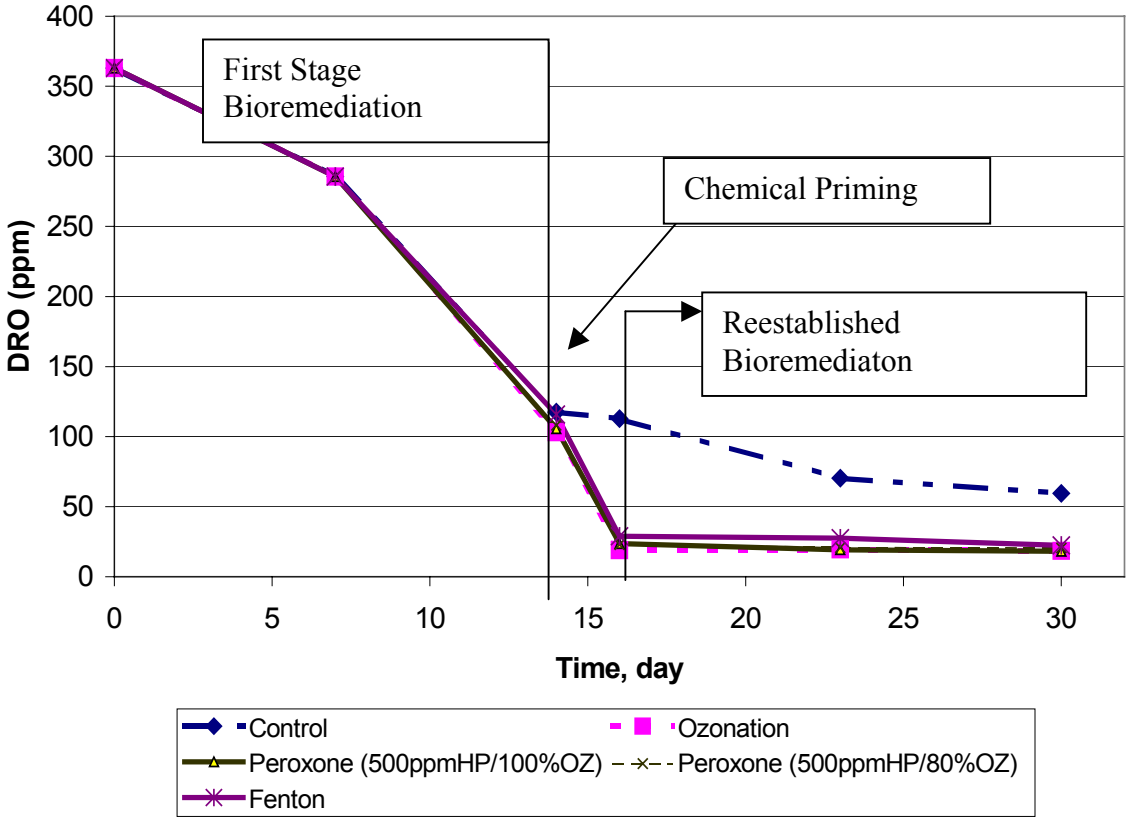

Figure 5.8. BIO/OX/BIO Treatment of the LMW-Soil

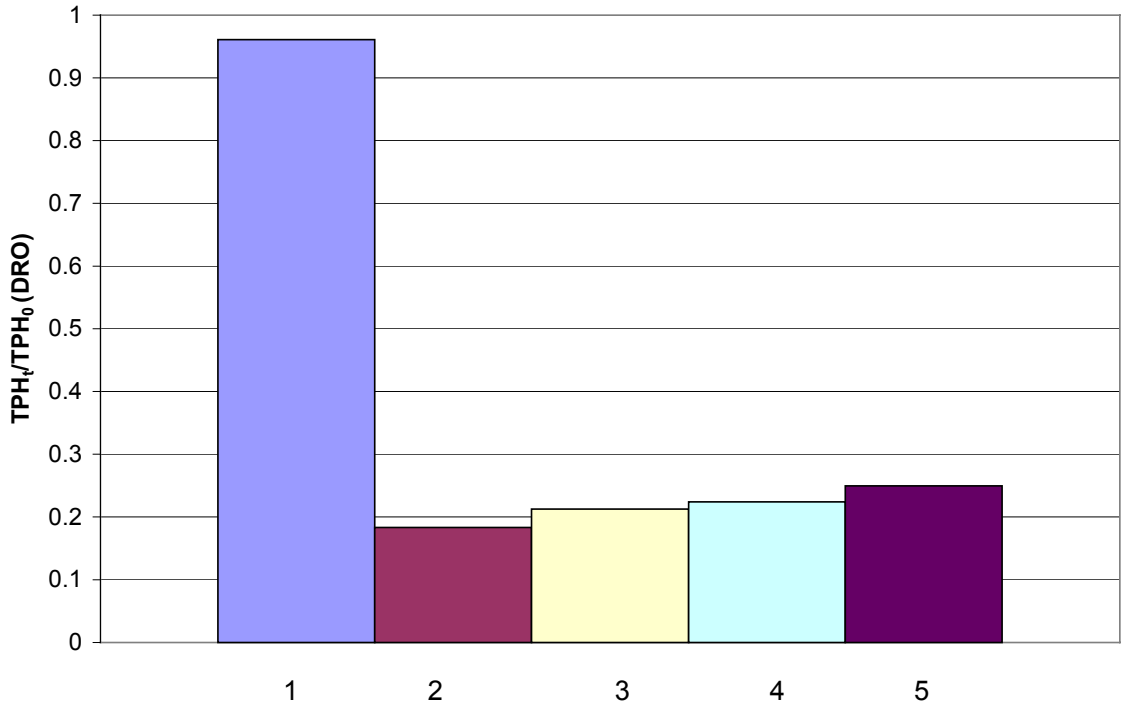

Figure 5. 9. Chemical Priming Experiments Results – LMW-Soil in Packed Soil Column

1. Control: Bioremediation, oxygen (90% (v/v) @ 1.5 scfh
2. 2. Ozonation : 2.5%(v/v) ozone @ 1.5 scfh, 2 hours
3. 3. Peroxone 1: 4.5% ozone @1.5 scfh/500 mg/kg H_2O_2, 2 hours
4. 4. Peroxone 2: 2.5% ozone @1.5 scfh/500 mg/kg H_2O_2, 2 hours
5. 5. Fenton: 20,000 mg/kg H_2O_2/2,000 mg/kg $FeSO_4$, 24 hours.

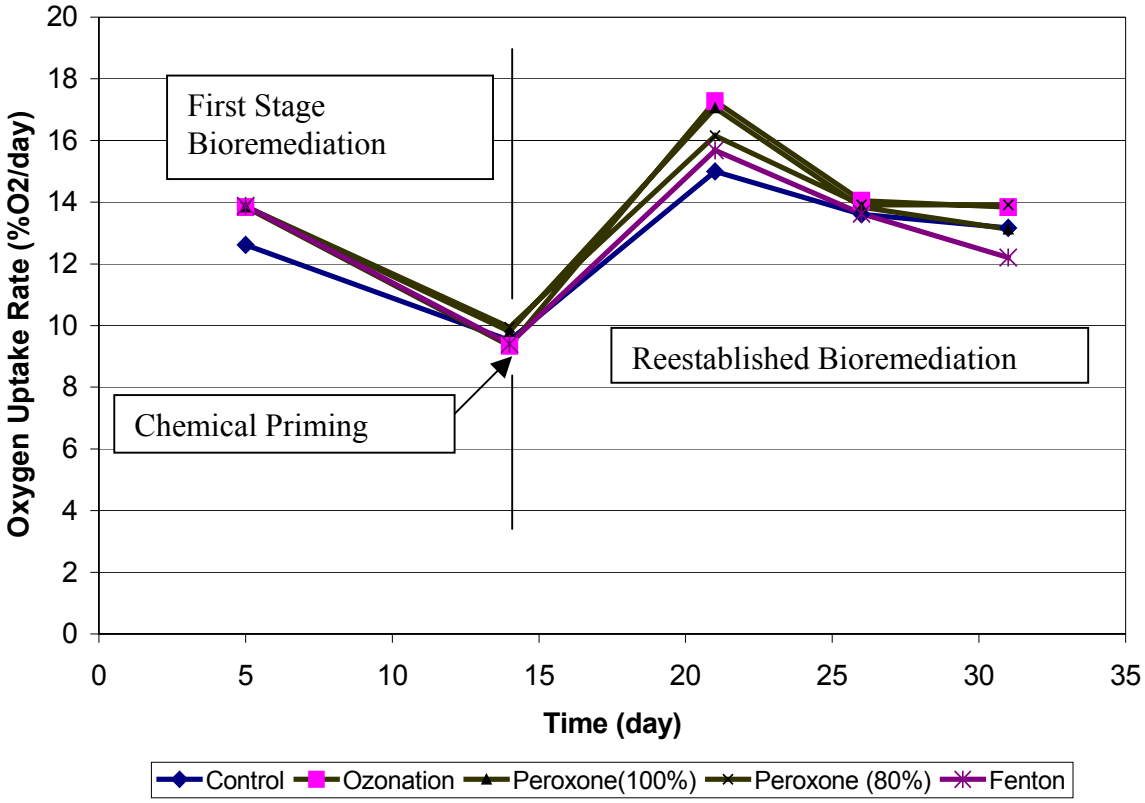

Figure 5.10. O_2 Evolution from BIO/OX/BIO Treatment of the LMW-Soil

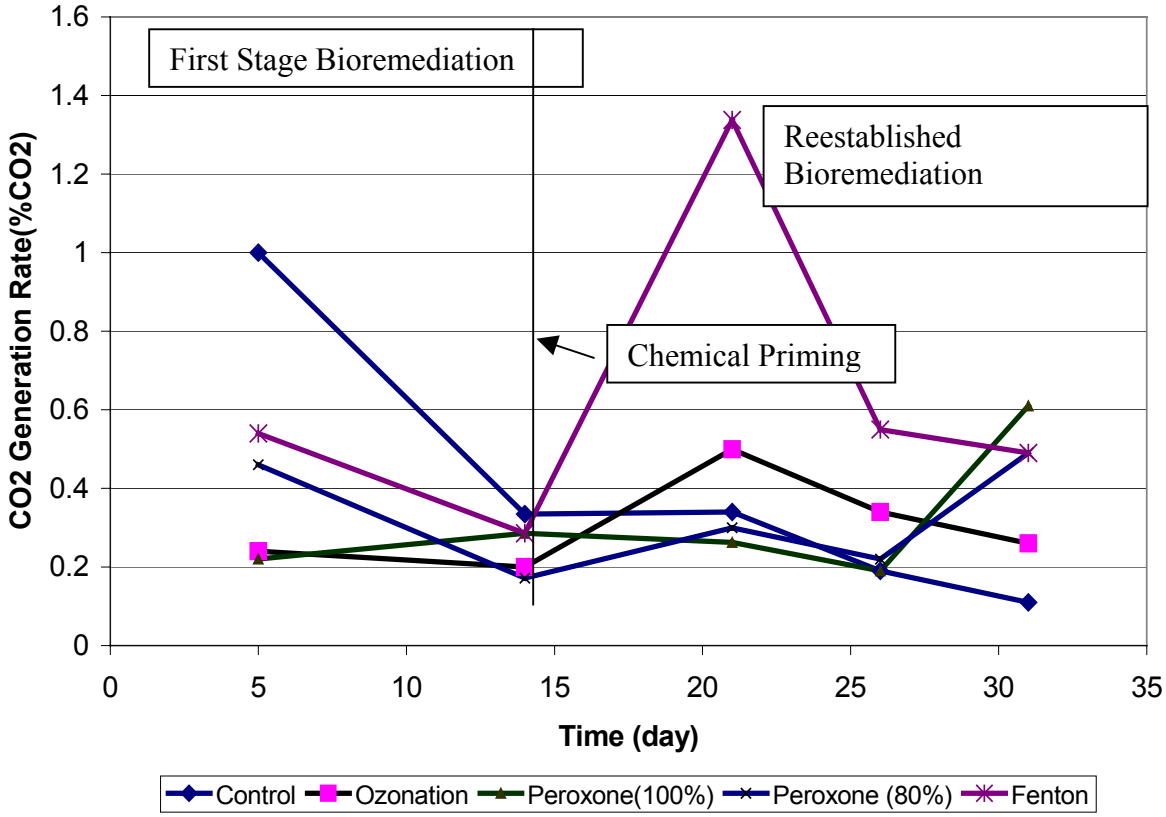

Figure 5.11. CO_2 Evolution from BIO/OX/BIO Treatment of the LMW-Soil

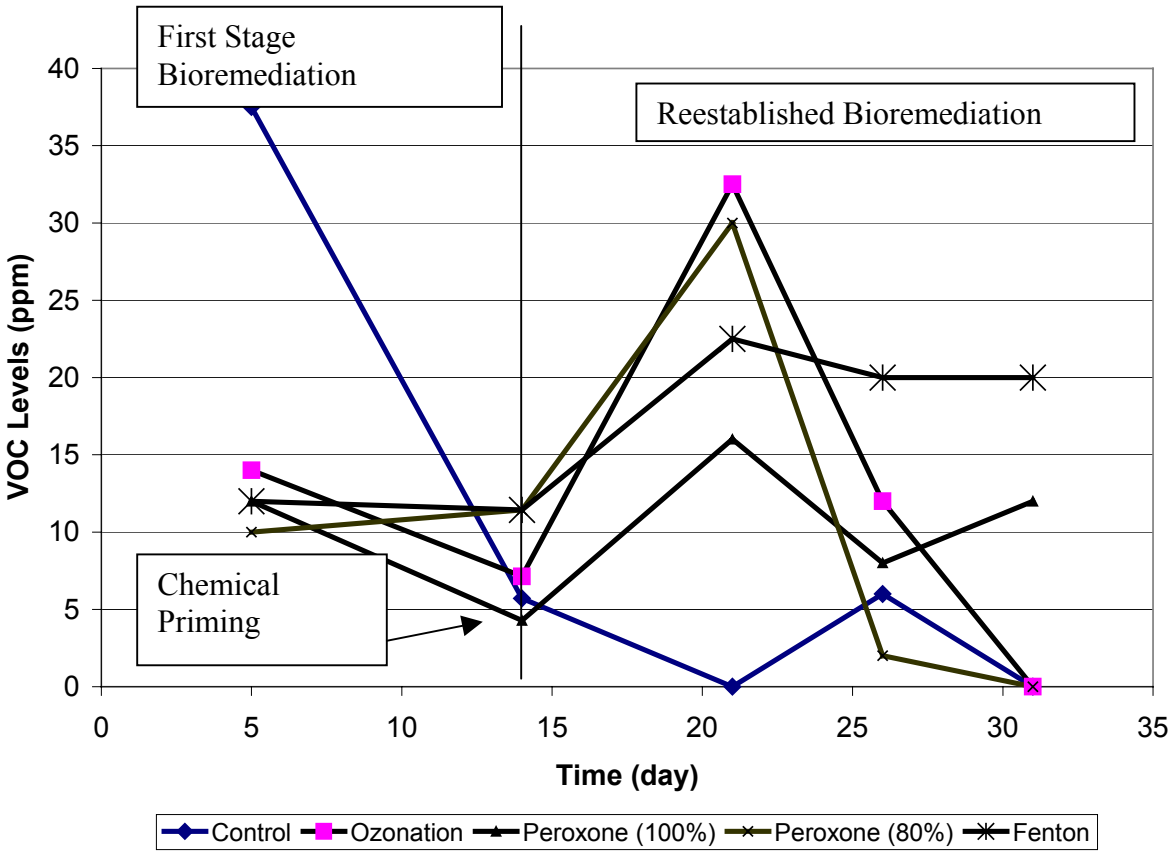

Figure 5.12. Headspace VOC Levels in BIO/OX/BIO Treatment of the LMW-Soil

CHAPTER VI

CONCLUSIONS AND ENGINEERING SIGNIFICANCE

The results of this study provide sufficient evidence for the feasibility of chemical oxidation enhanced bioremediation of TPH contaminated soils. Several conclusions can be made as an outcome of this investigation.

Slurry Phase Experiments

Firstly, chemical priming, performed in both slurry and packed soil systems, is capable of increasing the rate and extent of TPH removal within soil matrices. In slurry systems, Fenton's Reagent carried under acidic conditions yielded greater TPH degradation efficiencies than at neutral pH. Higher Fenton's Reagent dosages achieved more TPH removal during the chemical priming stage than lower Fenton's Reagent dosages. However, results from reestablished bioremediation attempts indicate that all chemical primed systems, regardless of Fenton's Reagent dosages, have the similar overall final TPH removal efficiencies. Compared with the Control Set, the chemical primed slurries achieved more than 32% additional removal of the heavy TPH for the HMW-Soil and 26% for the LMW-Soil during the reestablished bioremediation stage. This suggests that Fenton's Reaction, regardless of the dosage level, increases the

biodegradation potential of the soil TPH and makes unavailable or biorecalcitrant compounds more susceptible to biotreatment. Hence, these data verify that chemical priming in the form of Fenton's Reagent treatment is responsible for increased treatment rate and extent of TPH percent removal from the soils. Fenton's Reagent using 5,000 ppm hydrogen peroxide and 500 ppm ferrous sulfate under a pH of 2.5 was a comparably more effective, and possibly a more economical treatment condition, than the higher dosage conditions. This conclusion is based on the fact it achieved the same TPH removal enhancement efficiency during the reestablished bioremediation at lower hydrogen peroxide and ferrous sulfate dosages. Severe foaming problems were observed during this study for peroxone and ozonation applied in soil slurries. Management of foaming in the slurries remains unresolved. As such, ozonation and peroxone were not tested in slurry systems.

Soil Column Experiments

In the packed soil column experiments, results confirmed the Phase I data in that all three types of proposed chemical oxidation priming processes successfully increased the biodegradation potential of the contaminants in previously biologically treated soils. For the HMW-Soil, ozonation appeared to have provided the best enhancement to the reestablished bioremediation. Approximately 30% additional removal of the heavy TPH over the residual heavy TPH from first stage bioremediation was achieved during ozonation chemical priming stage. An additional 39% was removed during the

reestablished bioremediation stage. The peroxone process had the highest total TPH removal capability during the oxidation stage with 90% of total residue TPH being removed. However, little subsequent TPH removal (an additional of 2%) was observed during the reestablished bioremediation. Fenton's Reagent treatment did not appear to have the expectantly high enhancement capacity for the reestablished bioremediation stage. Only 10% additional heavy TPH removal was observed during the reestablished bioremediation stage for Fenton's Reagent.

The TPH in the LMW-Soil appeared to be very biodegradable at the onset using aeration, activated sludge seeding, and nutrient amending providing treatment. About 70% of the initial TPH was removed within the first 2 weeks of incubation. An additional 23% of the TPH was removed during the chemical priming stage. Unlike the HMW-Soil, the peroxone processes did not achieve a greater TPH removal than ozone and Fenton's Reagent. All of the oxidation conditions yielded similar TPH removals during the chemical priming stage. An additional 2% of the TPH was typically removed during the 2 weeks of reestablished biological treatment. This is likely the result of limited degradable TPH within the soil system after oxidation. The total proposed approach (BIO/OX/BIO) yielded a 20 ppm final TPH level in the LMW-Soil. Conversely, the Control Set after 4 weeks of biological treatment alone, resulted in a final 84% TPH removal (TPH level of approximately 60 ppm).

The proposed BIO/OX/BIO scheme was proven to be successful in treating the two contaminated soils tested (especially the HMW-Soil). Chemical priming was observed to

be more advantageous with the HMW-Soil that was contaminated with heavy refractory petroleum hydrocarbons, versus the LMW-Soil, which was contaminated with lighter diesel range organics. For the more biodegradable LMW-Soil, a BIO or BIO/OX treatment appears to be a sufficient option for the remediation of this soil.

In summary, this study indicates that petroleum hydrocarbon contaminants, especially the high boiling point hydrocarbons, can be treated by chemical oxidation primed bioremediation using a BIO/OX/BIO technique. However, additional investigations on chemical oxidizer dosage effects and the influence of oxidizers on soil fabric and microorganism health are needed. Biological treatment and chemical oxidation process integration techniques should be further studied before fielding this innovative concept.

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