THE EFFECTIVENESS OF TREATING OIL CONTAMINATED SOILS THROUGH COMPOST PROCESS

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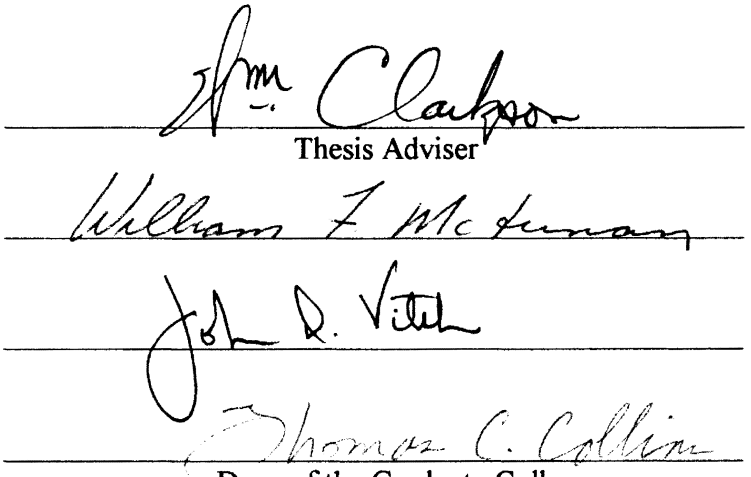

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PREFACE

During the summer and fall of 1992 and 1993, research with windrow composting for the biological remediation of oil contaminated soils was conducted. Contaminated soils were excavated and amended with nutrients and bulking agents, then placed in rows three feet deep and eight to ten feet wide on an impermeable surface. The source of contamination was lubricating oil leaks and spills from around natural gas pipeline compressor engines. The soils were treated in an empty warehouse at Transok's Crescent Natural Gas Processing Plant. Compost piles were aerated physically by turning with a backhoe with any additional water or nutrients being added at that time. The purpose of this research was to determine if this contaminated soil could be effectively bioremediated in field-scale batches of 100 cubic yards using only indigenous bacteria. Also to determine the techniques and conditions that would allow this process to be successfully applied to other sites and other types of hydrocarbon contaminants.

I wish to express my sincere gratitude to Dr. William Clarkson, my thesis advisor, for his invaluable assistance and guidance during the entire duration of this research and testing. I am also grateful to the other committee members; Dr. Jack Vitek for his counsel on developing my plan of study for this program and Dr. Bill McTernan for the direction he provided in developing this report. Special thanks also to my supervisor, Luis Rodriguez, and Fred Dennis and Mark Sproull at the Crescent Plant. Without their support and efforts this project would not have been possible.

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CHAPTER I

INTRODUCTION

Statement of Problem

The nature of operations in the oil and gas industries continually generates hydrocarbon contaminated soils. These are hydrocarbons in the form of crude, lubricating oils, transmission fluids, hydraulic fluids, natural gas condensate, gasoline and other refined volatiles. Historically, the source of this contamination is from leaks or spills at transfer connections at tank batteries, production facilities, compressor engines, underground storage tanks, machinery/equipment storage areas, maintenance centers and as a result of pipeline leaks or ruptures. In the past these soils were excavated and spread on roads for dust control, left in place and covered with clean soil or gravel, or landfill or treated. The Resource Conservation and Recovery Act (RCRA), Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA, i.e. Superfund) and the Clean Water Act now require remediation of these soils and have also severely restricted disposal options. The industry is discovering the only economical and practical method of remediating these large quantities of oil contaminated soils is through bioremediation processes.

The basis for the research and experiments of this study is the bioremediation of soil contaminated with lubricating oil from around compressor engines on natural gas pipelines. Almost all these types of engines leak lubricating oil, at various connections, seals, and gaskets. Some companies commonly install these engines on concrete foundations without any gutters, troughs, rails or other measures to collect this oil along with any contaminated storm or engine wash-down water. The oil and contaminated water runs off the foundation into the surrounding soils. Typically, depending on the type

of engine and how long it has been at the site, the contaminated soils extend to a depth of 18 to 24 inches and to 4 to 5 feet around the perimeter of the foundation. With an average size foundation being 8 feet by 20 feet this results in approximately 16 cubic yards of contaminated soils at each engine. Thousands of these engines operate on various pipelines, processing plants and refineries in Oklahoma.

Compost Treatment Processes

At most facilities with compressor engines, the best approach is the *ex situ* bioremediation of these soils. The remote, unmanned, nature of most of these sites makes maintenance and operation of a treatment area difficult. Moreover, the engine continues as a contamination source to an *in situ* treatment area, although to significantly lesser degree if controls are installed. Also a gravel cover is typically used around these engines to prevent erosion. An effective *in situ* process would require some type of aeration through tilling or other means which would displace the gravel cover. Because of the relatively small volume of soil around the engines, it can usually be excavated easily and transported to a treatment site or be treated *ex situ* at that site. Studies have shown the most cost effective means of treating soil is through a windrow-type composting processes (Jackson, Hammer, Hoffman, Gorman, 1993; Fyock, Nordum, Fogel, Findlay, 1991; Barnhart, Myers, 1989).

Composting systems are generally divided into three categories: windrow, static pile, and in-vessel. All involve excavating the soils and placing them in a treatment area. In the windrow approach, sludge (or contaminated soils) and bulking agent mixture is composted in long rows (or windrows) that are aerated by convection air movement and diffusion. Windrows are turned periodically by mechanical means to expose organic matter to ambient oxygen. In the static pile (or forced-aeration) approach, piles of a sludge/bulking agent mixture are aerated using a forced-aeration system installed under the piles to maintain a minimum oxygen level throughout the compost mass. In-vessel composting takes place in partially or completely enclosed containers in which

environmental conditions can be controlled. Bench and pilot-scale treatment studies from the oil and gas industries with hydrocarbon contaminated soils have been using windrow composting systems exploiting indigenous bacteria species. This is because system operations and maintenance costs are low and effective results have been demonstrated (McMillen, Kerr, Gray, Findlay, 1992; Fyock, Nordum, Fogel, Findlay 1991).

Project Objectives

The objectives of the research and experiments conducted during this project were to determine:

1) If soils contaminated with lubricating oil and other long-chain hydrocarbons could be effectively remediated, through windrow composting systems, on a full-scale basis using only indigenous bacteria.
2) The environmental and operating conditions, and the types and populations of bacteria, that promoted effective degradation of these hydrocarbons.

The majority of the studies and research conducted to date on this type of contaminant have involved laboratory beaker, bench-scale or pilot-scale testing. This project involved a full-scale, ongoing remediation project of soils contaminated with lubricating oil under field conditions. No inoculum preparations of exogenous or indigenous bacteria have been provided. These experiments provided substantive data on the effects of changes in environmental conditions (moisture, nutrients, oxygen, soil type, etc.), the ability to manipulate them and operate the system, and the difficulties encountered. The experiments include specific analysis on the types of bacteria which were most the aggressive degraders of the contaminant, response of the bacteria to changes in environmental conditions, and changes in population levels over the duration of the project.

CHAPTER II

LITERATURE REVIEW

Microbial Growth and Substrate Utilization Kinetics

Thermodynamic Relationships

Energy for microorganisms is obtained mainly through oxidation-reduction (redox) reactions and, to a lesser extent, photosynthesis. Microorganisms mediated redox reactions are carried out by catalysts which increase the velocity of the overall reaction. Catalysts, which are basically protein enzymes generated by the microbial cells, modify the reaction pathway so that the reaction may proceed with a lower activation energy (General Physics, 1990). Successful bioremediation of complex substrates involves having a sufficient microbial diversity to produce the proper enzymes for catalyzing the desired reactions. For all redox reactions a flow of electrons must occur between substances. Oxidation is the removal of electrons from the substance being oxidized and reduction is the addition of these electrons to a substance. The nature of the electron acceptor establishes the microbial metabolism mode and, therefore, the type of reaction. Redox reactions consist of the oxidation half reaction and the reduction half reaction. Successful bioremediation (mineralization of the contaminant) requires the addition of adequate quantities of nutrients and appropriate electron acceptors, including an energy source, in a controlled manner (Grady & Lim, 1980).

The appropriate electron acceptor must be selected to obtain the desired redox reaction which is governed by the energy yielding conditions of the substrate. Microbial populations will exploit the type of redox reaction that will yield the greatest energy

(General Physics, 1990). The amount of free energy that a microorganism can obtain from the coupled redox reaction establishes the preferred electron acceptor. Those organisms which can bring about the transfer most rapidly and can capture released energy more efficiently in a given environment will tend to dominate because the rate of growth will be the greatest (Riser-Roberts, 1992).

The Gibbs free energy is a part of the total energy that is available to perform useful work (McCarty, 1975). The amount of free energy that can be obtained by microorganisms from redox reactions is directly proportional to the electrical activity (pE) of the redox system (General Physics, 1990). The pE values of energy producing reactions of microbial significance indicate the mode of metabolism, or redox reaction that will occur.

Typical electron acceptors for bacterial mediated reactions are oxygen, nitrate, sulfate and carbon dioxide. When oxygen is used as the electron acceptor, the energy released is a maximum, whereas when carbon dioxide is used, the energy released is a minimum (Sims, Sims & Matthews, 1989). In addition, nitrite formed during denitrification can be used as an electron acceptor. When it is, the energy release is greater than from oxygen usage (Leahy & Colwell, 1990). The presence of oxygen is inhibitory to those organisms which can mediate reductions of sulfate, nitrate and carbon dioxide. Nitrate is also inhibitory to the organisms using sulfate and carbon dioxide as electron acceptors if it is present in significant quantities (Buday, Gergely, Torok & Szoboszlay, 1989). These inhibitory effects are part of the selective pressures which determine microbial population composition in a particular environment.

Stoichiometric Equations for Aerobic Growth

Reactions for the development of stoichiometric equations for the breakdown of contaminant substrate have been established (McCarty, 1975). The total reaction must include the organic substance being oxidized, the electron acceptor being reduced, and the major nutrients for cell growth. These would include nitrogen and phosphorus. The

equations assess if adequate quantities of nutrients and appropriate electron acceptors exist for successful contaminant degradation. To obtain the nutrient requirement, it is necessary to establish that portion of energy necessary for cellular growth. A factor must be included for the distribution of energy between cell synthesis and other needs (McCarty, 1975). These factors are represented by:

fe = fraction of organic compounds oxidized for energy

fs = fraction associated with conversion to microbial cells

fe = 1-fs

The overall reaction can be given in general terms by:

$$R = Rd - feRa - fsRc$$

where:

Rd = the half reaction for the electron donor

Ra = the half reaction for the electron acceptor

Rc = the half reaction that provides nutrient requirements for cell synthesis

One of the oldest and most widely accepted empirical formulas to represent the organic composition of microbial cells is $C_5H_7O_2N$. Phosphorus is also necessary nutrient but is only needed in trace amounts. The cell growth yield for *Pseudomonas* organisms (most common hydrocarbon degraders) is approximately 0.37 of new grams of cells produced for each gram of chemical oxygen demand (COD) removed (Gary & Lim, 1980). This results in a fs value of 0.44 and a fe value of 0.56. In the following stoichiometry equation for microbial growth, the half reaction equation for the electron donor (Rd) is that typically of oils, the electron acceptor (Ra) is oxygen, and the cell synthesis (Rc) will be provided from nitrate as the nitrogen source.

$$C_8H_{16}O + 6.13\ O_2 + 0.70\ NO_3 = 0.70\ C_5H_7O_2N + 5.52\ H_2O + 4.04\ CO_2$$

Which means for each mole of $C_8H_{16}O$ (oil) removed under these growth conditions:

* 6.13 moles of oxygen (O_2) are needed (1.53 grams O_2 per gram oil).
* 0.70 moles of nitrate (NO_3) are needed.
* 0.70 moles of microbial cells are formed.
* 5.52 moles of water and 4.04 moles of carbon dioxide are formed.

See Appendix A for detail of half reactions and equations for this problem. To approximate the heavier, longer-chained hydrocarbons typical of lubricating oil, this balanced normalized equation should be multiplied by three.

Biological Factors Affecting Hydrocarbon Biodegradation

Synergism

One microbe type in a population may be unable to synthesize a particular enzyme response for a reaction, but will perform on the substrate when the activity of a second organism synthesizes the desired component. Frequently, microorganisms produce metabolites that may be self-inhibitory or inhibit the growth of other organisms (General Physics, 1990). Successful mineralization of a substrate is then dependent upon the activity of a second organism to remove compounds excreted as a result of growth from the first organism (Riser-Roberts, 1992).

The synergism between specific microorganisms is so important that the coupled associations are more like a microbial web (community). The rate of degradation of hydrocarbons in mixed cultures is much faster than by the combined rates of each separate organism collectively. The increased rate of degradation is probably a result of combined metabolic attack at different sites on the organic contaminant, increasing overall degradation rates (Leahy & Colwell, 1990).

Co-Metabolism

Co-metabolism is defined as "the degradation of a compound only in the presence of other organic material which serves as the primary energy source" (Sims, Sims & Matthews, 1989). Enzymes generated by an organism growing at the expense of one substrate can also transform a different substrate that is not associated with that organism's energy production or cell growth. Co-metabolism (or co-oxidation if the transformation involves an oxidation reaction) may be critical for the mineralization of many recalcitrant substances, especially for more recalcitrant longer-chained hydrocarbons and xenobiotics (Leahy & Colwell, 1990)

Aerobes

The most commonly isolated organisms in areas of aerobic degradation of hydrocarbon contamination are the heterotrophic bacteria of the genera *Pseudomonas, Achromobacter, Arthrobacter, Micrococcus, Vibrio, Acinetobacter, Brevibacterium, Corynebacterium, Flavobacterium, Mycobacterium* and *Nocardia* (Riser-Roberts, 1992). *Pseudomonas* species appear to be the most prevalent and most adaptable to the different hydrocarbon contaminants. *Corynebacterium* species may be major agents for decomposing heterocyclic compounds and hydrocarbons in contaminated aquatic environments (Buday, Gergely, Torok & Szoboszlay, 1989). *Pseudomonas* have been found to be capable of biodegrading/biotransforming the more recalcitrant alkanes (C_{19} to C_{40}) as would be found in sludges and lubricating oils (Prince & Sambasivam, 1993).

Loss of oxygen as a metabolic electron acceptor induces a change in the activity and composition of the microbial population in soils. Facultative anaerobic organisms, which can use oxygen when it is present or can switch to alternative electron acceptors such as nitrate or sulfate, and obligate anaerobic organisms become the dominant populations. This is significant during composting because oxygen in the piles can be

depleted and switching electron acceptors, especially to nitrate, allows microbial activity to be sustained until oxygen is restored.

Anaerobes

Anaerobic degradation is performed mainly by bacteria utilizing either an anaerobic respiration or fermentation processes. The end products of anaerobic degradation of some hydrocarbons are reduced compounds, some of which are toxic to other microorganisms and plants. Petroleum can be degraded anaerobically when sulfates and nitrates are used as the electron acceptors. An alkane dehydrogenase is proposed to be the initial enzyme involved in the production of alkene as the first intermediate compound (Prince & Sambasivam, 1993). Alkanes shorter than C_9 can be degraded anaerobically, whereas some alkanes with longer chains may only be transformed into napthalenes, pyrene and other polycyclic aromatic hydrocarbons (Leahy & Colwell, 1990).

Catabolism of aromatic compounds can occur under anoxic conditions and in the presence of nitrate. *Pseudomonas* strain PN-1 can use benzoates but not phenol to grow under nitrate-reducing conditions (Riser-Roberts, 1992). Some facultative microorganisms retain low levels of oxygenase activity when grown in the presence of aromatic compounds, even under anaerobic conditions. A nitrate-respiring *P. stutzeri* is capable of using phenol as a substrate (Riser-Roberts, 1992). Microorganisms have been found to degrade xylenes and toluenes under denitrifying conditions, such as in lake sediments and sludge digestors. Sulfate-reducing microorganisms are strict anaerobes (Riser-Roberts, 1992). Reducible sulfur compounds (e.g. sulfate, thiosulfate) serve as terminal electron acceptors. These bacteria are most commonly found in aquatic environments but can also be found in soil and include *Desulfovibrio, Desulfonema magnum* and *Desulfovibrio vulgaris*. Sulfate-reducing bacteria alone have not been found to effectively degrade hydrocarbons (Atlas, 1991).

Hydrocarbon Degrading Microbe Communities

The fraction of the total heterotrophic community represented by hydrocarbon-utilizing bacteria and fungi is highly variable, with reported frequencies from 6% for soil fungi, 0.13% to 50% for soil bacteria, and 0.003% to 100% for marine bacteria (Buday, Gergely, Torok, Szoboszlay, 1989). The most consistent and reasonable estimates for soils appear to be 1% in unpolluted environments and 1- 10% in historic oil polluted ecosystems (Sims, Sims & Matthews, 1990).

Mixed populations of these organisms with overall broad enzymatic capabilities are required to degrade complex mixtures of hydrocarbons, such as crude oil in soil. Twenty-two genera of hydrocarbon-degrading bacteria have been isolated from soil environments (Atlas, 1991). Based on a number of published reports, the most important hydrocarbon-degrading bacteria in both marine and soil environments are those previously identified above in the section on "Aerobes" (Sims, Sims & Matthews, 1990).

Optimization of Soil Factors for the Biodegradation of Hydrocarbons

Nutrients

Microbial metabolism and growth is dependent on adequate supplies and proper ratios of nitrogen and phosphorus and other micro-nutrients or trace elements (i.e. sodium, potassium, calcium, magnesium, iron, etc.). During bioremediation processes (e.g. composting), microorganisms consume carbon for growth and also especially nitrogen for protein synthesis. The carbon to nitrogen (C:N) ratio depends upon the rate and extent of degradation of the contaminant involved. Common C:N ratios used for oily sludges and waste oils in soils has been 10:1 and 25:1 (Jackson, Haby, Hammer, Hoffman, Gorman, 1993; Kane, 1991). The sole carbon source in most inorganic predominant soils will be the hydrocarbon contaminant or the total petroleum hydrocarbon level. If an inadequate supply of nitrogen exists, then the organisms must work through more growth

cycles to develop enough enzymes to break down the excess carbon (Clarkson, 1993). All the available mirobial energy is being used for the degradation of the substrate and not for new cell growth and production of enzymes. If nitrogen levels are too high, then excess nitrogen will be lost as ammonia. At high enough levels, ammonia can retard the breakdown process (Sims, Sims & Matthews, 1989).

Phosphorus additions are not as critical as nitrogen because of it's micro-nutrient requirements which typically already exist in soil environments. However, a phosphorus to nitrogen ratio of 0.2 has proven effective in this study and others (Kane, 1991). Overall, the microbial requirements for nutrients are approximately the same as the composition of the cells. One additional consideration is to add nitrogen in the form of ammonium nitrate. Nitrate can be utilized as an additional electron acceptor by facultative anaerobes if oxygen supplies are decreased (Riser-Roberts, 1992).

Moisture

The moisture content of soil, or soil water, is the transport medium by which nutrients and organic constituents are adsorbed onto the microbial cell and by which waste products are removed. The moisture content also affects oxygen uptake of the cells, amount and availability of soluble materials and the pH of the soils. The optimum moisture content for aerobic remediation in this study and others cited has been 50 to 60 percent of field capacity, or approximately 6 - 8 percent by weight of inorganic based soils. However, field capacity will no longer be applicable when soils are excavated and bulking agents are added. The percent by weight basis should be used or physical observation to ensure the soils are moist but have not free drainage or slurries which would be typical of a 50 percent field capacity measurement. These levels are important to optimize biodegradation rates but are difficult to maintain under field conditions. Too much moisture can result in toxic anaerobic conditions developing and too little has been found to severely limit degradation rates (Fyock, Nordum, Fogel & Findlay, 1991).

The greatest diversity and activity of microorganisms and the highest population densities are consistently observed in the sandy water-bearing strata, whereas the dense, dry-clay layer zones have the least microbial activity (Riser-Roberts, 1992). Generally, with decreasing water potentials, fewer organisms are able to grow and reproduce; bacterial activity is usually greatest at high water potentials (wet conditions). When soils become too dry, many microorganisms form spores, cysts, or other resistant forms, whereas many others are desiccated (Atlas, 1991). The porosity and composition of the soil are the significant factors in water holding and draining capacities. It should also be noted that treated muncipal or rural water supplies should not be used unless they are de-chlorinated.

Temperature

Microbial utilization of hydrocarbons occurs at temperatures ranging from 5° to 70°C (General Physics, 1990). Although biodegradation occurs at a temperature of 5°C, hydrocarbons degraded much more slowly at these lower temperatures. Most soil microorganisms are mesophiles and exhibit maximum growth in the range of 20 to 35°C (Sims, Sims & Matthews, 1989). The majority of hydrocarbon utilizing bacteria are most active in this range (Riser-Roberts, 1992).

Temperature also affects biodegradation rates by its effect on the physical nature and chemical composition of the oil. Particularly affected are the surface area available for microbial colonization and the nature of hydrocarbons remaining for metabolic attack after any volatilization (Leahy & Colwell, 1990). At low temperatures, the viscosity of the oil increases and solubility is decreased which causes the oil to adsorb tightly to the soil particles. This provides less surface area available for microbial utilization and delays the onset of biodegradation (Leahy & Colwell, 1990). The volatilization of any lighter, short-chain alkanes is also reduced.

Oxygen

The biodegradation of most organic contaminants requires approximately two parts oxygen to completely metabolize one part of organic compound (Atlas, 1991). The complete oxidation of 1 mg of hydrocarbon to carbon dioxide and water requires 3 to 4 mg of oxygen, which is comparable to 4.59 mg calculated strictly from stoichiometry of oil (approximated at three times for lubricating oil), as presented earlier (Riser-Roberts, 1992). The optimum level for aerobic metabolism is greater than 0.2 mg/l of dissolved oxygen and a minimum air-filled pore space in the soils of 10% (Riser-Roberts, 1992). These oxygen levels in soil systems can be maintained by: the prevention of saturation with water, presence of sandy and loamy soils or a bulking agent (e.g. wood chips), moderate tilling, avoidance of compaction of soil, and limited addition of additional carbonaceous materials. This is for passive (e.g. windrow composting, landfarming, shallow in-situ) bioremediation type processes.

Soil pH and Composition

Soil pH also affects the activity of micro-organisms and the solubility of nutrients. The solubility of phosphorus is maximized at a pH value of 6.5 (Sims, Sims & Matthews, 1989). Typically, for aerobic degradation of hydrocarbons, the optimum pH is near neutral (between 6.5 to 7.5). Hydrocarbon contaminated soil pH can be highly variable in petroleum refining or production areas. Soil systems from these areas may require management with some lime additions to maintain these levels above 6.0. The lime has not been found to have a detrimental effect on microbial metabolism (McMillen, Kerr, Gray & Findlay, 1992). A pH level of greater than 6.0 has been found to minimize any hazardous metals transport in the soil (Fyock, Nordum, Fogel & Findlay, 1991).

Soil composition influences infiltration rate and permeability, water holding capacity, and adsorption capacity for various waste components and oxygen diffusion. The predominance of clay and silt particles in finer textured soils results in a very small

pore size with a slow infiltration rate of water (low porosity) and a low diffusion rate of oxygen. Clay soils tend to retain a higher moisture content which can further restrict oxygen diffusion. Coarse soils of sand and gravel with large interconnecting pores allow sufficient oxygen diffusion and rapid water movement. However, if the soils are excessively drained, nutrients in the added material will move too rapidly to be sufficiently adsorbed on the soil.

A bulking agent addition has been found critical for successful remediation of hydrocarbon contaminated soils in prepared bed and composting remediation processes regardless of soil types but especially for cohesive clay-based soils. The bulking agent increases or maintains porosity of the soil for adequate diffusion of oxygen and distribution of nutrients, heat and waste gases. These agents have included wood chips, straw, and saw dust. A typical soil to bulking agent ratio (per volume) used is 4:1 with an overall porosity rate of approximately 40 percent (Moore, 1992; Newton 1990). Typically, a trial and error method will be needed to find a ratio for specific soil types to maintain proper oxygen, moisture and porosity rates.

Effectiveness and Use of Indigenous and Exogenous Bacterial Strains

The microbial community in soil usually includes a significant hydrocarbon utilizing component, which readily increases in response to hydrocarbon contamination. These indigenous microbial populations are also highly adapted to a particular soil environment. Exogenous or seeded microorganisms cannot compete successfully and survive; for this reason, soils are not widely considered to be amenable to improvements in rates of biodegradation through seeding alone (Buday, Gergely, Torok & Szoboszlay, 1989). Other potential problems associated with the inoculation or seeding of soils with exogenous organisms include: the presence of inhibitory substances, predation, preferential metabolism of competing organic substrates, and insufficient movement of the seed organisms within the soil.

The addition of selected pure cultures of bacteria to soil has been found to increase the degradation of certain pesticides (e.g. DDT & parathion) (Atlas, 1991). The best results from seeding experiments, however, have been reported in studies in which the environment is controlled to some extent, such as in fermentors and chemostats (Riser-Roberts, 1992). The advantages of these systems are clear: competition with other microflora is reduced or nonexistent, and system parameters can be optimized to highest rates of biodegradation. The disadvantages are economic costs and impracticability to field applications.

Production and Effect of Biosurfactants

Hydrocarbons in soil systems are often difficult to remove because they will adsorb to surfaces of inorganic and especially organic soil particles. Biosurfactants are produced by microorganisms during growth on substrates that are insoluble in water, including petroleum hydrocarbons. These surfactants can enhance biodegradation of the contaminant by raising the solubility of this substrate, thereby making more readily available for degradation and facilitating transport of the substrate across the cell membrane (Falatko & Novak, 1992). This also explains a common phenomenon that occurs during the initial stages of bioremediation: an apparent increase in contaminant levels. The action of the surfactant enzymes releases the contaminants from the soil particles to which they have a high attraction, making them available for degradation, but also more easily detected by laboratory analysis (Jackson, Haby, Hammer, Hoffman & Gorman, 1993).

Long chain hydrocarbons C_{25}-C_{40} (lubricating oils and sludges) have a high attraction for adsorption to soil particles resulting in a lower availability for biodegradation (General Physics, 1990). Naturally produced biosurfactants are typically lipids with properties resulting from polar and apolar groups on a single molecule. Biosurfactants have been credited with facilitating cellular uptake and use of generally insoluble

substrates, especially longer chain hydrocarbons (Buday, Gergely, Torok & Szoboszlay, 1989). When associated with the cell, they promote transport across the membrane and, as extracellular compounds, they solubilize the substrate. The effectiveness of synthetic surfactants in enhancing biodegradation of hydrocarbons has been shown to be variable and dependent on the chemical formation of the surfactant (Falatko & Novak, 1992). Although considerably less toxic than they have been in the past, synthetic surfactants have been shown to inhibit microbial processes (Riser-Roberts, 1992).

CHAPTER III

COMPOSTING PROCESS TREATABILITY STUDIES AND REGULATORY CONSIDERATIONS

Kane Laboratory Study (Kane, 1991)

A treatability study involving laboratory experiments and bench-scale studies was performed on contaminated soils from a heavy equipment storage area. These soils consisted of motor, hydraulic, gear and transmission oil contaminants with total petroleum hydrocarbon (TPH) levels of 76,000 milligram per kilogram (mg/kg) from composite samples. The contaminants were extracted (washed) from the soils using distilled water. Microbial populations were cultured in lab reactors from sewage sludge using glucose as the substrate. The contaminant extract was slowly substituted for the glucose until the microbes were acclimated. A total of seven different reactors were set up in the lab in which predetermined and varying amounts of contaminant extract and acclimated seed were combined as well as nutrients and buffer solutions. One additional "blank" reactor was set up with acclimated inoculum, nutrients, and buffer but with no extract. The only variable in the reactors was the different levels of extract (substrate). The reactor vessels were four-liter Pyrex flasks fitted with aeration equipment, consisting of an air compressor delivering air through diffuser stones providing the oxygen needs of the culture and for mixing the reactor. An inorganic nutrient solution was added to provide a biochemical oxygen demand (BOD), nitrogen, phosphorus (BOD:N:P) ratio of 100:5:1. Other trace elements such as magnesium, potassium, selenium, chromium and molybdenum were also added.

Respirometer equipment was installed on the reactors to measure the amount of oxygen consumed by the process, and the oxygen supply was carefully regulated and computer monitored. The temperature of the reactors was maintained at 25^0 C. Initial test runs were analyzed to compare BOD levels of batches with contaminant extract and to those without. These first runs indicated no appreciable differences in BOD between live and blank runs. Two assumptions were made: that the actual BOD_5 of the extract was much lower than previously measured, and/or nutrients from the acclimating reactors were being transferred into the test reactors. Only after washing the acclimated "seed" bacteria were appreciable differences in BOD levels observed. The test runs were 120 hours, and batches without extract had generally consumed less than 50 mg/l BOD while live batches (with extract) had BOD consumption that increased to over 400 mg/l. The seven reactors were set up with the extract, nutrients and buffer solution and seed bacteria. A buffer solution was used to maintain a pH between 6.5 to 7.5. As stated, the only variable was the amount of contaminant extract added to the reactors. A gas chromatography (GC) hydrocarbon analysis was taken at the beginning and end of the 120 hour tests. Chemical oxygen demand (COD) analysis was also taken at the beginning and end of this process and BOD readings were taken continually by the respirometer equipment.

The time versus BOD data for all seven loaded reactors indicated that BOD increased proportionally to the amount of extract available. The BOD at 120 hours ranged from 875 mg/l with 340 ml of initial extract to 325 mg/l with 25 ml of extract. The greater the volume of extract the higher the initial chemical oxygen demand (COD) and greater increase of oxygen consumption over the test period of 120 hours. The initial GC analysis of the reactor solutions indicated hydrocarbon spikes. No hydrocarbon spikes were evident on samples after the test run indicating that the hydrocarbons were degraded.

Jet Fuel Contamination Project (Moore, 1992)

A static-pile bioremediation composting approach was used to treat soils from an underground jet fuel storage facility. This project involved 3,500 cubic yards of contaminated soil with total petroleum hydrocarbon (TPH) levels of 3,500 to 7,500 mg/kg. No introduced strains of bacteria were used in this process. A bulking agent of wood chips was added to the soils at a ratio of 4 parts soil to 1 part wood chips. Several types of bulking agents were tried, including gravel, wood chips and peat moss. Through trial and error, the wood chips were found to be most effective. Two cells were constructed that were 150 feet long by 65 feet wide, and 6 to 7 feet high each. A PVC liner was used with a collection sump downgradient of the cells to collect water and nutrients for recycling. A 1.5 foot lift of soil was placed on the bottom of the cells, then 2-inch perforated PVC pipes were placed on this at 10-foot centers. This was covered with gravel and connected to a 4-inch manifold with a vacuum blower to draw air through the system. Another 4-foot lift of soil was placed on top of the aeration system, followed by nutrient supply piping and a final 1.5 foot lift of soil mix. The entire cell was covered by PVC liner.

Nutrients consisting of nitrogen and phosphorous were mixed with water and connected to pumps to keep the moisture levels between 50 to 85 percent of field capacity. The carbon to nutrient ratio was not provided in the report on this project. Pressure in the aeration system ran at negative 1.5 psi. The aeration system calculations showed that 300 cubic feet of air was moving through each biocell per minute or the equivalent of two changes of air in the cell pore spaces per hour. Each cell was divided into six zones to monitor effectiveness of the treatment and ensure even distribution of nutrients throughout the cell. The system was run from October through mid-December, during which time the average TPH fell from 3,500 mg/kg to 2,500 mg/kg. In mid-March the unit was reactivated and by the end of May average levels had been reduced to 1,000 mg/kg. By the middle of August the average TPH in each cell was below 35 mg/kg.

Because this was below the state regulatory level of 50 mg/kg, the soils were returned to the excavation pit with no further treatment.

Port Stanley Pilot Study

(Barnhart & Myers, 1989)

In Port Stanley, Ontario an oil gasification site was used from the 1920's to the 1950's. The waste oil tar was stored in two open pits on-site and subsequently filled with dredge material resulting in the spreading of the oil tar over surface soils. A pilot-scale, on-site remediation of approximately 4,800 cubic meters of oil tar contaminated soil commenced in August and ran four months. The contaminants consisted of benzene, toluene, xylene (BTX), oil and grease, and polycyclic aromatic hydrocarbons (PAHs). A 500-foot by 200-foot biotreatment pad was constructed using compacted clay and compacted clay-faced berms. The excavated soil was prepared for bacterial application (additions to indigenous strains) by the addition of nutrients. This included nitrate nitrogen, phosphorous, potassium, ammonia nitrogen, and calcium. The nutrients were dissolved in water and sprayed onto the soil. The ratio of the nutrients, or nutrient load, was not provided in reports on this study. A bacterial suspension was prepared including nutrients sufficient for their rapid growth. Bacteria were applied approximately four days a week throughout the treatment period. This application consisted of 1,200 total gallons bacterial suspension, which consisted of approximately one-third cell mass. The suspension was applied through a high pressure distribution system to the soil.

The soil was tilled on a daily basis to a depth of 24 inches. The depth of the soil rose from 25 inches when it was first leveled in the facility to 33 inches as a result of the tilling operation. No bulking agent was added as the daily tilling probably sufficed to keep the soil adequately porous and aerated. The sampling regime of this project was relatively intense and consisted of an initial sampling round and four subsequent rounds performed at two week intervals. Twenty zones were established in the treatment area in which the samples were taken for nutrient, moisture and contaminant levels. No mention was made

in the report on this project of moisture levels although moisture was obtained through nutrient and bacterial applications, and it is assumed to be relatively high. The pH levels fluctuated between 6.0 and 7.5 with the average being 7.0. The initial BTEX levels were 13.2 ppm and were reduced to 3.6 ppm at the end of the four-month treatment period and were assumed to be volatilized during the treatment. Oil and grease levels were initially 1,538 mg/kg and were reduced to 990 mg/kg, and overall PAHs were initially 335 ppm and were reduced to 45.5 ppm due to biodegradation.

Chevron's Petroleum Sludge Composting Project

(Fyock, Nordum, Fogel & Findlay, 1991)

This pilot-scale windrow composting project sought to determine if indigenous bacteria could biodegrade petroleum production pit sludges. A laboratory treatability investigation was first carried out to provide information for the process design. Soil from the dike or berm surrounding the pit was tested for its ability to provide a bacterial inoculum for the process. The soil was found to contain high levels, 10^7 colony forming units per gram (CFU/g) from initial plate counts on an unknown growth medium, of acclimated bacteria capable of growing on sludge vapors as their sole source of carbon. Because of these high levels, the soil was used directly as the inoculum. The bulking agent selected for the process was locally available saw mill waste. Air permeability, moisture transfer, and plate count tests were conducted on mixtures of the sludge and bulking agent to determine optimum quantities of sludge to bulking agent needed to support growth of the bacteria.

The bulking agent was laid in rows, and the sludge applied at the rate of 20, 30 or 40 percent by volume. The sludge, a small amount of soil inoculum, about 500 mg/kg fertilizer, and water were then thoroughly mixed into the bulking agent using an auger mixer. The compost was aerated by mechanical/physical mixing once or twice a week. Water was added at intervals to maintain the desired 40 percent moisture content (of field capacity). Composite samples were taken once a week and nutrients added to maintain 50

mg/kg nitrogen and 20 mg/kg phosphate in the compost. Temperature was taken daily and the compost cooled by mixing when the temperature exceeded 135° F.

The number of sludge-degrading bacteria in two samples of starting compost were 10 x 10^6 to 20 x 10^6 CFU/g. These were good base line levels for initiating the treatment process and correlated to the initial soils levels prior to mixing of 10^7. After about 2 and 5 days of composting, the numbers increased to 180 x 10^6 and 240 x 10^6, respectively. The initial petroleum hydrocarbon fingerprint analysis consisted of a distinctive pattern of linear alkanes (C_9 to C_{32}). During composting, the extent of degradation was estimated by analyzing for total petroleum hydrocarbons (TPH) using a modification of the EPA Method 418.1 which uses an infrared spectrophotometer with a freon extraction process. The TPH in the compost containing 20 percent sludge decreased from about 100, 000 mg/kg (10%) to 27,000 mg/kg (2.7%) in the first 10 days of composting. The TPH then decreased gradually during the next 30 days to less than 10,000 mg/kg. A portion of the residual TPH may be biomass and partially oxidized hydrocarbons due to the analytical method selected. Operation and maintenance costs for a 330 cubic yard treatment cell are anticipated to be $30 per cubic yard of sludge.

Exxon's Petroleum Sludge Composting Project

(McMillen, Kerr, Gray & Findlay, 1992)

A bench-scale composting project sought to determine if petroleum production pit sludges could be effectively biodegraded with local indigenous bacteria. These sludges contain mostly heavy crude oils as well as some workover fluids and produced water. The goal was to reduce the total hydrocarbon content to less than 1 percent or 10,000 mg/kg (a regulatory limit in several states for production waste). The sludge was analyzed and found to contain 10.8 percent (108,000 mg/kg) total petroleum hydrocarbons (TPH) and showed a distinct pattern of linear alkanes, but virtually no compounds lighter than C_{10}. This indicates that few volatiles were present. Also, little biodegradation of the crude oil

had seemingly occurred while stored in the pit, since straight-chained alkanes are usually the most readily biodegraded hydrocarbons.

Wood chips were added as a bulking agent and barn yard manure was added as a nutrient source. Native site soil and the impoundment berm soil were analyzed for the presence of hydrocarbon-degrading bacteria to determine if either were suitable sources of inoculum for the compost mixture. The native soil was found to contain the highest bacteria levels with 7 x 10^6 CFU/g of degraders and was therefore used as the inoculum. The sludge was mixed with varying ratios of wood chips and manure to determine the optimal mixture for composting. The following mixture was found to incorporate the maximum amount of sludge while maintaining good air permeability and moisture retention: (volume ratios) sludge 6.5; bulking agent, 24; water, 6; manure, 4; and native soil, 0.5. The resulting compost had TPH levels of 5.9 percent, and had a moisture content of 39 percent and a pH of 7.0.

Fertilizer was added initially to give 300 mg/kg nitrogen (as urea), 217 mg/kg phosphate and several other trace elements. Nitrogen and phosphorous were monitored during composting and adjusted as necessary to maintain these levels. The compost mixture was aerated by mixing in a stainless steel bowl three times a week which also served to keep the temperature within mesophilic conditions. Active composting was maintained for approximately four weeks. The number of specific hydrocarbon degrading bacteria increased from 0.05 x 10^6 CFU/g of compost initially to 1 x10^6 on day three of composting, and 27 x 10^6 on day ten.

The TPH decreased from 5.9 percent to a final concentration of 0.47 percent, or approximately 92 percent biodegraded. Gas chromatography analysis indicated that the normal alkanes C_{10} through C_{30}+ were not detected.

Enogex's Lubricating Oil Composting Project

(Jackson, Haby, Hammer, Hoffman
& Gorman, 1993).

This bench-scale composting project assessed if lubricating oil contaminated soils could be effectively bioremediated with indigenous bacteria. These oil-saturated soils originated from around pipeline compressor engines which leaked lubricating oils for many years. Three treatment cells were constructed with 40 gallons of contaminated soil each. No bulking agents were added to the soils. The moisture was to be maintained at 50 percent of field capacity in each cell. No nutrients were added to Cell 1 (control cell), only moisture, Cell 2 was fertilized with a C:N ratio of 12, and Cell 3 was fertilized to maintain a C:N ratio of 25. The soils were aerated by thoroughly tilling twice a week.

During the second month of the composting, the soil began to agglomerate and it became difficult to maintain the soils consistency and adequate porosity. The soil was then tilled every day from this point. Also, half the soil was removed from Cell 1 and mixed with an equal part of sand. Another cell was then added with the other half of soil and sand mixture. Moisture content was also reduced to approximately 40 percent (of field capacity) to improve oxygen transfer capacities, and ammonium nitrate was added as an additional nitrogen source. Nitrate was added because the conversion of urea nitrogen to nitrate was inhibited by the limited amount of oxygen present in the soil. The nitrate also provided an alternative electron acceptor for the oxidation of the hydrocarbons.

The initial analysis of the contaminated soils indicated total petroleum hydrocarbons of approximately 80,000 mg/kg and plate counts of 2×10^4 CFU/g. Four distinct bacterial colonies were initially identified in the soil, *Pseudomonas putida*, *Pseudomonas flourescens*, *Salmonella typhi* and *Enterobacter cancerogenous* with *Pseudomonas* species being the known hydrocarbon degrader. The TPH concentration was measured by EPA method 418.1. At the end of 120 days, the TPH in Cells 1, 2, and 3 had been reduced to 45 to 60 percent of the initial levels and the TPH in Cell 4 to 25 percent of initial concentrations. The average C:N ratio for Cell 2 after fertilizer was

added was 15. The average C:N ratio for Cell 3 was 20 and the average C:N for Cell 4 was 25.

The highest levels of bacterial populations measured in the cells were: Cell 1: 4.8×10^8, Cell 2: 9.2×10^7, Cell 3: 1.5×10^8 and Cell 4: 4.5×10^7. Through the first 100 days of operation, three to four types of bacteria were detected in each cell. After this period, the number of types increased in several samples with five, six and seven different types of bacteria identified. As indicated above, cells with greater C:N ratios did not result in higher plate counts. On the contrary, Cell 1 in which no nutrients were added had the highest counts. There was also a temporary rise in TPH levels in all cells 60 days into the experiment. This may be due to the action of surfactant-type enzymes released by the petroleum degrading bacteria in the soil. These type of enzymes released the contaminants from the soil particles to which they had a high attraction, making them available for degradation, but also more easily detected by laboratory analysis.

Regulatory Considerations

Under the Resource Conservation and Recovery Act (RCRA) treatability exclusion rule, bench and pilot tests to determine hazardous-waste treatability using bioremediation approaches are exempt from RCRA listing, generating, and transporting regulations. The rule allows for the generation or collection of samples and standards for treatability studies for 1 kilogram of acute hazardous waste and 1,000 kilograms of non-acute hazardous waste, although the regional administrator or authorized state may grant requests for additional volumes of soils, water and other contaminated debris. The applicant must obtain a demonstration permit, which allows the applicant to build and operate the treatment site for 1 year and is renewed annually thereafter.

The majority of crude and lubricating oil contaminated sites are not RCRA regulated sites. The contamination is either non-hazardous by RCRA definitions or is an exempt waste under the oil exploration and production exclusion. However, these sites may be hazardous, and thus regulated, if there are sufficient levels of heavy metals or

halogenated compounds present; as may be expected in engine lubricating oils or as a results of cross-contamination with cleaning solvents. RCRA hazardous wastes must follow proper disposal guidelines through incineration or hazardous waste landfilling if the contaminant is not banned from landfilling. Treatment of the hazardous waste must be permitted and administered under state and federal approved guidelines.

Justification or mandates for remediation of oil contaminated soils, and consideration for design of the treatment area, are also provided through the regulation of contaminated storm water. On September, 9, 1992, the EPA issued final rules establishing a permitting regimen for storm water discharges that are classified as "associated with industrial activity" pursuant to 40 Code of Federal Regulations 122.26(b)(14) under the Clean Water Act. Any of the industry types listed, or any other industry that has had a reportable quantity spill in the past, or has the potential to contaminate storm water, must apply for a permit and include controls to reduce these pollutants. A reportable quantity spill for oil is defined as any amount that results in a sheen on the surface water.

Target contaminant levels for remediation of oil contaminated soils is typically 10,000 mg/kg of TPH. This is the action level several states have established for oil and gas production sites and related facilities for remediation of surface pits, impoundment dikes and other sources of contaminated soils. Amounts less than 10,000 mg/kg can be left in place or landfilled while amounts greater must be treated as a hazardous or special industrial waste depending on composition. Other states and regulated activities have action levels as low as 1,000, 500 or 50 mg/kg of TPH.

CHAPTER IV

EXPERIMENTS

Nature and Type of Contaminant and Soil

Soil contaminated by lubricating oil was excavated from around natural gas pipeline compressor engines for treatment. These soils may have also been contaminated with ethylene glycol which is used as a coolant. During the course of these experiments, three batches of contaminated soil were treated. The soil in the first batch was analyzed for hazardous constituents under RCRA toxic characteristic leachate procedure (TCLP). These tests included analysis for heavy metals, volatiles and semivolatiles. The results indicated that all of these constituents were less than the detection limits except for barium of 0.14 mg/kg. The pH was not tested but based on the known nature of the contaminant and inorganic content of the soil, it was presumed close to neutral. All of the batches were tested initially and throughout the experiment for total petroleum hydrocarbons (TPH) as the indicator parameter for the contaminant. All of the soils were excavated from around compressor engines in a common area; all had been exposed to the same type of lubricating oils. The target remediation level was 50 mg/kg TPH.

TPH Analysis

TPH was analyzed using EPA Method 8015 for non-volatiles. This method uses a gas chromatography analysis with methane or hexane extraction to chart hydrocarbon signatures or spikes by molecular weight. Typically methane extraction is used for non-volatiles in which the contaminant is solubilized and extracted from a wet sample (not dried prior to extraction). The first signatures from the chromatography run will be the

lighter volatiles followed by the heavier non-volatiles or extractables. The gas chromatography can be run for volatiles only, or with the use of other extraction and column injection techniques, run longer for the non-volatiles. The non-volatile 8015 method, which will detect hydrocarbons from C_{10} to C_{50}, was specified for these tests. The lubricating oil contaminant in these soils will be detected in this range. Any volatile hydrocarbons less than C_{10}, which existed in the soil or were a result of the biodegradation of the longer hydrocarbons, were not monitored because they typically would be volatilized during aeration of the soils.

Another TPH analysis method used in the past is EPA Method 418.1. This method uses an infrared spectrophotometer with a freon extraction process. The problem with this method is that it can only be expected to detect 50% of the volatile range of hydrocarbons (according to EPA's preamble to the testing protocol) and, more importantly for this research, it does not accurately discriminate between organic carbon (biomass, humus, etc.) and heavier hydrocarbons. As a result, Method 418.1 typically overstates the levels of hydrocarbon contamination. Remediation projects and other studies conducted within the last two years have begun to use the 8015 or similar analysis methodology.

Moisture/Nutrient Analysis

Moisture of the soil was determined according to the Standard Methods for the Examination of Water and Wastewater, 16th Edition, Method 209A. Volatile solids were determined according to Method 209G; ammonia nitrogen according to Method 417C which includes nitrogen in the sorbed and aqueous phases and phosphate phosphorus and nitrate nitrogen according to Method 429 of this same reference.

Soil Type

The Soil Mechanics Laboratory in the School of Civil and Environmental Engineering at Oklahoma State University performed a sieve and mechanical analysis of

the soils on September 7, 1993. A composite sample representative of the soils from the three batches treated, was analyzed. The soils were comprised of 42% gravel, 50.4% sand, and 7.6% clay or silt by weight. The high gravel content is not unusual because of successive layers of gravel used over the years around these engines as a ground cover. The high sand percentage was also expected because the engines were located in the Cimarron River valley.

Biological Activity

During the testing of the third batch of soils, samples were taken and shipped to Microbe Inotech Laboratories, Inc. in St. Louis, Missouri for analysis of biological activity. This initially consisted of a total plate count, bacterial identification and an endpoint assay. Subsequent samples were cultured for total plate counts and compared to the initial identification analysis to determine the specific counts of bacteria types within the population.

Standard Bacterial Plate Count

A standard spread plate method was used for this process. Aliquots from each sample were checked for weight and then serially diluted. Each dilution was then transferred in a sterile form to a laminar flow biological cabinet and placed on a previously prepared and dried trypticase soy broth agar (TSBA) medium in Petri plates. Observations for colony forming units (CFU) were made at 24 and 48 hours on incubation at 28°C for each sample. The different types of bacterial colonies formed were noted after 48 hours of incubation. These were counted and compared to the specific bacterial identification made below and the results reported.

Bacterial Identification

Two different analysis methods were used to identify the bacterial strains. The first method is the Gas Chromatography Fatty Acid Methyl Ester (GC-FAME) System and the second is the Biolog Microplate System. The GC-FAME method identifies bacteria based on unique fatty acid profiles. These profiles are identified by gas chromatography and are compared to a database consisting of more than 60,000 profiles of strains. The Biolog Microplate method characterizes bacteria by carbon source utilization tests. There were 95 wells on a microplate which contained a carbon food source and a tetrazolium dye. As the bacteria consumed the carbon source in a well, the dye turns purple. Each species of bacteria creates a distinct pattern of purple dots that is recognized by the automated microplate reader and compared to the Biolog database of Microbe Inotech Laboratories.

The species characteristics in the GC-FAME and Biolog databases are an "average" of the characteristics of hundreds of tested bacteria of the same species. The similarity and distance coefficients of the organism are calculated based on a hypothetical 'mean' organism in the databases. The database organism has a similarity coefficient of one and a distance of zero. The closer the strain is to one and zero the more closely it matches the mean organism in the database.

Endpoint Assay

The endpoint - kinetic assay measures the effectiveness of specific bacteria found in soil or water to break down hydrocarbon contaminants. After the plate counts, specific bacteria strains were isolated and grown for 18 hours on TSBA. The bacteria were then separately loaded into 96-well microtiter plates in which an undisclosed growth medium (mineral salts, vitamin mix , buffer), without a major carbon source, was added. The wells also contained a tetrazolium dye which was activated by the microbes oxidation of the carbon source. Free phase contaminant from the site was then added to selected wells to

serve as a the major carbon source. The bacterial strains were then allowed to grow and consume the contaminant as their carbon source. Based on the initial identification, counts were then made of each specific strain type. TSBA served as a positive growth control. The bacterial strains were also cultured on the oil and the TSBA separately as their sole carbon sources. The purpose was to determine their preferential substrate source and growth rates on each per strain. Bacterial growth was measured by color intensity of the dye after 24 hours of incubation.

Treatment Methodology

Three batches of soil contaminated by lubricating oil were treated from May 1992 through November 1993. The first batch comprised 133 cubic yards of soil, the second batch 89 cubic yards and the third 75 cubic yards of soil. These soils were treated in an abandoned warehouse at a natural gas processing plant site. A bench scale test was conducted simultaneously on the first batch and was used to confirm residual TPH levels and nutrient requirements. A bench scale test was also conducted simultaneously with the third batch of soils to monitor biological activity. This bench scale test comprised two cells of approximately 0.5 cubic yards of soil each. One cell contained contaminated soils from the third batch and the other "control" cell had uncontaminated soils from the same plant area. The soils were mixed with a nitrogen fertilizer and bulking agent of wheat straw, and water was added from a well supply. The windrow compost was aerated by turning with a backhoe every 7 to 10 days. The TPH, nutrient and moisture levels were sampled and analyzed approximately every 14 - 18 days. The warehouse was covered, had a concrete floor, and was large enough to accommodate the largest batch at a wind-row depth of three feet. A concrete floor, or other impermeable layer, prevents leaching of the contaminant into the surface soils and the roof or other cover prevents contaminated storm or treatment water from running off the site. The bench cells were turned by hand shovel and sampled and watered on approximately the same frequency.

Compost Process

The bulking agent was mixed in the soils in the approximate ratio of 1 part wheat straw to 4 parts soil. Ammonium nitrate was added as the nitrogen source and ammonium phosphate as the phosphorous source. No other nutrients or trace elements were added. A carbon, nitrogen, and phosphorus ratio (C:N:P) of 10:1:0.2 was initially established. This was accomplished by nitrogen and phosphorous analysis and calculating deficiencies based on the volume of soil and level of contamination. Because the soils were primarily inorganic (sands and gravel), the total petroleum hydrocarbon (TPH) levels were used as the basis for the total organic carbon content in the soil. Water was added to the soils from a well supply to keep the soils saturated to a point that no free liquids, drainage or slurries were produced. Water was added when the soils were turned to allow proper mixing and consistency. It was estimated that the moisture content of the soils was approximately 40 to 50% of a theoretical field capacity based on observations and moisture weight percent. The soils from all three successive batches were spread out into two piles (or rows) in the warehouse. The piles were 40 feet by 10 feet by 2 to 3 feet deep. A 10 foot aisle in the center of the piles was left open for access by the backhoe.

Maintenance

The windrows were turned every 7 to 10 days with a backhoe for aeration. Water was also added at this time if needed. It required approximately two labor hours to complete. Ammonium nitrate and ammonium phosphate would also be added at this time, if needed, based on the results of the latest analysis. These fertilizers were obtained at the local farmer's cooperative at a cost of less than $10 per 50 pound bag. The first batch treated had the greatest nutrient deficiencies which corresponded to the higher levels of TPH contamination in this batch. Samples were usually also collected at this time for analysis.

Sampling and Analysis Program

Sampling and analysis was performed for TPH, moisture, volatile solids, ammonia nitrogen (NH_3-N), nitrate nitrogen (NO_3-N), and phosphate (PO_4). The bench test associated with the third batch was analyzed initially for total heterotrophic plate count, bacterial identification and endpoint assay; subsequent samples were taken for plate counts only. These plate counts were correlated to the initial identification to determine the population of the hydrocarbon degrading species. Composite samples were taken which consisted of soils from least four different areas and depths in the warehouse (field) and bench study. The location of these samples for the composite were randomly selected.

TPH. For the first batch, TPH was sampled at the start of the treatment process and was not sampled again until five months later. At this time it was discovered that detectable TPH levels were not present. Samples were not taken more frequently because of difficulties in setting up the project, adding the proper amount of nutrients and moisture, and establishing procedures and responsibilities for sampling. Samples were obtained and analyzed approximately every 14 days for the second batch and approximately every 14 days for the bench study of the third batch. TPH samples were analyzed about once every 30 days for third "field" batch. One TPH analysis was performed on the uncontaminated "control" bench cell.

Moisture, Nutrients, Volatile Solids. Samples and analysis for moisture, nutrients and volatile solids were taken approximately every 30 days for the first and third batches and every 14 to 30 days for the second batch. For the third batch, samples were only taken from the field project and not the bench. The results from the field were correlated to the bench test. Although volatile solids analysis was performed, the results were inconclusive and did not correlate to the increases in cell mass or other factors. As such, they were not relied upon as indicator parameters or incorporated into the results or conclusions of this study. In addition, the results of the moisture analysis method selected

reflected the moisture content as percentage of weight and not as percentage of field capacity correlated to these excavated soils with a bulking agent addition. Subsequently, these results were not used to assess moisture requirements of the soil. This was made by physical observation and assessment alone to maintain the soils at speculated optimum moisture conditions.

Results

The results of the analyses performed for the compost treatment of batches one through three and the bench study are identified below with reference to appropriate tables and figures.

Batch One Table I on the following page summarizes the treatment results from batch one. Table I-A on Page 36 identifies the detail of the analysis results from this testing. This table identifies three separate composite samples (site #1, #2 & #3) that were taken for the nutrient and other parameters and one composite sample for TPH. Total nitrogen is comprised of ammonium nitrogen (NH_3) and nitrate nitrogen (NO_3). A simultaneous bench scale test was also being performed on a sample of these soils which followed the same treatment approach (i.e. aeration, nutrients, watering, etc.) as the field. The resulting TPH levels in the two cells from this test are also reported in this table.

TABLE I

SUMMARY OF TREATMENT RESULTS
BATCH ONE

Volume of Soil:	133 cubic yards
Initial TPH Level:	9659 mg/kg (PPM)
Ending TPH Level:	< 1 mg/kg (field)
Duration of Treatment:	6 months (5/92 - 11/92)
Nutrient Additions:	700 lbs. ammonium nitrate 350 lbs. ammonium phosphate
Estimated Cost:	$1,700 ($800 labor, $400 material, $500 analysis)

The lubricating oil in the contaminated soil in this batch was successfully remediated to non-detectable levels within a period of six months. The total nitrogen levels reflect an increase in microbial activity with a significant consumption of nitrogen during this process. The total nitrogen levels taken on September 17th were 1,857 mg/kg and approximately 30 days later were 486 mg/kg. Although TPH levels were only identified at the beginning and end of the treatment process, it can be assumed that the oil degradation rate increased rapidly sometime after September 17th. Since this process began in May of 1992, it could be concluded based on subsequent batches and other studies cited in this review, that the nitrogen addition made between July and September of that year was a critical factor for the success of this treatment.

TABLE I-A

BATCH ONE - NUTRIENTS / TPH ANALYSIS RESULTS

SAMPLE DATE	NUTRIENT / OTHER PARAMETER	SAMPLING LOCATIONS SITE #1	SITE #2	SITE #3	AVERAGE
12-Jul-92	Moisture	6.79	5.24	5.48	5.84
17-Sep-92	(% by weight)	11.15	13.23	–	12.19
23-Oct-92		7.87	16.14	8.84	10.95
11-Dec-92		8.53	8.21	8.37	8.37
12-Jul-92	Volatile Solids	–	–	–	–
17-Sep-92	(% wet)	4.23	4.33	–	4.28
23-Oct-92		3.62	5.17	5.08	4.62
11-Dec-92		4.51	4.60	4.55	4.55
12-Jul-92	Volatile Solids	5.79	5.14	4.69	5.21
17-Sep-92	(% dry)	4.88	4.88	–	4.88
23-Oct-92		3.93	6.17	5.57	5.22
11-Dec-92		4.93	5.01	4.97	4.97
12-Jul-92	NH3-N	401.00	352.00	322.00	358.33
17-Sep-92	(mg/kg)	179.00	329.00	–	254.00
23-Oct-92		445.00	334.00	527.00	435.33
12-Jul-92	NO3-N	–	–	–	–
17-Sep-92	(mg/kg)	1470.00	1735.00	–	1602.50
23-Oct-92		111.80	11.50	29.40	50.90
10-Jul-92	Total N	401.00	352.00	322.00	358.33
17-Sep-92	(mg/kg)	1649.00	2064.00	0.00	1856.50
23-Oct-92		556.80	345.50	556.40	486.23
10-Jul-92	PO4-P	0.00	0.04	0.00	0.01
17-Sep-92	(mg/kg)	0.00	0.00	–	0.00
23-Oct-92		257.00	370.00	411.00	346.00
21-May-92	TPH	9569.00	(Composite Samples)		9569.00
06-Nov-92	(mg/kg)	< 1.00			< 1.00
25-Nov-92		< 1.00			< 1.00
21-Dec-92		56.00	(Bench)		56.00
21-Dec-92		82.00	(Bench)		82.00

No phosphorous was initially detected in the soils and none was added until after September. It is not known what impact the phosphorus had on the microbial activity. The next sample on October 23, 1992 indicated levels of 346 mg/kg but no further analysis was performed after that. Based on similar compost treatment studies, it could be assumed that a noticeable phosphorus loss probably did not have a significant effect on microbial growth and activity since it is should be present in excess of bacterial requirements. However, the results of batch two of this study indicate a steady phosphorus loss correlated to decreased TPH levels, indicative of healthy increases in microbial activity and consumption of phosphorus. No other sources of phosphorus loss have been identified in the testing or literature review of this study.

Batch Two Table II below summarizes the treatment results from batch two. Table II-A on page 39 identifies the detail of the analysis results from this testing. This table identifies four separate composite samples (site #1, #2, #3 & #4) for nutrient and other parameters and one composite sample for TPH. Total nitrogen is comprised of NH_3 and NO_3.

TABLE II

SUMMARY OF TREATMENT RESULTS
BATCH TWO

Volume of Soil:	90 cubic yards
Initial TPH Level:	3006 mg/kg
Ending TPH Level:	48 mg/kg
Duration of Treatment:	4 months (3/93 - 6/93)
Nutrient Additions:	200 lbs. ammonium nitrate
	0 lbs. ammonium phosphate
Estimated Cost:	$1,600 ($800 labor, $100 material, $700 analysis)

The lubricating oil in the contaminated soil was successfully remediated below the target levels of 50 mg/kg during the three month treatment process. The total nitrogen

consumption corresponded with the hydrocarbon degradation rates as evidenced by total nitrogen levels of 678 mg/kg on 21 April 1993 and 226 mg/kg on 13 May. Additional nitrogen was added at the end of May following this analysis and was reflected by the level of 1,380 mg/kg indicated in June. The correlation between the increased nitrogen levels and the decrease in TPH levels between March and May is clearly evident. As the total nitrogen decreases in late April and May, the decrease in TPH becomes less dramatic and levels out in May to at or below target levels. Figure 1 on page 40 identifies the changes and relationships of TPH, nitrogen and phosphorus over the treatment period all in units of mg/kg. This is based on data from Table II-A.

Interestingly, the phosphorus levels showed a steady decline which also correlated to the decreases in the TPH and is indicative of an increase of microbial activity. The initial levels in March were 1,667 mg/kg and declined steadily to 742 mg/kg at the end of the treatment period in June. However, the loss rate of phosphorus above background or nutrient requirements cannot be directly correlated to an increase in microbial activity and degradation of the contaminant. It does appear that phosphorus loss is proportional to the amount available.

TABLE II-A

BATCH TWO - NUTRIENTS / TPH ANALYSIS RESULTS

SAMPLE DATE	NUTRIENT/OTHER PARAMETER	SAMPLING LOCATIONS SITE #1	SITE #2	SITE #3	SITE #4	AVERAGE
18-Mar-93	Moisture	10.30	9.19	8.87	8.61	9.24
21-Apr-93	(% by weight)	10.87	10.06	10.34	10.49	10.44
13-May-93		13.37	16.34	15.06	-	14.92
18-May-93		14.31	14.92	14.58	-	14.60
07-Jun-93		8.51	8.95	6.71	10.94	8.78
18-Mar-93	Volatile Solids	3.82	4.52	4.60	2.25	3.80
21-Apr-93	(% wet)	3.84	3.94	3.88	4.12	3.95
13-May-93		4.06	3.58	3.72	-	3.79
18-May-93		4.43	3.09	3.63	-	3.72
07-Jun-93		3.76	4.19	4.57	3.84	4.09
18-Mar-93	Volatile Solids	4.25	4.98	5.05	2.47	4.19
21-Apr-93	(% dry)	4.31	4.39	4.33	4.62	4.41
13-May-93		4.71	4.28	4.38	-	4.46
18-May-93		5.17	3.64	3.63	-	4.15
07-Jun-93		4.11	4.61	4.89	4.31	4.48
18-Mar-93	NH3-N	112.97	17.91	175.66	57.88	91.11
21-Apr-93	(mg/kg)	126.87	244.36	477.81	425.87	318.73
13-May-93		20.93	189.09	23.13	-	77.72
18-May-93		170.94	124.71	15.73	-	103.79
07-Jun-93		75.79	15.69	-	25.73	39.07
18-Mar-93	NO3-N	47.66	14.43	103.26	28.64	48.50
21-Apr-93	(mg/kg)	216.11	267.47	706.19	245.31	358.77
13-May-93		259.98	11.39	172.29	-	147.89
18-May-93		48.16	152.99	204.93	-	135.36
07-Jun-93		1318.76	1600.31	1489.19	993.04	1350.33
18-Mar-93	Total N	160.63	32.34	278.92	86.52	139.60
21-Apr-93	(mg/kg)	342.98	511.83	1184.00	671.18	677.50
13-May-93		280.91	200.48	195.42	-	225.60
18-May-93		219.10	277.70	220.66	-	239.15
07-Jun-93		1394.55	1616.00	1489.19	1018.77	1379.63
18-Mar-93	PO4-P	2156.48	51.78	2884.17	1576.41	1667.21
21-Apr-93	(mg/kg)	644.38	661.67	1916.87	1614.07	1209.25
13-May-93		1071.74	1574.69	989.75	-	1212.06
18-May-93		538.63	1601.36	809.25	-	983.08
07-Jun-93		756.86	538.85	674.83	996.48	741.76
18-Mar-93	TPH	3006.00	(Composite Samples)			3006.00
14-Apr-93	(mg/kg)	698.00				698.00
23-Apr-93		324.00				324.00
28-Apr-93		338.00				338.00
05-May-93		38.00				38.00
24-May-93		55.00				55.00
07-Jun-93		104.00				104.00
18-Jun-93		48.00				48.00

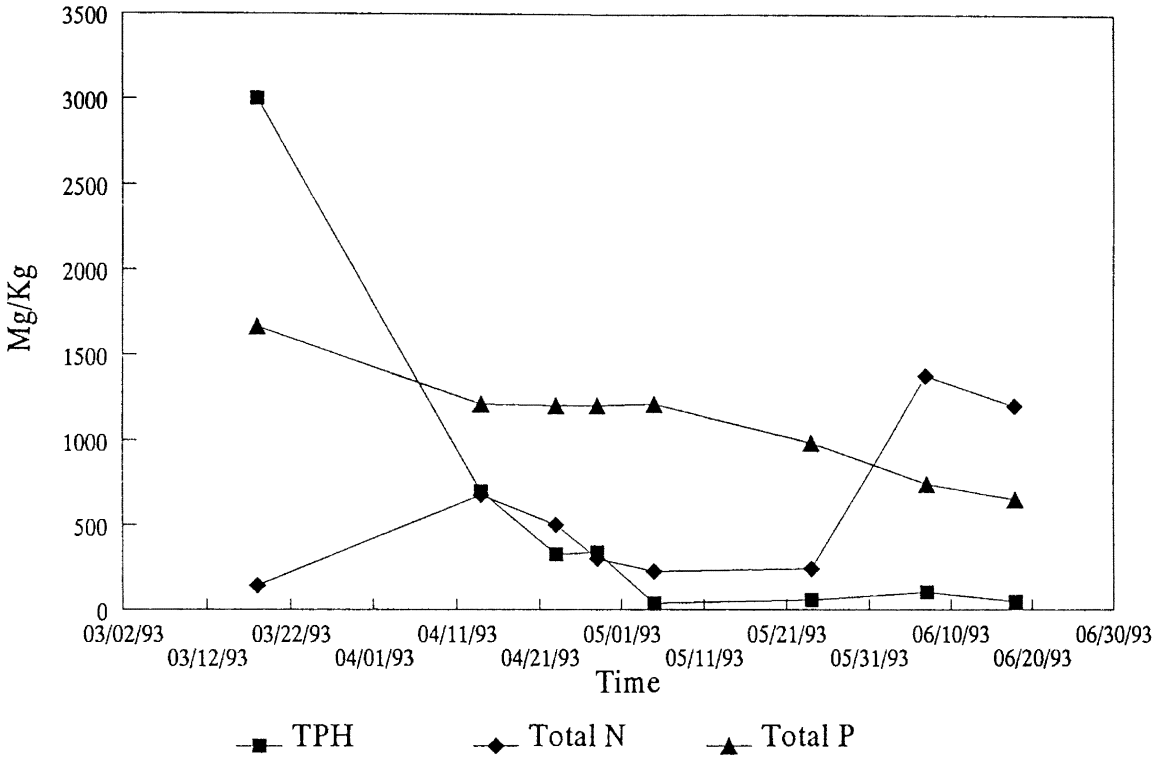

Figure 1. Batch Two - TPH / Nitrogen / Phosphorus Levels

Batch Three (w/bench test) Table III below summarizes the treatment results from batch three. Table III-A on the following page identifies the detail of the analysis results from this testing. This table identifies two separate composite samples for nutrients and other parameters and one composite sample for TPH. The nutrient and other parameters sampled are taken from the field location and TPH is taken from both the field and bench study.

TABLE III

SUMMARY OF TREATMENT RESULTS
BATCH THREE

Volume of Soil:	75 cubic yards
Initial TPH Level:	1601 mg/kg
Ending TPH Level:	175 mg/kg
Duration of Treatment:	3 months (7/93 - 10/93)
Nutrient Additions:	200 lbs. of ammonium nitrate 0 lbs. of ammonium phosphate
Estimated Cost (field):	$1,000 ($600 labor, $100 material, $300 analysis)
Estimated Cost (bench):	$1,500 ($200 TPH, $500 plate counts, $800 endpoint assay)

The lubricating oil contaminant was degraded from initial levels of 1,601 mg/kg to 175 mg/kg. The duration of the treatment process was three months. The target level of 50 mg/kg was not reached during this time because of the onset of cold weather and the probable resulting decrease in microbial metabolic activity in this low temperature range. The treatment and monitoring of this batch will continue in the spring of 1994. Figure 2 on page 43 identifies the changes and relationships of TPH, nitrogen and phosphorus levels over the treatment period measured in units of mg/kg. The results from the TPH, nitrogen and phosphorus are from samples from the field study as reported in Table III-A.

TABLE III-A

BATCH THREE - NUTRIENTS / TPH
ANALYSIS RESULTS

SAMPLE DATE	NUTRIENT/OTHER PARAMETER	SAMPLING LOCATIONS SITE #1	SITE #2	AVERAGE
14-Jul-93	Moisture	9.18	6.53	7.86
24-Aug-93	(% by weight)	10.21	–	10.21
21-Sep-93		11.94	–	11.94
	Volatile Solids			
14-Jul-93	(% wet)	3.10	2.87	2.99
24-Aug-93		6.97	–	6.97
21-Sep-93		6.90	–	6.90
	Volatile Solids			
14-Jul-93	(% dry)	3.41	3.07	3.24
24-Aug-93		7.75	–	7.75
21-Sep-93		7.82	–	7.82
	NH3-N			
14-Jul-93	(mg/kg)	223.74	236.39	230.07
24-Aug-93		26.10	–	26.10
21-Sep-93		22.29	–	22.29
	NO3-N			
14-Jul-93	(mg/kg)	1140.32	434.94	787.63
24-Aug-93		66.96	–	66.96
21-Sep-93		25.46	–	25.46
	Total N			
14-Jul-93	(mg/kg)			1017.70
24-Aug-93				93.06
21-Sep-93				47.75
01-Oct-93		(ESTIMATED – N ADDED 10/1)		500.00
	PO4-P			
14-Jul-93	(mg/kg)	3307.39	2178.81	2743.10
24-Aug-93		365.92	–	365.92
21-Sep-93		392.29	–	392.29
	TPH	(Bench)	(Field)	
10-Aug-93	(mg/kg)	1601.00	1601.00	n/a
25-Aug-93		5410.00	–	n/a
06-Sep-93		4333.00	657.00	n/a
20-Sep-93		2654.00	356.00	n/a
28-Oct-93		215.00	175.00	n/a
Control Cell:				
20-Sep-93		0.00		

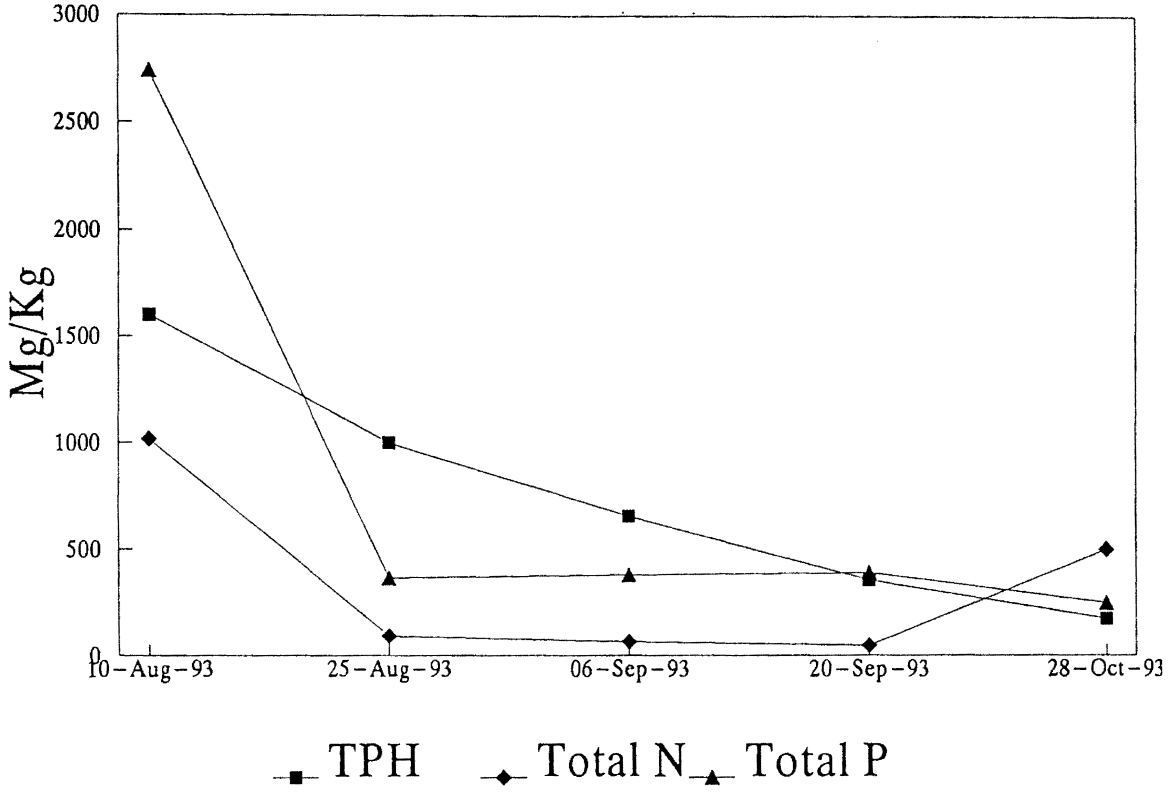

Figure 2. Batch Three - TPH / Nitrogen / Phosphorus Levels

The nitrogen and phosphorus levels decreased dramatically from July to August. The initial levels were 1,017 mg/kg and 2,743 mg/kg respectively in July which decreased to 93 mg/kg and 366 mg/kg in August. Although this may be due in part to sampling anomalies, it does correlate to changes in TPH levels and increases in microbial activity.

A significant increase in TPH levels of 1,601 mg/kg on August 10th to 5,410 mg/kg on August 25th occurred from bench test samples. This increase was not evident in the field scale project which showed steady declines. One possible explanation for this would be a biosurfactant effect of the microbe population and differences in population composition between the field and bench study. Other laboratory (flask) and bench scale projects have also shown increases in TPH levels in the early stages of treatment. During the early stages of degradation, the microbe populations produce surfactant-type enzymes which release contaminants from soil particles to which they have a high attraction. The surfactants solubilize the contaminant which is then more readily available for microbial consumption and also more easily detected by laboratory analysis. The enzyme surfactants are suspected to be more complex and efficient than the extraction method used by the laboratory analysis.

A possible explanation of why this was not evidenced in the field study is because of differences in microbial composition. The bench study, due to its smaller scale, provided oxygen, nutrients and water more readily to the microbes at first. This allows a rapid growth of certain species which dominated the population for that time and produce a surfactant effect on the contaminant and soil particles. Other species which produce the enzymes and other proteins necessary for completion of the degradation process have not yet been allowed to fully develop. The field study may support a more diverse population producing earlier the variety of necessary enzymes to complete the degradation process.

Batch Three (Biological Data) The soils from the two bench test cells were sampled for biological activity and TPH levels. One cell contained soil from batch three of the field test and the other contained uncontaminated soil from the same general plant area. Both cells were treated with water and aerated identically. Nutrients were also added to both cells based on sampling and deficiencies identified from the soils in the field test. The total plate counts taken on August 10, 1993 identified substantial populations of hydrocarbon degrading bacteria in both cells. These initial six strains are identified in Table IV on the following page. The identification process was explained earlier in this text and was accomplished by comparing the results of two different methods. These were the GC-FAME method and the Biolog method. In each method the profiles of the strains are compared to database profiles of known strains to arrive at a 'mean' organism. This database organism has a similarity coefficient of one and a distance of zero. A good match is considered one with a similarity coefficient greater than 0.5 and a distance coefficient of less than 7.0 according to Microbe Inotech Laboratories. The total count of these six strains of bacteria identified was 7.81×10^8 colony forming units per gram (CFU/g) in the cell with contaminated soil.

Table V on page 47 identifies the results of plate counts from the bench samples taken during the first two months of treatment. During August there were two composite samples taken (sample A & B) and these were averaged. During September only one composite sample was taken. Also during September the control cell (with the uncontaminated soils) had one composite sample taken. During the two month treatment period 11 different strains were identified. The first six were specifically identified from the bacterial identification testing. Table V also shows the proportion of the total count for each strain.

TABLE IV

BACTERIAL IDENTIFICATION

Summary of GC-FAME/Biolog Analyses							
Strain Name	**Primary Identification by GC**	**Sim. Coef.**	**Dist Coef.**	**Primary ID by Biolog™**	**Plate Type**	**Sim. Coef**	**Dist. Coef**
2337-1	*Escherichia coli*	0.268	5.246	*Klebsiella pneumonia A*	GN	0.668	5.015
2337-2	*no ID closest species Bacillus megaterium*	0.49	3.859	*no ID closest species Staphylococcus simulans*	GP	0.463	7.639
2337-3	*Enterobacter sakazakii*	0.252	5.365	*No ID closest species Citrobacter freundii*	GN	0.158	15.463
2337-4	*Gordona bronchialis*	0.522	3.685	*no ID closest species Rhodococcus erythropolis*	GP	0.182	15.883
2337-5	*Pseudomonas aeruginosa*	0.287	6.851	*Klebsiella pneumoniae A*	GN	0.866	2.153
2337-6	*Pseudomonas aeruginosa*	0.661	4.222	*Pseudomonas citronellolis*	GN	0.515	4.834

Strain #	**Closest Match**	**I.D. Method**
#1	*Klebsiella pneumonia*	Biolog
#2	*Bacillus megaterium*	GC-FAME
#3	*Enterobacter sakazakii*	GC-FAME
#4	*Gordona bronchialis*	GC-FAME
#5	*Klebsiella pneumoniae*	Biolog
#6	*Pseudomonas aeruginosa*	GC-FAME

TABLE V

BIOLOGICAL ACTIVITY OF PLATE COUNTS PER STRAIN

Sample Date	Sample #	Total Count (CFU/g)	Strain Type/ Proportion of Total Count #1	#2	#3	#4	#5	#6
08/10/93	A	3.62E+08	0.50 1.81E+08	0.10 3.62E+07	0.30 1.09E+08	0.00	0.00	0.10 3.62E+07
	B	1.20E+09	0.50 6.00E+08	0.03 3.60E+07	0.15 1.80E+08	0.02 2.40E+07	0.02 2.40E+07	0.20 2.40E+08
	AVG.	7.81E+08	0.50 3.91E+08	0.07 3.61E+07	0.23 1.44E+08	0.01 1.20E+07	0.01 1.20E+07	0.15 1.38E+08
08/26/93	A	3.94E+08	0.50 1.97E+08	0.05 1.97E+07	0.10 3.94E+07	0.20 7.88E+07	0.00	0.15 5.91E+07
	B	2.44E+09	0.50 1.22E+09	0.20 4.88E+08	0.05 1.22E+08	0.10 2.44E+08	0.05 1.22E+08	0.10 2.44E+08
	AVG.	1.42E+09	0.50 7.09E+08	0.13 2.54E+08	0.08 8.07E+07	0.15 1.61E+08	0.03 6.10E+07	0.13 1.52E+08
09/08/93	A	2.18E+09	0.10 2.18E+08	0.20 4.36E+08	0.00	0.50 1.09E+09	0.00	0.05 1.09E+08
	CONTROL	5.00E+10	0.05 2.50E+09	0.05 2.50E+09	0.00	0.50 2.50E+10	0.00	0.00
09/28/93	A	1.13E+08	0.00	0.20 2.26E+07	0.00	0.00	0.00	0.25 2.83E+07
	CONTROL	5.32E+07	0.00	0.10 5.32E+06	0.00	0.20 1.06E+07	0.05 2.66E+06	0.20 1.06E+07

Sample Date	Sample #	Strain Type/ Proportion of Total Count #7	#8	#9	#10	#11
08/10/93	A	0.00	0.00	0.00	0.00	0.00
	B	0.00	0.00	0.00	0.00	0.00
	AVG.	0.00	0.00	0.00	0.00	0.00
08/26/93	A	0.00	0.00	0.00	0.00	0.00
	B	0.00	0.00	0.00	0.00	0.00
	AVG.	0.00	0.00	0.00	0.00	0.00
09/08/93	A	0.05 1.09E+08	0.05 1.09E+08	0.05 1.09E+08	0.00	0.00
	CONTROL	0.20 1.00E+10	0.02 1.00E+09	0.03 1.50E+09	0.05 2.50E+09	0.10 5.00E+09
09/28/93	A	0.30 3.39E+07	0.20 2.26E+07	0.05 5.65E+06	0.00	0.00
	CONTROL	0.20 1.06E+07	0.15 7.98E+06	0.10 5.32E+06	0.00	0.00

The endpoint assay testing concluded that strains #1, #3 and #6 were the most aggressive hydrocarbon degraders. The strains were cultured on an optimum growth medium of TSBA and also on the oil contaminant as the substrate to compare growth. Figure 3 on the following page of the endpoint assay identifies that strains #1, #3, and #6 consumed oil as the carbon source almost as readily as the TSBA medium and their growth was greater than the other three strains. Microbial activity is correlated with carbon dioxide production, which in turn is measured by a colorimetric analysis of a tetrazolium dye (redox indicator) at the wavelength of maximum intensity for detecting the dye.

The six strains were also cultured on the oil contaminant as their only substrate source. Their growth was measured with the same redox indicator and compared after a 24 hour period. Figure 4 on page 50 of the endpoint assay identifies the results from this test. Strains #1, #3 and #6 can again be seen as having the highest growth rate on this substrate. These three strains dominated the population with 88% of the total count during the first month of treatment and with total plate counts increasing during this time. At the beginning of the second month, strains #2 and #4 then began to dominate with 70% of the total population with only moderate increases in total growth. At the end of the second month strains #6 and previously unidentified strains #7 and #8 dominated with 75% of the total population with decreases in total population growth also resulting.

The "control" cell was sampled at the beginning of the second month and indicated 5.00×10^{10} CFU/g. The dominant strains in this population were #4 and #7. By the end of the second month the total population of the "control" cell dropped to 5.32×10^{7} with #4, #6 and #7 dominating with 60% of the population. Figure 5 on page 51 identifies the composition of the various strains in proportion to the total population over the treatment period. Figure 6 on page 52 identifies the total count per strain over the treatment period.

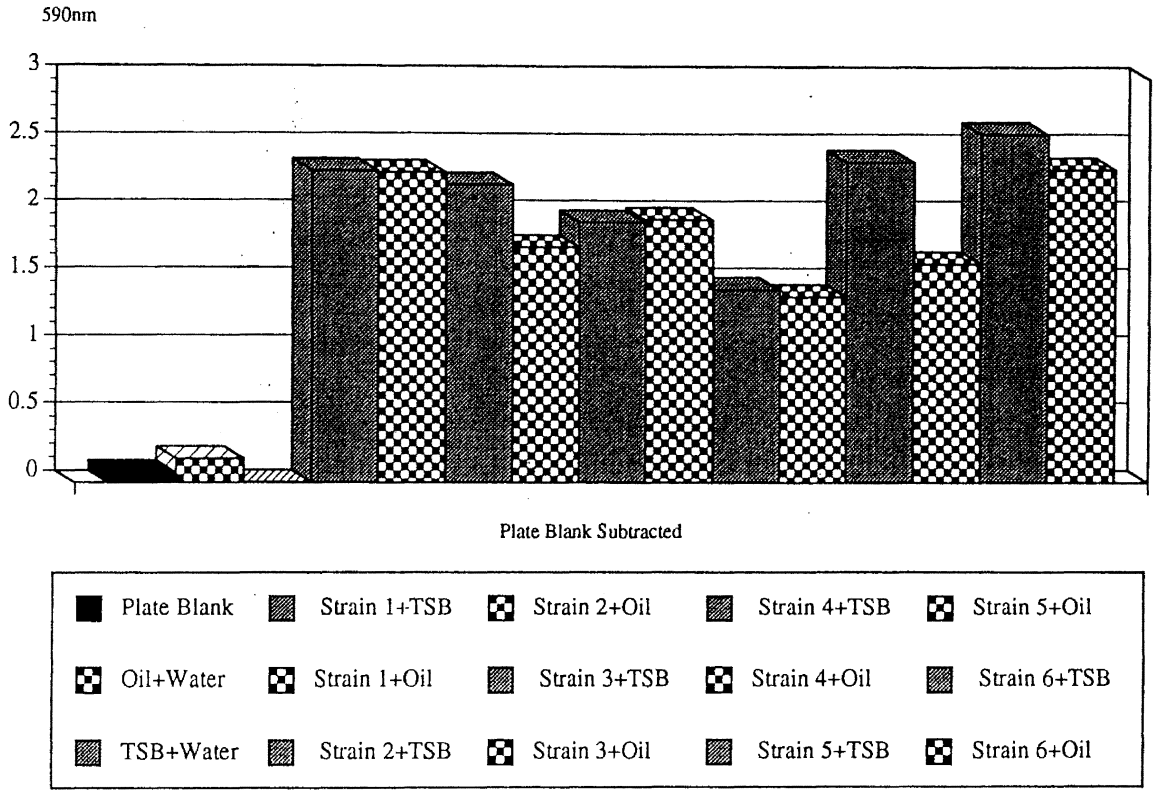

Figure 3. Batch Three - Endpoint Assay of Strain Growth on Oil and TSBA (24 Hrs.)

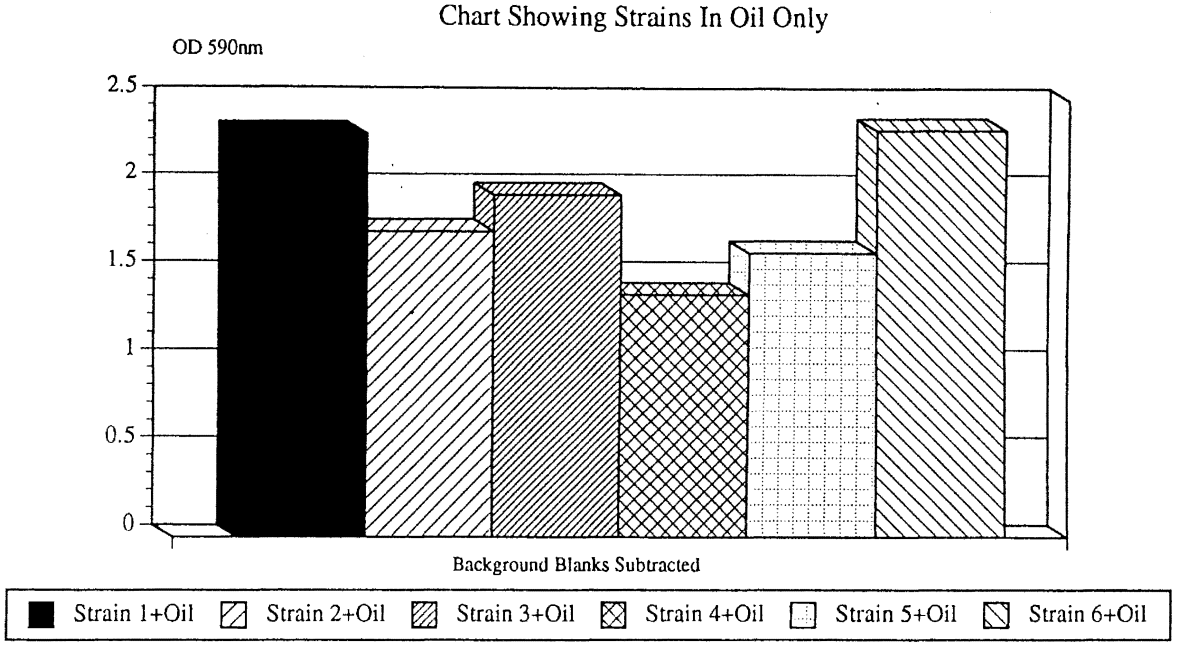

Figure 4. Endpoint Assay of Strain Growth on Oil (24 Hrs.)

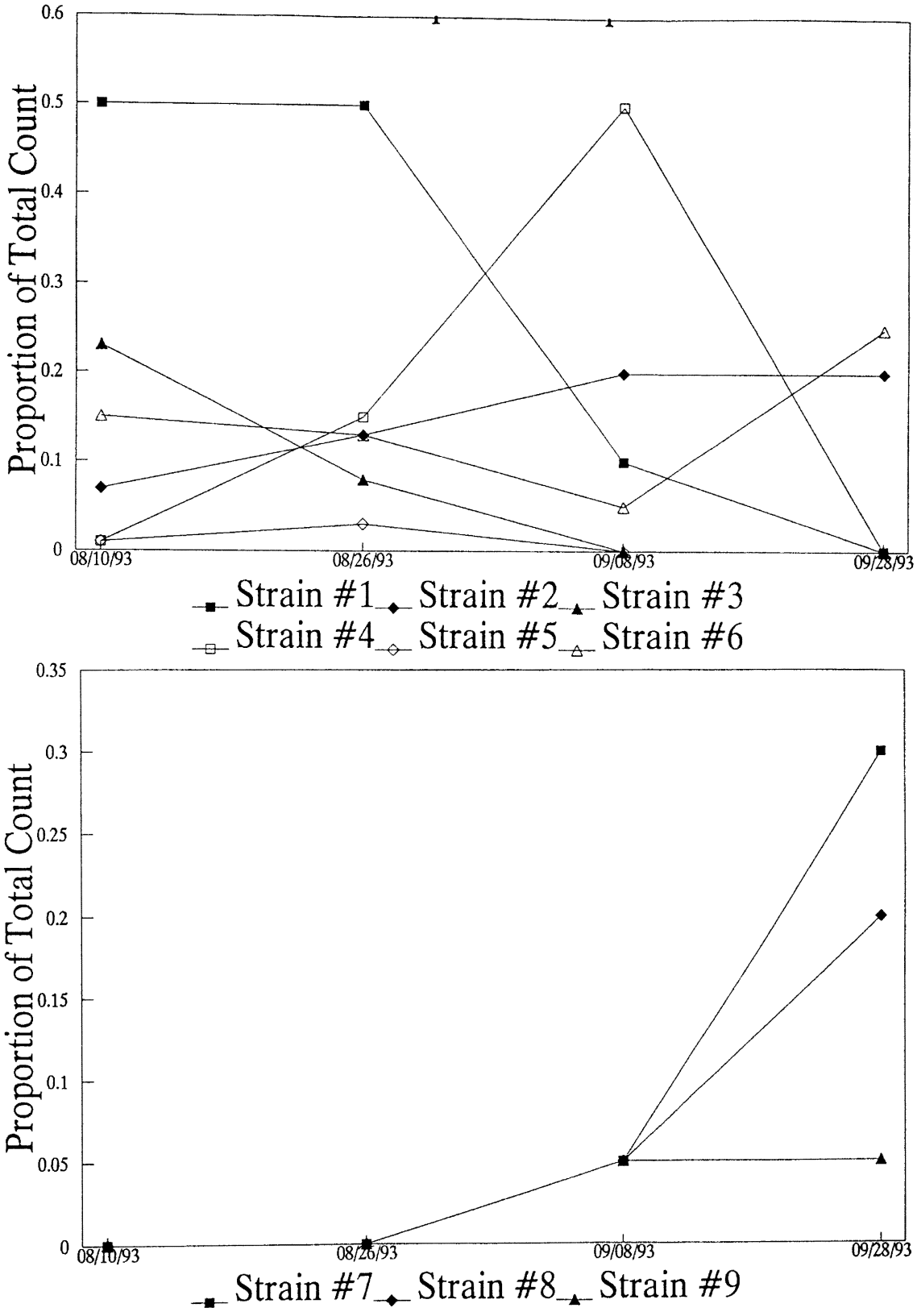

Figure 5. Proportion of Strain Growth to Total Population

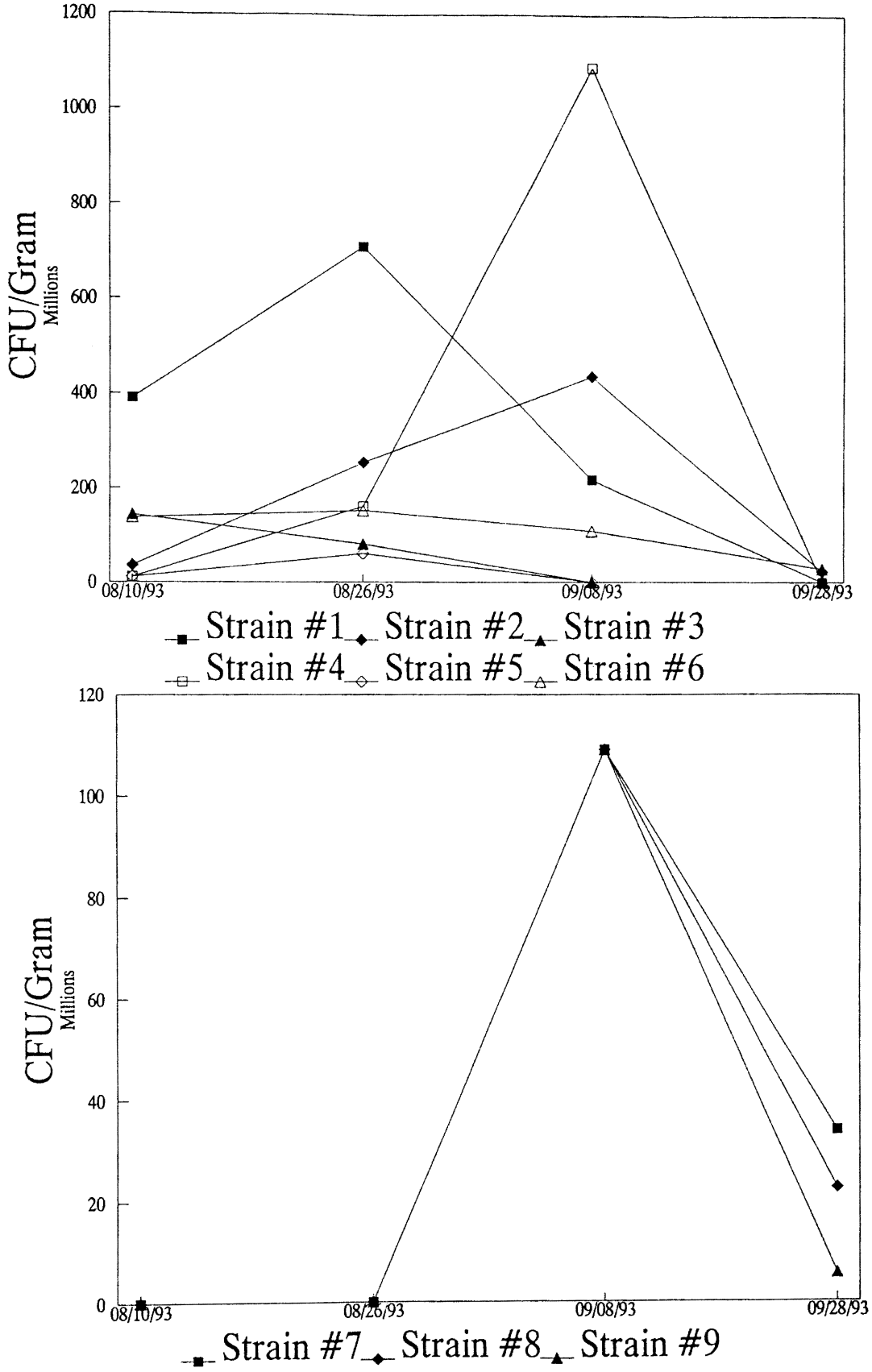

Figure 6. Total Count per Strain

Increases in total plate count activity corresponded to decreases (consumption) of nitrogen and phosphorus. Figure 7 on the following page identifies this correlation between nitrogen and phosphorus consumption and total plate count activity. This figure clearly shows the increase in total plate count activity during the first month and corresponding decreases of nitrogen and phosphorus during this same period of time. However, the biological samples were taken from the bench cells and the nutrient analysis was taken on the field test which may not accurately represent this correlation. Decreases in TPH levels also corresponded to rising total plate count levels. Figure 8 on page 55 compares total plate counts and the TPH levels from bench samples over the two month treatment period. The decrease in TPH levels after September 8th began as total plate counts were reaching their highest levels and continued as total plate counts also began to decline.

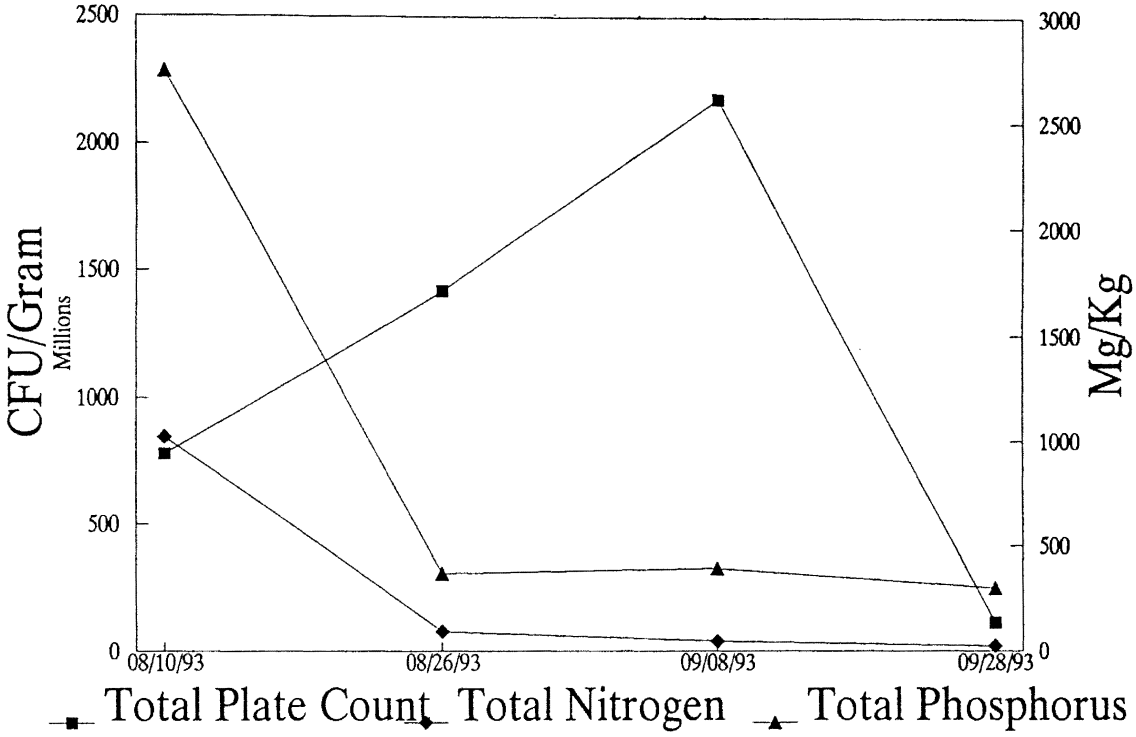

Figure 7. Total Plate Count / Nitrogen / Phosphorus Levels

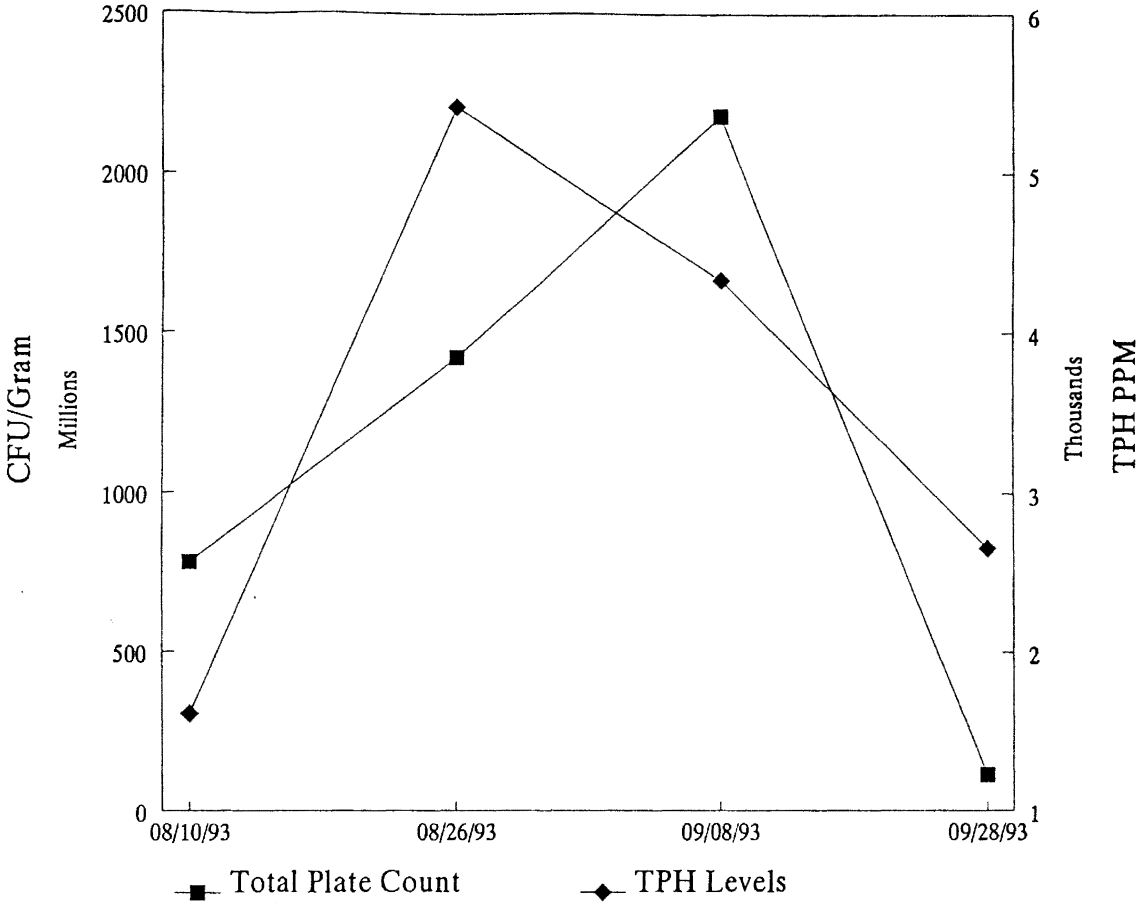

Figure 8. Total Plate and TPH Levels

Counts of hydrocarbon degrading microbes greater than 10^6 CFU/g are considered necessary for successful remediation and total counts greater than 10^4 CFU/g are considered adequate baseline populations (Riser-Roberts, 1992). The beginning levels for this project have been identified as 7.81×10^8 and were 1.13×10^8 and the end of the second month of treatment. One explanation for the high initial bacterial counts is the historical nature of the contaminant. The oils have been present in the soils for at least eight years with the indigenous microbes acclimating and prospering on this substrate source.

It was during the second month that most of the hydrocarbon degradation was observed (from the field analysis) with increases in total population counts correlating to decreasing TPH levels. There was also a noticeable shift in strain domination and composition over the treatment period. This can be expected because of the substrate changes due to the degradation of this complex mixture of longer chain hydrocarbons. The utilization of these new substrates is preferred by other microbes. The consumption or losses of nitrogen and phosphorus also corresponded to increases in microbial activity and growth.

CHAPTER V.

CONCLUSIONS

This testing has demonstrated that inorganic based soils contaminated with lubricating oils of up to 10,000 mg/kg can be remediated to less than 50 mg/kg within three to four months. Although the TPH level in batch three was 175 mg/kg at the end of this test, it is expected, as evidenced by testing of the other two batches and the trend of decreasing levels during treatment, that further treatment during warmer months will further reduce these levels to below 50 mg/kg. The treatment period for batch one was six months. However, the TPH levels were not monitored closely during this time and it is anticipated that the target level would have been reached in half the time if the nutrient and moisture additions and sampling regime had commenced earlier. This is based on the results demonstrated from batch two and other case studies cited in this text.

Other studies have shown that this same type of treatment process should also be effective for other heavier, long-chained hydrocarbon contaminants in different types of soils (Fyock, Nordum, Fogel & Findlay, 1991; McMillen, Kerr, Gray & Findlay, 1992). The results of this testing also indicate the oil contaminant was removed by biological degradation and not by other processes. Other processes could be volatilization or solubilization and dispersion in the treatment water. Long-chain hydrocarbons such as these are not conducive to volatilization even during mechanical aeration of these soils because of their heavy empirical weights and high boiling points. This is also evidenced by the fact that surface spills of these hydrocarbons are prevalent for many years even after constant exposure to sunlight, heat and oxygen.

For this treatment process it is also not practical to assume that the hydrocarbons solubilized and dispersed during the three to six month treatment period of this testing.

This is because neither treatment or storm water ran off the site or leached into the ground. There was no collection of water or leachate that formed under the treatment piles, nor was there evidence of leachate formation. The laboratory analysis method selected would have continued to detect a soluble TPH in the samples even more readily than non-soluble forms. Even more conclusive is that the endpoint assay and plate count testing also confirm the presence and growth of hydrocarbon degrading bacteria. The first batch was tested and was found to contain no contaminants that should be toxic to microbial growth (e.g. heavy metals & chlorinated compounds). Since the soil from all three batches was taken from the same area and exposed to the same type of operations and contaminants, it was assumed that successive batches would have the same results.

A bulking agent of wheat straw proved very effective with these types of soils. If more cohesive soils such as clay or organic based soils are treated, straw may be more effective if first chopped in a leaf shredder or other apparatus. The straw did not decompose significantly over the six month treatment process and was an important conduit for moisture and oxygen transfer. This was especially critical since the soils were not turned or watered except every 7 to 10 days. Other studies that have not used a bulking agent or other type of soil treatment had difficulty in maintaining adequate porosity of the soil to keep it from agglomerating . As a result, the soil has to be aerated much more frequently to keep anaerobic conditions from occurring and to provide effective treatment (Jackson, Haby, Hammer, Hoffman, Gorman, 1993).

The addition of moisture based on physical observation proved effective by maintaining moisture levels just to a point of any free drainage or slurries (mud) from forming. It is not known how this moisture level corresponds to field capacity since this measurement is not applicable to excavated soils with a bulking agent addition. It is suspected that this level corresponds to the reported optimum level of 50 percent of field capacity based on reported observations from other studies cited in this text.

The stoichiometric equation for microbial growth introduced in this text indicated that for a hydrocarbon substrate of this nature, utilizing oxygen as the electron acceptor

and nitrate as the nutrient source, that microbial growth and substrate degradation could occur. This also indicated that the oxygen and nutrient requirements were reasonable to obtain for this type of windrow composting process. The phosphorus requirement was not considered in this equation because of the cells' requirements for this nutrient being typically not a limiting factor.

The initial carbon to nitrogen ratio (C:N) used in this testing was 10:1. This was used because it corresponds approximately to the chemical composition of a typical cell. Other studies have shown effective results with much higher C:N ratios of 25:1 (Kane, 1991; Jackson, Haby, Hammer, Hoffman, Gorman, 1993) as evidenced by increases in plate count activity and decreases in TPH. Typically, no other nitrogen was added during this testing unless total nitrogen dropped below levels of 500 mg/kg. The overall nitrogen level during testing of the three batches was maintained closer to the 25:1 ratio than the initial 10:1. The nitrogen uptake or loss rates did directly correspond to increases in microbial growth in batch three. Although the biological activity was not monitored in batch one and two, the nitrogen losses did correspond to decreases in TPH levels. There is no other reasonable explanation for this nitrogen loss except through microbial consumption from this testing.

Noticeable phosphorus losses were also identified from the three batches. The loss rate appears to be proportional to the amount of phosphorus available. This study has shown that phosphorus levels as small as 300 mg/kg and as large as 2,000 mg/kg resulted in apparently the same degradation levels of the contaminant. The optimum levels to maintain biological activity were not determined from this study. However, phosphorus is a key nutrient needed for cell synthesis and enzyme production. The majority of the other studies cited in this text provided no phosphorus additions or monitoring. This leads to the conclusion that background levels of phosphorus in most soil environments are probably sufficient to promote microbial growth. However, additions of phosphorus above any background levels have not inhibited growth and will probably enhance it. This

may be especially important to sustain high microbial levels needed to degrade complex or high concentrations of contaminants.

The endpoint assay testing concluded that three of the six bacteria found in the soils were aggressive hydrocarbon degrading species. This is typical of most soil environments reported where up to 50% of the bacteria are hydrocarbon degrading species (Buday, Gergely, Torok, Szoboszlay, 1989). The prevalence of these acclimated microbes, with preference of this contaminant as their substrate, can be explained by the historic nature of these contaminated soils with the microbes being exposed to this contaminant for several years. Also the soil environment was amenable to microbial growth because of its natural porosity and relatively high moisture conditions. Although strains #1, #3 and #6 were the dominant strains during the first month of treatment comprising 88 % of the total population, their population then diminished with other strains dominating after the first month. This is typical of preferential substrate use by other microbes. As the contaminant was broken down other microbes with the capacity to utilize this characteristically different substrate then began to flourish.

The cost of this windrow composting approach, assuming 100 cubic yard batches of soil and minimal TPH, nutrient and biological monitoring, would be approximately $15 per cubic yard. This compares to approximately $30 for "forced air" static-pile compost processes, $50 to $100 for special industrial waste landfills and enclosed "bioreactor" methods and $1,000 per cubic yard for hazardous waste landfills.

Topics For Future Research

Topic I: Determining soil conditioning methods to reduce the cohesiveness and increase the porosity of predominant clay and other silty based soils for more effective bioremediation in compost systems.

Topic II: Identifying the most common types of surfactant-type enzymes and the microbes that produce them in various contaminated soil systems and determining approaches to optimize their use.

Topic III: Evaluate the use of commercial surfactants for their ability to enhance bioremediation processes of oil contaminated soils.

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APPENDIX A

STOICHIOMETRIC EQUATION FOR MICROBIAL GROWTH AND SUBSTRATE UTILIZATION (R)

Applying the overall stoichiometric equation of **R = Rd - feRa - fsRc** where:

fs = (Y grams volatile solids formed/gram COD removed) (8 grams COD equivalent) / 5.65* grams volatile solids equivalent

fs = 1.42 Y

Y = 0.37 gram cells/gram COD removed for Pseudomonas organisms

Y = 0.37 (0.85)** = 0.31 (observed cell yield)

* Using $C_5H_7O_2N$, one mole of cells weighs 113 grams and one equivalent is 1/20 of a mole, or 5.65 grams of volatile (organic) microbial solids.

** Assumes microbial cells are approximately 85% volatile.

fs = 1.42 (0.31) = 0.44; fe = 1.00 - 0.44 = 0.56

$Rd = 0.022\ C_8H_{16}O + 0.326\ H_2O = 0.174\ CO_2 + H^+ + e^-$

$Ra = 0.50\ H_2O = 0.25\ O_2 + H^+ + e^-$

$Rc = 0.036C_5H_7O_2N + 0.393H_2O = 0.036NO_3 + 0.185CO_2 + 1.036H^+ + e^-$

Applying the equation results in:

$$R = 0.023\ C_8H_{16}O + 0.141\ O_2 + 0.016\ NO_3$$
$$= 0.127\ H_2O + 0.093\ CO_2 + 0.016\ C_5H_7O_2N$$

Normalize to one mole of oil by dividing through by 0.023.

$$\mathbf{R = C_8H_{16}O + 6.13\ O_2 + 0.70\ NO_3}$$
$$\mathbf{= 5.52\ H_2O + 4.04\ CO_2 + 0.70\ C_5H_7O_2N}$$

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