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**A BENCH-SCALE INVESTIGATION OF LAND TREATMENT
OF SOIL CONTAMINATED WITH DIESEL FUEL**

**A Thesis Submitted to The Faculty of Graduate Studies And
Research in Partial Fulfilment of The Requirements For The
Degree of Master of Applied Science in
Environmental Systems Engineering
University of Regina**

By

Chad Micheal Taylor

Regina, Saskatchewan

December, 1998

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ABSTRACT

A bench-scale investigation (soil pan testing) was conducted with the objective of studying degradation rates of diesel contaminated soil (2,500 and 10,000 ppm by weight of total petroleum hydrocarbons (TPH) to dry weight of soil) under different treatment conditions over a 17 week testing period. A total of three treatment conditions were simulated. Each experimental cell (pan) contained 3 kg of contaminated soil. A control cell was established to provide confidence in the test results. The first experimental cell simulated the control cell but without suppression of microbial activity. The third experimental cell was spiked with diesel fuel and amended with nutrients. The fourth experimental cell was similar to the third but was not amended with nutrients. The cells were sampled on weeks 3, 5, 9, 13 and 17 of the experiment. Each test consisted of determination of TPH concentration and moisture content per cell. The pH of soil in each cell was determined at weeks 9 and 17. The microbial concentration of soil in each cell, as determined by heterotrophic plate count, was determined at the commencement of the experiment and at weeks 9 and 17.

The tests revealed the effectiveness of the various treatment methods. The greatest degradation of the diesel contaminated soil was obtained with the addition of nutrients in the form of commercially available granular fertilizer ($C_0 = 10,000$ ppm of TPH). A degradation rate constant (k) of 0.19 week^{-1} was calculated from the experimental data for this treatment condition. The corresponding degradation rate constant (k) for soil not amended with nutrients was calculated as 0.07 week^{-1} ; this condition would require 33 weeks to reach the regulatory objective of 1,000 ppm.

The use of sodium azide to suppress the biodegradation of diesel to validate the testing results (control cell) was ineffective and duplicate analysis of TPH in each cell at week 9 revealed the inherent limitations in soil sampling and analysis variability, even in the bench-scale methods employed. The control cell ($C_0 = 2,500$ ppm of TPH) exhibited a relatively low uniform degradation of TPH over the duration of the experiment.

Experimental cell EX-2 ($C_0 = 2,500$ ppm of TPH without microbial suppressant or nutrient amendment) exhibited a considerable decrease of TPH concentration from 2,500 ppm to 750 ppm from the commencement of the experiment to week 3. Thereafter, this cell exhibited variable concentrations of TPH in the range of 380 to 840 ppm. It is likely that this experimental cell simulated TPH degradation as a substrate limiting condition.

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NOMENCLATURE

APHA	American Public Health Association.
ASTM	American Society for Testing and Materials.
BART™	A biotector system.
BTEX	Benzene, toluene, ethyl benzene, xylenes.
C	See final concentration.
C_0	Initial concentration. The concentration of diesel fuel (by TPH) which is present in the soil prior to treatment.
C_u	Coefficient of uniformity. A crude soil particle shape parameter. Defined as D_{60}/D_{10} .
CCME	Canadian Council of Ministers of the Environment.
cfu	Colony forming units.
CSA	Canadian Standards Association.
D_{10}	Grain diameter (in mm) corresponding to 10% passing, by weight, in a hydrometer grain size analysis.
D_{60}	Grain diameter (in mm) corresponding to 60% passing, by weight, in a hydrometer grain size analysis.
<i>dd</i>	Days of delay. Data provided by the BART™ biotector system. It can be interpreted that the shorter the <i>dd</i> the greater the microorganism population and size and activity level.
DGTA/R	Dangerous Goods Transportation Act and Regulations.
ESA	Environmental site assessment.
GC/FID	Gas chromatography using flame ionization detector.
Heterotrophic	Those bacteria which are able to utilize organic materials as the principal sources of energy and carbon for survival, growth and synthesis.

HSWDGR	Hazardous Substances and Waste Dangerous Goods Regulation.
IR	Infra-red spectroscopy.
k	First order degradation rate constant (week^{-1}).
K	Rate constant (day^{-1}).
K_m	Maximum substrate utilization rate (day^{-1}).
K_s	Half velocity coefficient (mg/kg).
MPN	Most probable number. A statistical method for the prediction of a microbial population in a liquid by the presence/absence of growth in various dilutions.
PAH	Polycyclic aromatic hydrocarbon.
PCB	Polychlorinated biphenyl.
ppm	Parts per million. The abbreviation ppm measured by the weight of diesel to dry weight of soil is used throughout the thesis and should be considered equivalent to the units $\mu\text{g/g}$ and mg/kg .
RX	Reaction type. Data provided by the BART™ biodetector system. The RX obtained gives an indication of the types of microorganisms present.
SERM	Saskatchewan Environment and Resource Management.
t	Time (days).
TPH	Total petroleum hydrocarbons.
TRPH	Total recoverable petroleum hydrocarbons.
TSH	Total semi-volatile hydrocarbons.
TVH	Total volatile hydrocarbons.
USEPA	United State Environmental Protection Agency.
UST	Underground storage tank.

W_d	Weight of dry soil sample including weight of the dish.
W_w	Weight of wet soil sample including weight of the dish.
X	Microbial concentration (mg/kg).

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1.0 INTRODUCTION

1.1 Background

The characterization of a contaminated site is typically conducted following a phased approach. A Phase I environmental site assessment (ESA) is conducted when there is uncertainty regarding the current environmental status of a site (i.e., no recent spills). The Phase I ESA may be initiated by property transactions, environmental regulatory agency requests, detection of offsite or onsite impact, or site development. In Canada, a standard has been developed for conducting Phase I ESAs (Canadian Standards Association (CSA), 1994). The standard involves the evaluation and reporting of existing site information collected through a records review, site visit, and interviews with personnel familiar with the site history and operations. Its purpose is to identify actual and potential site contamination. The Phase I ESA may assist in reducing uncertainty about potential environmental liabilities and may provide a basis for further investigation of the site. The standard does not involve the selection or implementation of physical site characterization or remediation activities. Such activities are typically referred to as Phase II and/or Phase III ESAs.

The purpose of a Phase II ESA is to confirm and delineate, or to demonstrate the absence of, contamination at a site identified through the Phase I ESA procedure. In some cases, a Phase II ESA may be undertaken to establish a quantitative baseline for environmental conditions at the site. Phase II ESAs are typically undertaken prior to a remedial investigation and can provide the initial data requirements for performing an environmental risk assessment if required. The key feature that distinguishes the difference between Phase I and Phase II ESAs is the use of quantitative sampling and analytical

techniques in the Phase II ESA. The scope, duration and cost of a Phase II investigation are dependent upon the following factors: the methods employed; the size of the site; the number, type, and identity of suspected contaminants; the level of confidence desired in analytical results; and the environmental matrices to be sampled. Field sampling and analysis programs are developed on a site-specific basis. The sampling and analysis program may include elements such as: borehole drilling and/or test pitting; geophysical survey; soil vapour survey probe installation; surface water sampling; groundwater sampling; indoor air sampling; outdoor air sampling; sediment sampling; subsurface vapour sampling; and analysis of the various environmental matrices of interest for concentrations of organic and inorganic chemicals. A Phase II ESA may itself proceed in phases. Phase II ESAs may commence with screening surveys and coarse sample spacing. More detailed sampling is frequently necessary to delineate areas of contamination or define parameters essential for impact/risk assessment or remedial investigation. The Canadian Council of Ministers of the Environment (CCME) have provided details on additional aspects of site characterization (CCME, 1993a; CCME 1993b; CCME, 1994; the United States Environmental Protection Agency (USEPA), 1991; and Breckenridge and Keck, 1994).

A remedial investigation is typically conducted whenever a Phase II ESA reveals soil and groundwater contamination that is in contravention of local guidelines, standards, or regulations, considered dangerous to human health (via the environmental risk assessment) immediately or in the long term, or deemed unacceptable for any other reason. The purposes of a remedial investigation are as follows: to identify several feasible approaches to remedy the contamination, including in situ monitoring or risk management; to critically compare

these approaches by considering factors such as risks to workers and the surrounding population, and economic and technical feasibility; to determine the extent of remedial measures required; to estimate remediation costs; and to select criteria against which the success of the remedial measures can be evaluated. Remedial investigation programs are developed on a site-specific basis. The program may include elements such as: the assessment of storage, removal, disposal, and treatment alternatives for contaminated materials; quantitative risk assessment for a single contaminant and contaminant mixtures; human and/or environmental exposure estimation; computer modelling of environmental fate and transport processes; the socioeconomic impact analysis of remedial options; the review of rationales for relevant environmental guidelines, standards, and regulations; and the development of site-specific criteria to ensure protection of human health and the environment and to allow evaluation of the success of the remedial measures. If remedial action is warranted, subsequent phases may include: treatability studies (bench-pilot scale); detailed design; contract document preparation/tendering; implementation; monitoring/confirmation; environmental regulatory agency liaison/permitting; and public consultation.

A variety of technologies are available for the remediation of contaminated sites. The technologies can be classified as follows: bioremediation, solidification/stabilization, thermal treatment, or physicochemical based on the fundamental processes involved. Details related to remedial technology selection are provided by Anderson (1990) and Sims (1990).

Bioremediation is a term used to denote the application of microbiological processes for the remediation of contaminated soil, sediment, sludges and groundwater. The use of

bioremediation for remediation of contaminated sites has increased considerably in the last ten years and has spawned a subject that is both diverse in its application and nomenclature. The application of bioremediation is shown in Figure 1.1. Land treatment is synonymous with land farming as shown in the figure.

The most frequent application of bioremedial techniques is the use of *ex-situ* facilities to decontaminate soil excavated from the zone of contamination. In general, the basic strategy is to supply indigenous or introduced microorganisms with nutrients and oxygen in a controlled environment.

Land treatment is the practice of spreading contaminated soils or wastes on the surface of the ground to facilitate natural microbial degradation of contaminants. Land treatment was the first method used for bioremediation of soils and sludges and has been successfully applied by the petroleum industry for disposal of petroleum-refinery wastes. Land treatment is typically used in areas where air emissions would not pose unacceptable health risks and would not require collection and treatment.

1.2 Design Criteria

A common approach for the design of a land treatment facility is the use of soil pan testing on the contaminated soil. Soil pan testing, or pan microcosm testing, is a process where a representative volume of the contaminated soil is taken to the laboratory and spread in a pan. The soil is analyzed for nutrient concentration and total petroleum hydrocarbon (TPH) concentration. Nutrients are added to the soil to bring the ratio of carbon: nitrogen: phosphorous: potassium (C:N:P:K) up to predetermined values. In addition, the soil is

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Figure 1.1: Application of Bioremediation (Cookson, 1995)

analyzed for other factors such as pH, metals concentration, and salinity, which may require adjustment. Next, the soil is mixed (to simulate tilling) and irrigated at predetermined time intervals. By comparing the degradation rates of the different pans simulating various treatment conditions, the optimal treatment schedule can be implemented on the full scale facility with greater confidence that the contaminated soil will be degraded as demonstrated in the soil pan testing.

Conditions in soil pan testing are, however, easy to control. Actual field conditions such as precipitation, temperature and uniformity of contaminated soil will vary considerably compared with the controlled environment of a laboratory. Soil pan testing is still important because it indicates optimal degradation rates that can be reached in the field under ideal conditions. Before constructing expensive full scale facilities, smaller test plot studies should be conducted in the field in order to obtain results that are based on actual field conditions. The test plots should be designed and constructed with the same specifications as the full scale facility, and the critical environmental factors should be considered during the design, construction, operation and testing of both the test plot and the full scale facility.

1.3 Objectives of the Study

The microbial degradation of hydrocarbons has been extensively researched and is well documented. The use of land treatment is well documented and its success is often attributed to the activity of indigenous soil microorganisms. However, physicochemical processes including volatilization, sorption, chemical degradation, migration and photodecomposition may also influence the reduction of hydrocarbon concentration. The

objective of this study was to examine and verify, through bench-scale methods, the microbial processes in land treatment of a hydrocarbon contaminated soil from a project site (an industrial site under decommissioning) located in southeastern Saskatchewan. The specific objectives of the study included:

- an examination of the project site and collection of samples for use in this study;
- the determination of the character and concentration of hydrocarbons;
- the determination of the presence and population of soil microorganisms;
- the determination of the ability of soil microorganisms to degrade hydrocarbons; and
- an investigation of the degradation of hydrocarbons by soil microorganisms under nutrient amendment conditions.

1.4 Scope of the Study

The scope of the study included a review of literature on the land treatment of hydrocarbon contaminated soils and bench-scale methods (treatability studies) to evaluate the likelihood of success of the technique including optimization of the process. Bench-scale testing was used to determine the degradation rate constant (k) and time (t) to reduce hydrocarbon concentrations to acceptable levels (i.e. regulatory objective of 1.000 ppm TPH) under various initial hydrocarbon concentrations and nutrient amendment conditions. The bench-scale testing was conducted under controlled/stable temperature and soil moisture content and represented ideal operating conditions.

2.0 LITERATURE REVIEW

2.1 Management of Hydrocarbon Contaminated Soils in Saskatchewan

In the Province of Saskatchewan, three alternatives are provided to property owners/managers for the management of risks at petroleum contaminated sites (Saskatchewan Environment and Resource Management (SERM), 1995). The alternatives include: the decommissioning of storage tanks and remediation of affected soil and groundwater to pre-established criteria (remediation based on future land use); the decommissioning of storage tanks and remediation of affected soil and groundwater to site-specific criteria (remediation based on site specific sensitivity); or performance of a site specific risk assessment and development of site specific remediation criteria and risk management methods appropriate for the specific set of site conditions.

Typically, hydrocarbon contaminated soils are excavated and transported to the local municipal landfill for land treatment and ultimate disposal. This is practised when the concentration of hydrocarbons in the soil are within specified criteria (usually <20,000 ppm TPH). Soils exhibiting hydrocarbon concentrations in excess of this criteria pose a dilemma to the contaminated site manager. The contaminated soil must either be remediated on-site or removed and transported to a hazardous waste treatment facility located out of province at high cost (i.e transportation charges). Alternative on-site remediation/risk management options for petroleum contaminated sites include: *in-situ* vapour extraction; *in-situ* bioremediation; on-site aeration; and liquid recovery/groundwater treatment.

The excavation and land treatment of hydrocarbon contaminated soil off-site is often chosen because the liability and risks are removed from the site and the costs of land

treatment are low (minus excavation and transportation costs) when compared with other alternatives.

2.2 Guidelines for Land Treatment of Hydrocarbon Contaminated Soil in Saskatchewan

The land treatment method can take advantage of naturally occurring soil microorganisms to biodegrade petroleum hydrocarbons in addition to the volatilization of shorter chain petroleum hydrocarbons. The SERM document "Guidelines for the Remediation of Above/Underground Petroleum Storage Sites and Disposal of Petroleum Contaminated Soils in Saskatchewan" outlines suitable site and soil characteristics, land treatment procedures, soil sampling requirements and necessary approval requirements for sites receiving a single, one-time application of petroleum contaminated soil (SERM, 1993). A summary of the guidelines follows, in Sections 2.2.1 to 2.2.3.

2.2.1 Site and Soil Characteristics

Land treatment site characteristics should include:

- a maximum site slope of six percent;
- a minimum distance to surface water of 30 metres; and
- a minimum distance to residential property lines or buildings of 100 metres.

Soils with moderate to high levels of fertility are preferable. Unsuitable land treatment sites include gravel pits, surface mine lands or quarries, and sites situated within one in ten year floodplains.

2.2.2 Land Treatment Procedures

The acceptable procedures for land treatment are outlined below.

- Contaminated soils should be spread only when the land is tillable, but not earlier in the year than April 1 and no later than November 1.
- The petroleum loading rate should not exceed two percent (2%) or 20,000 parts per million (ppm) total petroleum hydrocarbons (TPH) (purgeable and extractable) as fuel oil or gasoline in the soil to be land treated. Lead levels must be below 375 ppm.
- Surface vegetation and litter should be removed from the area. Contaminated soil should not be applied at thicknesses exceeding 0.15 m. The minimum area required for land treatment based on a maximum spreading thickness of 0.15 m may be calculated as follows:

$$\text{Area in hectares} = \text{Volume of soil to be land treated (m}^3 \times 0.00066)$$

- An earthen dyke or berm should be constructed around the treatment area in order to control runoff. Signage should be erected around the area.
- The proposed land treatment site should be marked with stakes and/or flags to form a grid prior to, or at the time of the site inspection. The stakes must remain in place during application of the contaminated soil and until all follow up monitoring requirements are fulfilled.
- Land treated soil should be incorporated (mixed) with the upper 0.10 to 0.15 m of native soil within 48 hours after application. Immediate incorporation (within 24 hours) is desirable. If fertilizers are added, they may be broadcast either prior to or following soil spreading, but prior to incorporation.

- To enhance hydrocarbon breakdown, the soil should be disced weekly or every second week until hydrocarbon contamination reaches acceptable levels. Less frequent tillage may not provide adequate aeration and mixing particularly on heavy-textured soils and, therefore, may slow hydrocarbon breakdown. Soil monitoring should continue until contamination is below acceptable levels.

2.2.3 Soil Sampling Requirements

2.2.3.1 Contaminated Stockpiled Soil

Soil samples must be taken to evaluate and document the contamination levels in the soil to be treated. Soil samples must be grab samples (not composite samples). The grab samples from contaminated stockpiled soil must be collected from the interior of the pile no closer than one foot from the pile surface. Soil samples should be analyzed for total petroleum hydrocarbons (TPH) (purgeable and extractable), benzene, toluene, ethylbenzene and xylenes (BTEX) and lead.

2.2.3.2 Initial Land Treatment Site Sampling

Initial sampling of the site must be undertaken prior to the application of contaminated soil. This will provide a baseline for the site. The sample must be a composite sample and must be taken from a representative area intended to be used. The composite sample should be analyzed for TPH (purgeable and extractable), BTEX and lead.

2.2.3.3 Follow-up Monitoring

To monitor hydrocarbon breakdown, follow-up soil samples must be taken. These samples should be composite samples. The number of composite samples taken in the land treatment area is based on the volume of soil actually land applied. Each composite sample must consist of individual samples taken from 16 separate locations. The areas where composite samples will be collected must be identified by the use of stakes and/or flags positioned around the border of the site and representing the individual sampling locations. The same locations must be sampled during each sampling event using the same sample identification. Soil sampling should be completed at the times outlined in Table 2.1 until TPH results are below 1,000 ppm.

Table 2.1: Soil Sampling Schedule During Year of Land Treatment

LAND TREATMENT DATE	SAMPLING REQUIRED IN FIRST YEAR
Before July 1	Once in August and once in October
July 1 to September 15	Once in October
After September 15	None

In subsequent treatment years composite samples should be collected in June, August and October until results are below 1,000 ppm TPH (purgeable and extractable).

2.3 Additional Considerations for Design and Operation of Land Treatment Sites

Land treatment is designed to optimize degradation by application of an aerobic biological process while using the soil as inoculum and support medium for biological

growth (Cookson, 1995). Biodegradation is optimized by aerating the soil and working in nutrients with agricultural techniques. Tilling procedures serve to condition the soil to enhance biodegradation of the contaminants. General guidelines for soil preparation include:

- Soils should be conditioned to obtain a relatively uniform mix by removing larger rocks and other debris.
- Additives (e.g. lime for pH control, nutrients, microbial seed) should be applied by using mixing techniques that achieve at least 90 percent contact between soils and additives.
- Inorganic nutrients generally benefit solid-phase bioremediation. They are generally added in mass ratios of 100:10:1 with respect to carbon:nitrogen:phosphorous. Trace amounts of potassium may be beneficial. Single-dose applications should be limited to 3 lb of nutrients per cubic yard of soil to prevent undesirable osmotic effects. Higher doses should use time-release nutrients or repeated applications.
- The porosity of the soil should be increased as much as possible. The porosity can be increased either by mixing during earth-moving activities or by addition of a bulking agent (e.g. wood chips, sand, etc.). Compaction should be avoided during soil placement.
- Soils with a high clay content usually benefit from a bulking agent such as sand, sawdust, or wood chips. Gypsum can be used to reduce moisture content of clayey soils. Additions typically range from 10 to 30 percent by volume.
- The soil's pH can be adjusted with either lime, alum or phosphoric acid, as required. The optimum pH range is 6 to 8.

Process performance for land treatment is established by a program of scheduled monitoring. Degradation of contaminants in soil systems is established by monitoring their disappearance in soil through time.

2.4 Land Treatment as a Bioremediation Process

Land treatment of hydrocarbon contaminated soil is one of the simplest of the bioremediation processes (Figure 1.1). A bioremediation process typically involves stimulating indigenous or introduced microorganisms (e.g. sewage sludge, microbial inoculum, etc.) to metabolize hydrocarbons into cell biomass and non-toxic by-products including CO₂, H₂O and inorganic salts. The basic requirements for a successful aerobic bioremediation include (Rowell *et al.*, 1992; Huesemann, 1994; and Major and Cox, 1992):

- presence of petroleum degrading bacteria;
- presence and accessibility of organic food sources (petroleum products);
- oxygen supply;
- inorganic nutrients;
- moisture; and
- suitable temperature and pH.

Factors affecting land treatment can be classified into three categories (Grasso, 1993): substrate limiting factors; environment limiting factors; and biological consortia limiting factors. The factors are important in estimating the rate and degree of completion of the degradation of hydrocarbons. Each is described in the following subsections.

2.4.1 Substrate Limiting Factors

Substrate limiting factors can be described in terms of the biodegradability and toxicity of the contaminant. The organic substrate must be biodegradable, under aerobic conditions. Some compounds, such as polychlorinated biphenyls (PCB's) are extremely bioresistant and hence are not suitable for land farming techniques. For contaminated soils containing bioresistant compounds, pretreatment is required. Pretreatment schemes include supercritical extraction, solvent washing, and chemical dechlorination (e.g. if PCB's present). Some organic compounds are toxic to the microorganisms above a limiting concentration level. In some the cases substrate toxicity can be controlled by dilution or pretreatment.

Petroleum products can serve as the primary carbon and energy source for bacterial growth and reproduction. However, the nature of the chemical components in a petroleum product will influence the rate and degree of completeness of the biodegradation process.

2.4.2 Environment Limiting Factors

Environment limiting factors include temperature, pH and nutrients. Temperature is an important environmental limiting factor. For successful land treatment, temperatures favourable for microbial growth (8°C to 40°C) are required. The pH of the soil should be maintained between 6-8 for effective treatment. Microorganisms require nutrients to form cellular material for growth and reproduction. Nitrogen, phosphorus and potassium are the typical rate limiting nutrients. For land treatment operations, a C:N:P ratio of 100:10:1 has been found to be successful.

Bioremediation proceeds aerobically when oxygen is used as the terminal electron acceptor. It is also essential to have the optimum moisture content. Low moisture content causes bacterial desiccation. Elevated moisture content reduces the oxygen supply by reducing soil-air flow and so decreases the biodegradation rate.

2.4.3 Microbiological Consortia Limiting Factors

The diversity of microorganisms present allows a wide variety of organics to be degrade successfully. The diversity of microorganisms present at the land treatment site will control the rate of bioremediation and the kind of contaminant that can be treated. Acclimation processes will delay the onset of biodegradation. However, natural evolutionary processes will result in acclimation of indigenous microbes. Although indigenous bacteria capable of degrading organic waste are usually present at sites, their capacity for degradation and rate of activity might not be sufficient. If this occurs, an alternative microbial source could be considered.

2.5 Additional Solid Phase Treatment Techniques

An *ex-situ* bioremediation facility is a facility that is designed to treat contaminated medium above the ground surface. Treatment facilities can range from very simple land treatment operations noted above to more complex structures often termed “biopiles”. The volume of the soil, space available, type of contaminant, and the remediation goals typically influence the level of complexity required. *Ex-situ* bioremediation techniques have evolved from simply spreading the contaminated soil on the ground surface and periodically tilling

into a well defined methodology that includes containment barrier construction, tilling, watering, nutrient and bacterial culture application. Essentially, all *ex-situ* treatment facilities will have the following attributes:

- a liner to ensure that contaminants in the soil do not contaminate underlying soils;
- a liner support structure to contain the contaminated soil;
- a leachate collection and recirculation system;
- a nutrient delivery system; and
- a cover to reduce volatile gas emission, water infiltration or excessive evaporation of moisture.

A biopile is created by spreading contaminated soil on a lined treatment bed. Air and/or nutrients dissolved in water are passed through the soil pile to enhance biodegradation of the contaminants present in the soil. A schematic of a typical biopile system layout is shown in Figure 2.1.

There are two types of biopiles:

- Oxygen and nutrients are delivered to the bacteria by water movement through the soil pile. The water is added by an irrigation system and the bed is equipped with a drainage collection system. The drainage from the soil pile, or leachate, is treated in a liquid phase bioreactor and recirculated through the soil pile. The soil pile may be totally enclosed if volatile-emissions control is necessary.

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Figure 2.1: Schematic of Typical Biopile System Layout

- Nutrients are mixed with the soil when the soil pile is created and oxygen is delivered by pumping air through the soil. The air is delivered by pipes placed at the bottom and middle of the soil pile. A plastic liner is placed on top of the pile to prevent rainwater from permeating into the pile.

2.6 Mass Transport in the Solid Phase

Bioremediation in solid phase such as in soils and sediments, involves the transport of the contaminant from the solid phase to the microorganisms. The contaminant desorbs from the solid surface. The rate of desorption depends on the type of chemical and the nature of its interaction with the solid phase. Usually, the solid phase contains pores and the contaminant diffuses out of the pores into the liquid or gas phase. The microorganisms can be present as a suspension in the liquid phase within saturated pores or attached to the solid phase either inside or outside the pores.

Diffusion in the pores of a solid phase, such as soil or sediment, has several complexities, including (Grasso, 1993):

- The diffusion coefficient is confined to the pores of the solid phase, since the contaminant does not diffuse through the solid phase itself-therefore the path is tortuous and unpredictable due to heterogenous distributions of pore sites.
- The effective diffusion coefficient is not independent of concentration.
- Adsorption and desorption on the solid phase surface occurs.
- The diffusion coefficient depends on the properties of the soil including mineral composition, organic matter content, bulk density, moisture content, and temperature.

2.7 Treatability Studies

The factors outlined in Sections 2.4 and 2.5 are often evaluated by laboratory bench-scale treatability studies prior to the design of an *ex situ* bioremediation facility. The use of treatability studies in a structured technology selection process are provided by Korfiatis and Christodoulatos (1993).

2.7.1 Aerobic Solid Phase Bioremediation Bench-Scale Treatability Study Method

A bench-scale method to assess the feasibility of biological remediation of soil and/or groundwater impacted by hydrocarbons has been proposed by Saberiyan, *et al.* (1995). The method consists of two phases: characterization and experimentation. The first phase includes the physical, chemical and biological characterization of a contaminated soil sample. This phase determines soil parameters, contaminant type and the presence and population of indigenous hydrocarbon-degrading microorganisms. The second phase is an experimental portion of the method and consists of a respirometry test to indirectly determine microbial growth/respiration via measurement of carbon dioxide (CO₂) levels and contaminant loss. An overview of the method is provided below.

2.7.1.1 Contaminant Characterization

The rate of biodegradation is dependent on the nature of the contaminant, the number of carbons and the chemical C-C bond. Identification of the contaminant of concern and the most representative carbon chain (C_{xx}) is important for data interpretation based on the stoichiometric relation between contaminant reduction and CO₂ production. The

identification and determination of the most representative carbon chain can be achieved by: hydrocarbon identification scan (Oregon Department of Environmental Quality (ODEQ) Method TPH-HCID; Hydrocarbon Identification); total petroleum hydrocarbons by infrared spectroscopy (IR) (United States Environmental Protection Agency (USEPA) Method 418.1 Modified); and gas chromatography (GC) methods (USEPA Methods 3810/8020 and 3550).

2.7.1.2 Microbial Enumeration and Characterization

Microbial enumeration and characterization analysis is conducted by mixing 10 g of contaminated soil with 100 mL sterile water and placing in a Waring blender for approximately one minute. One mL of the supernatant (liquid) is mixed with nine mL of sterile Bushnell-Haas broth. The sample is ten-fold serially diluted to a 10^{-4} dilution. Samples from each dilution are plated in triplicate on Bushnell-Haas media by plating 0.1 mL from each dilution tube. The formulation of the broth was 0.2 g/L magnesium sulphate; 0.02 g/L calcium chloride; 1 g/L monopotassium phosphate; 1g/L ammonium phosphate dibasic; 1 g/L potassium nitrate; and 0.05 g/L ferric chloride. The plates are amended with petroleum products (e.g. gasoline, diesel fuels, oils, etc.). The plates are incubated for 15 days at room temperature. Following the incubation period, bacterial colonies are enumerated on each plate. Plates containing between 30 and 300 colonies are selected. The counts are averaged to determine the number of colony forming units per mL (CFU/mL).

In addition to the procedure noted above, the indigenous microbial community in the contaminated soil sample is characterized using standard microbiological and biochemical techniques (ie. standard methods for genus and species identification).

2.7.1.3 Respirometry Test

Growth of bacteria in the presence of petroleum products, although a good indicator, is insufficient to demonstrate the rate of contaminant reduction at the site with indigenous bacteria. To confirm the biodegradation rate, it is necessary to monitor disappearance of the petroleum product and the generation of CO₂ (final by-product) over time. This phase of the study investigates the contaminant biodegradation rate under different nutrient, bacterial, and environmental conditions. Total contaminant reduction is monitored throughout the study. CO₂ accumulative concentrations are monitored periodically to confirm biological activities.

The method typically consists of one control cell (amended with sodium azide (NaN₃) to suppress microbial activity) and several experimental cells. Each cell contains approximately 11 kg of soil and is sealed on the top with one point air injection line and an effluent line. Two (2) sample ports are constructed on top of each cell for soil sample collection. Influent air for the cells is passed through a moisture separator. KOH traps are used in the influent line to eliminate the introduction of ambient CO₂ to each cell. Ambient air contains CO₂ concentrations of 300 to 700 ppm which would interfere with correct evaluation of the amount of CO₂ production as the final product of bioremediation.

2.7.1.4 CO₂ Generation

In order to mitigate both sampling and analytical variations inherent in TPH analysis, CO₂ liberated from soils undergoing bioremediation are monitored periodically to confirm biodegradation and its intensity in each cell. CO₂ is the final product of a complete bioremediation process. The presence of the KOH trap in the influent line proves that the

primary source of CO₂ in each cell comes from aerobically metabolizing organisms in the soil. Stoichiometric details are provided in Saberiyan, *et al.* (1995).

2.7.1.5 Contaminant Reduction

Contaminant concentrations are monitored periodically throughout the study. A simple oxidation-reduction reaction cannot be used for biodegradation analysis. Since some of the petroleum product degrades to be used as a cell component, there is a more complex stoichiometric relationship between the contaminant reduction and final by-products. A stoichiometric relation is calculated to represent the complete biodegradation reaction. The chemical structure of the biomass is assumed to be C₅H₇O₂N per mole.

A mathematical model (based on the Monod equation) is used to evaluate substrate removal attributable to biodegradation processes (Section 2.8).

2.7.1.6 Nutrient Analysis

A ratio of 100:10:1:0.1 of C:N:P:K is typically necessary for biological activity. These levels are the accepted values required for efficient bioremediation processes (Saberiyan, *et al.* ,1995; Cookson, 1995).

2.7.2 Pan Microcosm Testing

Cullimore (1993) defines a microcosm as a small portion of an environmental system that is separate and operates as a distinct biological entity. A microcosm usually involves very small volumes where conditions are optimized for the growth of a particular species or

consortium of microorganisms. Pan microcosm testing most closely simulates the land treatment process and represents the conventional test procedure for the biological land treatment of contaminated soils (Rogers *et al.*, 1993; Huesemann, 1994; USEPA, 1991; and USEPA, 1993). Pan microcosm testing is a process where a representative volume of the contaminated soil is taken to the laboratory and spread in a pan.

Rogers *et al.* (1993) recommends, for the case of hydrocarbon contaminated soil, that the test should be conducted by mixing contaminated soils with an appropriate amount of clean soil to produce a mixture that has oil and grease levels no greater than 1 to 2 percent by weight. This mixture is placed in a pan reactor with typical dimensions of 20 cm by 30 cm with 10 cm depth. Nutrients and water are then added to the soil to enhance the activity of the indigenous bacteria, in amounts determined by the initial soil characterization. Periodically, the pan soils are mixed, and nutrient, pH, and moisture levels are measured and if necessary, adjusted. Optimal moisture content levels are 15% on a percentage basis by weight or 80% of field capacity (Cookson, 1995). Samples of the soils are taken at the start of the experiment and every week for 2 to 3 months. The hydrocarbon concentration-time profiles that are generated are used to estimate contaminant removal kinetics and the basic design parameters for the land treatment process.

U.S.EPA (1993) states that because soil pans are typically small, operating parameters (e.g., nutrient availability, pH, moisture, oxygen, and temperature) are relatively easy to control and study costs are relatively low. Generally, oxygen addition can be provided by tilling or mixing the soil one to three times per week, while moisture is monitored and amended either daily or weekly. Since conditions are usually so easy to

control, these studies are more likely to reflect ideal operating conditions rather than less-than-perfect conditions typically experienced during field applications.

2.7.3 Suppression of Microbial Activity in Control Cells

Treatability studies should include controls to measure the impact of physicochemical processes, such as volatilization, sorption, chemical degradation, migration, and photodecomposition. Inhibited controls can be established by adding formaldehyde, mercuric chloride, sulfuric acid, or sodium azide to suppress microbial activity (USEPA, 1993).

Contaminant concentrations are measured in both the experimental and control cells at the beginning of the study, at intermediate times, and at the end of the study. The mean contaminant concentrations in both the control and experimental cells at the end of the study can be compared to their initial concentrations to see if a statistically significant change in concentration has occurred. The decrease in the control cells may be attributed to abiotic mechanisms, while the decrease in the experimental cells would be a result of abiotic and biotic processes. The difference in mean contaminant concentrations between the experimental cells and the inhibited controls at each time interval sampled will reveal whether there is a statistically significant reduction in contaminant concentration due to microbial activity. Care should be taken to assess the effects that the different suppressing agents can have on the chemical behavior of the soil-contaminant system. For example, formaldehyde has the potential to act as an electron donor, while sulfuric acid will impact the pH. Sodium azide can, under certain circumstances, promote spontaneous explosive

reactions, while mercuric chloride may complex certain hydrocarbons, leading to artificially low hydrocarbon concentrations. Placing the media in an autoclave may result in desorption of volatile contaminants. Finally, suppression agents may modify soil structure.

Complete suppression of microbial activity in soils can be difficult to accomplish. Incomplete mixing of suppression agents with soils can result in pockets of surviving microbes in soil pores. In some cases, microbial populations can transform and detoxify suppressing agents. Additional suppressing agents can be provided during the study to maintain reduced biological activity. The effectiveness of suppressing agents can be measured by techniques such as microbial enumeration, respirometry, and enzyme analysis. Unless these or similar techniques show no microbial activity, it may not be possible to distinguish between removal of contaminants by abiotic and biological processes in the control cells. However, complete suppression of the controls is not necessary provided biological activity is inhibited sufficiently so that a statistically significant difference between the experimental and control cells can be determined. If suppression is not complete, substantial degradation in the control can mask the occurrence of biodegradation in the experimental cells. Both during and at the end of the study, plate cultures can be performed to determine whether microbial activity in the control cells were adequately suppressed.

2.8 Enumeration of Soil Microorganisms

2.8.1 Introduction and Overview

Soil is a unique medium that contains a diverse community of organisms, representing many morphological and physiological types. In attempting to characterize

these organisms, one must be cognizant of both numbers and activities. Some organisms exist at relatively low levels numerically but can exert profound influences on some biological processes (American Society of Agronomy, 1982).

Organisms in soils are never static in numbers or activity. Therefore, the enumeration of some population or microbial activity represents a point in time for that particular population that is in dynamic equilibrium with its physical, chemical, and biological environment. Variation of microbial numbers and activities can occur with depth and soil type. Soil structure, texture, and moisture levels can drastically affect aeration within the soil environment from one characterized as aerobic to one of anaerobic properties, thus influencing the distribution of physiological types.

The diversity of organisms in soil is also dependent on the type of plant and proximity of the organism to the plant root itself. It is well known that the numbers and activities of organisms vary with the distance from the root, with greatest numbers and activities in the soil most intimately associated with the root surface. The effect of the root on soil organisms decreases proportionately as the distance in space from the root increases.

2.8.2 Principles

No microbiological technique is as widely accepted in enumerating soil organisms than the plate count using a variety of media. The underlying principles consist of: dispersing the sample, distributing an aliquot to an appropriate medium, incubating under suitable conditions, and finally, counting the developed colonies. Despite this apparent simplicity, important assumptions underlie the utilization of this technique (American

Society of Agronomy, 1982).

For the technique to be valid, the sample must be uniformly dispersed so each cell has an equal opportunity to express its presence when it encounters an appropriate medium. Fortunately, this objective is reasonably achieved if the investigator is careful during sample preparation. Standardization of shaking for uniform dispersion is achieved through the use of mechanical shakers or through strict attention to the shaking motion and time of shaking. Wetting agents are frequently added to the diluent to facilitate the dispersion process. It is further assumed that there is no hyphal fragmentation during sample dispersion. However, in environments with a large proportion of mycelial-forming organisms, the more vigorous and harsher the dispersion, the greater the potential for an overestimate of the population.

An additional assumption underlying this technique is that a medium specific for the organism, or organisms, the investigator wishes to study exists. This assumption imposes a distinct limitation on the technique, because few media that are specific to only one generic group have been devised. In some cases, the chemical or physical environment selected for the group may contribute to the specificity of the media. Examples include alteration of acidity, energy source, or temperature.

One of the biggest assumptions involved with the technique deals with the apparent lack of interaction among organisms when they are growing on a suitable medium. However, competition and other interactions among organisms are well documented. Antibiotics produced by numerous soil organisms may have a profound effect on other organisms, and numerous well-defined antagonisms exist among soil organisms.

Difficulties with dispersion, media formulation, or even absorption of microbes to walls of pipettes can be dealt with by standardizing procedures and rigorously following the same protocol throughout for all samples. However, other assumptions seriously limit the application of the technique to soil systems. Therefore, utilization of this procedure should have an adequately defined set of objectives, whereby the investigator understands and accepts the limitation imposed by utilization of the technique.

The plate count method is considered acceptable because media that are specific for fixed physiological groups have been developed and it is assumed the incubation conditions approach the natural environment. In utilizing the technique, it must be remembered that numbers are not synonymous with importance. As with all enumeration techniques, it must be recognized that organisms are not uniformly distributed throughout the soil environment but rather are found in a point distribution frequently dependent on a localized feature that allows for the maximum expression of that particular organism.

2.8.3 Pour Plate Technique

In the pour plate technique, suspensions of microorganisms are added to melted agar tubes that have been cooled to approximately 42-45 °C (Prescott *et. al.*, 1990). The bacteria and agar medium are mixed well and the suspensions are poured into sterile petri dishes using aseptic technique. The agar is allowed to solidify, trapping the bacteria at separate discrete positions within the matrix of the medium. Although the medium holds bacteria in place, it is soft enough to permit growth of bacteria and the formation of discrete isolated colonies both within the fluid and on the surface of the agar. The pour plate technique is

often used for the quantification of numbers of viable bacteria. The facts that agar solidifies below 42°C and that many bacteria survive at these temperatures ensure the success of this isolation technique. However, in some cases significant numbers of bacteria can be killed by the heat shocking so that this method cannot always be used.

After the agar plates are appropriately inoculated, the plates must be incubated under suitable environmental conditions. If conditions are favorable the microorganisms will reproduce until they form a colony. Various environmental parameters are controlled in the laboratory for cultivating microorganisms. Among the most critical parameters that influence microbial growth are temperature and oxygen concentration.

Incubation periods will vary depending on study objectives. In enumeration studies, samples for most bacteria and fungi generally may be assayed after 4 to 7 days, whereas samples for actinomycetes will take on the average of 10 to 14 days before they will be suitable for counting. Some organisms may require sufficiently longer periods and may require incubation for at least 21 to 28 days.

After incubating, the plates should be removed from the incubator and inspected. The dilution that yielded from 30 to 300 colonies/plate should be selected. Plates with colonies obscured by large spreading organisms should be excluded from the final count. The colonies can be counted with the aid of a Quebec colony counter or low-power microscope.

Despite precautions to assure a thoroughly mixed suspension, duplication between replicate samples may be difficult to achieve. For the decimal dilution series, subsequent dilutions should differ from the preceding by a factor of approximately 10. Also, where replicate plates differ by more than one dilution factor, difficulties have occurred during the

preparation phases. In these instances, dilutions and plating should be repeated with a fresh sample. In addition, one should strive to achieve uniformity during the shaking process.

2.8.4 Microbial Community Patterning

A limitation of the more traditional analytical testing procedures such as the pour plate method is that it is typically very labour intensive, and therefore expensive. For these reasons, biological community patterning using microplates has been employed as an alternative means of evaluating biological activity with respect to its application to bioremediation (Quinn and Reinhart, 1996).

The biological community patterning approach to the evaluation of bioremediation effectiveness is based on direct inoculation of whole environmental samples into microplates, and the subsequent absorbency analysis of colour development in the microplates as a result of metabolic respiration. This technique characteristically identifies or “fingerprints” the community at the point in time when the absorbency evaluation takes place, and does not rely on laboratory enumeration plating techniques to describe the microbial population. The “fingerprint” is determined by the wells that exhibit colour development and the degree of colour development in these wells.

In microbial community patterning using microplates, there is no quantification or qualification associated with a single element that has changed, but instead the degree of change or degree of difference in the entire microbial community is evaluated. This is in contrast to microbial enumeration studies that attempt to both quantify and qualify change with numbers and species type. When attempting to add validity to bioremediation as the

mechanism that accomplishes a remedial effort, what becomes significant is not necessarily the numerical bacteria counts nor the indicator of species type, but the relative changes during and after in the counts or in the species that are dominating the community's activities. These relative changes in the community can be evaluated more expeditiously and more economically with the use of biological community patterning using microplates.

2.8.5 Biological Aggressivity and the Biological Activity Reaction Test

The patented BART™ biological activity reaction test system (Droycon Bioconcepts Inc., Regina, Canada) can be employed to determine the presence or absence of microbial populations; identification of the types of microorganisms, if present; and level of aggressivity of microorganisms, if present.

The tests form a simple method to determine the activity potential of various groups of bacteria by their potential aggressivity. The method is described in detail by Cullimore (1993).

The BART™ biodefectors correspond to selected groups of bacteria and include: HAB-BART™ (total aerobic bacterial); IRB-BART™ (iron related bacteria); SRB-BART™ (sulphate reducing bacteria); FLOR-BART™ (fluorescing pseudomonads); SLYM-BART™ (slime-forming bacteria); and DN-BART™ (denitrifying bacteria).

The data obtained by the method include days of delay (*dd*) and the reaction type (RX) which occurs. In general, the shorter the *dd* the greater the population size and activity level. The RX obtained gives an indication of the types of bacteria present in the sample.

Although originally designed for testing of drinking water supplies, the BART™ systems can be employed to evaluate microbial activity in soils. 100 mL consisting of 50 mL sterile water and 50 mL of soil are added to a measuring cylinder. The mixture can then be poured into a stomacher bags. The bags can then be placed in a Lab-Blender 400 Stomacher and agitated for 10 minutes.

1:15 and 1:150 dilutions of the prepared samples can be placed in BART™ biodetectors. For example, 14 mL of sterile water and 1 mL of prepared sample would be required for a 1:15 dilution. 15 mL of sterile water and 0.1 mL of prepared sample would be required for a 1:150 dilution. The biodetectors are stored at room temperature and observed daily for ten (10) days for RX and *ddl*. The likely combinations of microbial growth and level of aggressivity can be determined with the aid of a HACH BART™ Biological Activity Reaction Tests Reaction Comparator Chart (HACH, 1995).

As an example, a SLYM-BART™ result may indicate the presence of *Pseudomonas*. This genus is well known as a hydrocarbon degrader (Cookson, 1995).

2.9 Methods for Determination of Total Petroleum Hydrocarbons

2.9.1 Infrared Spectroscopy

The total recoverable petroleum hydrocarbons analytical procedure (USEPA Method 418.1), commonly known as the TRPH analysis, is an analytical procedure that can provide an initial indication of the possible presence and extent of organic contamination (Lee III et. al., 1993). More specifically, this test is widely used to investigate sites known or suspected to be contaminated with petroleum products. TRPH is also useful in assisting remediation

efforts. For example, TRPH results can help identify contaminated soils in the initial cleanup (i.e. excavation) of petroleum contaminated soils. As such, TRPH provides a general indication of the presence of such contaminants in a cost-effective manner; often additional testing must be performed (such as Method 8010/8020, volatile organic compounds) in order to quantitate specific contaminants.

The TRPH procedure is basically an infrared spectrophotometric method that quantifies fluorocarbon-113 extractable petroleum hydrocarbons from environmental samples, including surface and groundwater, saline waters, soils, and industrial and domestic wastes. Typical method detection limits are 1 mg/L for aqueous samples and 5 mg/kg for soil samples. TRPH is similar to USEPA Method 413.2 (Total Recoverable Oil and Grease), with the latter being broader in scope. Both procedures use fluorocarbon-113 (1,1,2-trichloro-1,2,2-trifluoroethane) to extract organic materials from the sample. The TRPH procedure incorporates an additional step, using silica gel to remove the polar constituents. In theory, the TRPH procedure would determine the presence of petroleum hydrocarbons only, whereas the oil and grease procedure would include both petroleum hydrocarbons and non-petroleum hydrocarbons such as vegetable oils, animal fats, greases, and related materials.

2.9.2 Gas Chromatography

There is an emerging consensus that total petroleum hydrocarbons should be determined as the sum of purgeables plus extractables where purgeables are arbitrarily all compounds detected below decane and extractables all compounds above (Loescher, 1995).

This is a positive step in that methods which involve only extraction lose and/or mask the lighter hydrocarbons, generating biased low data for instances of gasoline contamination. Two separate analyses are required, however, which means increased costs. Data users should always make certain which procedure a lab is using. Exceptionally low costs likely mean only extractables are being determined. Variability in purgeable data comes from four main sources. The first is sample preservation, where substantial losses of volatiles through biodegradation are observed in unpreserved samples. Thus, methanol preservation, either in the field or immediately on receipt at the lab is strongly recommended. Second, the USEPA allows either purging a 1 g sample of soil in 5 ml of water or a 0.1 to 0.2 mL methanol extract of the soil in 5 mL of water for volatiles analysis. The methanol extracts have been shown to provide much higher results, particularly in clay soils.

Third, GC/MS, GC/PID/FID and GC/FID are all accepted instrumental techniques, each responding somewhat differently across the range. The fourth and perhaps greatest source of variability is calculation method. A typical sample may contain hundreds of peaks, each with its own intrinsic response factor. Response also varies across the range with GC conditions. The sum of all peaks is defined as total purgeables and is quantitated against a single peak or select few peaks of known concentration. The potential for bias and between lab variability is enormous. These last two issues apply to both purgeables and extractables.

Extractables raise additional problems. Extraction temperature and extraction solvent are major sources of variability. For example, our in house data shows soxhlet extraction/concentration give lower recoveries of C_{10} - C_{17} hydrocarbons than cold extraction. Different polarity solvents also have different extraction efficiencies.

2.10 Rate Constants and Their Applicability

2.10.1 Introduction and Overview

Numerous standard methods exist for determining the biodegradability of a compound in the aquatic environment. These methods produce results in terms of percentage removal after a fixed time period. The kinetics of biodegradation are described by a variety of mathematical expressions, ranging in complexity and applicability. A review of some of the commonly used expressions is provided below and their applicability to solid phase systems is examined.

2.10.2 First Order Reactions

The Monod equation can be used to investigate the degradation of a single substrate (contaminant). The model takes advantage of the fact that the biodegradation rate is a function of substrate concentration and is shown below in Equation 2.1.

$$\frac{dC}{dt} = \frac{K_m X C}{K_s + C} \quad (2.1)$$

When the substrate concentration (C) is very small compared to K_s , it takes the form,

$$\frac{dC}{dt} = -K_m \cdot \frac{X}{K_s} \cdot C \quad (2.2)$$

Integrating Equation 2.2 and setting $C = C_0$ at $t = 0$ yields,

$$\ln\left(\frac{C}{C_0}\right) = -K_m \cdot \frac{X}{K_s} \cdot t \quad (2.3)$$

or simply a first order equation which can also be expressed as,

$$\ln \frac{C_0}{C} = k \cdot t \quad (2.4)$$

where,

$$k = K_m \cdot \frac{X}{K_s} \quad (2.5)$$

and K_m , X , and K_s are constants for the system and where,

C_0	=	initial contaminant concentration (mg/kg)
C	=	contaminant concentration at time t (mg/kg)
k	=	degradation rate constant (day^{-1})
K_m	=	maximum substrate utilization rate (day^{-1})
K_s	=	half velocity coefficient (mg/kg)
X	=	microbial concentration (mg/kg)
t	=	time (days)

It is possible to determine the rate constant K by plotting $\ln(C/C_0)$ versus time and performing a regression analysis.

Care should be exercised when using the half-life equation for describing biodegradation since, at low concentrations of the chemical, the rate of substrate utilization can become limiting.

The rate of biodegradation may also be dependent on the concentration of microorganisms responsible for the biodegradation. However, if the concentration of microorganisms is assumed to be constant in the test system (ie. if the test chemical

concentration is so small that growth of microorganisms can be ignored) then the rate constant, K , in the equation incorporates the constant biomass concentration value. Variations in the quantity of biomass can change the rate constant, K , and this is usually the reason for large variations in the reported values of the rate constant, K , between different test sites.

2.10.3 Second Order Reactions

When the concentration of the microorganisms does not remain constant in the test system (because of growth), the rate of biodegradation may be described by second-order equations. The use of second-order equations in the literature is limited.

The second-order equation implies that the rate of biodegradation increases with both the concentration of the test chemical and the biomass present. However, the rate of biodegradation eventually becomes constant with increases in the test chemical concentration. A possible explanation of this phenomenon is, that as the substrate concentration is increased, it reaches a point where the environment becomes too toxic for microorganisms to reproduce. Hence, the second-order equation is valid only in a narrow range of test chemical concentration (Grasso, 1993).

It should be noted that the second-order equations involve the concentration of the microorganisms. This microbial concentration can be obtained from either plate count tests or other enumeration methods. It is generally assumed that:

- Plate counts (or other enumeration methods) detect similar percentages of the total microbial population in different samples;

- All, or a constant proportion, of the enumerated bacteria are transforming the chemical; and
- Transformation rates per cell are approximately the same for all sites examined.

Generally, the microbial consortium present in natural populations are not equally active in the degradation of a specific test chemical. Therefore, the use of a second-order equation, wherein the microbial concentration value is used as the concentration of the entire microorganism population is not always valid, and may lead to large site variations for the kinetic rate constant.

2.10.3 Applicable Rate Constants Reported in the Literature

First order degradation rate constants for TPH are limited in the literature. The rate constants reported in the literature were for specific organic compounds such as benzene and specific polycyclic aromatic hydrocarbon (PAH) compounds. However, the kinetic constants reported by Saberiyan *et. al.* (1995), Demque (1994), and Demque *et. al.* (1997) were considered. The rate constants for TPH reported by Saberiyan *et. al.* were obtained using the respirometric techniques described in Section 2.7.1. This method is considered to be considerably more sophisticated than that of soil pan testing. The rate constants for TPH reported by Demque (1994) and Demque *et. al.* (1997) were obtained through field studies (i.e. pilot scale studies) and involved a level of sophistication greater than that attempted in this study. Nonetheless, literature data are used to provide a benchmark for the study results, these are described in greater detail in Section 4.5.3.

3.0 METHODS

3.1 Description of Project Site and Contaminated Soil

The decommissioning of a diesel fuel underground storage tank (UST) was commenced in August of 1995 at a project site (an industrial site for decommissioning) located in southeastern Saskatchewan. The work began with excavation and removal of the UST. Examination of the tank upon removal showed extensive corrosion and the failure of welds. The dimensions of the excavation were approximately 6 m x 7 m x 4 m. Groundwater was observed seeping into the excavation from the north and west sidewalls. Visual and olfactory evidence of the presence of diesel fuel was noted at the excavation sidewall soils and in groundwater seepage. Further excavation of contaminated soil was not feasible because of the following physical constraints: an above ground structure to the west; a gravel road to the north; and scrap laydown to the south and east. The groundwater seepage/free product was removed and disposed of by vacuum truck and in accordance with the provincial *Hazardous Substances and Waste Dangerous Goods Regulations (HSWDGRs)* (SR 25/92 s3) and *Dangerous Goods Transportation Act/Regulations (DGTA/R)* (SS 1984-85-86, D-1.2).

The analysis results of soil samples collected from each of the excavation sidewalls were below the provincial criteria (commercial/industrial) for hydrocarbon parameter concentrations in soil with the exception of the west sidewall and the stockpiled soil (Table 3.1 and Figure 3.1). The TPH concentrations for these samples ranged from 1,300 to 4,100 ppm. In addition, one of the stockpiled soil samples exhibited toluene and xylene concentrations above the provincial criteria.

Figure 3.1: Project Site and UST Excavation Sampling Locations

Table 3.1 UST Excavation Soil Analytical Results

Sample Location	Benzene	Toluene	Ethylbenzene	Xylene	TVH	TSH	TPH
Detection Limits	0.005	0.005	0.005	0.005	0.005	1	10
EX-W-NW	4.4	5.8	7.6	21	74	3,400	3,474
EX-N-NW	0.98	0.62	1.4	6.6	23	250	273
EX-E-NE	0.55	0.52	2.1	9.8	19	1,200	1,219
EX-S-SE	0.17	0.4	0.8	4.8	12	350	362
EX-B-E	<0.005	<0.005	<0.005	<0.005	0.022	<1	0.022
SP-101	0.11	0.28	0.56	3.8	18	1,300	1,318
SP-102	28	62	24	120	500	3,000	4,100
COMP-1	0.03	0.037	0.19	0.74	1.9	720	722
COMP-2	0.022	0.024	0.18	0.67	1.9	300	302
BACK-1	<0.005	<0.005	<0.005	<0.005	0.027	<1	0.027
SERM (1995) Interim Risk Management Criteria							
Residential/Parkland	0.5	3	5	5	-	-	1,000
Commercial/Industrial	5	30	50	50	-	-	1,000

Note: Units in µg/g (ppm) unless otherwise noted.

Excavation and relocation of the UST backfill to a local municipal landfill operation for land treatment was conducted in September of 1996. The approximate volume of soil that was transported to the municipal landfill operations was 400.4 tonnes (37 loads, approximately 370 m³). The excavation was backfilled with clay fill material. Sub-samples of the contaminated UST backfill were obtained and used in the study. An overview of the experimental work is provided below.

3.2 Introduction and Overview of Experimental Work

The experimental work consisted of two phases: microbial and physical characterization of the hydrocarbon contaminated soil and land treatment site soil; and experimentation (soil pan testing). Sample collection, analytical methods, experimental

procedures and data analysis are described in the following sections. Results for the characterization and experimental phases of the study are presented in Section 4.

3.3 Bulk Sample Collection

Samples of the hydrocarbon contaminated soil (diesel fuel UST backfill) and the land treatment site soil were collected on September 18, 1996 from a project site located in southeastern Saskatchewan (Section 3.1). Bulk sample containers of 26 U.S. gallon (98.4 L) capacity (Rubbermaid Roughneck container) were rinsed with 1 L of sodium hypochlorite solution prior to filling with a sample to avoid microbial cross-contamination of the bulk soil samples. One (1) container was filled to approximately 3/4 capacity with the hydrocarbon contaminated soil and a second container was filled to approximately 3/4 capacity with surficial soil from the land treatment site. Vegetation was removed prior to collection of the land treatment site surficial soils. Sub-samples for microbial and contaminant characterization were collected from each bulk sample container. The sub-samples for microbial characterization were placed in sterile soil bags (2 from each container) (Section 3.4.1). A sub-sample of the hydrocarbon contaminated soil for contaminant characterization was placed in a certified laboratory clean 250 mL glass jar with Teflon lined lid (Section 3.5.4). The lids were affixed to each bulk sample container and the containers were transported to Regina on September 20, 1996 and were stored outdoors until December 12, 1996. The bulk sample containers were brought indoors at that time and allowed to acclimatize for approximately one (1) week. The experimental phase of the study commenced on December 17 and 18, 1996 (Section 3.5).

3.4 Characterization Phase of Study

3.4.1 Heterotrophic Plate Count

The procedure for conducting the heterotrophic plate count followed the *Standard Methods for the Examination of Water and Wastewater* method 9215 B (Pour Plate Method) (American Public Health Association (APHA), 1994). The count is indicative of the presence and population of aerobic soil bacteria which are able to grow on nutrients present in ordinary bacteriological culture media.

The sterile soil bags were squeezed by hand to break down the soil samples into finer particles. Ten grams of the soil were mixed with 90 mL of sterile phosphate buffer (dispersion medium). Phosphate buffer was used rather than distilled/deionized water as it is less likely to induce osmotic shock on the soil microorganisms. The slurry was shaken by hand at least fifty times to displace microbes from soil particles to the liquid phase, and allowed to stand for a minimum of thirty (30) minutes. The slurry was briefly shaken prior to inoculation in serial dilution tubes of melted agar that had cooled to 42-45 °C. The extraction was vortexed into each tube (of serial dilutions) and the mixture was poured into petri plates. After incubation, the number of colonies that developed were counted and the concentration of microorganisms in the original suspension was calculated.

3.4.2 Microbial Hydrocarbon Utilization Screen

The contaminant utilization screen generally followed the standard methods for heterotrophic plate counts and most-probable number (MPN) methods except that Bushnell-Haas Broth was used as the bacteriological culture media and this media was amended with

the hydrocarbon of interest (i.e. diesel fuel). The Bushnell-Haas Broth is a minimal medium containing magnesium, calcium, potassium and iron salts. The absence of a carbon source can be used in evaluating the ability of microorganisms to decompose hydrocarbons. Microorganisms capable of degrading hydrocarbons should show growth in Bushnell-Haas Broth supplemented with a hydrocarbon source.

For the pour plate method, the soil sample slurry (described in Section 3.4.1) was serially diluted into Bushnell-Haas Broth and agar was added at 15 g/L for solid media. The mixture was amended with sterile weathered diesel fuel to 1% concentration and then poured into petri plates. After incubation, the number of colonies that developed were counted and the concentration of microorganisms in the original suspension was calculated.

For the MPN method, the soil sample slurry (described in Section 3.4.1) was serially diluted into three (3) tubes containing the Bushnell-Haas Broth amended with sterile weathered diesel fuel to 1% concentration. The tubes that showed growth were recorded as positive and used to calculate the MPN of colony forming bacteria in the sample. The MPN was determined from MPN tables provided in *Standard Methods for the Examination of Water and Wastewater* (APHA, 1994).

3.4.3 Physical Characterization of Contaminated Soil

A particle size analysis of the hydrocarbon contaminated soil was conducted following the American Society of Testing and Materials (ASTM) method D 422 (ASTM, 1994).

3.5 Experimental Phase of Study

3.5.1 Soil Pan Testing

The growth of bacteria in the presence of petroleum products, although a good indicator, is insufficient to demonstrate a rate of hydrocarbon degradation with indigenous bacteria. To confirm a degradation rate, it is necessary to monitor the reduction of TPH concentration in soil over time. This phase of the study investigated the hydrocarbon degradation rate through soil pan testing. One control cell designated EX-1 and three experimental cells designated EX-2, EX-3 and EX-4 were established by placing 3 kg $\pm$ 0.001 kg of the hydrocarbon contaminated soil in each pan. The dimensions of each pan were 40.2 (L) x 28.8 (W) x 7.7 cm (H). A schematic of the experimental set-up is shown in Figure 3.2. In summary, the control and experimental cells had the following properties:

- EX-1 Sodium azide (NaN_3) solution (25 g purified NaN_3 mixed in 100 mL of water) was added to the soil in the control cell (EX-1) to suppress microbial activity. The initial TPH concentration was estimated to be 2,500 ppm (Section 3.5.4).
- EX-2 The first experimental cell (EX-2) was used to investigate the indigenous microbial activity in the absence of nutrient amendments. The initial TPH concentration was estimated to be 2,500 ppm (Section 3.5.4).
- EX-3 The second experimental cell (EX-3) was used to investigate the indigenous microbial activity in the presence of higher concentrations of TPH and nutrient amendment (Section 3.5.2). The initial TPH concentration was estimated to be 10,000 ppm (Section 3.5.4).

Figure 3.2: Experimental Setup

EX-4 The third experimental cell (EX-4) was used to investigate the second experimental cell (EX-3) in the absence of nutrient amendment. The initial TPH concentration was estimated to be 10,000 ppm (Section 3.5.4).

The experimental phase of the study commenced on December 18, 1996 and was conducted for a duration of 17 weeks.

3.5.2 Determination of Soil Nutrients and Nutrient Amendment of Cell EX-3

A sub-sample of the contaminated soil was analyzed for determination of soil nutrients at the commencement of the experiment. The parameters included available nitrate - nitrogen, available phosphate - phosphorous and available potassium and determination followed McKeague (1978b, 1978c and 1978d) methods 4.34, 4.43 and 4.51 respectively.

The determination of available nitrate - nitrogen consisted of preparing a 1:1 soil:water suspension by adding 20 mL of distilled water to 20 g of air dry soil in a suitable container and then stirring intermittently for 30 minutes. A standard nitrate solution was prepared. The standard and sample were read with a pH meter with nitrate-calomel electrode assembly. The electrode tips were rinsed carefully after each reading. A graph of mV vs. ppm N was prepared and $\text{NO}_3\text{-N}$ in soil was obtained directly from the graph.

The determination of available phosphate - phosphorous consisted of measuring approximately 1.0 g of air dry soil into a 50 mL Ehrlenmeyer flask. Seven mL of extracting solution was added and the suspension was shaken for 40 seconds on a mechanical shaker. The solution was allowed to settle for several minutes or filtered. Available phosphate -

phosphorous was then determined by colourimetric methods (i.e. titration with appropriate reagents).

Available potassium was determined by displacing the ions from soil colloids with NH_4 . This was achieved by shaking the soil with 1 M NH_4OAc adjusted to pH 7.0. The filtered extract was analyzed for potassium using emission spectrophotometry.

Commercial fertilizer (Esso Engro™ 15-30-10 granular fertilizer) was added to experimental cell EX-3 to increase the available nitrate - nitrogen levels to above 50 $\mu\text{g/g}$ but no more than 100 $\mu\text{g/g}$. If the inorganic N levels are above 50 $\mu\text{g/g}$, no additional fertilizers need to be added (Huesemann, 1994). The granular fertilizer was mixed by hand into the soil and eventually dissolved by maintenance of moisture content (Section 3.5.4).

3.5.3 Enumeration of Soil Microorganisms

Each experimental cell was sampled for microbial enumeration at the commencement of the experiment, at week 9 and at the conclusion of the experiment. This was conducted only as a periodic check to confirm microbial presence in the experimental cells. Enumeration values were not used in any specific analysis. Microbial characterization consisted of heterotrophic plate counts as described in Section 3.4.1.

3.5.4 Monitoring of TPH Concentrations, Moisture Content and pH

Initial characterization of the contaminated soil described in Section 3.3 consisted of determination of: benzene, toluene, ethyl benzene and xylenes (BTEX); total volatile hydrocarbons (TVH); total semi-volatile hydrocarbons (TSH); lead (Pb); pH and moisture

content. The procedure for determination of BTEX, TVH and TSH followed the United States Environmental Protection Agency (USEPA) *Test Method for Evaluating Solid Waste* methods 3810/8020 and 3550 Modified (USEPA, 1986a and 1986b), (APHA, 1994), (AEC, 1992a and 1992b). The procedure for determination of lead (Pb) followed the USEPA *Test Method for Evaluating Solid Waste* method 3050/6010 (USEPA, 1986c). The procedure for determination of pH followed the McKeague (1978a) method 4.12. Briefly, the pH of the soil was determined by weighing 25 g of soil into an Ehrlenmeyer flask, adding 25 mL of distilled water and shaking the mixture for 30 minutes on a reciprocating shaker. The pH of the suspension was measured using a pH meter.

The EPA methods 3810/8020 for BTEX and TVH involved headspace extraction with a gas chromatograph/photo ionization detector/flame ionization detector (GC/PID/FID) system. The volatile BTEX and TVHs with low water solubility are volatilized from an aqueous matrix sample and are analyzed by Gas Chromatography/Photoionization detector then Flame Ionization detector. Identification and quantification of the compounds in the samples were achieved based on retention time and response factor of each compound in the standards analyzed under identical instrumental conditions.

The EPA method 3550, upon which the bulk of the study analytical results were obtained is described below.

Lead content, (EPA method 3050/6010), was measured by the inductively coupled plasma (ICP) method after acid digestion of the sample. The digested sample is nebulized and the resulting aerosol is transported to the plasma torch. Element -specific atomic-line emission spectra are produced by a radio-frequency ICP.

The analytical results for the hydrocarbon contaminated soil were as follows: benzene (0.024 $\mu\text{g/g}$); toluene (<0.005 $\mu\text{g/g}$); ethyl benzene (0.14 $\mu\text{g/g}$); xylenes (0.54 $\mu\text{g/g}$); TVH (1.7 $\mu\text{g/g}$); TSH (700 $\mu\text{g/g}$); lead (Pb) (24.5 $\mu\text{g/g}$); moisture (28.2 %); and pH (7.16). The resultant TPH value (TVH + TSH = 702 $\mu\text{g/g}$) for the sample was deemed to be low and therefore soil placed in experimental cells for the experimental phase of the study were spiked with fresh diesel fuel to several estimated concentration ranges.

Nine grams by weight of fresh diesel fuel (obtained from local commercial source) was added to experimental cells EX-1 and EX-2. This would theoretically yield a concentration of 3,000 mg/kg (ppm) of diesel fuel in soil. A conversion factor of 0.83 was used to estimate the initial TPH concentration in the cells (ie. 2,500 ppm). This conversion factor was estimated from the chromatograms of the diesel fuel. Review of the chromatograms (at varying concentrations) showed the presence of approximately 20 % volatile (< C_9) and 80% semi-volatile hydrocarbon compounds (C_9 - C_{30}). Thirty-seven and a half (37.5) grams by weight of the fresh diesel fuel was added to experimental cells EX-3 and EX-4. This would theoretically yield a concentration of 12,500 mg/kg (ppm) of diesel fuel to soil. Utilizing the conversion factor noted above, the initial TSH concentration in the cells was estimated to be 10,000 ppm.

The procedure for determination of TSH, (EPA method 3550), utilizes a known weight of soil sample that is mixed with methylene chloride in an ultra sonic bath. The extract is decanted, dried and concentrated. Gas chromatography is used for detection and quantification. The detailed procedure for determination of TSH was as follows. First, the moisture content of each sample was determined. For most analytical procedures involving

soil analysis, the results are reported on a dry weight basis. Since much of the analysis was conducted on a wet sample, a moisture correction was necessary.

The appropriate number of labelled aluminum pans were weighed out to 0.01 g. Approximately 10 to 30 g of soil were placed in each pan and the pans were reweighed to 0.01 g. The pans were placed in a drying oven set at 105 ± 5 °C overnight. The samples were cooled and reweighed for moisture loss. The moisture content expressed as a percentage is given by:

$$\%moisture = \frac{W_w - W_d}{W_d} \quad (3.1)$$

where,

W_w = weight of wet sample and pan (g)

W_d = weight of dry sample and pan (g)

The extraction procedure consisted of the following. Three or more core samples of soil were removed from the sample jar using a 1 mL plastic syringe. The tip of the syringe was cut to create a coring device which was pushed into the soil. The soil core was then extracted using the syringe plunger. Approximately 2 g of soil were transferred into a 25 mL vial. The soil was mixed with anhydrous NaSO_4 to absorb water and break up the clay. About 20 mL of methylene chloride and 10 μL of the appropriate surrogate standard were added and the vial capped with a Teflon screw cap. The vial was placed in an ultra sonic bath and sonicated for 20 minutes.

The contents of the vial were filtered through #4 Whatman filter paper, with anhydrous sodium sulphate crystals in the bottom of the filter cone. The filtration was conducted into a Micro Kuderna-Danish concentrator with a 4 mL vial attached. The residue on the filter paper and the 25 mL were rinsed a few times with dichloromethane. The funnel was removed from the filtering apparatus and placed in a water bath set between 80 to 90 C. The filtrate was allowed to evaporate until the volume was 1 mL.

The extract volume was made up with dichloromethane to 1 mL. The extract was then transferred to a 1 mL autosampler vial for analysis by a Varian 3400 Gas Chromatograph equipped with an FID detector. The individual compounds were identified by retention time and quantified according to the response factor of the nearest standard component.

The sum of the peak areas of the sample were obtained from the chromatogram and compared to the corresponding value of the standard.

Each experimental cell was sampled for determination of TPH concentration at weeks 3, 5, 9, 13 and 17 of the experiment. The time frequency of sampling was increased from an initial value of every 2 weeks to 4 weeks to ensure that the final TPH concentrations fell below the regulatory objective within the budget constraints of the experimental work. The determination of TPH concentration followed that as described above. Moisture content was determined in conjunction with the TPH analysis. pH was determined at weeks 9 and 17. The pH of soil in each cell was expected to remain stable and, therefore, was only measured at the commencement, midpoint, and cessation of the experiment. The determination of moisture content and pH followed the procedure described previously.

Moisture content of the soil was maintained by adding 140 g of distilled water to each pan every second day for the duration of the experiment. The amount of water was adjusted as soil was removed from the experimental cells during sampling.

4.0 RESULTS AND DISCUSSION

4.1 Initial Microbial Characterization of Soil

The heterotrophic plate count results for the two samples of the contaminated soil were 1.3×10^7 and 1.4×10^7 cfu/g. The results for the two samples of the land treatment site soil were 6.5×10^6 and 3.6×10^6 cfu/g. It was interesting to note the higher population (by order of magnitude) of microorganisms in the contaminated soil versus the land treatment site soil. It is likely that the presence of diesel fuel (i.e. carbon source) contributed to the higher microbial enumeration.

4.2 Microbial Hydrocarbon Utilization Screen

4.2.1 Pour Plate Method Results

The plate count results for the two samples of the contaminated soil were 6.7×10^4 and 8.3×10^4 cfu/g. The results for the two samples of the land treatment site soil were 4.5×10^5 and 1.6×10^5 cfu/g. It was interesting to note the higher population (by order of magnitude) of microorganisms in the land treatment site soil versus the contaminated soil in this variation of the pour plate method. These results suggest, semi-quantitatively, an affinity of soil microbes not accustomed to the presence of diesel fuel to exhibit higher microbial enumeration than the soil microbes from the contaminated soil sample.

4.2.2 MPN Method Results

The MPN values for the two samples of the contaminated soil were 5.4×10^4 and 3.5×10^4 MPN/g. The results for the two samples of the land treatment site soil were 1.6×10^5

and 1.6×10^4 MPN/g. It was interesting to note the lower population of microorganisms in this variation versus the pour plate method variation. The lower value for the MPN method may indicate a lower affinity of the soil microbes to exist in the liquid phase versus the solid phase.

4.3 Physical Characterization of Contaminated Soil

The laboratory visual classification of the soil was a CH-CI clay following the Unified Classification System (ASTM method D 2487-85 (ASTM, 1994). Descriptors of the soil included: clay and shales (various colors), some silt, trace sand, moist, stiff, pieces of coal. The hydrometer grain size analysis yielded D_{60} and D_{10} values of 0.034 mm and approximately 0.0005 mm, respectively. The resultant coefficient of uniformity, C_u , was 68 indicating a well-graded soil in the clay size particle range.

4.4 Initial Nutrient Concentrations of Contaminated Soil

The analytical results for the hydrocarbon contaminated soil were as follows: available nitrate - nitrogen ($2.5 \mu\text{g/g}$); available phosphate - phosphorous ($2.9 \mu\text{g/g}$); and available potassium ($371 \mu\text{g/g}$).

4.5 Experimental Phase of Study

4.5.1 Microbial Characterization of Soils

The results of microbial characterization of soil in each experimental cell are shown in Table 4.1. Generally, there was an increase in microbial population for experimental cells

EX-2, EX-3 and EX-4. The results for the control cell (EX-1) indicate that microbial activity was still present but at a reduced level. This shows that a complete sterilization could probably not be achieved in EX-1.

Table 4.1: Heterotrophic Plate Counts for Soil in Experimental Cells

TIME (WEEKS)	DATE	EX-1	EX-2	EX-3	EX-4
0	96/12/18	1.5×10^7	1.5×10^7	1.5×10^7	1.5×10^7
9	97/02/19	1.8×10^5	2.3×10^7	5.0×10^7	4.3×10^7
17	97/04/16	1.0×10^5	3.1×10^7	3.2×10^7	4.3×10^7

Units reported in cfu/g except for time and date.

4.5.2 TSH Concentrations, Moisture Content and pH

The TPH concentration and pH of soil in each experimental cell are shown in Table 4.2. The TPH versus time plots for each experimental cell are shown in Figure 4.1. The use of sodium azide to suppress the biodegradation of diesel to validate the testing results (control cell) was somewhat successful and duplicate analysis of TPH in each cell at week 9 revealed the inherent limitations in soil sampling and analysis variability, even in the bench-scale methods employed. The control cell (EX-1) was compared with an experimental cell of 2500 ppm initial concentration of TPH without nutrient amendment (EX-2). The control cell (EX-1) exhibited a reduction in TPH content over the duration of the experiment from 2,500 ppm to 950 ppm. The experimental cell (EX-2) exhibited a considerable decrease of TPH concentration from 2,500 ppm to 750 ppm from the commencement of the

Table 4.2: TPH Concentration, Moisture Content and pH values for Soil in Experimental Cells

TIME	DATE	EX-1			EX-2			EX-3			EX-4		
		TPH	Moisture	pH	TPH	Moisture	pH	TPH	Moisture	pH	TPH	Moisture	pH
0	96/12/18	2,500*	-	-	2,500*	-	-	10,000*	-	-	10,000*	-	-
3	97/01/08	2,000	21.4	-	750	25.4	-	6,700	22.1	-	4,800	26.0	-
5	97/01/22	2,000	21.0	-	740	24.7	-	3,500	23.2	-	9,700	18.0	-
9	97/02/19	1,500	18.8	9.14	840	16.7	8.18	1,800	21.5	8.22	5,200	19.4	8.24
		(1,000)	(18.8)	-	(780)	(16.7)	-	(3,800)	(21.5)	-	(5,500)	(19.4)	-
13	97/03/19	570	22.1	-	380	19.3	-	990	23.9	-	3,000	19.3	-
17	97/04/16	950	17.1	8.71	720	15.3	8.35	360	15.6	7.81	4,200	16.7	8.27

Notes: * denotes estimated concentration (Section 3.5.4)
 () denotes duplicate analysis
 Units for TPH = ppm
 Moisture Content expressed as %
 Units for pH = pH units

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Figure 4.1: TPH versus Time for Each Experimental Cell

experiment to the third week respectively. Thereafter, this cell exhibited variable concentrations of TPH in the range of 380 to 840 ppm. It is likely that this experimental cell simulated TPH degradation as a substrate limiting condition.

The greatest reduction of TPH was obtained with the addition of nutrients (EX-3). The corresponding reduction in TPH was not as great in the cell without nutrient amendment (EX-4). Furthermore, the TPH concentrations for this cell exhibited considerable variability over the duration of the experiment as evidenced by the measured TPH concentrations.

4.5.3 Calculation of Rate Constants

Values of $\ln (C/C_0)$ for each cell were plotted versus time and are shown in Figure 4.2 (EX-1 and EX-2) and Figure 4.3 (EX-3 and EX-4). The “best fit” lines for the data are also shown in the figures. The first order degradation rate constants were calculated in accordance with the following equation:

$$\ln \frac{C}{C_0} = -k \cdot t \quad (4.1)$$

where,

- C_0 = initial contaminant concentration (ppm)
- C = contaminant concentration at time t (ppm)
- k = first order degradation rate constant (week^{-1})
- t = time (weeks)

Figure 4.2: Degradation Rate Constants (k) for EX-1 and EX-2

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Figure 4.3: Degradation Rate Constants (k) for EX-3 and EX-4

The correlation coefficients obtained using the data were not high with the exception of experimental cell EX-3 (Table 4.3). There was excellent correlation for the control cell and experimental cell EX-3 at the 5 percent level of significance. There was poor correlation for experimental cells EX-2 and EX-4 at the 5 percent level of significance. Low correlation, however, is a characteristic of biological systems and need not always be construed as a poor curve fit because of the inherent difficulties associated with the use of any kinetic equations to growth of microorganisms. It is also well established that the metabolic activity of microorganisms may vary even under controlled conditions.

Table 4.3: Regression Statistics for Calculation of Rate Constants

	EX-1 (Control)	EX-2	EX-3	EX-4
Constant	0.035	-0.618	0.044	0.056
Std Err of Y Est	0.320	0.515	0.130	0.301
r^2	0.742	0.436	0.991	0.756
r	0.861	0.660	0.995	0.869
No. of Observations	6	6	6	5
Degrees of Freedom	4	4	4	3
k (week ⁻¹)	0.08	0.06	0.19	0.07
Std. Err of Coef.	0.022	0.036	0.009	0.023
$t_{v=4, \alpha=0.05}$	0.811	0.811	0.811	-
$t_{v=3, \alpha=0.05}$	-	-	-	0.878

The rate constants (k) and the time (t) to reach the regulatory objective of 1,000 ppm are summarized in Table 4.4.

Table 4.4: Summary of Rate Constant (k) and Time to Reach Regulatory Objective (t)

	EX-1 (Control)	EX-2	EX-3	EX-4
k (week ⁻¹)	0.08	0.06	0.19	0.07
t (weeks)	12	15	12	33

The control cell (EX-1) exhibited a relatively low uniform degradation ($k = 0.08$ week⁻¹) of TPH over the duration of the experiment with reasonable first-order kinetic regression statistics. The experimental cell (EX-2) exhibited a considerable decrease of TPH concentration from 2500 ppm to 750 ppm from the commencement of the experiment to week three respectively. Thereafter, this cell exhibited variable concentrations of TPH in the range of 380 to 840 ppm and poor first-order kinetic regression statistics.

The greatest degradation of the diesel contaminated soil was obtained with the addition of nutrients ($C_0 = 10,000$ ppm of TPH). A degradation rate constant (k) of 0.19 week⁻¹ was calculated from the experimental data for this treatment condition ($C = 360$ ppm of TPH; $t = 12.1$ weeks). The corresponding degradation rate constant (k) for soil not amended with nutrients was calculated as 0.07 week⁻¹; a 63% reduction. This treatment condition simulation would require 32.9 weeks to reach the regulatory objective of 1,000 ppm TPH.

First order degradation rate constants for TPH are limited in the literature. The rate constants reported in the literature were for specific organic compounds (e.g. benzene, PAH compounds, etc.). However, the kinetic constants reported by Saberiyan *et. al.* (1995), Demque (1994), and Demque *et. al.* (1997) were considered. The values from their studies and the results from this study are compared in Table 4.5.

Table 4.5: Summary of Study Results (k) and Results from Literature

Saberriyan <i>et. al.</i> (1995)							
	#1	#2	#5				
k	-0.46	-0.39	-0.49				
Demque <i>et. al.</i> (1997)							
	Untreated			Biostimulated/augmented			Undrained
	a	b	c	a	b	c	c
k	-0.28	-0.28	-0.25	-0.43	-0.51	-0.49	-0.69
Sudy Results							
	EX-3						
k	-0.19						

Notes: a - no tillage

b - monthly tillage

c - semimonthly tillage

The kinetic constant obtained for experimental cell EX-3 was lower than those reported by Saberriyan *et. al.* (1995) using respirometric techniques (i.e. 0.4-0.5 week⁻¹). This may be attributable to the clay soil used in the experiment. The presence of clay sized particles would likely inhibit the movement of oxygen, moisture and nutrients even through constant simulation of tilling of the soil by hand. It is believed that the respirometric technique provides a more accurate representation of microbial activity and directly relates oxygen consumed by the cells to the kinetic constants. In classical wastewater treatability studies, reduction in substrate and biomass growth rates are generally measured analytically. Some of the reductions may be due to other mechanisms. In the respirometric technique,

however, the kinetics are derived based only on oxygen consumed by the microorganism, which is a direct representation of the biodegradation process.

The kinetic constant obtained for experimental cell EX-3 was also lower than those reported by Demque *et. al.* (1997) in field trials. However, it should be noted that the soil used in those experiments was sand. Sand sized particles are less likely to inhibit the movement of oxygen, moisture and nutrients than clay sized particles.

The rate constants for TPH reported by Saberiyan *et. al.* were obtained using the respirometric techniques described in Section 2.7.1. This method is considered to be considerably more sophisticated than that of soil pan testing. The rate constants for TPH reported by Demque (1994) and Demque *et. al.* (1997) were obtained through field studies (i.e. pilot scale studies) and involved a level of sophistication greater than that provided herein. Nonetheless, these data provide a reasonable comparison for the study results.

4.5.4 Application of Research Results

This study has confirmed that the greatest degradation of the diesel contaminated soil was obtained with the addition of nutrients. Acceleration of the land treatment process would reduce monitoring and associated costs of the management of a contaminated soil. The bench-scale methods employed in this study reveal the relative ease at which degradation rate constants can be estimated. The methods used in this study can also be applied to a wide range of contaminated soil situations (i.e PCBs, PAHs, herbicides/pesticides etc.).

This study has also confirmed that degradation of the diesel contaminated soil will also take place even without the addition of nutrients. However, a lower degradation rate

constant and greater time to treat the contaminated soil would result. This type of treatment simulation could be used to demonstrate the natural attenuation mechanisms in a variety of situations.

5.0 CONCLUSIONS

The principal conclusions that can be drawn from the study are summarized below:

- 1) The greatest degradation of the diesel contaminated soil was obtained with the addition of nutrients ($C_0 = 10,000$ ppm of TPH). A degradation rate constant (k) of 0.19 week^{-1} was calculated from the experimental data for this treatment condition ($C = 360$ ppm of TPH; $t = 11.5$ weeks). The corresponding degradation rate constant (k) for soil not amended with nutrients was calculated as 0.07 week^{-1} ; a 63% reduction. This condition would require 33 weeks to reach the regulatory objective of 1,000 ppm.
- 2) The control cell ($C_0 = 2,500$ ppm of TPH) exhibited a relatively low uniform degradation of TPH over the duration of the experiment. Experimental cell EX-2 ($C_0 = 2,500$ ppm of TPH without microbial suppressant or nutrient amendment) exhibited a considerable decrease of TPH concentration from 2500 ppm to 750 ppm from the commencement of the experiment to week 3 respectively. Thereafter, this cell exhibited variable concentrations of TPH in the range of 380 to 840 ppm. It is likely that this experimental cell simulated TPH degradation as a substrate limiting condition.

- 3) The use of sodium azide to suppress the biodegradation of diesel to validate the testing results (control cell) was ineffective and duplicate analysis of TPH in each cell at week 9 revealed the inherent limitations in soil sampling and analysis variability, even in the bench-scale methods employed.

5.1 Recommendations for Further Research

This study has revealed the effectiveness of the various land treatment methods. A review of the wealth of literature on the subject shows that a vast knowledge base exists. In order to maintain optimal biodegradation conditions and monitor the land treatment process, environmental parameters including contaminant concentration, moisture, nutrients and pH should be determined periodically.

The following recommendations are made for further research:

- 1) Bench-scale tests should be designed and constructed to monitor reduction in TPH due to volatilization alone.
- 2) Bench-scale tests should be designed and constructed to isolate the degradation rates due to bioremediation by more effective sterilization techniques. Possible agents include formaldehyde, mercuric chloride, and sulphuric acid. Preferably, the techniques would not effect TPH testing during the studies.

- 3) The degradation rates of soil contaminated with higher concentrations of diesel fuel should be studied. Higher concentrations of diesel fuel may have a toxic effect on indigenous microorganisms, which may cause a reduction in microorganism activity.
- 4) The use of microbial aggressivity techniques (e.g. BART™ biodeetector systems developed by Droycon Bioconcepts Inc., Regina, Canada) in land treatment should be investigated. Two forms of data can be obtained by this method; they are days of delay (*dd*) and the reaction type (RX) which occurs. It can be interpreted that the shorter the *dd* the greater the microorganism population size and activity level. The RX obtained gives an indication of the types of microorganisms present. These systems may provide greater flexibility in the field rather than the cumbersome traditional techniques such as the plate count method. The investigation should establish the correlation, if any, between *dd* and the reduction of TPH.

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