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Biological Treatability of Flare Pit Waste

by

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ABSTRACT

A laboratory study is developed and undertaken to assess the applicability of biological methods of flare pit remediation, based the regulatory limit of 1000 mg/kg Mineral Oil and Grease. Slurry-phase biological treatment is selected for screening level experiments investigating the effects of inoculation, petroleum spiked soils, salinity, and petroleum composition (by component classes of saturates, aromatics, and polar compounds) on the rate and extent of biodegradation. With the removal of other limiting factors, the extent of petroleum biodegradation in the slurry-phase is found to depend on hydrocarbon composition, in a manner similar to that demonstrated in the literature for solid- phase treatments. Numerical estimates of the biodegradability achievable for flare pit sites are developed from literature data on the composition of Alberta crude oils and the biodegradabilities of component classes. When these estimates are applied to contaminant levels from 436 sites, roughly 15% of sites had levels of contamination exceeding the 1000 mg/kg regulatory limit, that might be reduced to that concentration by bioremediation.

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LIST OF ABBREVIATIONS

AEUB	Alberta Energy and Utilities Board
AEP	Alberta Environmental Protection
ANOVA	Analysis of Variance
API	American Petroleum Institute
AUGFWM	Alberta User Guide for Waste Managers
BTEX	Benzene, Toluene, Ethylbenzene and Xylene
CCME	Canadian Council of Ministers for the Environment
CFU	Colony Forming Units
DCM	Dichloromethane (CH ₂ Cl ₂)
DOUR	Dissolved Oxygen Uptake Rate
EC	Electrical Conductivity
FDMS	Field Desorption Mass Spectrometry
GC-FID	Gas Chromatograph with Flame Ionization Detector
GC/MS	Gas Chromatograph Mass Spectrometry
GRI	Gas Research Institute
kg	Kilograms
ln	Natural logarithm
meq	Milliequivalents
mg	Milligrams
min	Minutes
mi	Minutes (in GC-FID chromatograms)
mL	Milliliters
MOG	Mineral Oil and Grease
pA	PicoAmps
PAH	Polynuclear Aromatic Hydrocarbons
pH	Hydrogen ion activity
ppm	Parts per million (by mass)
O&G	Oil and Grease

S	Siemens
SAR	Sodium Adsorption Ratio
SQG	Soil Quality Guidelines (of CCME)
TPH	Total Petroleum Hydrocarbons
UCM	Unresolved Complex Mixture
WCR	Waste Control Regulation
z	Hydrogen deficiency number

1.0 INTRODUCTION

1.1 The Flare Pit Problem

Flare pits are earthworks associated with oil and gas production in Alberta usually constructed as unlined pits with berms made from excavated soil. The chief purpose of these pits' construction was to provide an area to burn unwanted gas, or to store and/or burn produced fluids. The produced fluids disposed of in these pits can contain liquid hydrocarbons, process chemicals, crude bitumen, and formation brine. Contaminated soils at these sites thus present a complex combination of petroleum, metal, and salt contamination. Site contamination may be further complicated by the application of soil sterilants as a fire safety precaution to prevent growth of vegetation, or by use of flare pits as a site for disposal of waste from production operations. The lack of pit liners to control contaminant migration means these sites can pose significant risks to the environment and human health.

As of 1996, produced fluids may no longer be discharged to earthen pits in Alberta (AEUB, 1994a), and no new pits may be constructed (AEUB, 1996). Nonetheless, the past practice of using flare pits has left a significant number of contaminated sites. It is estimated that there are about 30,000 flare pit sites in Alberta (Speer, 1999). Remediation and decommissioning of these sites presents a challenge to the oil and gas industry.

1.2 Research Programme

The primary goal of the flare pit research programme is to develop a workable strategy to effectively minimize environmental and human health risks associated with flare pit waste in Alberta, and in the process, ensure that the upstream oil and gas industry has the tools to comply with the regulations laid out by Alberta Environmental Protection (AEP) and the Alberta Energy and Utilities Board (AEUB). It was intended to approach this objective through the development of a bioremediation technique specifically tailored to flare pit sites.

Such research must address challenges particular to these sites: the complex mixture of contaminants encountered, their variability from site to site, the need for a rapid treatment technique due to Alberta's short summer season, and the economics of developing cost-effective site-management technology for the relatively small volumes of contaminated soils encountered at these sites.

The variability of flare pit contamination is addressed in the first stage of this programme, with the development of a flare pit characterization database (Speer, 1999). The purpose of this is to identify the extent of characterization data available for these sites, and to categorize sites according to contamination. Categorization and treatability testing based on this database is seen as a necessary response to the variability of contamination between flare pit sites, where a treatment demonstrated for a particular site may not necessarily be representative of others.

The second stage of the research programme is the evaluation of the suitability of biological treatment for these sites. This thesis represents the research conducted in this second phase. Screening level slurry-phase biological treatment was applied to assess the effect of contaminant parameters (as identified by the database) on the achievable biodegradation of petroleum contamination.

1.3 The Treatability Approach

1.3.1 Objective

The objective of this project was to identify what portion of Alberta's flare pits are feasibly treatable using biological methods. This requires identifying the contaminant factors that will limit biodegradation at Alberta sites, separate from external factors that might be controlled by a bioremediation technique or technology. A means of predicting the ultimate biodegradability of the petroleum contaminant is sought, and the possible inhibition of bioremediation due to additional contamination such as salt, metal, or soil sterilants, must be

considered.

1.3.2 Purpose of Treatability Testing

Treatability testing is conducted prior to full-scale application of a bioremediation method in order to assess the expected outcome of treatment. The objective of treatability testing is to save money in the long run by identifying potential problems, quantifying operation parameters, and allowing for the comparison of alternative treatments prior to full scale application. Treatability testing prior to full-scale application is especially beneficial for flare pit bioremediation. Contamination of composting bulking material or landfarming soil by flare pit material entails a high cost of error if the treatment fails, producing more contaminated material for disposal.

Treatability testing for flare pit contamination must account for the fact that such contamination is highly variable from site to site. It is preferred to develop treatability generalizations that apply to all flare pit sites, to avoid the expense of site-by-site testing. Therefore, the research conducted in this treatability programme is directed at explaining the effect of differences in contamination, and developing generalizations about treatability that can be applied to all flare pit sites.

Case studies of bioremediation applied to flare pit waste and to petroleum contamination in general provide much preliminary information on the extent of treatability to expect, and the types of problems to be encountered in treatment. These are discussed in the literature review.

1.3.3. Overall Approach

The objective of the treatability approach was addressed by applying a slurry-phase screening study, with an aim towards developing treatability predictions based on site information available from the characterization database. Parameters of high significance (those with

many database entries) were emphasized in the experimental approach, and were considered for their effect on both the rate and extent of biodegradation. The rate of biodegradation is of concern for the development of a technology that can achieve the desired treatment within a limited time frame. The extent of biodegradation achieved determines whether the treatment can meet regulatory criteria.

For the purposes of laboratory study, slurry treatment offered close control of process variables as a means of identifying and assessing limiting factors. With mass-transfer limitations removed, treatment achieved completion in about 10 days. This fast treatment offers the potential of conducting a large number of designed experiments directed at developing scientific understanding of the primary and interaction effects of contaminant parameters. This approach is also applicable to the study of external environmental parameters such as variations in process temperature and nutrient supply, which may be optimized to improve the extent of biodegradation attainable.

For this project, the experimental programme began with simple trial runs. Each stage of experimentation involved further literature review as different aspects were found to be of importance. The slurry treatment was observed to achieve quick contaminant reductions, but was ultimately limited by the biodegradability of the petroleum contaminant. A synthesis of experimental results, database data and information in the literature was applied to develop an estimate of the prospects for bioremediation. This estimate is given in the discussion section of this thesis.

2.0 LITERATURE REVIEW

2.1 General Description of Flare Pit Sites

Contaminated soils at these sites can present a complex combination of petroleum, metal, salt, and other contamination. The composition of this contamination varies from site to site. This project considers the applicability of biological treatment methods for remediating flare pit sites. These methods are not able to address the metal and salt contamination at flare pit sites, but are applicable for multiple-treatment approaches for mixed contamination. Common to all treatment alternatives to be considered is the cost of treating small amounts of contaminated soil often at remote sites, and the need to achieve reasonable treatment endpoints quickly.

Bioremediation is considered for treatment of flare pit waste in light of successes in similar fields, but has received mixed reviews from lab studies and field experience. Preceding studies (CAPP, 1989, 1994) evaluated technologies on the basis of whether or not they are applicable to all sites, and did not find bioremediation to be a suitable alternative to physical or chemical treatment methods such as solidification or thermal desorption. This was based on the time required for treatment, which for bioremediation exceeded the three-month treatment screening criteria applied in the evaluation. With the development of a characterization database, it becomes possible to consider different categories of sites, based on contamination. Treatability testing using this decision- making tool allows the identification of sites suitable for treatment.

2.2 Remediation Objectives

For this project, the criteria for treatability were taken to be the generic criteria applicable to all land-uses, as given by Alberta Tier I criteria (AEP 1994). For petroleum contamination, the maximum level of residual petroleum allowable is 1000mg/kg, measured as Mineral Oil and Grease. In part because of the practical difficulty in achieving the 1000 mg/kg

requirement, industry favors a move away from generic criteria, towards risk-based criteria based on human health and ecological endpoints.

Tier 2 criteria are determined by the operator of the flare pit on a site specific basis, and are presented to AEP. The criteria are determined using risk analysis to demonstrate that the proposed criteria provide sufficient protection for the proposed future land use of the site. A typical risk-based approach involves two levels of analysis: Level 1 involves screening (assessing whether or not the contaminant is hazardous). Level 2 involves fate and transport modeling (assessing whether exposure to the contaminant will occur for human or ecological receptors). Such an approach requires definition of future site use, definition of receptors, and contaminants on a site-by site basis. Usually individual compounds and receptors are used as surrogates for a broad range of compounds and organisms, to simplify modeling. For the time being, risk-based remediation objectives present more expensive characterization requirements, but are possibly more cost-effective if the most suitable and achievable treatment can be identified (Moyer and Parsons, 1998).

New criteria based on ecological toxicity parameters may arise from the development of suitable methods of ecological risk-based characterization. If sufficient characterization data is developed for Alberta sites using these criteria, treatability may be reassessed in a manner similar to that by which it is addressed here. Whether the objective of bioremediation is risk reduction instead of contaminant mass reduction, it is expected that many of the principles developed will be the same.

2.3 Characterization Database

The flare pit characterization database prepared by Jennifer Speer was compiled from industry data for 436 flare pits, with multiple entries for some sites resulting in 616 entries (Speer, 1999). The sites included all major oil and gas producing regions of Alberta, as well as some from British Columbia and Saskatchewan. Entries represent both oil and gas

production, and sour (H_2S) and sweet (no H_2S) locations. Flare pits associated with batteries, wells, satellites, and gas plants were all represented. From visual and mathematical comparison of the database to a larger existing wellsite database and several randomly generated data sets, the flare pit characterization database entries were deemed to be representative of the total population (Speer, 1999).

The extent of characterization for these sites varied from a simple LSD site location entry to extensive physical, chemical and biological characterization of the contaminated site. A total of 210 parameters were considered between the various entries. By retaining only those parameters that were available for a significant number of site entries ($>15\%$), the number of parameters in the database was reduced to 48. These included general description data, such as pit use, soil type, and location (precise location was eliminated from the report to offer confidentiality to organizations which contributed data), as well as chemical composition regarding metals, hydrocarbons, soil sterilants and salinity (Speer, 1999).

These different potential contaminants are discussed in the sections that follow. Low levels of contamination, below which contamination was deemed to be of no concern, were established from provincial and federal designations for soil acceptable for all uses (Alberta Tier 1 and The Canadian Council of Ministers of the Environment Soil Quality Guidelines (CCME SQG) (AEP, 1994; CCME, 1997). High levels for some contaminants are based on Alberta Environmental Protection's Waste Control Regulation (WCR) and the Alberta User Guide for Waste Managers (AUGFWM) limits for landfill disposal, above which the contaminated soil is considered a hazardous material, limiting disposal options (AEP, 1993; AEP, 1995).

2.4 BTEX

BTEX contamination consists of benzene, toluene, ethylbenzene, and xylene. These compounds are known to be highly hazardous, both by acute and chronic toxicity. Benzene is

a known carcinogen (LaGrega *et al.*, 1994). Because their physical and toxicological properties have been extensively studied, they are often considered as surrogate compounds in risk assessment analysis. For flare pit sites, high BTEX levels can be taken as an indication of the presence of similar highly mobile low molecular weight petroleum compounds.

From a regulatory perspective, the four BTEX compounds are considered individually as well as collectively. Low levels from Alberta tier 1 and high levels from AUGFWM are listed in Table 1.

Table 1: BTEX Regulatory Limits and Available Data

	Regulatory limits	
	low (mg/kg)	high (mg/kg)
Benzene	0.05	1000
Toluene	0.1	1000
Ethyl Benzene	0.5	1000
Xylene	0.1	1000

(AEP, 1994; AEP,1993; AEP,1995)

These compounds are also considered to be a hazard if total BTEX exceeds 1000 mg/kg.

The BTEX compounds are known to be readily biodegradable by bacteria, and possibly by soil fungi as well (Cookson, 1995). Their volatility presents some challenges to treatment, as it is difficult to ensure that reductions are due to biodegradation rather than volatilization, which simply turns the soil pollutant to an air pollutant. Similarly, volatilization will affect the precision of the characterization data over time. (Cookson, 1995).

2.5 Petroleum

2.5.1 Petroleum: Contamination Concerns

Petroleum is a mixture of thousands of organic compounds, many of them known or suspected carcinogens (La Grega *et al*, 1994). Petroleum in soil is undesirable due to its potential toxicity, to both humans and other organisms, its inflammability, aesthetic nuisance, and its reduction of the ability of soil to hold moisture.

2.5.1.1 Means of Defining the Extent of Contamination

The most common measure of petroleum is a bulk gravimetric measure, such as Oil and Grease or Mineral Oil and Grease. In the case of Oil and Grease measurement, the petroleum is extracted from a sample mass of the contaminated soil with a solvent, and then the mass of the contaminant is quantified from the extract by weight after evaporation of the solvent. Mineral Oil and Grease measurement requires as an extra step treatment of the extract with silica gel to remove polar compounds. The polar component of petroleum is discussed below. This treatment also removes natural organic compounds such as pigments and fatty acids found in the soil (originating from organic matter in various states of decay), which may have been extracted from the soil along with the petroleum.

Extraction efficiency can vary with the solvent used, so caution is advised when comparing results obtained by different extraction methods. Further discrepancies in measurement have been shown to result from differences in the method of quantifying the petroleum in the extract. Danielson (1995) compares results achieved for 3 different extraction solvents and two different quantification methods conducted on the same soil:

Table 2: Example Contaminant Quantification

Extraction solvent	Percent petroleum	
	gravimetric measurement	GC-FID measurement
toluene	5.9	4.0
dichloromethane (DCM)	5.9	2.9
supercritical CO ₂	5.8	3.0

(source: Danielson ,1995)

Database entries for petroleum concentration represent measurements made by several methods, including diverse field-portable methods such as fluorometer measurement and the Hnu-Hanby test. Analyses of petroleum in soils includes oil and grease, mineral oil and grease, total extractable hydrocarbons (TEH), total petroleum hydrocarbons (TPH) and total hydrocarbons (TH). A comparative study by Alberta Environmental Protection concluded that “ comparisons between the various test methods cannot be made, but the knowledge of a hydrocarbon concentration will help determine reductions in concentrations as remediation techniques are applied on a site specific basis” (AEP, 1993); Often measurements show similar trends, but different values. Comparing field portable methods, Lambert and colleagues reported that "Results from field analysis methodologies and those from laboratory analysis are seldom numerically equivalent. This is a result of the fact that each technique employs different sample preparation procedures and each type of instrument has its own unique limitations (Lambert *et al.* 1996)." Since various methods of petroleum quantification were used throughout the database and there is no means of compensating for the differences, reported concentrations achieved by different methods were considered as a single parameter, converted to ppm petroleum. For the laboratory work conducted in this project, Oil and Grease (O&G) and Mineral Oil and Grease (MOG) measurements were achieved by soxhlet extraction using dichloromethane (DCM) and quantified gravimetrically. Gas Chromatograph with Flame Ionization Detector (GC-FID) analysis is also employed for

qualitative comparison. Component class analysis, separation of petroleum into saturates, aromatics, polars, and asphaltenes, is also applied in experimentation. A more complicated and expensive quantification, it nonetheless offers much explanatory capability to the analysis of bioremediation; these component classes are biodegradable to different extents. The separation of the petroleum extracted from a sample into component classes is achieved by precipitation of asphaltenes in pentane, then by separation of saturates, aromatics and polar compounds on a column of silica gel using solvents of different polarity (ASTM 1998a; ASTM 1998b).

Component classes considered in this study:

Saturates - (also referred to as alkanes) lack double bonds between carbons, and instead all bonds are saturated either with hydrogen atoms or single bonds between carbon atoms.

Aliphatics-includes linear and branched alkanes, as discussed below

Linear alkanes-(also paraffins, normal alkanes, n-alkanes) all of the carbons of these compounds are arranged linearly, without branches or rings.

Branched alkanes-(iso-alkanes) features branches at one or more points in the chain of carbon atoms. This category includes the isoprenoids pristane and phytane.

Cycloalkanes-(alicyclics, naphthenes) compounds in which a non- aromatic ring configuration occurs; multiple rings are possible, as are linear branches from these rings.

Identification of linear-alkanes and select iso-alkanes is undertaken by gas chromatograph (GC-FID) analysis, which also creates a boiling point profile that roughly shows the distribution of compounds within a sample by molecular weight. Qualitative comparison can be made between the amount of linear alkanes, which tend to emerge as distinct peaks, and the naphthenes, which appear as an unresolved complex mixture (UCM), or "hump". A gas chromatograph coupled with a mass spectrometer as its detector (GC-MS) can be employed to further identify compounds based on their functional groups, which allows more comprehensive characterization.

Aromatics are based on configurations of aromatic rings (6-carbon rings each with 3 double-bonds). This includes the BTEX compounds as well as compounds with multiple aromatic rings, collectively termed Polynuclear Aromatic Hydrocarbons, or PAHs. There are many possible molecular configurations of this type, so a chromatograph of the aromatic fraction of petroleum tends to form a UCM hump, although individual compounds may be present in sufficient quantities to form peaks. Further characterization is possible using specialized column chromatography to further separate the aromatic fraction based on the number of aromatic rings present in particular compounds. (ASTM 1998a; ASTM 1998b). GC-MS is often used in approaches which involve the quantification of specific PAHs for risk analysis or the regulatory requirements of other jurisdictions.

Polars are distinct from hydrocarbons in that molecules of these compounds contain other atoms in addition to hydrogen and carbon, these compounds have an increased polarity, causing them to sorb with greater strength to the columns used for class separation.

Asphaltics -comprised of both resins and heterocompounds

Heterocompounds -(also resins, NSOs) aromatic nuclei with alkyl- and alicyclic systems and heteroatoms (nitrogen, sulfur, and oxygen)

Asphaltenes-chemically similar to heterocompounds, asphaltenes are defined by their insolubility in pentane or heptane. These high molecular weight compounds are colloidal in crude oil, kept in suspension by an interaction between heterocompounds and aromatics (Speight and Moscopedis, 1981, Long, 1981)

Other Polars -other polar compounds such as alcohols and fatty acids, while not present in crude oil, may be present in flare pits as partially oxidized products of combustion, and as intermediates and products of biological degradation, or as part of soil organic matter.

Figure 1: Examples of Saturate Compounds

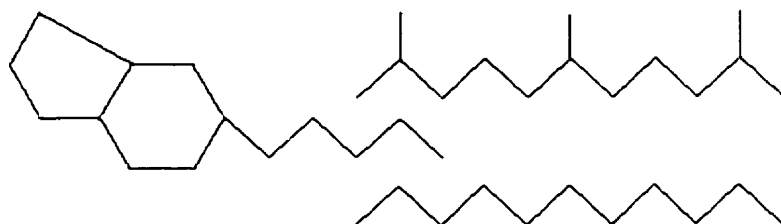


Figure 2: Examples of Aromatic Compounds

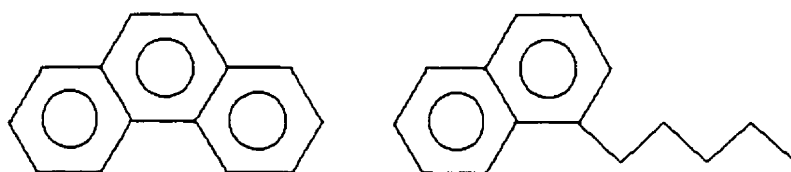
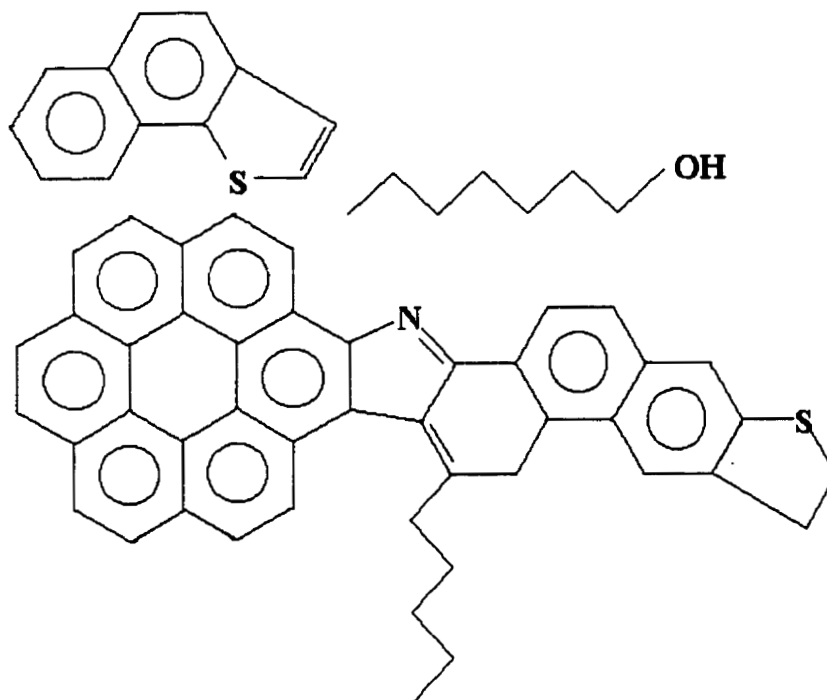


Figure 3: Example Polar Compounds

2.5.2 Petroleum: Regulatory Criteria

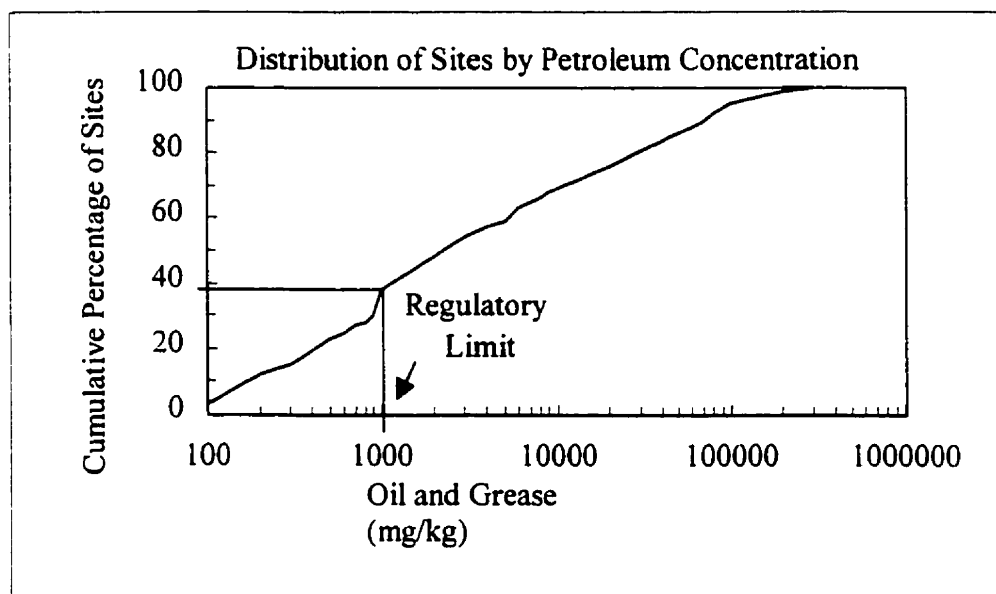
The Alberta Tier 1 criteria for Petroleum in soil is 1000 mg/kg MOG (or 0.1% dwt). Tier I criteria also specify acceptable concentrations for a range of inorganics, aliphatic and aromatic hydrocarbons, and organic pesticides, though characterization data on these specific compounds is more costly and hence missing from most of the database entries. WCR regulations do not list a maximum acceptable concentration for petroleum.

2.5.3 Petroleum: Available Data

At the time of writing, 485 entries in the site characterization database reported a measure of petroleum contamination. All measurements, including TPH, Oil and Grease, and other readings, were converted to a single petroleum concentration for simplicity of analysis. Measurements ranged from 10 ppm to 537300 mg/kg, or 0.0001% to 53.73% dwt. Of these

sites, 300 (62% of the 485 sites reporting a value) exceeded the 1000 mg/kg regulatory limit. (Speer, 1999). A cumulative distribution of the petroleum concentration for these sites is illustrated in Figure 4.

Figure 4: Distribution of Petroleum Concentrations in Database



(this figure prepared from data assembled by Speer, 1999)

2.5.3 Petroleum: Remediation

Several physical, chemical, and biological technologies have been developed to remediate petroleum contaminated soils. These are enumerated in CAPP (1994), Cartwright (1991), Cookson (1995), LaGrega *et al.* (1994), and Schleck (1990). In general, the technologies may be enumerated as follows

- landfilling
- thermal treatment (e.g. thermal desorption, incineration)
- solidification or capping
- aqueous leaching (using surfactant)
- solvent extraction

- bioremediation
- soil vapour extraction (for BTEX and volatile compounds)

The applicability of these treatments depends in large part on site characteristics. Bioremediation, the site management alternative considered in this project, employs biological processes, mainly the growth of petroleum-degrading soil bacteria and fungi, to reduce petroleum contamination to an acceptable level. While the particulars of each of these remediation methods are not discussed here, in general it can be said that for on-site treatment bioremediation is the least costly if it can be successfully applied. In practice, however, bioremediation can often take years to reach the regulatory cleanup objective. In a 1993 CAPP survey of treatment technologies (CAPP 1994), all bioremediation methods considered were rejected in pre-screening stage that required clean-up be achieved within a three-month time frame (i.e. a single summer season).

Much research (section 2.5.3.2) exists to suggest that petroleum can be bioremediated quickly, but the extent and the rate of biodegradation are known to be affected by many factors, and successful implementation requires a great deal of preliminary information. Thus in the following sections, the conditions for successful bioremediation are enumerated (section 2.5.3.1). The general bioremediation technologies used to establish these conditions are discussed (section 2.5.3.2), and a selection of case studies are presented as an indication of achievable results for bioremediation (section 2.5.3.3).

2.5.3.1 Conditions for Successful Bioremediation

Several environmental parameters affect the rate and extent of biodegradation (Cookson, 1995; Admassu and Korus, 1996; LaGrega *et al.*, 1994). They are arranged hierarchically by Cookson as follows;

- microorganisms capable of degrading contaminant
- energy source for growth
- carbon source for growth

- electron acceptor (oxygen for aerobic processes)
- adequate moisture
- pH
- nutrients for growth
- suitable temperature
- absence of toxicity
- removal of metabolites
- absence of competitive organisms

Bioremediation is performed by a consortia of microorganisms, which is to say a mixed community of different species of bacteria and fungi which individually metabolize different portions of the petroleum contaminant, interacting to degrade each other's metabolic byproducts. Ideally, the community will be such that all petroleum will be mineralized, transformed to CO₂ and H₂O, but some petroleum compounds are not known to degrade, or degrade only partially. Given sufficient acclimation time, soil contaminated with petroleum often has enough hydrocarbon-degrading microorganisms to support bioremediation (Bitton and Gerba, 1984, Cookson, 1995). However, flaring may serve to heat-sterilize the surface layers of flare pit soils, depleting the indigenous microbial population.

The substrate for growth in most cases is the petroleum itself. Raymond et al. (1990) ranked different fractions of petroleum in decreasing ease of biodegradability:

- Volatile saturates
- Volatile aromatics
- Heavy saturates and mono-aromatics
- Polynuclear aromatic hydrocarbons
- Asphaltenes, resins, and waxes

Inability of microbial enzymes to break multiple alicyclic and aromatic ring structures limits

the degradation of many compounds, and many are partially degradable as microbes remove linear side chains from branched and cyclic molecules (Boethling *et al.*, 1994, LaGrega *et al.*, 1994). It is hypothesized that the biodegradation of many of the more recalcitrant compounds is also limited by bioavailability; that their low solubility or hydrophobicity are such that they do not come into contact with hydrocarbon degrading microorganisms in significant amounts for biodegradation. It is a general limitation of bacteria that they may only metabolize dissolved or emulsified material that can cross their cell membrane by diffusion (Cookson, 1995). Bacteria have been shown to produce surfactants to emulsify petroleum compounds, and bioremediation can involve the addition of chemical surfactants (Shiebenbosen *et al.*, 1995). Fungi are known to produce extra-cellular enzymes which are capable of breaking down many PAH compounds, so much research is now being directed at using these organisms to expand the prospects for the degradation of heavy aromatics and other low solubility compounds (Fritsche, 1994; Barr and Aust, 1994; Evans and Buck, 1998)

Another uncertainty surrounding bioavailability is the effect that the concentration of the contaminants will have on the process of bioremediation. De Jonge and coworkers found that for high concentrations (>4000 mg/kg for the soil studied), biodegradation rates are a function of the aqueous-phase solubility of the contaminants (De Jonge *et al.*, 1997). Below this concentration oil is sufficiently scarce that biodegradation is limited by desorption of the oil from soil particles and diffusion to capable microbes. While the actual concentration at which this phenomenon occurs likely varies from soil to soil, as an order of magnitude estimate it suggests that sorption and diffusion limitations will be a concern when hydrocarbon concentrations approach the 1000 mg/kg MOG Alberta Tier I target. Studies show hydrocarbon bioavailability limitations to be a combination of low solubility, sequestration, and bonding to soil constituents, affected by aging, soil characteristics (mainly clay content, small-scale porosity and organic matter), and the composition of the contaminant (Malekani *et al.*, 1997, Lambert *et al.*, 1996, Chung and Alexander, 1998). The research at this point has been able to identify many contributing factors but is not yet able to predict their effect. It is expected that because of the nature of flare pit sites (heavy, aged

contamination and frequently high clay or organic matter content), soil sorption effects will limit the extent of bioremediation achievable.

Oxygen, moisture, nutrients, pH, and temperature are referred to as “environmental factors” in the body of this report. At different levels they affect the bioremediation process (Cookson, 1995; Danielson, 1993). Development of bioremediation technologies strives to optimize these conditions to ensure good petroleum degrading microbial activity. In many cases the “optimal” conditions are compromises between the ideal conditions for different microbial species (Cookson, 1995).

A study to differentiate between these several effects, was conducted by Fu and associates through remediation studies in soil slurry, soil wafer, and compacted soil tube with a phenol contaminant (Fu *et al.*, 1996). This study provided a good framework of the information required to model bioremediation in a soil system, developed with increasing sophistication beginning with slurry phase treatment. Fu and associates found that the experimental results fit closely with the predicted results for the model developed for phenol. The treatability screening applied in the flare pit project seeks to address the biodegradability achievable in absence of the technical problems presented.

For this project, slurry treatment was selected to afford control over these many factors, and in the long term to develop an understanding of their effects such that bioremediation may be optimized.

2.5.3.2 Petroleum Treatment Technologies

If conditions are adequate, remediation can be achieved *in situ*, which is to say without excavation of the contaminated material. With *in situ* treatment the contaminated soil is left in place, and amendments required to enhance microbial activity (such as oxygen and nutrients) are delivered through the soil pore spaces. Because removal, handling and

transport of the contaminated soil incurs additional risk and cost, it is preferable to treat contaminated soils *in situ* if possible, however the method presents several practical challenges. The transport of soil gases, in both the delivery of oxygen and the removal of carbon dioxide, must be balanced with the transport of water and water-borne nutrients. Limited pore space and permeability often preclude in-situ treatment. Treatment applied to sites of heterogeneous soil makeup may result in partial and inconsistent contaminant reduction (Cookson, 1995).

Ex situ treatment, involving removal of the contaminated soil and treatment on the land surface in some manner, allows greater control of the soil structure. Amendments and monitoring are technically easier to administer *ex situ*, but the expense in terms of energy, equipment, and handling is usually greater than *in situ*. An advantage to removal of the contaminated soil is that it allows for soil mixing to reduce the problems presented by soil heterogeneity, although the ease of handling will vary (Danielson, 1995). Land treatment involves mixing waste with soil over a large land area using disk harrows, rototillers or similar agricultural methods. Nutrients, water, and soil bulking amendments such as straw or wood chips are easily applied, and cultivation ensures aeration. Due to its simplicity and longtime use this land treatment has been favoured to this point, although it is coming under increasing scrutiny (AEUB 1994b). This method offers little control over volatile petroleum compounds. It is climate-dependent resulting in long treatment periods due to Alberta's dry climate and short summers. If significant biodegradation fails to occur, land treatment is effectively only landspreading; dilution of contamination in a larger volume of soil.

Composting and biopile treatment involves mixing the contaminated soil with nutrients and bulking agents to improve soil structure, and offer greater control over treatment conditions. With composting, easily degradable organic material is added to develop high microbial populations which often result in autothermal heat generation and conditions suitable for thermophilic bacteria. An early assessment of the applicability of this technology (CAPP, 1989) rejected composting as a suitable treatment, compared to established physical and

chemical methods. At that time it was not a proven technology, but since then several case studies have arisen leading to increased interest in the prospects for its application. These include a study by McMillen and associates achieving over 90% degradation using composting technology (McMillen *et al.*, 1992).

Slurry phase treatment involves preparing an aqueous suspension of soil particles either by mixing or by force of aeration. It is advantageous in that it provides chemical and biological homogeneity to a degree that is difficult to achieve in the solid phase, ensuring the supply of oxygen, water or nutrients does not limit the bioremediation process. The high energy input required to maintain solid particles in suspension and the sometimes lengthy periods needed to de-water slurry after treatment present difficulties for field application. (LaGrega *et al.*, 1994). It is, however, a favourable tool for research as it allows close control over the many factors affecting bioremediation.

A survey of results demonstrated for flare pit petroleum and crude oils:

- Huesemann (1995): This lab test of achievable biodegradation in soil includes extensive analysis and comparison of chemical composition of the petroleum contaminants tested.
 - oil, 21° API: 50% biodegradation
 - oil, 39° API: 66%
 - oil, (also 39°API): 67%
 - diesel drilling mud: 79%
 - weathered oil-contaminated site soil: 64%
 - 10W-30 motor oil: 66%
 - diesel oil no.2: 76%
- Wibowo (1996): Soil spiked with 2% Alberta sweet mix crude, treated in laboratory columns with forced aeration at mesophilic temperatures. Reductions of TPH of 42 to

58% with respect to abiotic control.

- McMillen *et al.*, (1992): "Composting of a Production Pit Sludge" A sludge containing 10.8 percent hydrocarbons was composted with wood chips, manure, water and inorganic fertilizer for 4 weeks at mesophilic temperatures. The reductions achieved:

Total	92.1%
Saturates	97.0
Aromatics	86.4
NSO's	78.8
Asphaltenes	30.3

- McMillen *et al.*, (1994): 0.5% crude oil mixed with soil, treated for 4 weeks in soil at mesophilic temperatures . Treatments for 17 crude oils of different API gravities yielded reductions of 10 to 63%, based on respirometry.
- Johnson and Danielson (1994): Solid Phase bioreactor, oil and brine contaminated topsoil (E.C 27cS/m, SAR 24, oil content 6.7%). 43% reduction in TPH in 11 months (5 summer and 6 fall-winter) in heated cells, 25 % in non-heated cells.
- Danielson (1995): Same solid-phase bioreactor as above, with flare pit waste (8.5 % hydrocarbons, EC.>30 dS/m) ; achieved 30% degradation in 7 months, no significant effects for the variables considered (aggregation, cultivation, inoculation).
- Hayes *et al.* (1997): Comparison of 6 week soil pan and slurry treatability tests on contaminated soil from manufactured gas site for Gas Research Institute (GRI). O&G and TPH reduced approximately 50%

These selected case studies present a broad range of reduction; (compare 92.1% with 30%). This is attributable to differences in operating conditions and differences in contamination.

2.5.3.4 The Composition of Alberta's Oils

The hydrocarbons typical of gas wells are condensates, liquid hydrocarbons dissolved in natural gas which condense when brought out of the formation. Contamination at these sites is predominantly BTEX and short chain hydrocarbons to C7, all highly volatile. As noted above, the degradation of compounds in this range is well understood. That hydrocarbons in this range can be successfully treated is a simplifying assumption fundamental to the laboratory project, and this affected how samples were selected (taken from oil sites), handled (without refrigeration) and treated (with high rates of aeration).

The non-volatile fraction of flare pit hydrocarbons composition is expected to be a modified version of produced oil, after burning, intrinsic biodegradation, and weathering. An extensive study of Alberta oil formations was undertaken by Deroo and colleagues (Deroo *et al.* (1977). The authors of this study classify Alberta oils into four groups, designated 1, 2, 3 and heavy. The relative composition of these four groups, in terms of proportions of saturates, aromatics, and resins/ asphaltenes, is illustrated in Figure 5, compared against a 90% confidence interval of 636 crude oils collected worldwide, by Tissot and Welte (1978). Oils of types 1 have a certain gross composition, attributed to similar organic source materials and formation conditions. Group 1 can be distinguished from group 3 by close inspection of the saturate fraction. GC-MS analysis presented in the report shows differences in the proportions of cyclic saturates between Groups 1 and 3, which is attributed to differences in the sedimentary deposition of source materials. Group 2 oils, which are more distinct in their composition are formed in similar manner but are theorized to have undergone a lesser level of thermal maturation in formation. Heavy oils have undergone significant biodegradation in formation, resulting in removal of the easily degradable saturate fraction, and an increased proportion of naphthenes, aromatics, resins and asphaltenes. Milner and colleagues explain the transformational effects by which differences in oil chemistry can develop in reservoirs (Milner *et al.*, 1977). Later studies based on closer analysis, further develop the categorization of Alberta oils (Brooks *et al.*, (1988); Allan and

Creaney (1991).

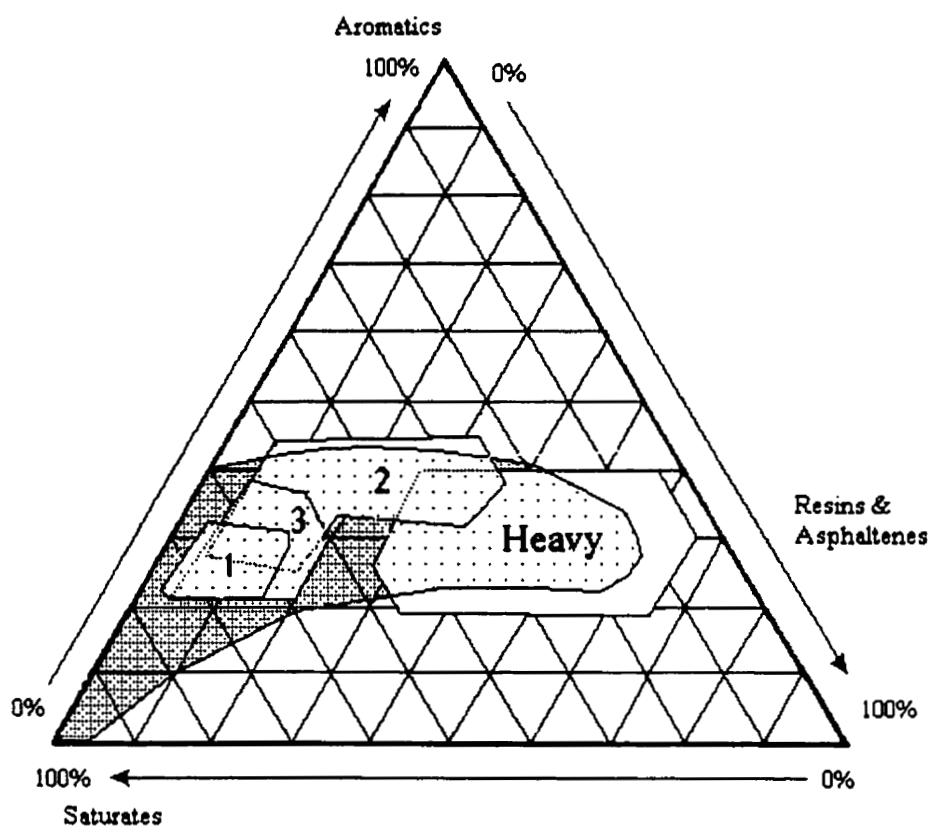
The distinction between heavy and conventional oils is generally made on the basis of density, rather than chemical composition. Using the American Petroleum Institute measure of the density of petroleum, °API, according to the formula

$$^{\circ}\text{API} = 141.5 \times (\text{specific gravity of oil at } 15.6^{\circ}\text{C}) - 131.5,$$

heavy oils are defined as those with densities 20 °API or lower (McMillen *et al.*,1994).

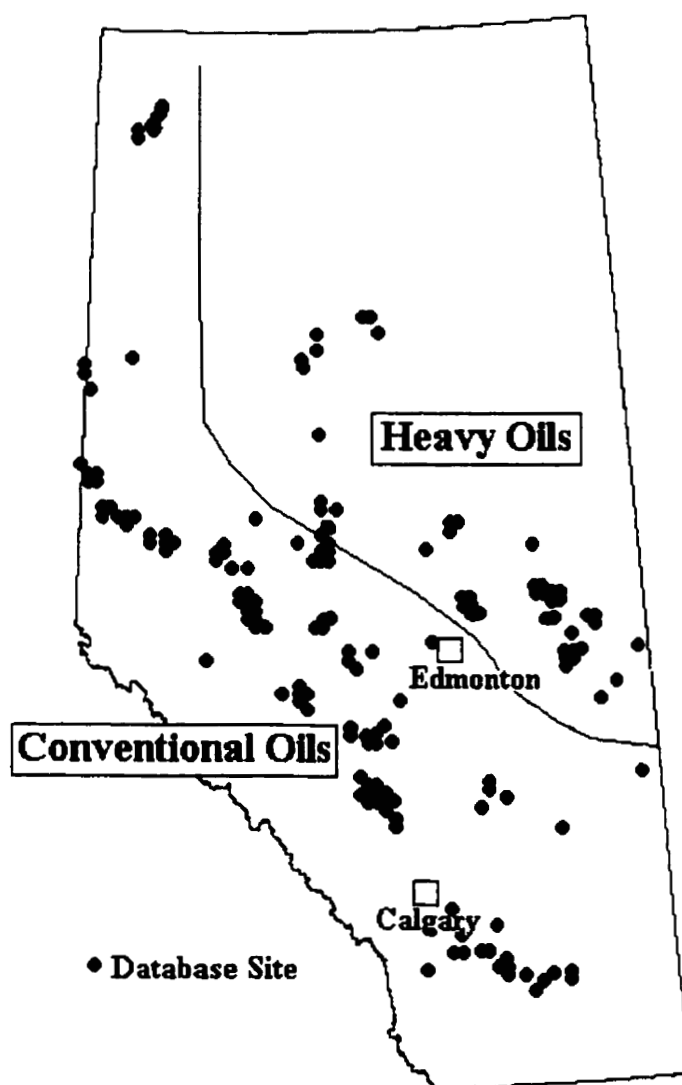
Figure 6 indicates the location of the database flare pit sites with respect to regions of heavy oil and conventional oil (Groups 1, 2, 3) production in Alberta. The distinction between the two regions is taken from Mossop and Shetson (1994), though it should be noted that the transition between heavy conventional and heavy oil occurs as a gradient rather than a distinct boundary. Within the conventional oils, Groups 1, 2 and 3 occur in overlapping distributions in the area indicated (Deroo *et al.* (1977))

Figure 5: Graph Showing Alberta Oil Groups



(adapted from Deroo *et al.*, (1977), with shaded area representing 90% confidence interval of 636 crude oils collected worldwide, by Tissot and Welte (1978))

Figure 6: Location of Flare Pit Sites



(Site location from Speer (1999); regions of heavy and conventional oil from Mossop and Shetson (1994))

2.6 Salinity: (E.C. and SAR)

Salinity at flare pit sites is a result of the disposal of produced brine from the formation. Excess soil salinity is harmful mainly by the osmotic pressure that it creates, causing a gradient that tends to draw water from the cells of plants and soil organisms. Salinity is most commonly measured as electrical conductivity (EC), which can be correlated to osmotic pressure, total cation concentration, and roughly correlated to salt concentration. The effect of salinity on plant growth is explained as follows in Seatz and Peterson (1964);

<u>EC</u>	<u>Plant response</u>
dS/m at 25°C	
0-2	effect negligible
2-4	Yield of very salt-sensitive crops restricted
4-8	Yield of salt-sensitive crops restricted
8-16	Only salt-tolerant crops yield satisfactorily
>16	Only a few very salt-tolerant crops yield satisfactorily

Sodium adsorption ratio (SAR) is a measure of the proportion of sodium ions to calcium and magnesium ions in soil (from Seatz and Peterson (1964)):

$$\text{SAR} = [\text{Na}^+] / ([\text{Ca}^{2+}] + [\text{Mg}^{2+}])^{1/2}$$

for concentrations in mmols/l

Excess sodium can cause swelling and dispersion of clays, affecting soil structure and the suitability of the soil for agriculture. Soil SAR is related to plant growth. Normal soils tend to have a maximum SAR of 13 while the limit of survival for most plants is in soil with a SAR of 40. (Brady and Weil, 1996)

For Alberta Tier 1 criteria, EC is not to exceed 2 dS/m, and SAR is not to exceed 6 (AEP

1993). Salt contamination is typically addressed by leaching the salt from the soil with water. This method requires some means of disposing of the water used. High SAR is dealt with by the application of gypsum or other calcium salts to the soil to adjust the proportion of sodium. This provides calcium ions for exchange of sodium, which is removed by leaching.

Soil salinity is considered a possible inhibitory factor in the bioremediation of flare pit sites. Salinity may complicate bioremediation by inhibiting the growth of petroleum degrading microbes (Beveridge and Doyle, 1989). Various microbial salinity tolerance mechanisms exist to deal with the salinity effect, but each requires an outlay of metabolic resources that could otherwise be used for growth, thus the rate of bioremediation is slowed. Nonetheless, studies by Macmillen and Danielson indicate that the inhibitory effect does not emerge until levels of 40 dS/m and 36 dS/m, respectively (Macmillen, 1994; Danielson, 1994). The specific effect of soil salinity composition of microbial activity may vary, but at this point its application is limited by the availability of such information in the site data available. Danielson compared the effects of NaCl and KCl on the laboratory biodegradation of crude oil, observing no significant difference (Danielson, 1994).

2.7 Metals

From a regulatory perspective, metal toxicity is addressed as a human health concern. The risk to human health associated with the presence of metals in soil depends on each metal's toxicity, concentration, and the route of human exposure. These factors were taken into account in the development of Alberta Tier I and CCME regulations. Alberta Tier I regulations list a single acceptable concentration suitable for all land uses. CCME regulations separate criteria for agricultural, parkland, commercial/residential, and industrial land use. The high regulatory cutoffs are based on the Waste Control Regulation (WCR) limitation for use as landfill cover material. Above this concentration the waste management option of landfill disposal is ruled out, and options for management of this contamination are severely limited.

Table 3: Regulatory Limits for Metals

Metal		Regulatory limits (mg/kg)	
		Low (Tier I)	High (WCR)
Arsenic	As	12	500
Barium	Ba	600	2000
Cadmium	Cd	1.4	100
Chromium	Cr	64	100
Cobalt	Co	20	200
Copper	Cu	63	20
Lead	Pb	70	500
Mercury	Hg	6.6	20
Molybdenum	Mo	4	----
Nickel	Ni	40	500
Vanadium	V	130	2000
Zinc	Zn	200	10000

(AEP,1994; AEP,1993; AEP,1995)

For the purposes of this project metals must be considered as a possible interference to the bioremediation of the petroleum contaminant. Anecdotal evidence exists that metals inhibit bioremediation of flare pits, when present at levels toxic to petroleum degrading microbes. The mechanisms of metal toxicity are particular to individual metals (Manahan, 1997). Cadmium, copper, lead and mercury compounds bind to cell membranes and interfere with membrane function, while other metals pass by the membrane without incident only to affect other cellular materials. Other bonding sites include carboxyl groups ($-\text{CO}_2\text{H}$) and amino groups ($-\text{NH}_2$) on intracellular proteins. As a mechanism of defense some microbes employ enzymes to convert metals to less toxic valences, or continuously transport metals outward, while others sequester or bind the metal within their cells before it interferes with vital cell

functions (Manahan, 1997).

A knowledge of metal concentrations at which the inhibition of bioremediation occurs would be beneficial to prediction of site treatability. In most cases these levels will be higher than the levels set by human health regulations, which are based on conservative estimates to afford a factor of safety. In the development of the database used in this project, numerous studies in the literature regarding measured effects of individual metals were assembled. Metal concentrations tolerated by select microorganisms are presented in Table 4 (from Providenti *et al.*, 1993).

Table 4: Metal Tolerances of Selected Microorganisms

Metal	Tolerated Concentration	Microorganism
Arsenic	0.4 mg/L	<i>Pseudomonas aeruginosa</i>
Cadmium	0.2 mg/L	<i>Staphylococcus aureus</i>
	0.4 mg/L	<i>Pseudomonas aeruginosa</i>
	0.01 mg/L	<i>Klebsiella aerogenes</i>
Chromium	1.6 mg/L	<i>Pseudomonas aeruginosa</i>
	1.5 mg/L	<i>Pseudomonas fluorescens</i>
Cobalt	100 mg/L	Heterotrophic Bacteria
	0.62 mM	Soil Microorganisms
	1.09 mM	Soil Microorganisms
Copper	100 ppm	<i>Pseudomonas spp.</i>
	20 nM	<i>Escherichia coli</i>
	10 ppm	<i>Klebsiella aerogenes</i>
	0.68 mM	Soil Organisms
	1.18 mM	Soil Organisms
Lead	100 mg/L	<i>Sediment bacteria</i>
	3.2 mg/L	<i>Pseudomonas aeruginosa</i>
	0.05 mg/L	<i>Sediment bacteria</i>
Mercury	0.1 mM	<i>Mycobacterium scrofulaceum</i>
	10 mg/mL	<i>Pseudomonas fluorescens</i>
Molybdenum	100 mg/L	<i>Sediment bacteria</i>
	102 mg/L	<i>Pseudomonas aeruginosa</i>
Nickel	25 mg/L	<i>Sediment bacteria</i>
Zinc	1600 mg/L	<i>Pseudomonas aeruginosa</i>

(from Providenti *et al.*, 1993)

Predicting the effect of metal contamination will be complicated by the fact that remediation of petroleum is dependent on consortia of microbes, with each species presenting a different tolerance and mechanisms of resistance to metal toxicity (Manahan, 1997). Furthermore, the precision to which a level of inhibition can be determined will be affected by many site variables other than its concentration. Speciation, the valence of ion that the metal tends to form in solution, can have a pronounced effect on the toxicity of metal ions (Manahan, 1997). Soil redox potential and pH interact in their effect on speciation; metals tend to form hydroxylated ions as pH increases (Hughes and Poole, 1989), though this effect occurs at different pH's for different metals. Beveridge compares studies in which copper and cadmium were found to increase in toxicity with increasing pH, whereas nickel has been found to decrease. Separate studies report both an increase and a decrease for zinc (Beveridge, 1989).

Toxicity is a function of the solubility of metal ions, which is affected by numerous environmental factors. (Hughes and Poole, 1989; Beveridge, 1989). Solubility is dependent on speciation. Soil water hardness, as well as the presence of other inorganic ions, tends to lessen the concentration of metals in solution. Since salinity is a known problem for many flare pit sites there may prove to be a significant interaction between salinity and metal toxicity if high salt content keeps metals out of solution. Studies concerning chloride and cadmium suggest that the chemistry of soil salinity may have a bearing on this effect (Naidu *et al.*, 1994, Bingham *et al.*, 1984). Clay minerals can adsorb metals, especially clays of high cation exchange capacity. Complexation or chelation of metals with soil organic matter will also affect the mobility of the compound.

Numerous parameters affect the precision with which the inhibition of bioremediation by metals can be predicted. It remains to be seen whether or not these parameters are significant at the levels encountered at Alberta flare pit sites. Even if these many factors are understood, their application will be limited by the availability of sufficient site characterization data.

Metal contaminants are treated either by leaching for removal, or by fixation to reduce their mobility. Lime treatment is a common practice (LaGrega *et al.*, 1994), the purpose being to raise the pH of the soil to reduce metal mobility.

2.8 pH

A pH range of 5.5 to 7.0 promotes the best availability of plant nutrients when considering major plant nutrients and the populations of soil organisms, but soil should ultimately be maintained with the range of 4 to 10. (Cookson, 1995). To meet Alberta Tier 1 requirements, pH values may not exceed 8.5 or be less than 6 (AEP, 1994). WCR classifies hazardous soil as having a pH less than 2.0 or greater than 12.5 (AEP, 1993a).

pH affects microbial activity by many mechanisms. Cookson (1995) recommends that for effective bioremediation pH generally be maintained near 7, within the range of 4 to 10. Extreme pH may be remedied by the addition of basic or acidic minerals, such as lime. This may alter the salinity of the soil to an extent that treatment for salinity is subsequently required.

2.9 Site Concerns Excluded From This Review

Remediation of a site requires consideration of many factors that are not addressed at depth in this project, either because

1. They are necessarily specific to individual sites and do not fit into the general approach applied (e.g. site groundwater characteristics) or
2. While they may be a problem for many sites, there is insufficient information upon which to base generalizations (e.g. soil sterilants)

3.0 EXPERIMENTATION

3.1 Overview

From a review of the literature, bioremediation is a proven technology for the volatile fraction of petroleum. For heavier contamination, results are mixed. Experimentation with flare pit waste has delivered biodegradabilities of 0% to 50% for the non-volatile portion of petroleum contamination (Visser *et al.*, 1999). Proceeding from previous flare pit research undertaken by Danielson (1995), the slurry treatment applied in this experimental programme was developed to address the resistance of flare pit sludge to biodegradation. Possible limiting factors for the degradation of residual petroleum remaining after treatment are (from Johnson and Danielson, 1993):

- recalcitrance of the contaminant,
- limited bioavailability of the contaminant,
- mass transfer limitation for oxygen and nutrients in the solid phase, or
- buildup of toxic metabolic by-products.

The treatment of the non-volatile fraction is limited by the numerous environmental conditions generally recognized for petroleum bioremediation, and a host of factors particular to flare pit sites. The treatability programme was set up to serve as a rapid laboratory-scale treatment capable of studying these effects prior to application of biotreatment on a larger scale.

A lab scale experiment affords the possibility of closely studying the effect of contaminant characteristics as to their effect on the limits of bioremediation. Alternatively it may be used to manipulate and investigate the many environmental conditions affecting the bioremediation process, as a means of developing optimal conditions for a working bioremediation technology. While the slurry apparatus was developed with both of these uses in mind, in this project it was applied in the service of the former application.

The characterization database was indispensable for taking into account the high variability of contamination from site to site, and the treatability lab programme was directed at those parameters deemed to be of greatest concern: hydrocarbons, salinity, and heavy metals. Hydrocarbons and salinity were actively manipulated in the experimental programme. Because of the complexity of the expected heavy metals effect (as addressed in the literature review), the soils tested were simply screened for heavy metals. Data collected from metal contaminated samples might be used to inform future attempts to understand this contamination.

Initial experimentation in this project considered the petroleum contamination as a gross parameter alone as Oil and Grease (O&G), because comprehensive characterization of hydrocarbon composition is rare within the database. Experimentation began with an initial run for the testing and development of monitoring procedures to be applied. O&G measurements showed an increase in petroleum concentration, but the monitoring and characterization methods applied at this stage offered little explanation for this effect. The monitoring protocol discussed below (in section 3.2.4.1) was developed and applied to subsequent experiments, wherein the apparent increase repeated itself.

Experiment Series 1, "Significance of Inoculation", was undertaken to assess whether or not addition of an inoculum of petroleum degrading microbes was necessary for laboratory testing. The interpretation of this experiment was complicated by an increase in measured O&G. Analysis of samples by an outside lab and application of more sophisticated methods of petroleum quantification (GC-FID and eventually component class analysis) were incorporated to explain this effect and to avoid it in further experimentation.

Experiment Series 2, "Petroleum Spiked Soils" was undertaken next, involving clean soils spiked with petroleum extracted from a flare pit soil. A decrease in petroleum was achieved for these treatments. Subsequently, the experiment "Salinity 1" was undertaken to ensure

that the slurry salinity arising from the addition of nutrient salts and NaOH or HCl for pH control was not interfering with the treatment process, and it was found that this salinity had no interfering effect. This experiment is presented together with other salinity experiments in Experiment Series 4, as explained below.

Experiment Series 3, "Petroleum Composition" was undertaken at this point, comparing the biodegradation of petroleum in 5 different flare pit soils of different petroleum concentration and composition (as determined by component class analysis). The results showed very rapid reductions could be attributed to the same specific classes of petroleum in each soil. Understanding and predicting limits to treatment required consideration of the component classes of the petroleum contaminant. Consequently, further literature review was undertaken at this point to determine the range of composition that might be expected at Alberta sites. Applying this information to the petroleum concentrations found in the database, an estimate was developed of the general biodegradability of flare pit contamination; this estimate is developed in the discussion.

Experiment Series 4 begins with "Salinity 1", as explained above. Second in this series is "Salinity 2", undertaken to locate a slurry salt concentration which inhibited bioremediation, followed by "Salinity 3". These experiments streamlined the monitoring and characterization methods, based on the findings of previous experiments.

3.2 Methodology

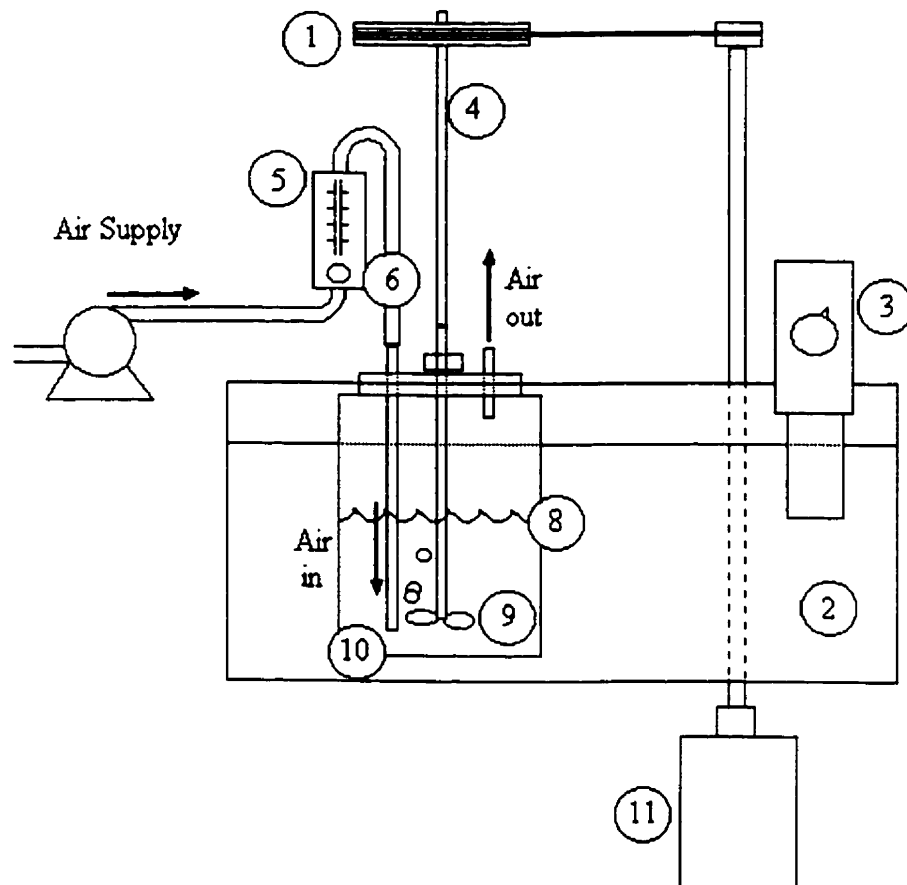
3.2.1 Slurry Apparatus

Slurry treatment was applied by means of a lab apparatus developed for this project. Reference to a "slurry reactor" in the context of this experiment refers to one of the ten individual treatment chambers of this apparatus. Each reactor was a glass jar 2 litres in volume, containing 1 liter of slurried soil and 1 litre of open headspace. For these experiments, the temperature of the reactors was maintained at 30°C by means of a water

bath. Continuous agitation was applied by a propeller-bladed mixing shaft in each reactor, and aeration by point source aeration with adjustable flow rate. The following features are indicated in Figure 7.

1. Mixer Belt Drive
2. Insulated Water Bath (temp 30°C for these experiments)
3. Heater/ Circulator
4. Mixer Shaft (~100rpm)
5. Air Flux Control (0 to 4 litres per minute)
6. Air Hose (1/4" I.D.)
7. Reactor Bracket
8. Jar (glass)
9. Mixing Propeller (1 1/2" diameter)
10. Submerged Aeration Tube
11. Mixer Motor (1/3 hp, 1725 rpm)

Figure 7: Schematic Diagram of the Slurry Reactor



3.2.2 Flare Pit Soil Sample Collection

Flare pit soil sample collection, conducted in liaison with industry representatives, concentrated on sites associated with oil production. Flare pit soil samples were acquired from pits near Edmonton, Red Deer, Drumheller, and Lethbridge. With the exception of two power-augured samples, contaminated soils were collected as surface grab samples with a metal trowel. Volumes of samples taken from individual sites ranged from 500mL to 20 L.

These samples were stored at room temperature in clean mason jars or plastic containers until needed for experiments. This represents a departure from the standard protocol for sample handling, which requires that samples be refrigerated and tested within 7 days to prevent losses due to volatilization and microbial activity. The simple grab samples utilized also represent a departure from standard sampling protocol. Effective site characterization typically achieves a more representative measure of contamination by collecting several samples at various depths, with some procedure to ensure randomization. These departures are acceptable for the purposes of this project since precise characterization of these individual sites was not a priority. Handling and storage was taken to be consistent with normal weathering. The characterization of the soils used for experimentation follows in Table 5. The experimental series for which each soil was applied is indicated at the bottom of Table 6.

Table 5: Characterization Data of Soils Tested in Laboratory Programme

Soil	A	D	E	F	H	I
O&G (%dw _t)	1.5	1.7	0.1	0.3	2.0	0.1
EC (dS/m)	1.6	3.9	2.2	2.6	0.9	1.3
pH	7.7	7.7	7.6	7.7	7.1	7.6
% Sand	24.3	16.0	20.0	11.4	45.0	69.0
% Silt	32.9	36.0	30.0	35.7	28.3	17.0
% Clay	52.9	48.0	50.0	52.9	26.7	14.0
Particle Size Analysis ¹	Clay	Clay	Clay	Clay	S.C. Loam	Sandy Loam
Metals ² (mg/kg)						
Aluminum	102000	82000	112000	87000	103000	74000
Arsenic	---	---	---	---	---	---
Barium	95	212	99	337	168	0
Cadmium	---	---	---	---	---	---
Chromium	---	---	---	---	25	---
Cobalt	---	---	---	---	---	---
Copper	29	32	30	39	25	
Iron	1575	1963	1737	1966	1509	774
Lead	---	---	---	---	---	---
Mercury	0.06	0.97	0.03	0.05	0.03	0.01
Selenium	---	---	---	---	---	---
Zinc	62	74	74	96	63	43

¹ Soil particle size analysis performed by outside lab.

² Metals characterized by atomic adsorption spectrophotometer analysis of nitric acid digest (ASTM 1998c) except for mercury, which was characterized by an outside lab.

Table 6: Soil Use in Experiments

	Soil					
Experiment:	A	D	E	F	H	I
Series 1	X					
Series 2	(Series 2: petroleum spiked clean clay and sand)					
Series 3		X	X	X	X	X
Series 4		X				

3.2.3 Sample Treatment

3.2.3.1 Soil Slurry

Prior to testing samples were passed through a 2-mm (10 mesh) sieve, and homogenized by kneading. A portion of each sample, approximately 100g dry mass, was added to the jar of a test reactor. Nutrients were added in solution as discussed below, then the volume was brought to 1 litre with deionized water. Soil concentration varied in the slurry due to normal operation; Slurry moisture evaporated due to aeration and was regularly replenished with deionized water. Soil solids were regularly removed for sampling over the course of an experimental run. The working slurry concentration was 5 to 10% contaminated soil by dry mass.

3.2.3.2 Nutrient Addition

Nitrogen and phosphorus were added in solution as 0.68g of monobasic ammonium phosphate ($(\text{NH}_3)_2 \text{HPO}_4$) per litre of slurry. This corresponds to solution concentrations of 140mg/l nitrogen as ammonia, and 160 mg/l phosphorus as phosphate. For a 100: 10: 1 C:N:P ratio, these is a sufficient nitrogen concentration and an excess of phosphorus for 1600 mg of $(\text{CH}_2)_n$ in the slurry, or 1.6%dwt for 100g of soil in 1L. Since the nutrient requirements vary with contaminant concentration, or nitrogen could be removed from solution as ammonia, nitrogen concentrations were monitored and replenished. Slurries were

monitored for their of nitrogen as ammonia approximately every 4 days using a Hach DR/2000 spectrophotometer, using method Method 8154 calibrated for the concentration in the slurry (Hach,1993).

3.2.3.3 pH

The pH of the slurries was monitored with a handheld pH meter, and maintained at neutrality (pH 7) by dropwise addition of HCl or NaOH.

3.2.3.4 Temperature

For the experiments conducted in this project, temperature was maintained at 30°C. The effects of cooling due to aeration and evaporation caused a fluctuation of $\pm 1^\circ\text{C}$

3.2.3.5 Surfactant

In some cases SimplegreenTM solution was added as a supplementary surfactant, typically 10 mL to the 1 litre slurry volume. Instances of addition are noted in individual experimental designs. Since this product is itself biodegradable, its addition results in increases in biological activity (as indicated by nutrient and oxygen uptake) that do not necessarily imply hydrocarbon degradation. Application of the surfactant towards the end of an experimental run, when activity had slowed, was used as a means of testing whether inhibitory conditions had developed, without altering the hydrocarbon composition of the sample. Assessment of hydrocarbons both before and after such addition showed whether increased activity was due to increased hydrocarbon availability, or simply use of the surfactant as substrate.

3.2.3.6 Antifoam

Dow Corning DB500 anti-foam was added dropwise to slurries to inhibit foaming. This was done once initially, and no more than one additional application was required in practice.

3.2.3.7 Abiotic Controls

Abiotic controls were run to determine the changes in contamination due to non-biological

(i.e. physical and chemical) aspects of treatment. Inhibition of slurry microbes was achieved using mercuric chloride, sodium chloride, and sodium azide. The particulars of the addition of these compounds are detailed in each experimental design.

3.2.3.8 Method for Electrical Conductivity (EC) of Soil Slurry

Soil sample EC was characterized by measuring the conductivity of a 1:1 soil water extract. For slurries, the test was performed on the slurry water, as separated from the solids by centrifuge (at 2000 G for 30 minutes). A handheld conductivity meter and a 10-mL graduated cylinder were used as a conductivity cell.

Materials

slurry sample (about 10 mL)

electrical conductivity meter (0 to 1999 μS)

10 mL graduated cylinder

conductivity standard solution (1.413 mmhos/cm at 25 °C)

thermometer

Procedure

1. Centrifuge slurry sample to settle solids
2. Allow sample to cool to room temperature, the same temperature as the standard solution.
3. Transfer 6 mL of aqueous sample to 10 mL graduated cylinder. This volume will be enough to fill the cylinder to overflowing upon insertion of probe.
4. Insert probe of conductivity meter into the graduated cylinder.
5. When readout has stabilized, record the cell conductivity of the sample, C_{sample} , in μS .
 - i. If the sample exceeds the range of the conductivity meter, repeat steps 3 to 6 with a dilution of the sample.
6. Perform steps 3 to 6 with the conductivity standard at the same level of dilution, and record the cell conductivity of the standard, C_{standard} , in μS .

Calculation: The electrical conductivity (EC) of the slurry is calculated as

$$E.C. = 1.413 (C_{\text{sample}} / C_{\text{standard}}) \text{ dS/m at } 25^{\circ}\text{C}$$

Frequently employed relations to other salinity parameters (from Soil Analysis 1965)

Total cation concentration (milli-equivalents per liter) = $10 \times \text{EC}$ (dS/m)

Osmotic pressure (atmospheres) = $0.36 \times \text{EC}$ (dS/m)

Salt concentration (mg/liter) = $\sim 640 \times \text{EC}$ (dS/m) (this correlation is not as strong as the first two)

3.2.4 Monitoring

Slurry concentration of ammonium-N was monitored to ensure sufficient nitrogen and was taken to indicate sufficient phosphorus, as both were added together. Temperature and pH were likewise measured on a regular basis, and corrections noted. Slurry volume lost due to evaporation and sampling was replaced with deionized water. Slurry that splashed onto the upper sides and lid was routinely rinsed back into the slurry with a spray bottle of deionized water.

A means of ongoing monitoring was sought so that the pace of biological activity in the slurry reactors could be followed over time. Preliminary testing assessed the following methods to indirectly estimate biodegradation rate:

- Ammonium-N and phosphate-P uptake
- CO_2 production by NaOH absorption
- Chemical Oxygen Demand (COD)
- Dissolved Oxygen Uptake Rate (DOUR)
- Fixed and volatile solids ignited at 550°C

Of these, DOUR was selected, and monitored for Experiment Series 2 through 4 in the experimental programme (the method is given in Appendix 2). Measurements were taken on a daily basis at the beginning of each experimental run, when activity was highest, then every

other day as activity slowed.

3.2.4.1 Method for Dissolved Oxygen Uptake Rate (DOUR)

This procedure is employed to measure the oxygen uptake rate of a slurry. It is adapted from Standard Method 2710B, 'Oxygen-Consumption Rate' (APHA, 1998a).

Materials

YSI dissolved oxygen probe and meter
stopwatch

Procedure

1. Oxygen probe and meter were calibrated according to manufacturer's instructions, by allowing the readout at the 'saturation' setting to equilibrate as the probe was exposed to air of 100% water saturation, then adjusting this readout to 100%.
2. Slurry to be tested was disconnected from mixing and aeration, and removed from water bath. This was done for ease of measurement, which was performed quickly so that change in temperature and homogeneity is minimal.
3. Probe was immersed in slurry and used to stir.
4. When dissolved oxygen meter readout equilibrates (in about 15 seconds), the reading was recorded and the time was noted.
5. Slurry is returned to water bath, and mixing resumed. Aeration was not reconnected.
6. After a time a second measurement is made, repeating steps 3-5. This time varies, and depends on the expected rate of oxygen uptake; slurries with high uptake will consume most of the dissolved oxygen within 10 minutes, while for less active slurries a pause of 40 minutes may be advised to affect a measurable decrease (a decrease of at least 2 mg/l was required between measurements, as explained below.)
7. After the second measurement the slurry was returned to the water bath, where mixing and aeration were reconnected.

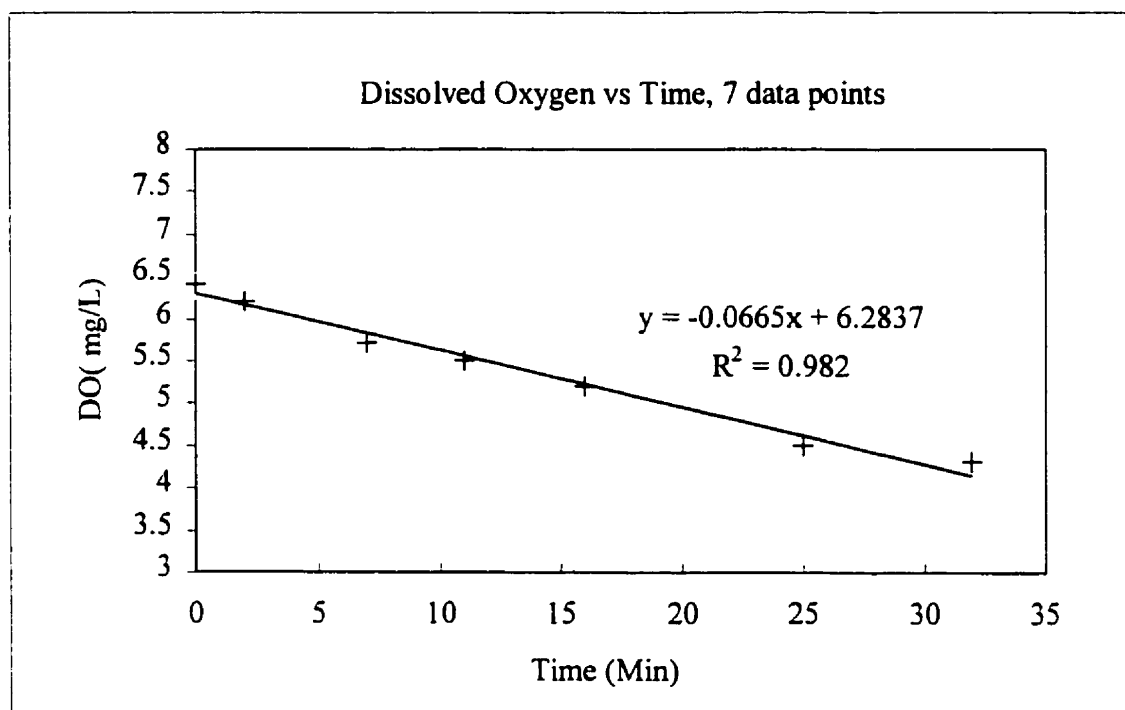
8. The dissolved oxygen uptake rate was calculated as the change in dissolved oxygen divided by the change in time.

$$\text{DOUR} = (\text{DO}_i - \text{DO}_n) / (\text{time}_i - \text{time}_n) \quad (\text{units: mg/L/hr})$$

Note on this adapted method:

The Standard Method advises 15 readings over 15 minutes, plotted as DO vs time. The slope of the best-fit line of this data is taken to be the dissolved oxygen uptake rate. Only 2 data points are used in this adapted method, to conserve time and simplify calculation. Because of this, measures must be undertaken to ensure the validity of this test method. Figure 8 presents a plot prepared from several measurements taken over 32 minutes, showing a linearity ($R^2=0.982$) for the range of measurement. The rate of dissolved oxygen uptake is taken from the negative slope of the graph,

$$\begin{aligned} \text{DOUR} &= 0.665 \text{ mg/l/minute} \\ &= 4.0 \text{ mg/l/hr.} \end{aligned}$$

Figure 8: Assessing DOUR Method Validity

From the data points, it is clear that the precision of the DO measurement (± 0.1 mg/l for the probe used) will affect the precision of the DOUR calculation, so as a point of the procedure a measured decrease of 2 mg/l between readings was required for calculations (with the exception of abiotic controls), limiting the error to $\pm 5\%$.

If the second reading is below 2mg/l the measured oxygen uptake rate is considered lower than actual; oxygen uptake is non-linear below this point (APHA, 1998a). Below 1 mg/l the probe is inaccurate. The slurry is re-aerated to saturation and the test is repeated with a smaller period of time separating the measurements. Keeping within these guidelines ensures that this method is suitable as a rough measure of microbial activity in this screening level testing.

3.2.4.2 Microbial Enumeration

The plate count method for hydrocarbon degrading microbes is taken from Clark's "Agar-Plate Method for Total Microbial Count" (Clark, 1965), using a crude oil media). It is used to compare the initial hydrocarbon-degrading populations of the inoculated and non-inoculated treatments in Experimental Series 2

Materials

petri dishes (9 for each soil to be tested)

10-mL pipet

autoclave

test tubes with sealable caps

deionized water

Erlenmeyer flask

hot plate

stirring rod

water bath

foil

microscope

Procedure

1. Preparation of serial dilutions:

- i. For each soil sample to be tested, 8 test tubes were autoclaved at 150 °C for half an hour, and allowed to cool.
- ii. A measured mass of wet soil (approximately 1g) was transferred to the first test tube.
- iii. The dry mass of soil added by moisture analysis of a separate sample of the soil was determined by the procedure for dry soil analysis in section 3.2.5.1.
- iv. 9mL of dionized water was added to the test tube. This established a 10^{-1} dilution.

- v. The test tube was capped and shaken vigorously.
- vi. 1mL was immediately pipetted from this test tube to another test tube to establish a 10^{-2} dilution. Disposable pipets were used to ensure sterility.
- vii. steps v and vi were repeated to provide a dilution series through 10^{-7} .

2. Preparation of agar media

- i. The following were stirred into 1 liter deionized water, heated in an Erlenmyer flask on a hot plate:
 - 2g of oil
 - 2 mL of Simplegreen TM Surfactant
 - 15 g of agar
 - $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 0.2 g
 - CaCl_2 0.02g
 - KH_2PO_4 1.0g
 - $\text{NH}_4 \text{NO}_3$ 1.0g
 - $\text{K}_2 \text{HPO}_4$ 1.0g
 - FeCl_3 2 drops
- ii. This mixture was pipetted in 15 mL portions into test tubes while still hot.
- iii. One test tube of this media was required for each pour agar plate to be prepared. Excess test tubes were prepared to compensate for spillage in the autoclave
- iv. Foil caps were placed on the test tubes.
- v. The tubes were autoclaved at 150°C for half an hour.
- vi. The tubes were cooled at 45°C in the water bath.

3. Poured plates

- i. The 10^{-7} dilution was agitated, and 1 mL pipetted onto each of 3 sterile petri dishes.
- ii. Step i was repeated for the 10^{-6} and 10^{-5} dilutions.
- iii. A test tube of agar media was poured into each seeded petri dish, and each was swirled to mix the media with the diluted sample.
- iv. The agar was left to solidify.

4. When the agar had solidified, the petri dishes were inverted and stored in a plastic bag to retain moisture.
5. The petri dishes were left undisturbed for 10 days at 21°C. In this incubation time hydrocarbon degrading microorganisms formed colonies visible as white specks.
6. With the aid of a low-power microscope and a transparent grid, the number of colony forming units (CFUs) was enumerated for each plate.
7. By multiplying the number of CFUs per plate by the dilution factor, the number of CFUs per gram of moist soil was calculated. From the three repetitions at each of three dilutions, an average was calculated.
8. By dividing this average by the grams of dry matter per gram of moist soil, as determined by the procedure for dry soil analysis, the number of CFU's per gram of dry soil was calculated.

3.2.5 Contaminant Analysis

Analysis of petroleum was undertaken before and after treatment, as well as during treatment for some experiments as noted in the experimental designs. The analysis of contamination before treatment involved analysis of a portion of the homogenized soil used to prepare the slurry.

Analysis at the end of treatment required dewatering of the slurry by centrifugation. The soil solids, typically concentrated to a muddy constituency, were then homogenized by thorough mixing. A portion of this mixture was used to determine the moisture content (by the method in Appendix 4), while another portion was prepared for Soxhlet extraction. The supernatant of the centrifugation process was discarded. In one instance, Slurry E in Experiment Series 3, liquid hydrocarbons were observed as a sheen on the surface on the slurry. A DCM extract of the supernatant performed using a separatory funnel yielded no petroleum in the gravimetric range of measurement for this slurry. Nonetheless, the slurry water represents a possible loss of contaminant, contributing to the reductions measured in the abiotic controls.

For slurry samples taken during a treatment, a 100mL sample of slurry was centrifuged at 200g for 30 minutes, then analyzed. Since stratification by particle size occurs within the slurry, the jar was capped and shaken vigorously and the samples taken immediately when the complete range of particle sizes were in suspension. The specific analysis of petroleum varied between experiments, and the methods used are listed in each experimental design.

3.2.5.1 Dry Soil Analysis

The amount of dry soil from which an extract is taken is assessed by this method

Materials

soil or slurry sample

porcelain dish

ovens capable of 105°C

analytic balance (0.0001g)

dessicator

Procedure

1. Take tare measurement of empty dish (empty)
2. Add soil or slurry sample to dish
3. Take mass measurement (wet mass)
4. Heat thimble and contents in at 105°C for 16 hrs, a time period which has been found to bring the sample to a constant mass
5. Cool in dessicator
6. Take mass measurement (dry mass)
7. Dry mass as fraction of wet mass is: $(\text{dry mass} - \text{empty}) / (\text{wet mass} - \text{empty})$
8. Apply this ratio to calculations based on soil and slurry to achieve figures based on dry mass

3.2.5.2 Soxhlet Extraction

A Soxhlet extractor is an apparatus used to extract solutes from solid matrices using a volatile solvent. The sample to be extracted is placed in the center chamber. Solvent heated in the reservoir vaporizes, condensing in the condenser, dripping on to the sample. When solvent fills the center chamber, siphon action returns it to the reservoir. Continual cycling of fresh solvent through the sample concentrates soluble matter in the reservoir. (Method from ASTM, 1998d)

Materials

soxhlet apparatus

water bath or suitable heat source for solvent reservoir

reagent grade solvent (dichloromethane-DCM)

cold water source for condensor

cellulose extraction thimble

soil or slurry sample in jar

granular sodium sulfate

mortar and pestle

analytic balance

centrifuge

Procedure

1. Sample preparation

- i. Contain the soil or slurry sample in a glass jar of known mass
- ii. If the sample is a slurry, concentrate the soil solids by accelerating settlement in a centrifuge. Discard supernatant. Treat hereafter as a soil sample.
- iii. Reserve a portion of the soil to analyze moisture content.
- iv. Measure the mass of the soil to be extracted and the jar in which it is contained.
- v. Mix sodium sulfate into the soil until the entire mixture is granular and extra added sodium sulfate is visible as white grains. The granular structure ensures

contact of solvent with soil.

- vi. Measure the mass of the mixture and the jar.
 - vii. Let the mixture sit for at least one-half hour.
 - viii. Using the mortar and pestle, grind the mixture until it passes a 10-mesh sieve (2mm openings). This can be assessed visually if all particles are clearly smaller than 2mm in diameter
2. Measure the mass of the extraction thimble
 3. Add prepared soil/ sodium sulfate mixture to thimble
 4. Measure mass of thimble and mixture
 5. Rinse glassware with DCM immediately prior to use
 6. Place the thimble in the soxhlet inner chamber.
 7. Fill the solvent reservoir with 400mL DCM. (Loss of solvent as is a normal operating phenomenon as the interior of the condensor is open to the fume hood. This volume ensures that enough solvent will remain in the apparatus to maintain the circulation process)
 8. Assemble the soxhlet apparatus.
 9. Activate flow of cold water through the condensor.
 10. Heat the solvent using the water bath. A bath temperature of 60°C results in 3 solvent cycles per hour; 2 days of circulation results in approximately 150 solvent cycles.
 11. After solvent has circulated, the extract and solvent are filtered through a 20cm column of anhydrous sodium sulfate to remove moisture.
 12. The extract is analyzed by gravimetric analysis (Section 3.2.5.3) or GC-FID (Section 3.2.5.5).

3.2.5.3 Gravimetric Analysis

Materials

extract in solvent

beaker

fume hood

mass balance

dessicator

Procedure

1. Take tare measurement of empty beaker
2. Transfer extract in solvent to beaker
3. Allow solvent to volatilize in fume hood
4. Place beaker in dessicator
5. Measure periodically until extract and beaker reach a constant mass
6. Mass of extract is mass of beaker and extract minus mass of beaker.

Calculation

Oil and Grease is achieved by combining the measurements made in dry soil analysis, soxhlet extraction, and gravimetric analysis. These calculations are also conducted to calculated individual fractions from component class separation.

Table 7: Sample Calculation for Oil and Grease (O&G)

	measurement	calculation	example
1	jar		111.7 g
2	jar+wet soil		148.7
3	jar+wet soil+dessicant		267.3
4	wet soil as fraction of mix	$(2-1) / (3-1)$	0.238
5	empty dish		25.7798
6	dish and wet soil		28.0567
7	dish and dried soil		26.8114
8	dry soil as fraction of wet	$(7-5) / (6-5)$	0.453
9	dry soil as fraction of mix	$8 / 4$	0.108
10	extraction thimble		11.4325
11	thimble and mix		109.3776
12	drysoil in thimble	$(11-10)*9$	10.552
13	volume of solvent		180 mL
14	volume of aliquot		25
15	mass of beaker		27.9724 g
16	mass of beaker and extract		28.0547
17	extract (aliquot)	$16-15$	0.0823
18	extract (total)	$17*(13/14)$	0.5926
19	Oil and Grease	$18/ 12$	0.056
			5.6 % dwt
			56000 mg/kg

Accuracy: A measure of the material extractable by the Soxhlet method was achieved by analyzing clean soils spiked with petroleum. Oil and grease measurements were made after 1 day of aging:

4 samples

mean= 88% recovery

std.dev= 0.21%

95% confidence interval (t-distribution)= $\pm 6\%$

While extractability is expected vary with time of soil contact, for flare pit soil samples it is assumed that that this effect has stabilized after prolonged contact.

Precision: Oil and Grease was measured for five replicates of a single homogenized soil sample

5 samples

mean= 1.66 % dwt

std.dev.= 0.06 % dwt

95% confidence interval (t-distribution)= $\pm 0.116\%$ dwt
($=\pm 7\%$ of mean)

Bias: Blank runs varied from 0.00% to 0.10%, averaging 0.05%

3.2.5.4 Method for Component Class Separation

O&G samples could be separated into saturate, aromatic, polar and asphaltene fractions using classical column chromatography with an activated silica gel column. In practice the measured distinction between asphaltenes and polar compounds was imprecise. The procedure for precipitating asphaltenes is given here, but in the experimentation they are reported together as “polars”.

A. Precipitation of Asphaltenes

Materials

sample of extracted oil in beaker (beaker 1)

n-pentane, chilled, 125mL

DCM, 1 mL

funnel

fast filter paper (sargent 500)

250mL beaker to receive filtered material (beaker 2)

Procedure

1. Measure mass of beaker 2 to tare for step 6.
2. Add sufficient DCM (~1mL) to fluidize the oil extract in beaker 1
3. Add 100 mL pentane to beaker 1, mix by swirling
4. Cover beaker 1 and let sit for 1/2hr
5. Filter contents of beaker 1 into beaker 2 using fast filter paper
6. Evaporate solvent and measure mass of residue in beaker 2. (The residue is maltenes, the non-asphaltene fraction of oil).
7. Asphaltenes are calculated as original oil minus maltenes.

B. Column separation of Saturates, Aromatics, and NSO's

Materials

pre-weighed beakers, 100mL

buret, 50mL

solvent-leached cellulose wool

15g silica gel 100-200 mesh, activated by heating at 250 degrees for 16 hrs

pentane

DCM (dichloromethane)

2-propanol

(HPLC Grade reagents were used)

beaker 2 with maltenes, (from procedure 1 above)

For this separation a chromatography column was prepared using a 50 mL glass buret. Plugs of steel wool and solvent-leached cotton wool at the bottom support the absorbent, activated silica gel.

Procedure

1. Prepare the column. (Silica gel is slurry-packed in the column to promote even flow of eluents)
 - i. With stopcock shut, add about 15 mL pentane to the buret.
 - ii. Add the silica gel slowly, tapping the side of the buret to cause it to settle in the pentane without air bubbles. Try to add continuously, so as to avoid discontinuities of settlement.
 - iii. As necessary, pour pentane down inside of buret to rinse down gel particles and raise level of pentane in buret
 - iv. When all of the silica gel is added, place a leached cotton plug in the column above the gel. This prevents addition of solvents from disturbing the gel
2. Introduce the sample (contents of beaker 2) into the column by re-dissolving it in a small amount of pentane.
3. Place a pre-weighed 100mL beaker beneath the buret and open the stop-cock.
4. Pass the sample through the column with 80 mL of pentane. This will elute the saturates, with the other components retained by the silica gel.
5. Place a pre-weighed 100mL beaker beneath the buret and open the stop-cock.
6. Elute the aromatic fraction with 80mL DCM.
7. Place a pre-weighed 100mL beaker beneath the buret and open the stop-cock.
8. Elute the NSO's with 80mL of 2-propanol
9. Allow solvents to evaporate in a fume hood then analyze gravimetrically or by GC-FID.

Regarding this method:

This method is used to measure Mineral Oil and Grease, which is the sum of the saturate and aromatic Fractions. This method was adapted from ASTM methods D1319 and D2549 (ASTM 1998a, 1998b). The standard methods for oil and grease of the American Public Health Association (APHA, 1998b) employs mixing of powdered silica gel with sample and subsequent filtration, as opposed to the column elution used here. The flow rate of column elution is adjusted to ensure the same contact time between sample and silica gel. APHA advises an allowance of 30g silica gel for every 1g of polar material to be absorbed. The column setup holds 15 g of silica gel, so to keep to the above allowance samples to separated did not exceed 0.5g of total oil, or if exceeding that measure were separated on two columns in sequence. The APHA methods employs a freon or hexane solvent, so results may differ. DCM is used in Alberta analytical lab procedures so its use is preferred here for correlation of lab data to accumulated field data.

3.2.5.5 GC-FID Quantification

Analysis was undertaken using an HP-6890 Gas chromatograph with the following attributes:

Column: HP-1 Methyl Siloxane Capillary 30.0m x 250 μ m x 0.25 μ m nominal

Carrier gas: Helium, 19.51 psi, 1.7 mL/min, average velocity 38 cm/s

1.0 μ L injection

Inlet: 320°C Detector: 325°C

Temperature program: Initial 75°C, hold 5 minutes

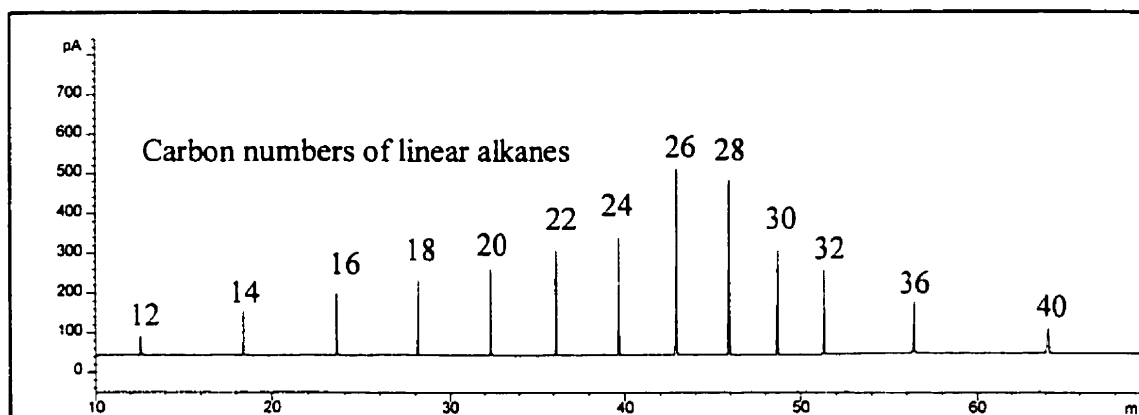
Ramp 5°C/min to 320°C

Hold 30 minutes

The gas chromatograph separates components of an injected sample along a narrow column by their boiling point, according to the temperature program. The output is the chromatogram, a graphical record of the of elution of the sample at the end of the column, as measured by a flame ionization detector. The horizontal axis of the chromatogram output is

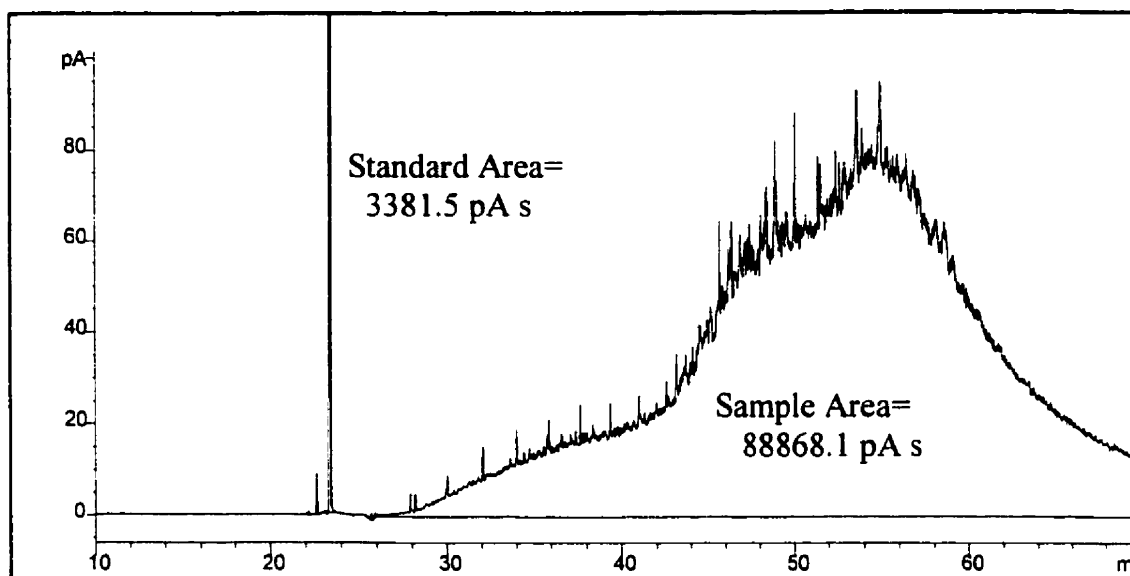
elution time in minutes, while the vertical axis is in pA (picoAmps), a measure of current at the flame ionization detector roughly proportional to the mass rate of elution at that time. The chromatograph of a wax standard is presented in Figure 12. This is used to establish elution times for linear alkanes in the boiling point profile.

Figure 9: Wax standard for GC-FID analysis



G.C. Quantification of the mass of a sample is achieved using hexadecane ($n\text{-C}_{16}\text{H}_{34}$) as an internal standard. To quantify the mass of a petroleum sample, a known mass of hexadecane is added. For most of the samples tested in this project, a mass of 0.000835g was added, diluted in a stock standard of DCM. The solvent, standard and sample were then mixed by swirling. This mixture was analyzed using the GC-FID by the program above. This quantification method was selected because the ratio of the standard to the petroleum would not vary with evaporation of the solvent during storage. An example chromatogram is presented in Figure 10.

Figure 10: Sample Chromatogram Illustrating Quantification



For petroleum samples, the chromatogram yielded a peak for the hexadecane standard and a series of peaks and a hump for the sample. The area of the sample peaks and hump are taken to be proportional to the sample mass by the same factor that the area of the standard peak is taken to be proportional to its mass (0.000835g).

$$(\text{area of n-C16 peak}) / 0.000835\text{g} = (\text{area of sample peaks}) / (\text{sample mass})$$

$$\text{sample mass} = (\text{area of sample peaks}) * 0.000835\text{g} / (\text{area of n-C16 peak})$$

For the sample in Figure 10, the mass of the measured extract would be

$$\begin{aligned} \text{sample mass} &= (88868.1 \text{ pA s}) * 0.000835\text{g} / (3318.5 \text{ pA s}) \\ &= 0.0224 \text{ g} \end{aligned}$$

For samples with sizeable n-C16 peaks prior to standard addition two samples were run, one

with and one without standard. In this manner a correction could be made whereby the natural C16 peak was subtracted from the standard.

Repeated tests on a single sample indicate this to be a method of modest precision; calculated quantities based on a single sample varied by up to 20%.

3.2.5.6 Simplified Extraction and Calculation of n-C17/Prystane Ratios

This method was applied to quantify the rate of degradation of n-alkanes, which was observed to proceed rapidly at the beginning of slurry treatment. The extraction and GC-FID analysis was simplified so that this test could be conducted daily. The method compares the slurry concentration of heptadecane (n-C17) to pristane (an easily identifiable branched saturate which degrades more slowly). The location of these compounds is indicated in Figure 14.

Materials

2 beakers

Funnel

Filter paper

DCM

Procedure

1. Sample preparation

- i.) Concentrate the soil solids of ~80 mL of slurry by accelerating settlement in a centrifuge
- ii.) Discard supernatant.
- iii.) Contain the soil or slurry sample in a glass jar
- iv.) Mix sodium sulfate into the soil until the entire mixture is granular and extra added sodium sulfate is visible as white grains.
- v.) Let the mixture sit for at least one-half hour

- vi.) Add sufficient DCM to submerge solids, and let sit for 2 hours
- vii.) Filter DCM into clean beaker
- viii.) Allow DCM to evaporate, concentrating the extract for GC-FID analysis

2. Sample analysis

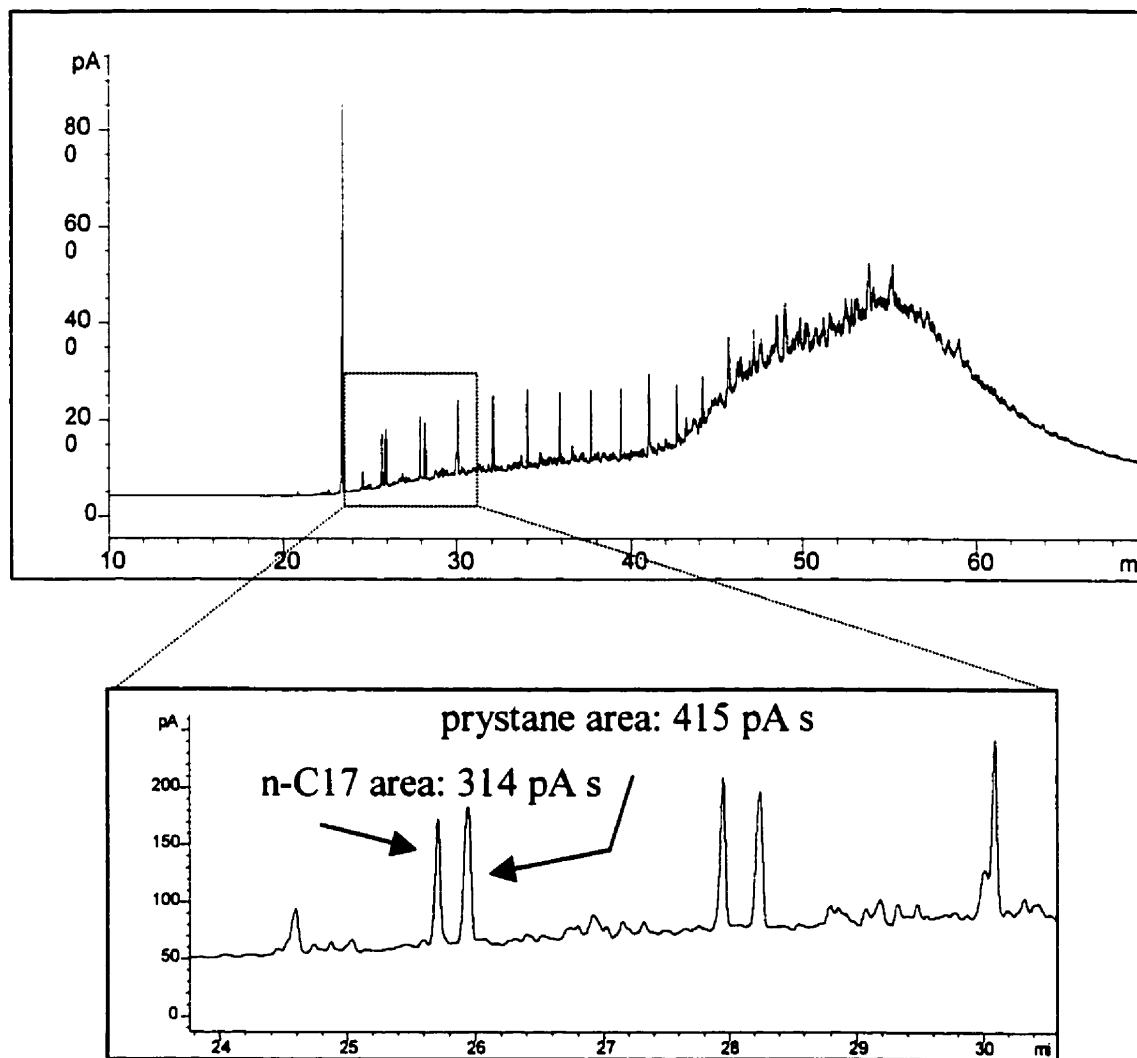
- i.) The extract is injected into the GC-FID
- ii.) From the resulting chromatogram, the peak of heptadecane (n-C17) (identified from its elution time compared to that of a standard) is integrated using the GC's analysis software
- iii) The peak of pristane (eluting directly after n-C17) is integrated in the same manner
- iv) The ratio of n-C17 to pristane is calculated as follows:

$$\text{n-C17/pristane} = (\text{area of n-C17 peak}) / (\text{area of pristane peak})$$

For the sample in Figure 14 the ratio is

$$\begin{aligned}\text{n-C17/prystane} &= (315 \text{ pA s}) / (415 \text{ pA s}) \\ &= 0.757.\end{aligned}$$

Figure 11: Location of n-C17 and Prystane in Chromatogram



3.3 Experimental Programme

3.3.1 Experiment Series 1: Significance of Inoculation

The purpose of this test was to determine if addition of an inoculum of petroleum-degrading microbes offered a significant advantage to the slurry test treatment. The results of this test were affected by a false positive in the contamination measurement by which the effect of inoculation could not be assessed. The experiment is presented here due to its importance in the evaluation of methods for measuring degradation.

3.3.1.1 Significance of Inoculation: Design

The inoculum used was cultured from a mixture of flare pit soils in slurry with added petroleum; the suspended solids in this slurry were plated to 8×10^7 CFU (colony forming units) per mL. (Agar Plate Method (Clark, 1965) with a crude oil media). Forty milliliters of this inoculum was added to each of three slurries, approximately 3×10^7 CFU per gram of dry soil. Plate counts of an inoculated slurry indicated 6×10^7 CFU/g dry soil, compared to 2×10^7 CFU/g dry soil for a non-inoculated slurry.

Table 8: Experiment Series 1: Details

Significance of Inoculation	
Run time	6 weeks
Temp.	30°C
pH	neutral
Soil used:	A (see Table 5)
Additives:	surfactant, antifoam, nutrients,
	microbial inoculum (varied)
Treatment	Status
1	abiotic control with mercuric chloride
2	abiotic control with mercuric chloride
3	inoculated
4	inoculated
5	inoculated
6	indigenous microbes alone
7	indigenous microbes alone
8	indigenous microbes alone

Oil and Grease was assessed by gravimetric analysis of a soxhlet extract of the slurry, both before and after treatment.

3.4.2.2 Significance of Inoculation: Results

Table 9: Contaminant Levels for Inoculation Comparison

Oil and Grease: % / dry weight				
Treatment		initial	2 weeks	3 weeks
abiotic	1	1.5%	14.4	5.6
	2	"	11.3	7.6
inoculated	3	"	30.9	9.7
	4	"	2.8	6.9
	5	"	7.9	---
indigenous	6	"	8.1	4.3
	7	"	8.5	7.4
	8	"	7.7	7.4

O&G measurements were conducted for samples 4 and 8 at the 3rd week by analysis at an outside environmental laboratory. Also tested were the slurry as initially prepared, and the soil from which it was prepared:

Table 10: Comparison of Experimental Results With Outside Lab Analysis

Sample	Oil & Grease (% dry mass)	
	Outside lab analysis	Results of this experiment
initial soil	0.45	1.5
initial slurry	2.40	--
4	2.92	6.9
8	3.50	7.4

3.5.2.3 Significance of Inoculation: Interpretation

The results of this experiment offer little insight into the benefit of inoculation for the slurry treatment. The results instead demanded focus on the method of quantifying degradation of O&G; the measurements show an increase in O&G, in varying amounts. Independent analysis of soils by an outside laboratory yielded a similar trend, with different results attributable to different methods of measuring the dry mass of soil; the outside lab uses soil air-dried to a constant weight (as opposed to oven dried), so the percentages of oil and grease reported are lower. From the outside lab results it is apparent that treatment over time is not required to cause these measurement increases; the act of creating slurry from a soil sample was sufficient to cause the increase.

A quick test shows Mercuric Chloride (HgCl_2) to be soluble in the DCM used for extraction, so this was a possible interference in the abiotic controls. Likewise the surfactant used was solvent extractable, but was not added in amounts large enough to be a significant positive interference. Treatment of a sample of extract with activated silica gel removed 75% of the extract as polar material, yielding a result closer to the initial measurement of 1.5% Oil and Grease.

It is hypothesized that the addition of this surfactant made slurry water more soluble in the solvent, increasing the readings. Soil organic matter of variable solubility may also contribute, but it seems unlikely that the soil contained as much as 30.9% of such material, as indicated in the results for sample 8.

GC-FID analysis of preserved samples of treatment 4 (inoculated) and 8 (indigenous microbes only) conducted several months later showed little degradation of n-alkanes in the chromatogram of the magnitude experienced in later experimentation, indicating that degradation was minimal in both samples. Experience from later experiments shows that these compounds usually degrade quickly in the slurry. In light of this, the aerobic activity observed may simply have been due to biodegradation of the surfactant.

3.3.2 Experiment Series 2: Petroleum Spiked Soils

In response to difficulties encountered in the measurement of oil and grease, this experiment was directed mainly at conducting the treatability test on a soil where the level of petroleum contamination was known with certainty. Different clean soil types were spiked with petroleum extracted from a heavily contaminated soil, as a test of the possible effects of soil type on biodegradation. From the literature review (section 2.5.3.1), it is acknowledged that there are many possible contributing factors to the effect of soil sorption. Limits to the soil characterization available in the database suggests a meaningful framework is available to fully apply the effects of soil sorption is unlikely. However, the findings are presented here as the beginnings of an approach to this phenomena that would strive to account for multiple soil characteristics and the possible interactions between them.

3.3.3.1 Petroleum Spiked Soils: Design

This test was designed as a 2x2 factorial, with the variables being clay content (0% clay/100% clay) and organic matter. Different levels of organic matter were achieved by igniting soils at 550°C to volatilize this material. Test samples are designated as "ignited" or "not ignited" to acknowledge the fact that while this procedure will volatilize most organic material, it may also cause other physical or chemical changes to the soil. Sufficient samples of each spiked soil were retained to conduct this test later, to include time of contact as a variable. All soils were heat sterilized and inoculated to ensure equal populations of hydrocarbon degrading microbes.

Table 11: Experiment Series 2: Details

Petroleum Spiked Soils				
Run time	6 weeks			
Temp.	30°C			
pH	neutral			
Soil used:	Calcium bentonite clay or fine Sand (0.16-0.315 mm) spiked with oil (All samples 7% O&G)			
Additives:	surfactant, antifoam, nutrients,			
	microbial inoculum, oil			
Treatment	Soil type	Name	Clay	Ignited
1	clay	C	+	-
2	ignited clay	IC	+	+
3	sand	S	-	-
4	ignited sand	IS1	-	+
5	2nd replicate	IS2	-	+
6	3rd replicate	IS3	-	+
7	abiotic	SC	-	+

Oil and Grease (O&G) was assessed by gravimetric analysis of a soxhlet extract of the slurry, before and after treatment.

3.3.2.2 Petroleum Spiked Soils: Results

Figure 12: Graph of DOUR over Time for Clay

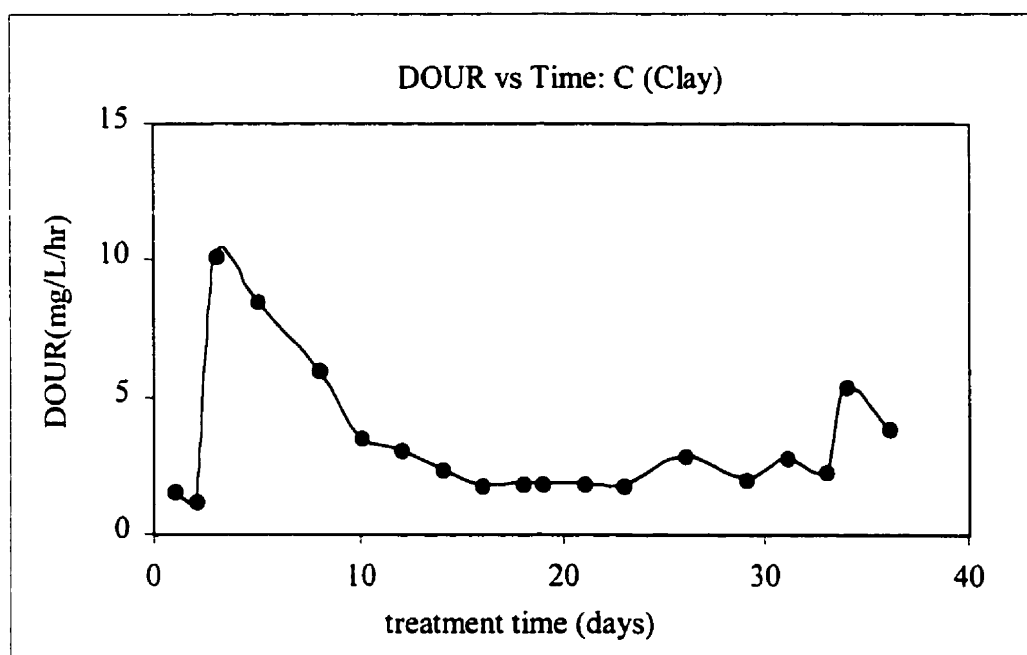


Figure 13: Graph of DOUR over Time for Ignited Clay

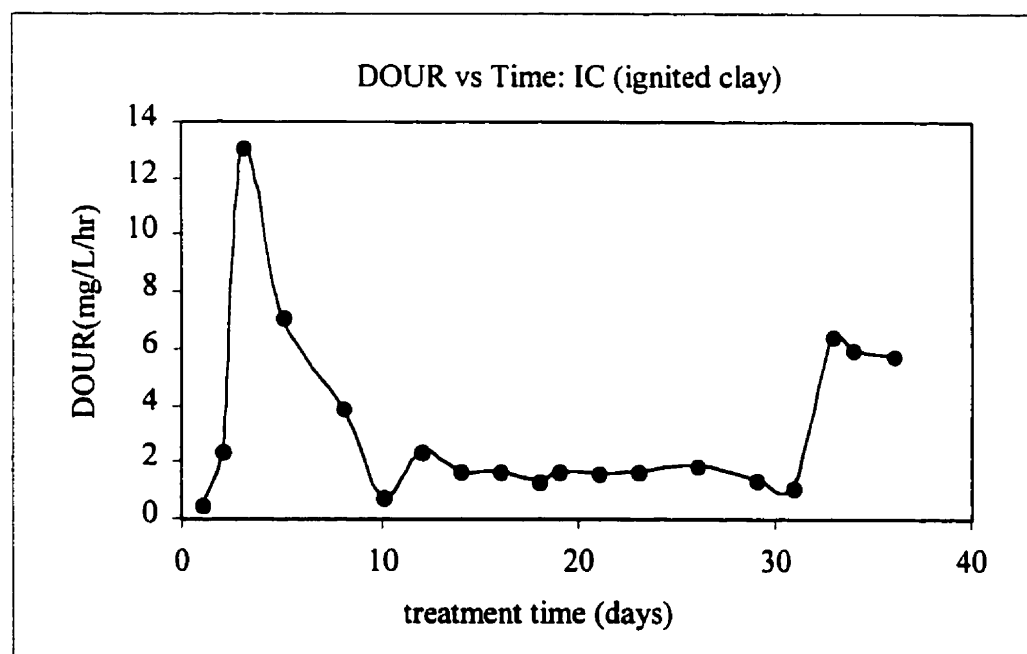


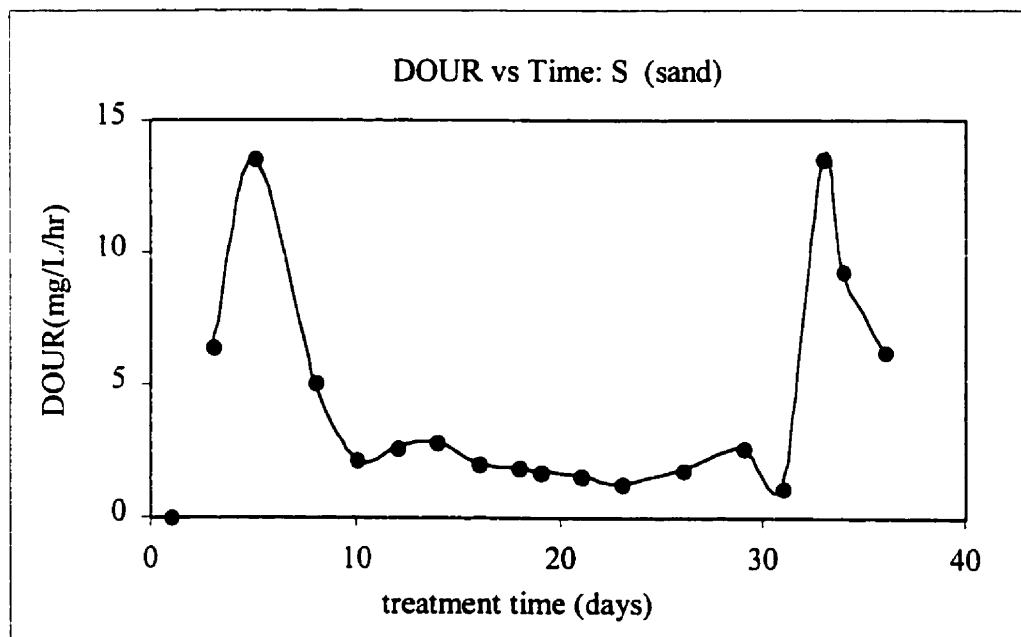
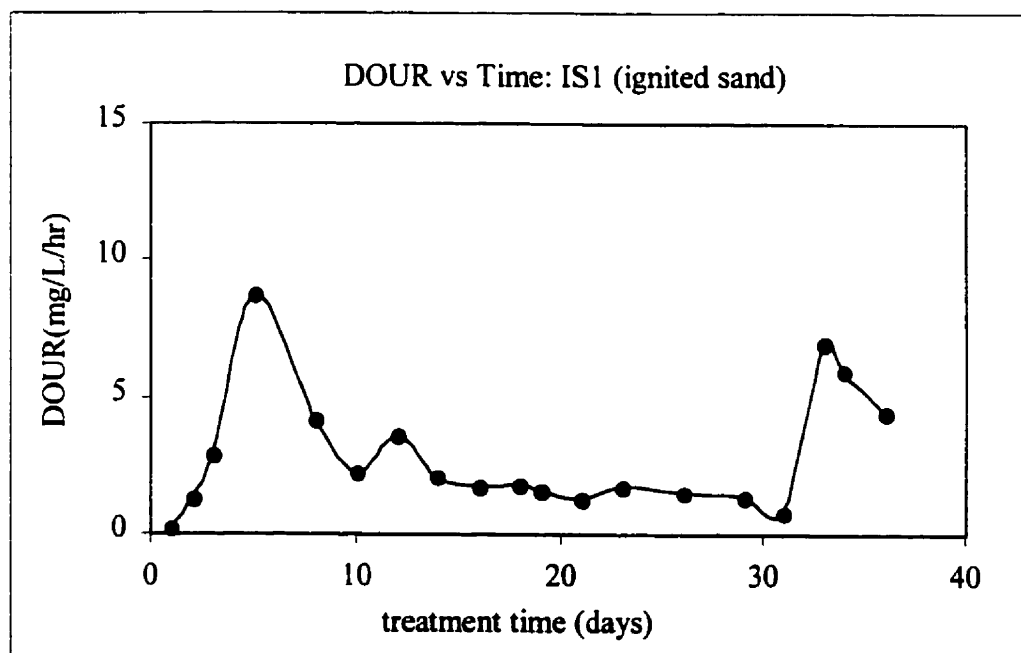
Figure 14: Graph of DOUR over Time for Sand**Figure 15: Graph of DOUR over Time for Ignited Sand (First Replicate)**

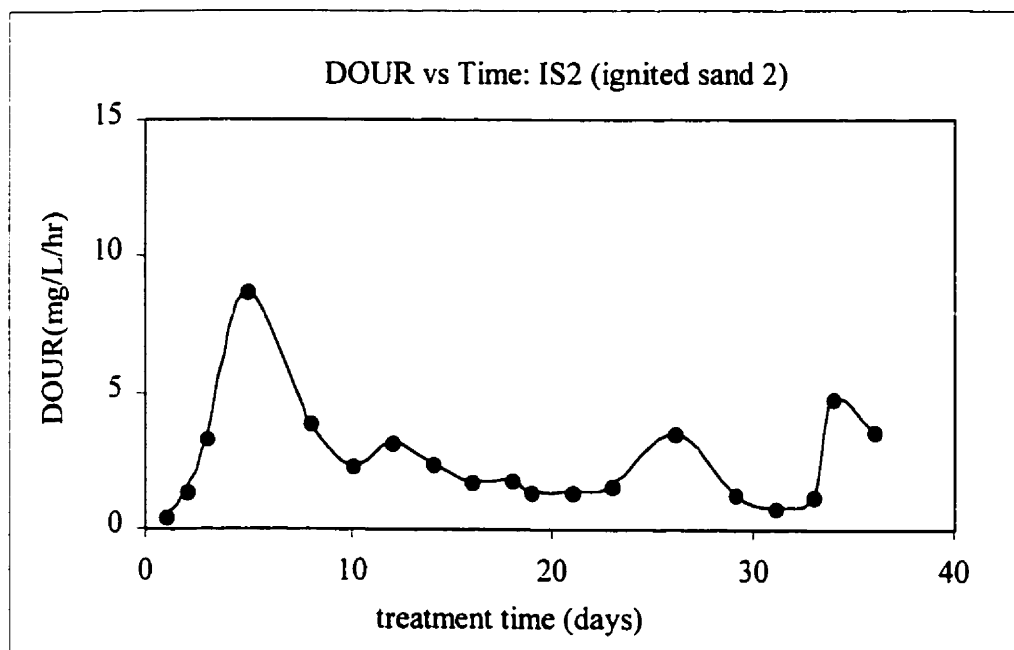
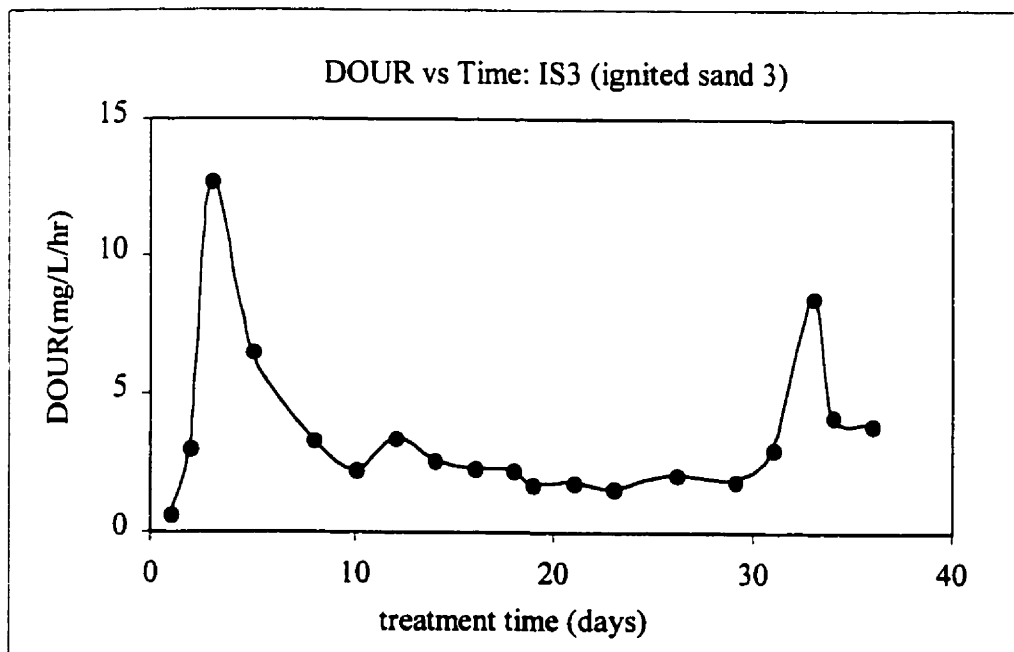
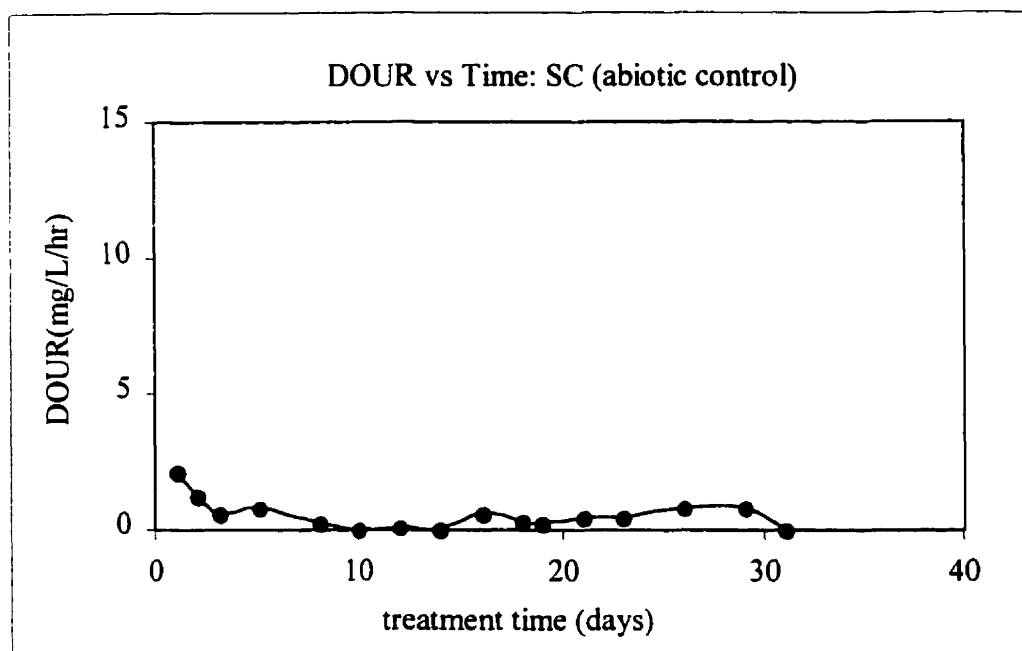
Figure 16: Graph of DOUR over Time for Ignited Sand (Second Replicate)**Figure 17: Graph of DOUR over Time for Ignited Sand (Third Replicate)**

Figure 18: Graph of DOUR over Time for Abiotic Control**Table 12: O&G Data for Spiked Soils after 6 Weeks**

Treatment	Name	O&G %dwt	Percent reduction with respect to initial concentration	Percent reduction with respect to abiotic control
1 clay	C	6.0	14	-7 (increase)
2 ignited clay	IC	4.9	30	13
3 sand	S	4.2	40	25
4 ignited sand	IS1	5.2	26	7
2 nd replicate	IS2	4.8	31	14
3 rd replicate	IS3	5.5	21	2
5 abiotic	SC	5.6	20	--
initial oil		7	--	--

3.3.2.3 Petroleum Spiked Soils: Interpretation

An analysis of variance (ANOVA) is applied to assess the significance of clay content and ignition in this experiment. The effect of varying clay or ignition factors is statistically analyzed to determine whether this difference is significantly larger than random variability. The three replicates performed for ignited sand are used to estimate random variability. The F-statistic calculated in the ANOVA is a measure of the magnitude of a hypothesized effect compared to experimental error. F-crit is determined from statistical tables for the appropriate degrees of freedom and the level of significance $\alpha=0.05$, and represents the maximum value of F that would occur for 95% of comparisons between variabilities that are due to error alone. Thus, if the F value for a given effect exceeds F-crit, it is likely that the variability exceeds error alone.

Table 13: ANOVA (%Reductions With Respect to Initial Contamination)

ANOVA					
Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	F	F-crit $\alpha=0.05$
Clay	20.25	1	20	1.9048	5.317645
Ignition	0.25	1	0.25	0.0238	5.317645
Interaction	72.25	1	72	0.6857	5.317645
Error	21	2	10.5		

Table 14: ANOVA (% Reductions With Respect to Abiotic Control)

ANOVA					
Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	F	F-crit $\alpha=0.05$
Clay	44.22	1	44.2225	1.2171	5.317645
Ignition	1.82	1	1.8225	0.0502	5.317645
Interaction	9.33	1	9.325	0.2566	5.317645
Error	72.76	2	72.76		

The analyses of variance show that neither clay nor ignition cause an effect on the extent of biodegradation that is greater than the variability of error. Reductions relative to initial are good while reductions relative to abiotic control are modest. Considering the dissolved oxygen uptake rate, it appears that the 4% salinity was sufficient to inhibit the abiotic control SC, and experiments to follow also suggest that this concentration will inhibit activity. Little volatilization loss is expected since the initial concentration is based on oil for which volatilization had stabilized. The decrease in the abiotic control is possibly a soil sorption phenomenon, with the contaminant decreasing in extractability over time. It is to be expected that such a phenomenon would repeat itself in the biologically treated samples, although perhaps to varying extents based on the type of soil.

No further experiments were conducted on the effect of soil components on the biodegradation process. While from the literature review it is expected that the nature of the soil will have an effect, the means of assessing and applying that affect are beyond the scope of this project. It would likely require comprehensive characterization and comparison of soil parameters; there are several different types of clay to be considered, and types of organic matter with widely different physical and chemical properties. Most research that is presently proceeding on the issue of soil sorption does so with single compound contaminants or simple mixtures, rather than petroleum. The application of findings would

be limited by the availability of site characterization data.

The peak at the end of each DOUR graph corresponds to the addition of extra surfactant. This may be due to an increase in the availability of the contaminant due to this addition, but in light of the findings of the previous test (high DOUR in the presence of surfactant, with no petroleum degradation), it is just as likely that the high DOUR at the beginning and end of this experiment is due to degradation of the surfactant. Analysis performed in the experiment to follow confirms this phenomenon by quantifying petroleum both before and after surfactant addition, and observing DOUR peaks without contaminant degradation.

3.3.3 Experiment Series 3: Comparing Petroleum Composition

Five contaminated soils were selected for this experiment to observe the degradation process for contaminants of different concentration and composition. Analyses of O&G, MO&G component classes and GC-FID boiling point profiles were undertaken at frequent intervals. The intention of this was to establish rates of biodegradation as well as endpoints based on these parameters, comparing the effects of composition and concentration between the five soils tested.

3.3.3.1 Comparing Petroleum Composition: Design

Table 15: Experiment Series 3: Details

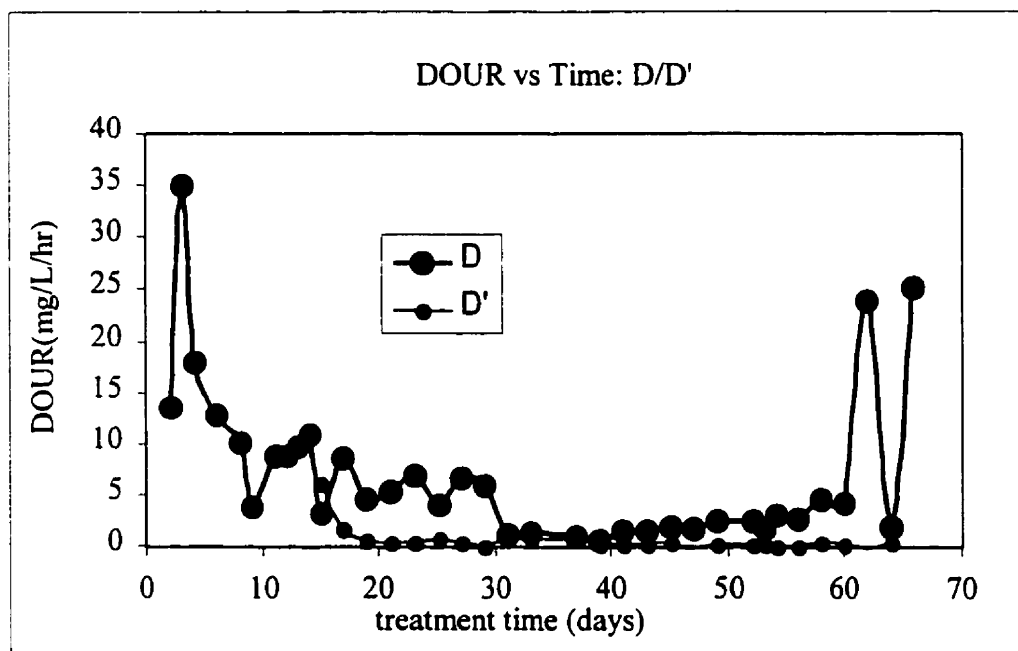
Comparing Petroleum Composition		
Run time	6 weeks	
Temp.	30°C	
PH	neutral	
Soil used:	D,E,F,H,I (see Table 5)	
Additives:	antifoam, nutrients,	
Treatment	Soil	Status
1	D	active
2	D'	abiotic * *4 g/L sodium azide added
3	E	active
4	E'	abiotic *
5	F	active
6	F'	abiotic *
7	H	active
8	H'	abiotic *
9	I	active
10	I'	abiotic *

For this experiment, Oil and Grease (O&G) was measured at about 10 day intervals to establish a rate of degradation over 2 months. At these intervals component class separation was performed on slurries D, F, and H, to determine saturates, aromatics, polars, and Mineral Oil and Grease (MOG). For slurries E and I, of lesser petroleum concentration, the class separation was performed only on the initial and final extract. Once gravimetric measurements were taken, GC-FID analysis was undertaken for each sample; selected chromatograms relevant to the interpretation are presented here.

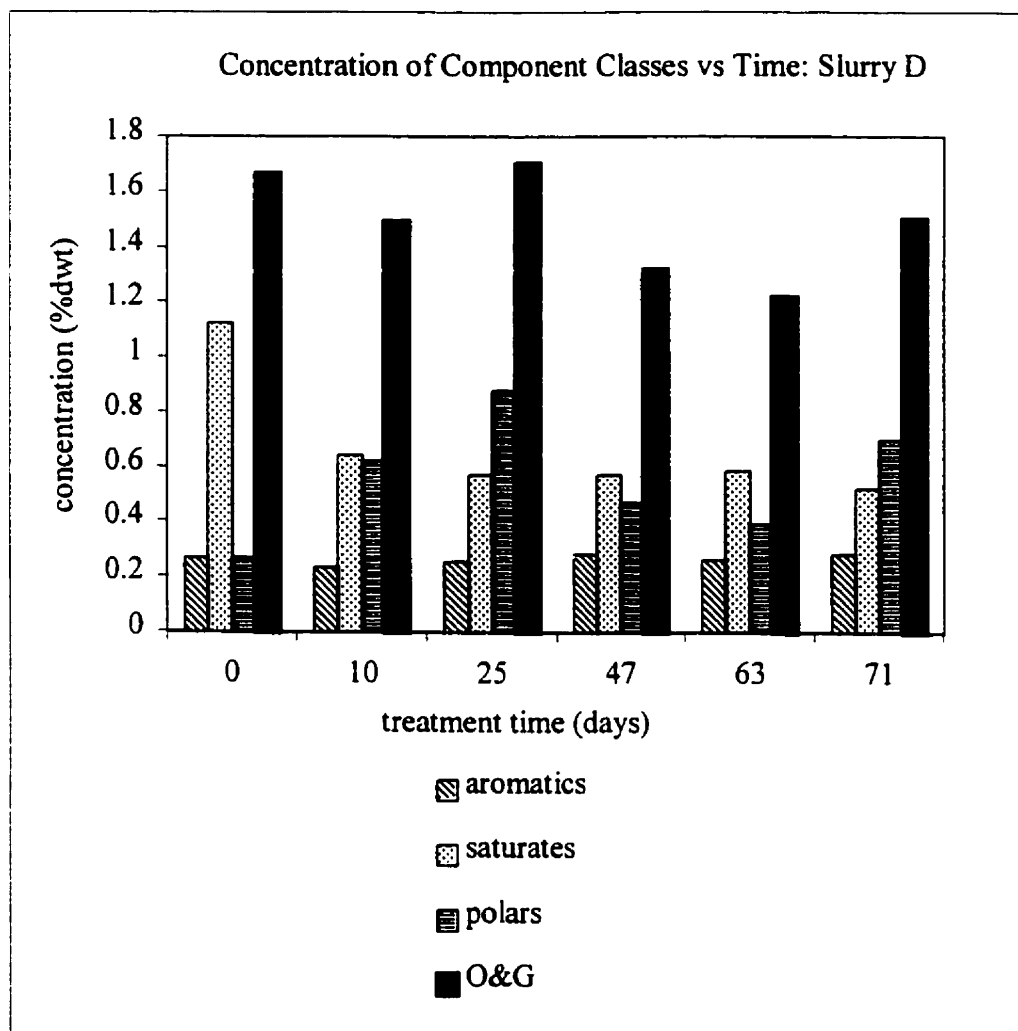
3.3.3.2 Comparing Petroleum Composition: Results

Results For D/D'

Results collected for slurry D and its corresponding abiotic control D' follow directly. Figure 22 presents the DOUR of slurries D and D' over the course of the experiment. Table 16Table 16 lists the contaminant concentrations as a percentage of dry mass for O&G measurements and individual fractions. Results for D' are presented as an average abiotic value. Contaminant concentrations for D are plotted in Figure 23. Table 17 presents the results of the GC-FID quantification for D and D' at the end of treatment. GC-FID chromatograms from the conclusion of treatment are presented for slurries D and D', for the saturate, aromatic and polar fractions, in Figure 24 through Figure 29.

Figure 19: Dissolved Oxygen Uptake Rate, D/D'**Table 16: Contaminant Levels, D/D'**

	Concentration (% of dry mass)						
	day	day	day	day	day	day	Average
	0	10	25	47	63	71	Abiotic
Saturates	1.12	0.64	0.57	0.57	0.58	0.52	0.93
Aromatic	0.27	0.23	0.25	0.28	0.26	0.28	0.28
Polars	0.27	0.62	0.88	0.47	0.39	0.7	0.39
MOG	1.39	0.87	0.82	0.85	0.83	0.8	1.24
O&G	1.66	1.49	1.70	1.32	1.22	1.5	1.85

Figure 20: Contaminant Levels, D/D'**Table 17: GC-FID Quantification, D/D'**

	Final Concentration (% of dry mass)	
	Treated (D)	Abiotic (D')
Saturates	0.27	0.68
Aromatics	0.11	0.16

Figure 21: Chromatograph of Slurry D' Saturates (Abiotic Control)

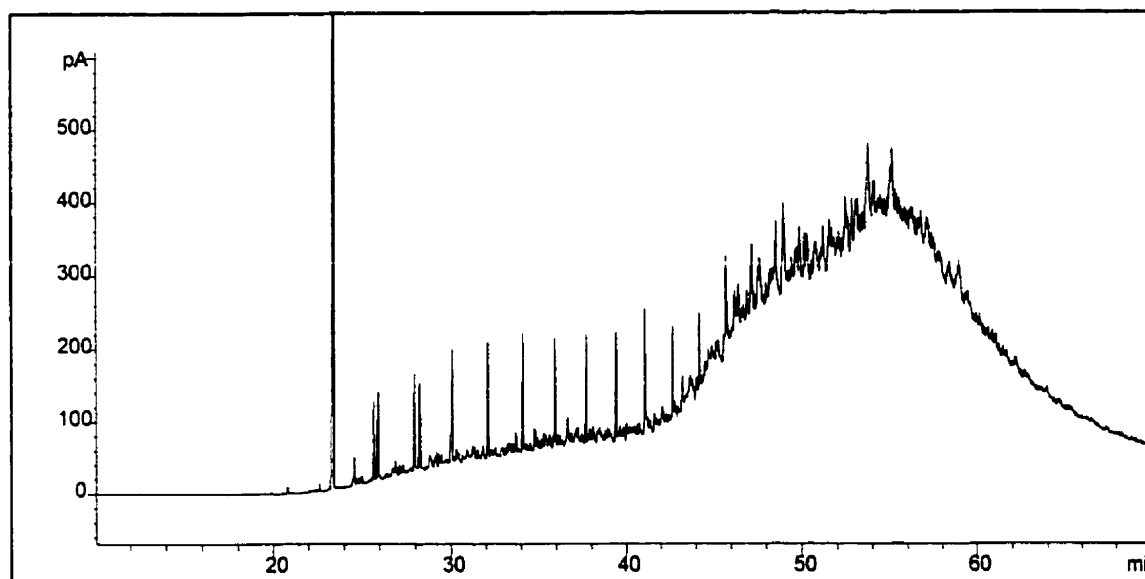


Figure 22: Chromatograph of Slurry D Saturates (Treated)

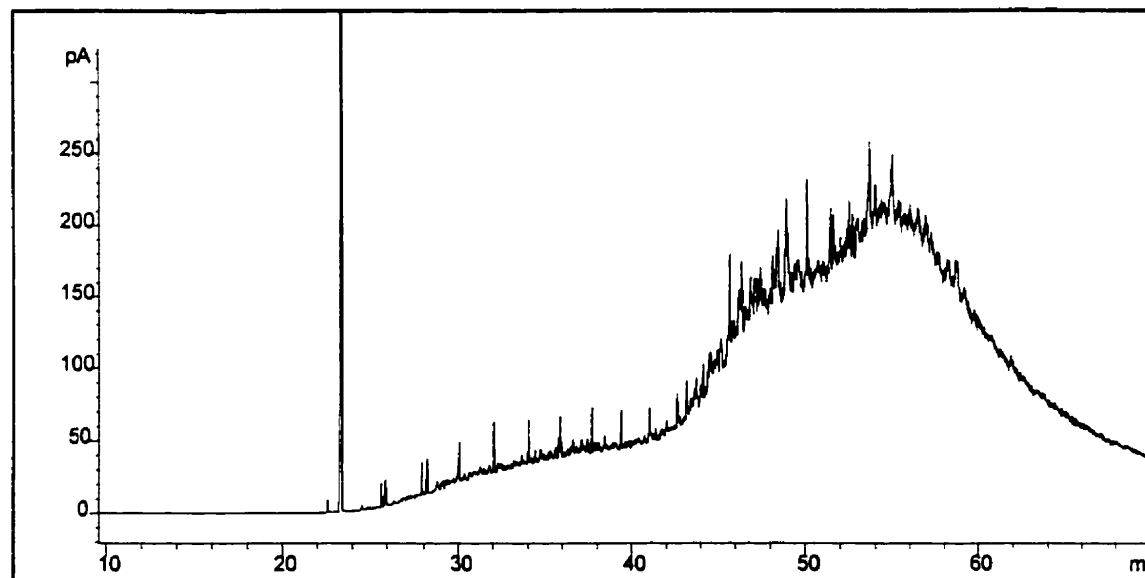


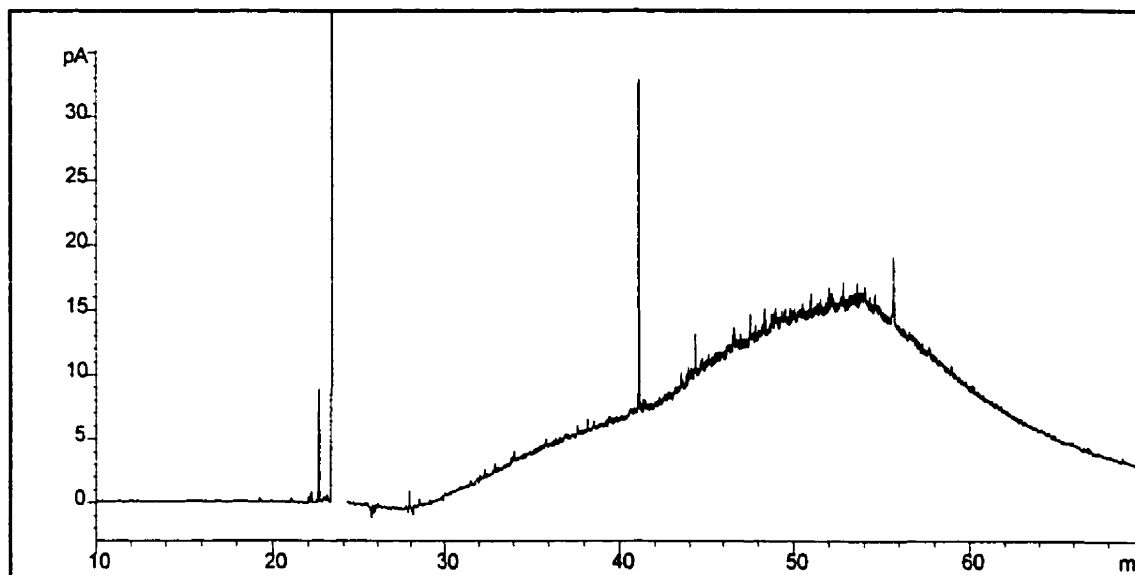
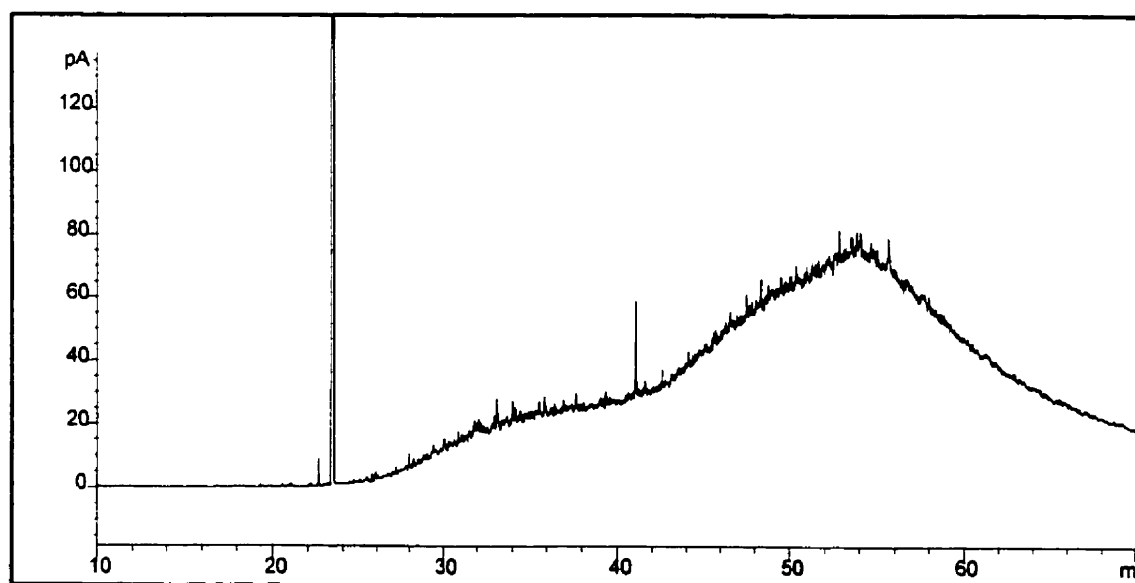
Figure 23: Chromatograph of Slurry D' Aromatics (Abiotic Control)**Figure 24: Chromatograph of Slurry D Aromatics (Treated)**

Figure 25: Chromatograph of Slurry D' Polars (Abiotic Control)

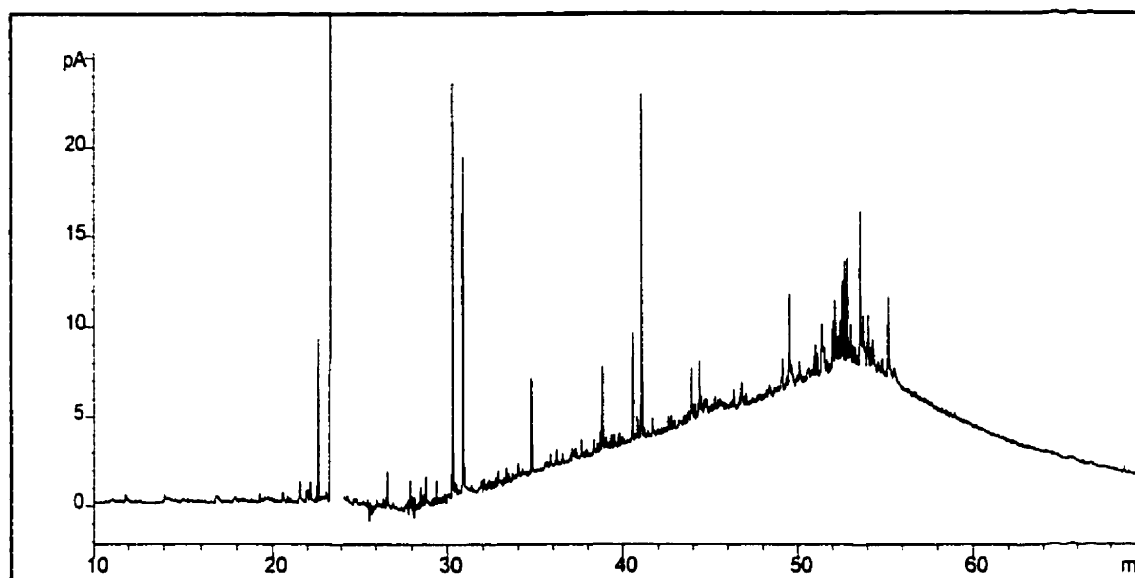
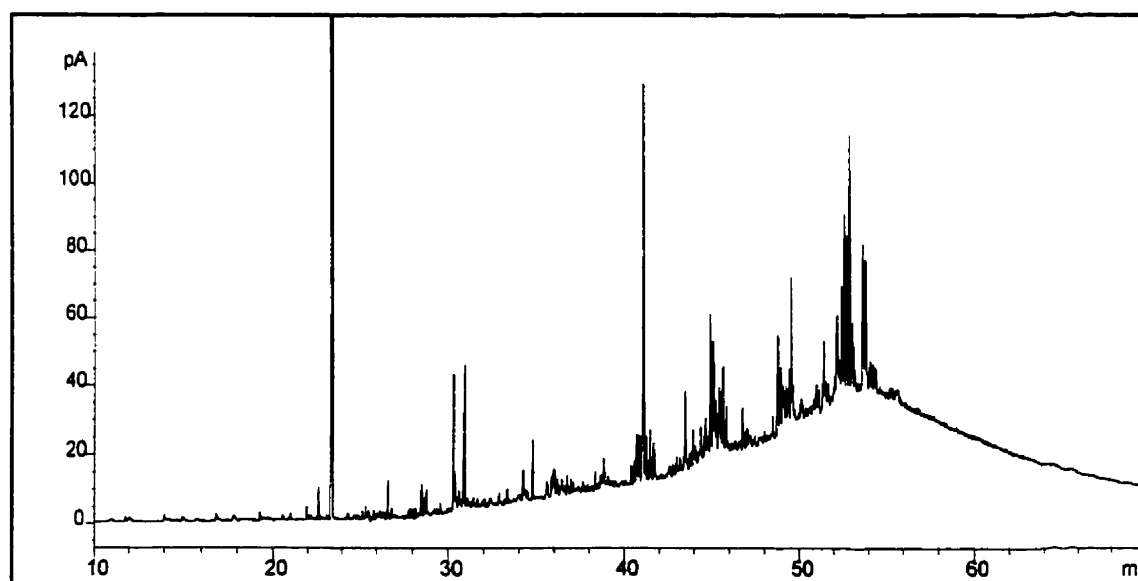
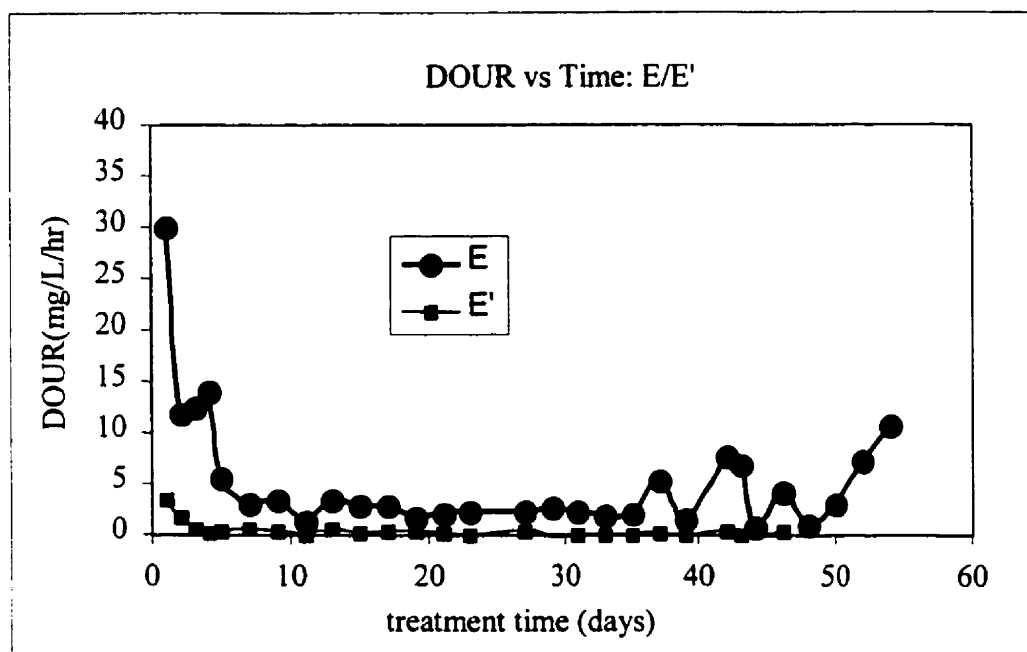


Figure 26: Chromatograph of Slurry D polars (Treated)

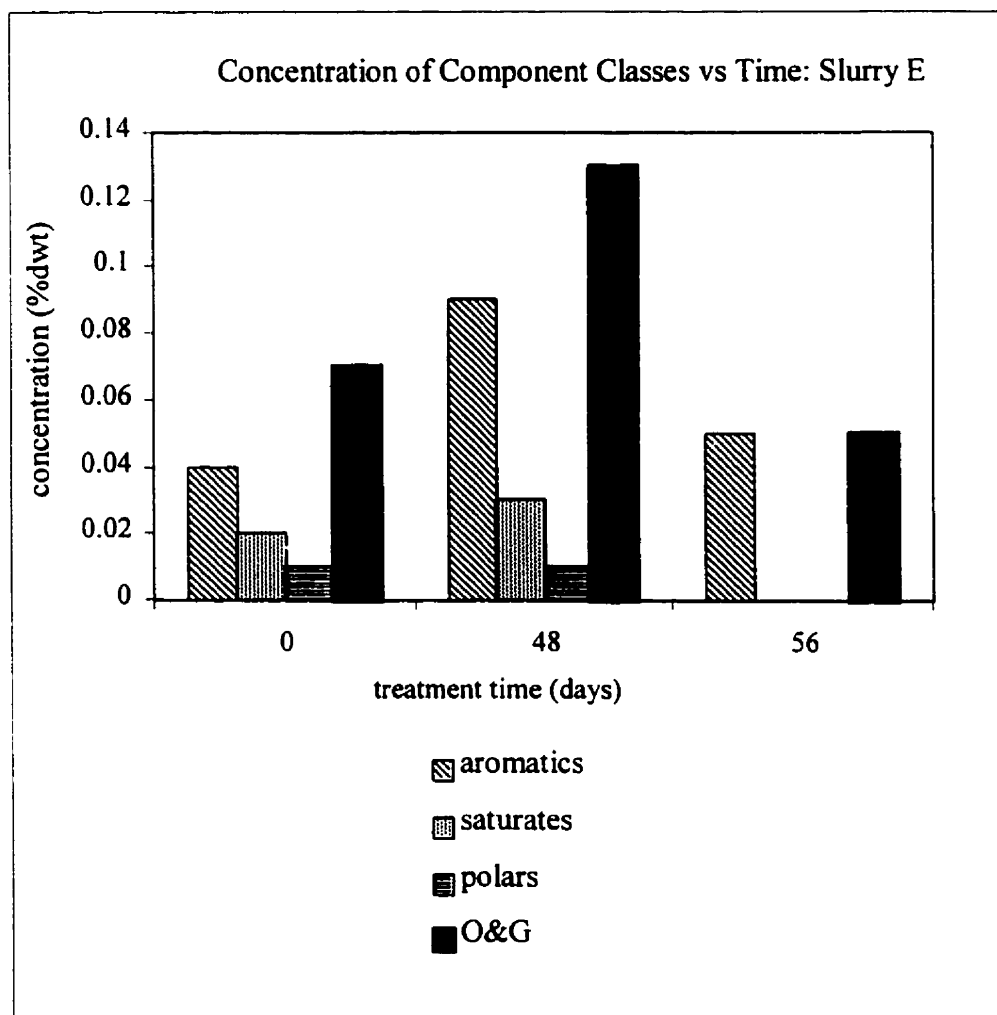


Results For E/E'

Results collected for slurry E and its corresponding abiotic control E' follow directly. Figure 30 presents the DOUR of slurries E and E' over the course of the experiment. Table 18 lists the contaminant concentrations as a percentage of dry mass for O&G measurements and individual fractions. Results for E' are presented as an average abiotic value. Contaminant concentrations for E are plotted in Figure 31. Table 19 presents the results of the GC-FID quantification for E and E' at the end of treatment. GC-FID chromatograms from the conclusion of treatment are presented for slurries E and E', for the saturate fraction, in Figure 32 and Figure 33.

Figure 27: Dissolved Oxygen Uptake Rate, E/E'**Table 18: Contaminant levels, E/E'**

	Concentration (% of dry mass)					
	day 0	day 10	day 35	day 48	day 56	Average Abiotic
Saturates	0.02	---	---	0.03	0.00	0.01
Aromatic	0.04	---	---	0.09	0.05	0.03
Polars	0.01	---	---	0.01	0.00	0.00
MOG	0.06	---	---	0.10	0.05	0.12
O&G	0.07	0.08	0.09	0.13	0.05	0.04

Figure 28: Contaminant levels, E**Table 19: GC-FID Quantification, E/E'**

	Final Concentration (% of dry mass)	
	Treated (E)	Abiotic (E')
Saturates	0.014	0.016
Aromatics	0.007	0.036

Figure 29: Chromatograph of Slurry E' Saturates (Abiotic Control)

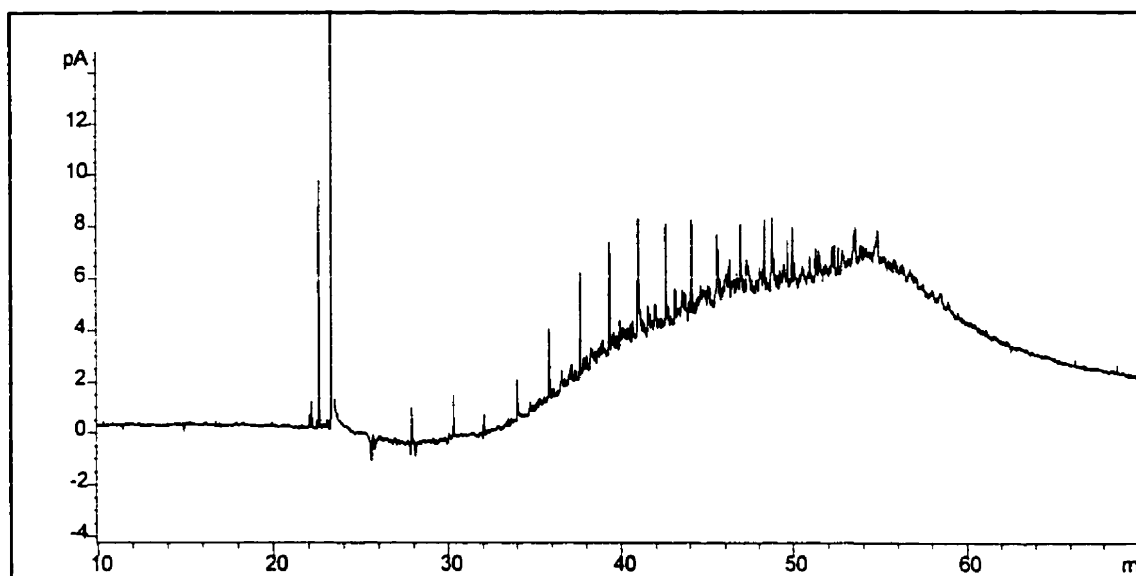
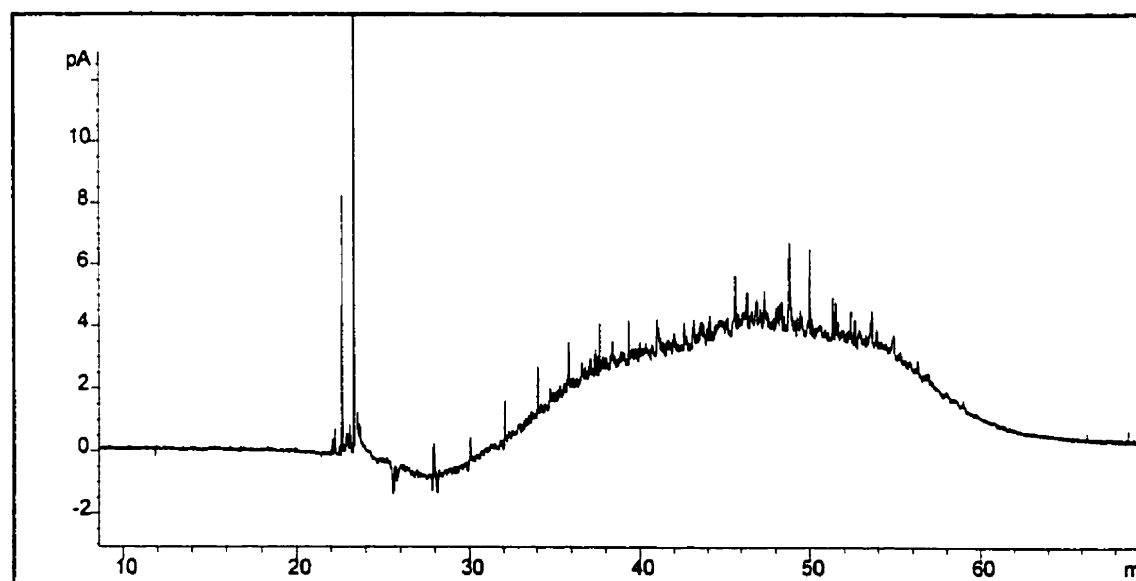
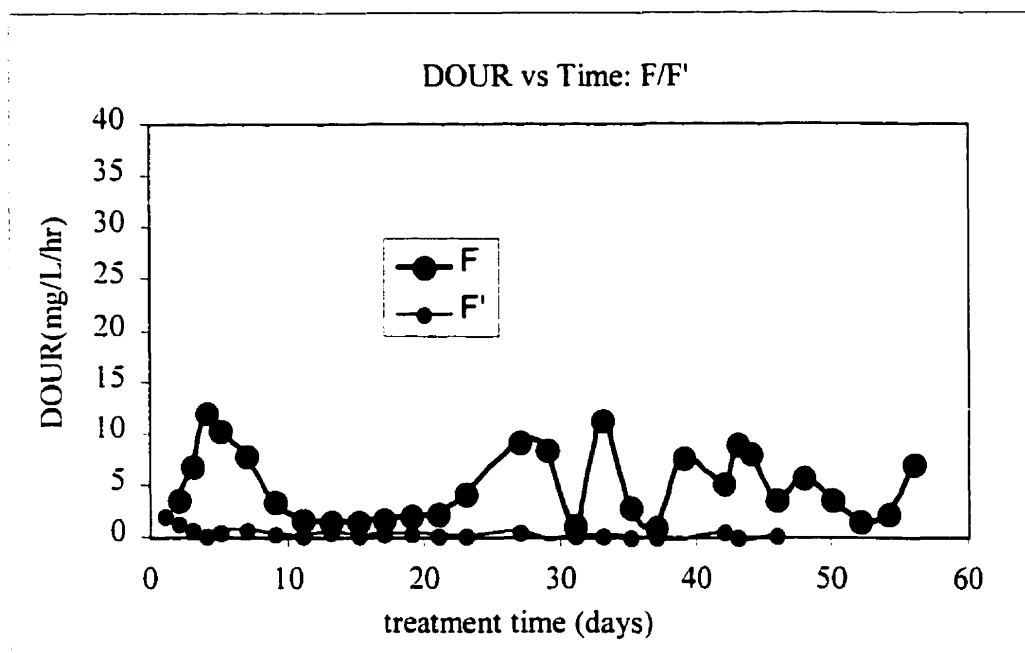


Figure 30: Chromatograph of Slurry E Saturates (Treated)

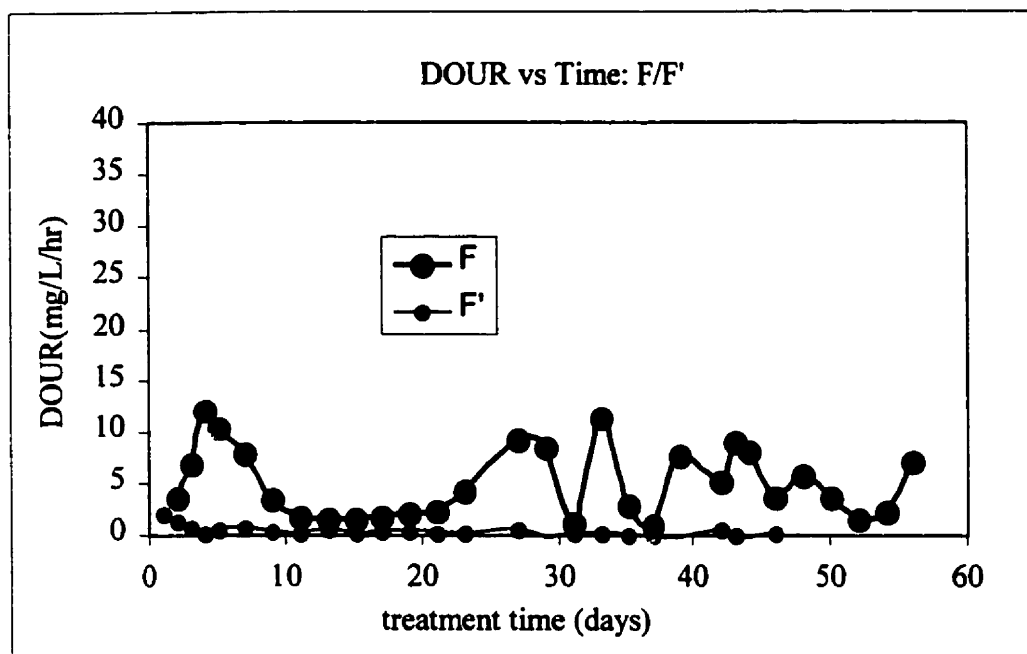


Results For F/F'

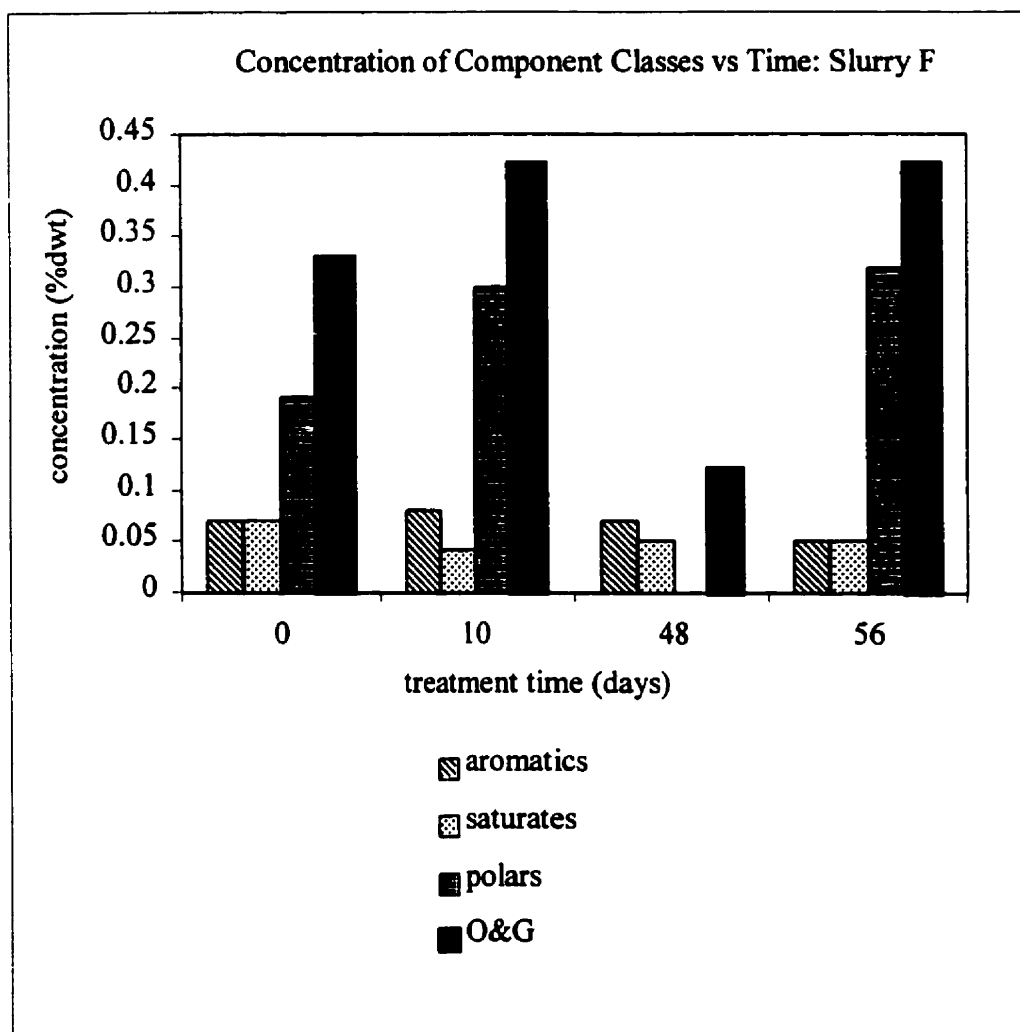
Results collected for slurry F and its corresponding abiotic control F' follow directly. Figure 34 presents the DOUR of slurries F and F' over the course of the experiment. Table 20 lists the contaminant concentrations as a percentage of dry mass for O&G measurements and individual fractions. Results for F' are presented as an average abiotic value. Contaminant concentrations for F are plotted in Figure 35. Table 20 presents the results of the GC-FID quantification for F and F' at the end of treatment. GC-FID chromatograms from the conclusion of treatment are presented for slurries F and F', for the saturate fraction, in Figure 36 and Figure 37.

Figure 31 : Dissolved Oxygen Uptake Rate, F/F'**Table 20: Contaminant levels, F/F'**

	Concentration (% of dry mass)					
	day 0	day 10	day 35	day 48	day 56	Average Abiotic
Saturates	0.07	0.04	---	0.05	0.05	0.08
Aromatic	0.07	0.08	---	0.07	0.05	0.09
Polars	0.19	0.30	---	0.00	0.32	0.25
MOG	0.14	0.12	---	0.37	0.11	0.17
O&G	0.33	0.42	---	0.12	0.42	0.42

Figure 31 : Dissolved Oxygen Uptake Rate, F/F'**Table 20: Contaminant levels, F/F'**

	Concentration (% of dry mass)					
	day 0	day 10	day 35	day 48	day 56	Average Abiotic
Saturates	0.07	0.04	---	0.05	0.05	0.08
Aromatic	0.07	0.08	---	0.07	0.05	0.09
Polars	0.19	0.30	---	0.00	0.32	0.25
MOG	0.14	0.12	---	0.37	0.11	0.17
O&G	0.33	0.42	---	0.12	0.42	0.42

Figure 32: Contaminant Levels, F**Table 21: GC-FID Quantification, F/F'**

	Final Concentration (% of dry mass)	
	Treated (F)	Abiotic (F')
Saturates	0.020	0.046
Aromatics	0.059	0.051

Figure 33: Chromatograph of Slurry F' Saturates (Abiotic Control)

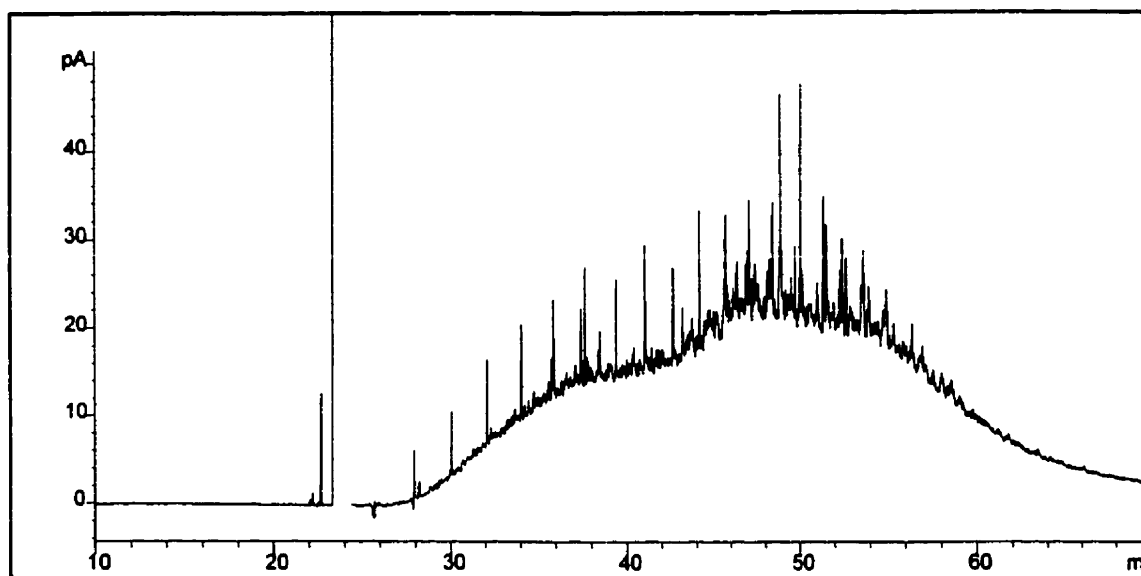
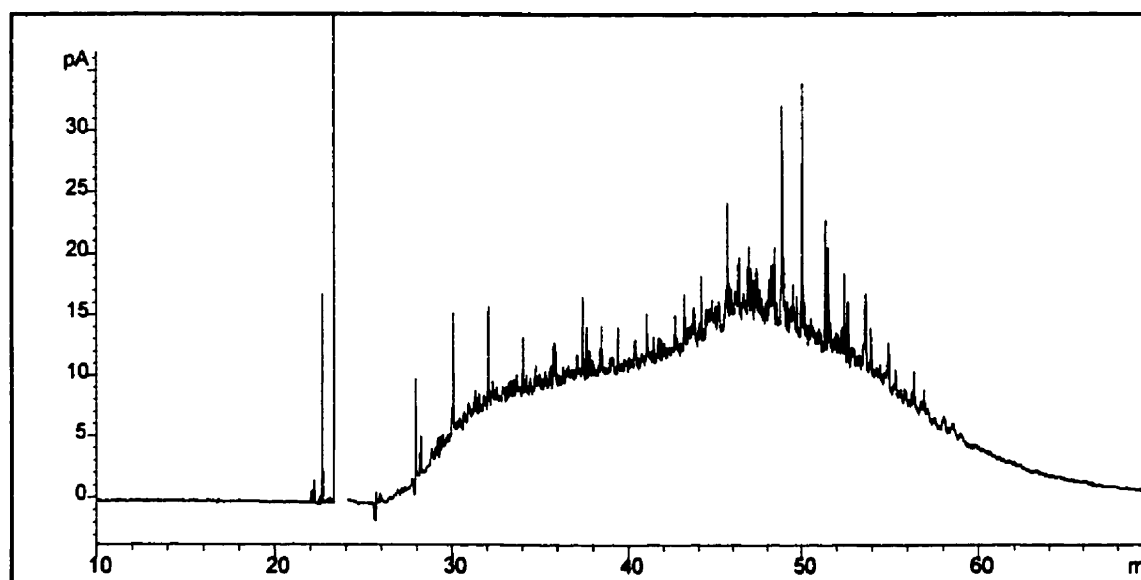
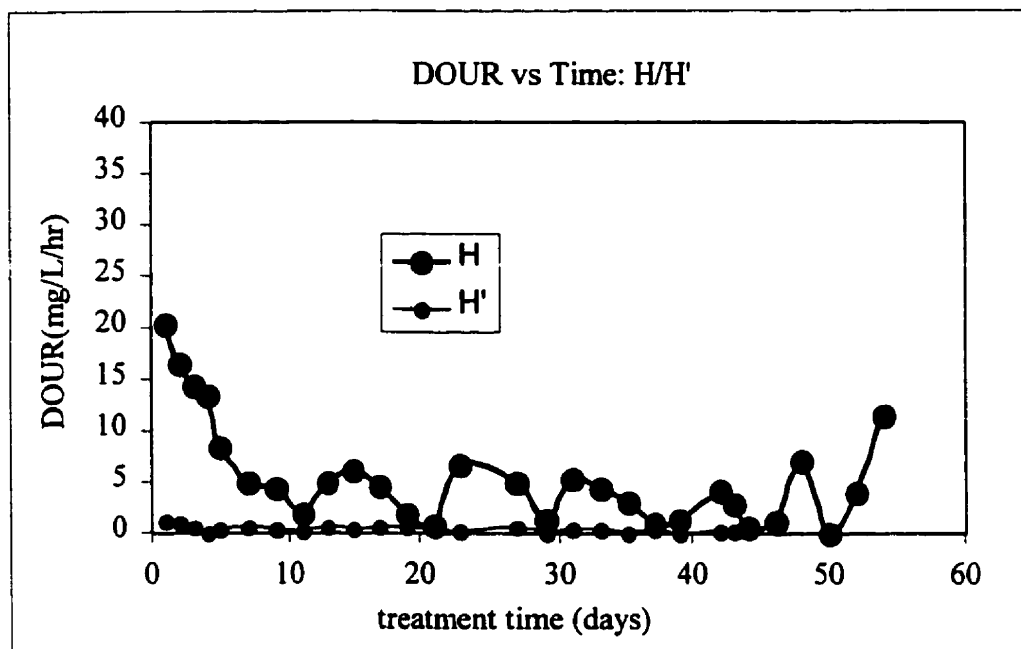


Figure 34: Chromatograph of Slurry F Saturates (Treated)

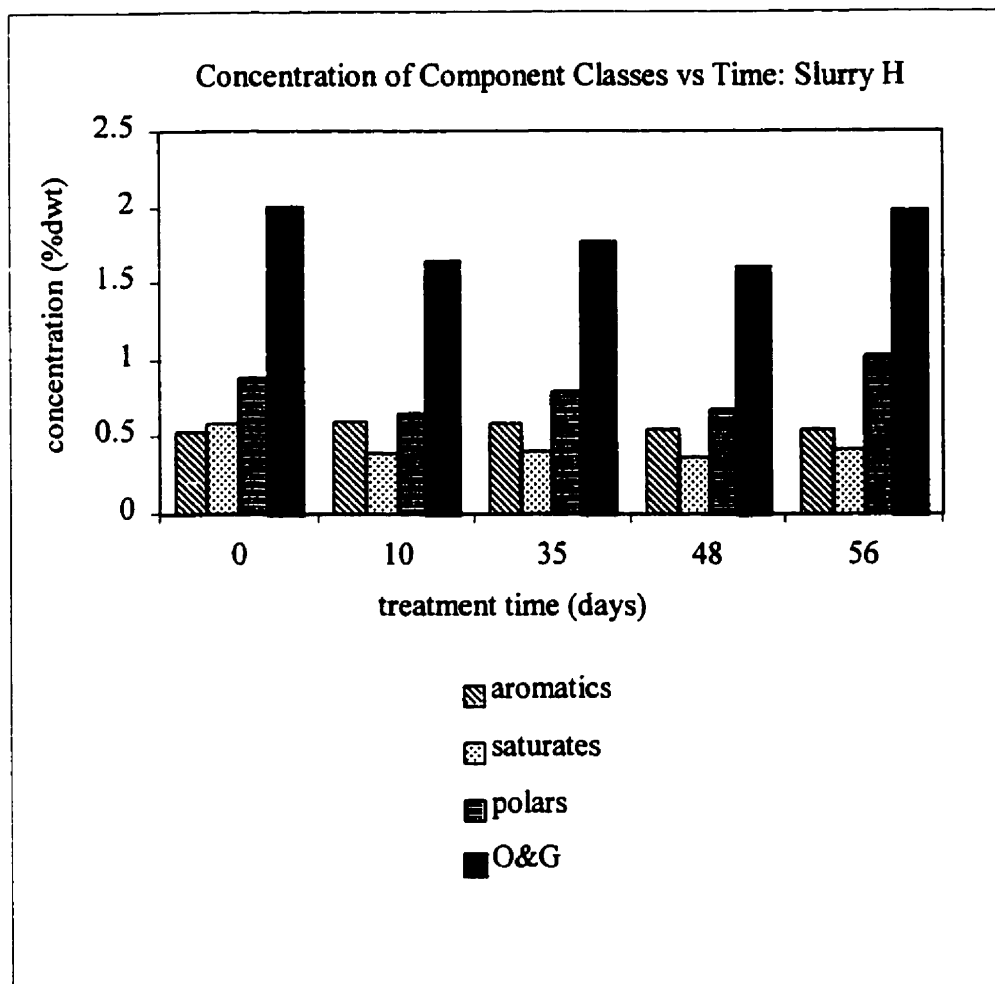


Results For H/H'

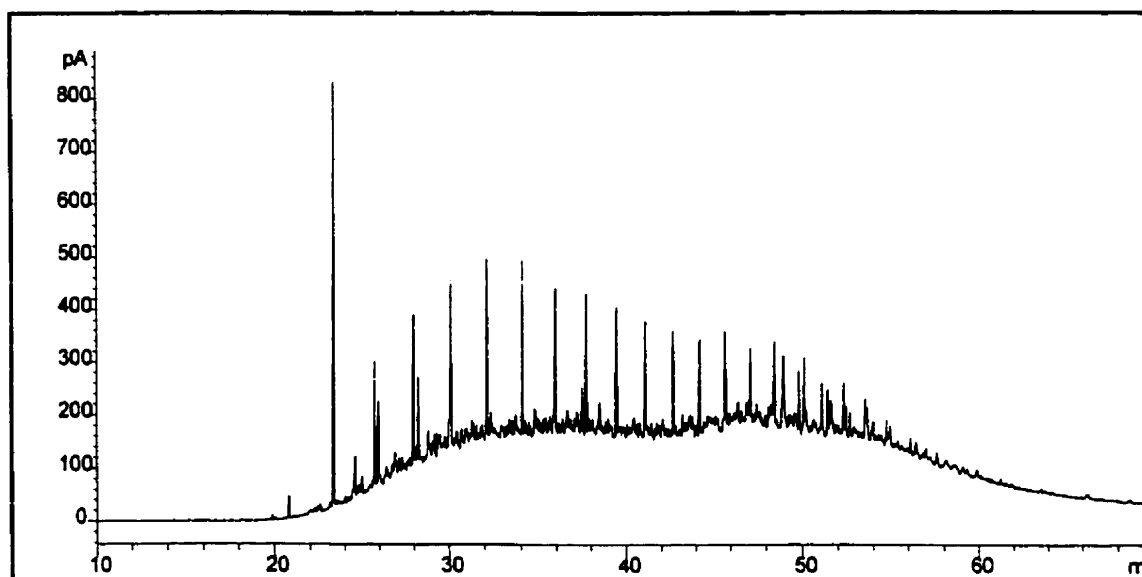
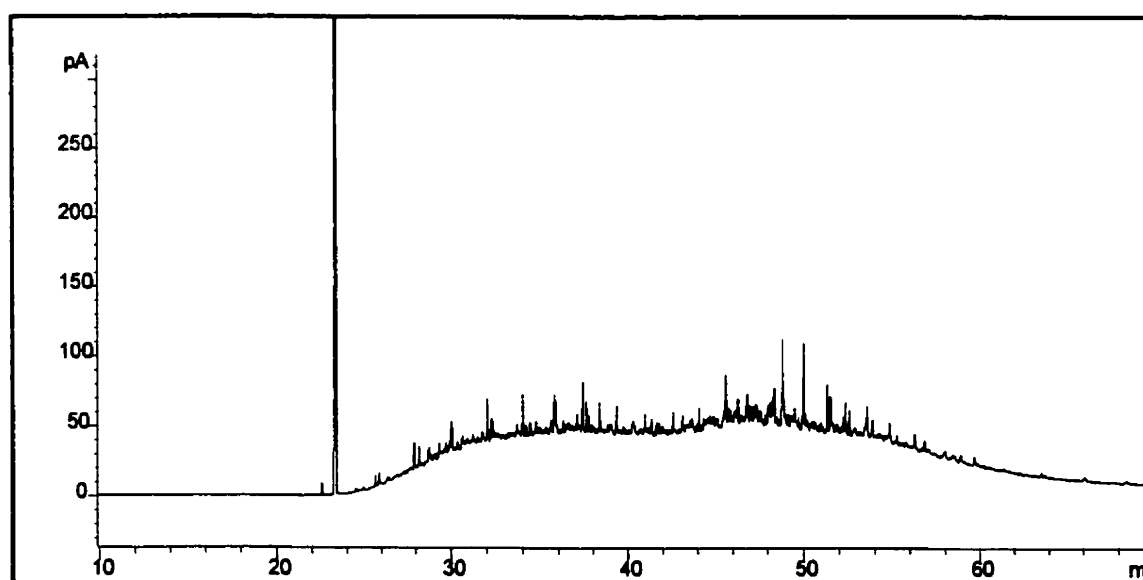
Results collected for slurry H and its corresponding abiotic control H' follow directly. Figure 38 presents the DOUR of slurries H and H' over the course of the experiment. Table 22 lists the contaminant concentrations as a percentage of dry mass for O&G measurements and individual fractions. Results for H' are presented as an average abiotic value. Contaminant concentrations for H are plotted in Table 39. Table 23 presents the results of the GC-FID quantification for H and H' at the end of treatment. GC-FID chromatograms from the conclusion of treatment are presented for slurries H and H', for the saturate fraction, in Figure 40 and Figure 41.

Figure 35: Dissolved Oxygen Uptake Rate, H/H'**Table 22: Contaminant Levels, H/H'**

	Concentration (% of dry mass)					
	day 0	day 10	day 35	day 48	day 56	Average Abiotic
Saturates	0.58	0.39	0.40	0.36	0.41	0.54
Aromatic	0.53	0.59	0.58	0.55	0.54	0.56
Polars	0.88	0.65	0.79	0.67	1.02	0.47
MOG	1.12	0.98	0.97	0.92	0.95	1.10
O&G	2.00	1.63	1.76	1.59	1.97	1.57

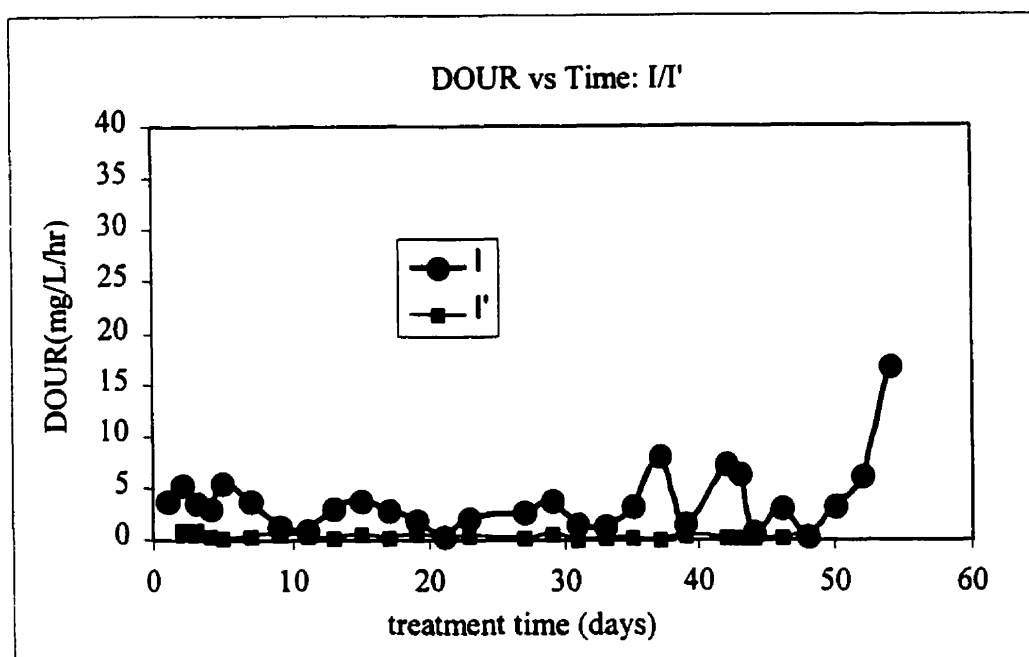
Figure 36: Contaminant Levels, H**Table 23: GC-FID Quantification, H/H'**

	Final Concentration (% of dry mass)	
	Treated (H)	Abiotic (H')
Saturates	0.27	0.68
Aromatics	0.11	0.16

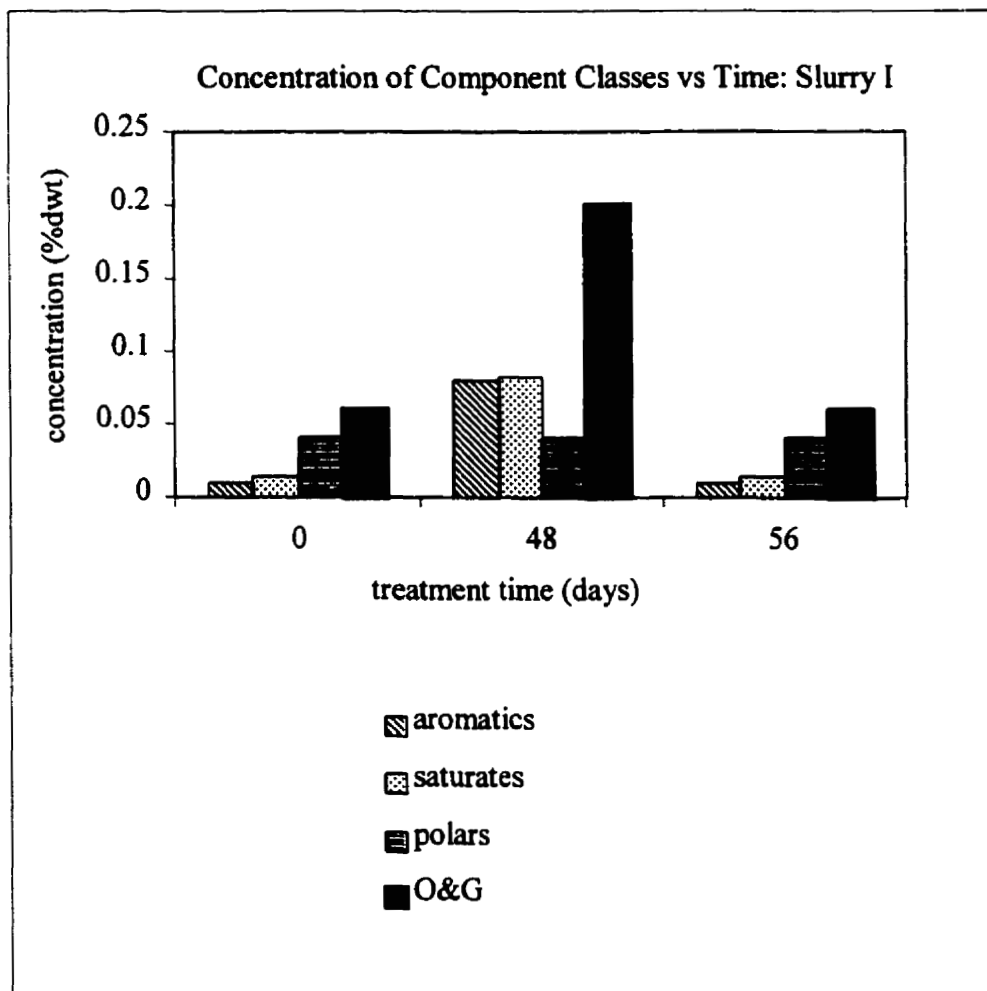
Figure 37: Chromatograph of Slurry H' Saturates (Abiotic Control)**Figure 38: Chromatograph of Slurry H Saturates (Treated)**

Results For I/I'

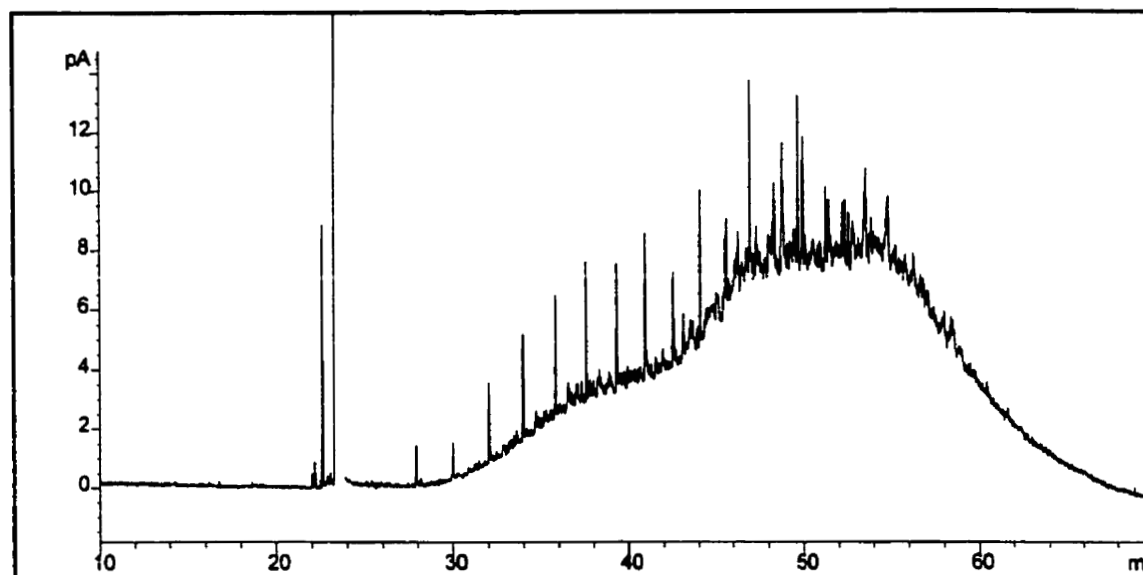
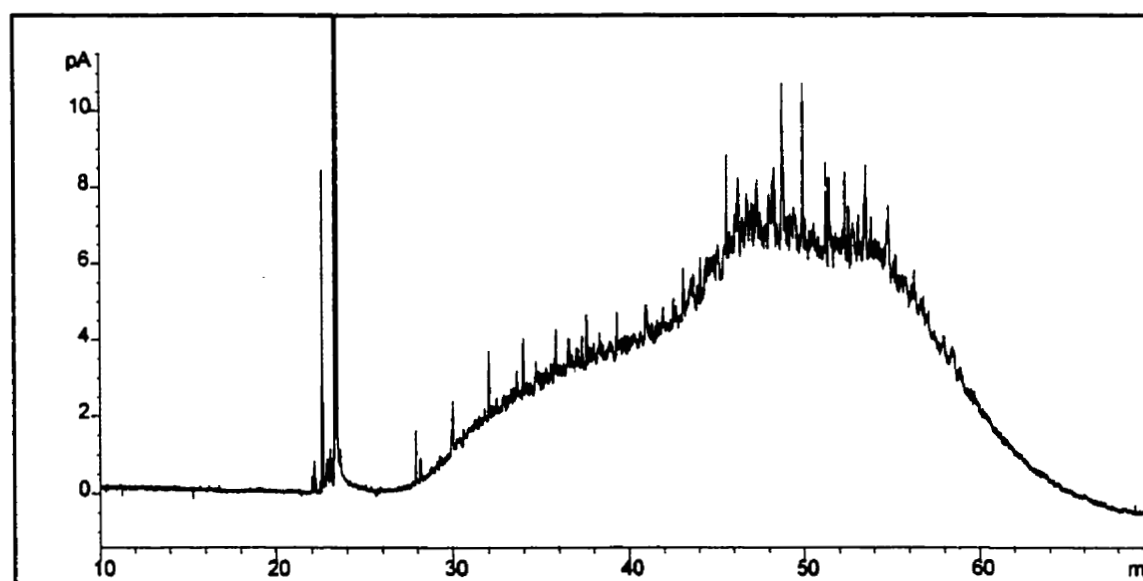
Results collected for slurry I and its corresponding abiotic control I' follow directly. Figure 42 presents the DOUR of slurries I and I' over the course of the experiment. Table 24 lists the contaminant concentrations as a percentage of dry mass for O&G measurements and individual fractions. Results for I' are presented as an average abiotic value. Contaminant concentrations for I are plotted in Figure 43. Table 25 presents the results of the GC-FID quantification for I and I' at the end of treatment. GC-FID chromatograms from the conclusion of treatment are presented for slurries I and I', for the saturate fraction, in Figure 44 and Figure 45.

Figure 39: Dissolved Oxygen Uptake Rate, I/I'**Table 24: Contaminant Levels, I/I'**

	Concentration (% of dry mass)					
	day 0	day 10	day 35	day 48	day 56	Average Abiotic
Saturates	0.01	---	---	0.08	0.01	0.01
Aromatic	0.01	---	---	0.08	0.01	0.02
Polars	0.04	---	---	0.04	0.04	0.02
Mineral O&G	0.02	---	---	0.16	0.02	0.03
O&G	0.06	0.04	0.04	0.20	0.06	0.06

Figure 40: Contaminant Levels, I/I'**Table 25: GC-FID Quantification, I/I'**

	Final Concentration (% of dry mass)	
	Treated	Abiotic
Saturates	0.005	0.006
Aromatics	0.002	0.002

Figure 41: Chromatograph of Slurry I' Saturates (Abiotic Control)**Figure 42: Chromatograph of Slurry I Saturates (Treated)**

3.3.3.3 Comparing Petroleum Composition: Interpretation

Dissolved Oxygen Uptake Rate

The DOUR data for slurries D, E, and H indicate a high rate of aerobic activity in the slurry during the initial week of treatment, slowing at the end of 10 days, and fluctuating for the remainder of the treatment. Slurries F and I exhibit more modest activity, attributed to the lower concentrations of petroleum present. Slurry E had a low measured concentration of petroleum, but it was observed that free product (liquid petroleum) occurred on the surface of this slurry during treatment. Biodegradation of volatile hydrocarbons as well as other aerobic activity (such as nitrification) contribute to the DOUR, and so this measurement is not directly related to the biodegradation of the contaminant of concern (non-volatile petroleum).

Addition of surfactant towards the end of each treatment run results in an increase in oxygen uptake, suggesting that the reduction in activity up to this point was not due to a build up of toxic metabolites. Comparison of contaminant levels before and after this addition reveal no change, suggesting that the increased DOUR occurs due to degradation of the surfactant, and not the intended further hydrocarbon biodegradation due to increased bioavailability.

Contaminant Levels by Gravimetric Analysis

For slurry D, the saturate fraction of the extract was reduced by 43% in the first ten days, and 54% by the end of the ten week treatment, with respect to the initial concentration. A loss of 13% of saturates was measured for the abiotic control, although some oxygen uptake at the beginning of the abiotic test run suggests at least part of this reduction is biological. The biological removal as a ratio of saturates remaining after abiotic losses is 31% in the first 10 days, and 44% by the end of treatment. No reductions were observed for aromatics either in the treatment or the abiotic control. MOG, the sum of the saturate and aromatic fractions, is reduced 37% in the first ten days and 42% over ten weeks overall, or 30% and 35% with

respect to the abiotic control. Gravimetric analysis of extracted oil does not suggest a clear trend for O&G measurement as the data points for this measurement fluctuate both above and below the initial concentration.

Similar results are found for slurry H. The saturate fraction of the extract was reduced approximately 33% with respect to the initial concentration, or about 28% with respect to abiotic losses. No reductions were observed for aromatics. For slurry H, MOG is reduced 15% overall, 13% with respect to the abiotic control. These reductions are achieved within 10 days, and percentages were calculated based on an average of the measured reduction from that point onward.

For slurry F there was a measured 35% reduction in MOG, but the measurements at this concentration are approaching the limit of precision. No reduction of contaminant can be inferred for slurries E and I from the gravimetric data.

GC-FID

Most of the GC-FID data indicates a decrease in both saturates and aromatics. This data is not taken to be a dependable measure of degradation at this point, due to the limited precision achieved with this measurement method. Quantitative GC-FID data was limited by its precision; by the method used calculated concentrations for a single sample could vary by as much as 20%, which is not sufficiently sensitive for the reductions in contamination achieved. It is presented here as a qualitative comparison of the nature of the contaminant with treatment and without. The chromatograms shown are those taken from the end of treatment, as these were prepared from larger slurry samples (i.e. about 25g of soil solids as opposed to 5 g), affording greater clarity in the analysis. Comparison of chromatograms over time shows that the reductions achieved occurred within the first weeks of treatment. Chromatograms of the abiotic controls closely resemble those of the original contamination prior to treatment, with the exception of the removal of material up to C-16, presumably due to volatilization.

Comparison of the saturate fraction of slurry D with that of the abiotic control D' indicates a depletion of n-alkanes in the treated sample with respect to the UCM hump. The isoprenoids pristane and phytane, which emerge as peaks beside n-C17 and n-C18 respectively, are reduced in the much the same ratio as the n-alkanes, although they are known to be more resistant to degradation. This may indicate that the n-alkanes that are remaining escaped degradation due to availability limitations. Without dependable quantification the UCM hump must be used here as the basis for comparison of peak degradation, so no inference can be made at this point about the degradation of the saturate compounds which comprise this feature.

Aromatic compounds form a similar unresolved chromatographic hump which does not lend itself well to qualitative comparison. The peaks that emerge in these chromatographs are variable, and can be shown to be artifacts of the experimental procedure; the peak eluting near the n-C25 reference is likely due to an aromatic compound in the laboratory air supply, as a GC-FID analysis of condensate from residue in the aeration system reveals a large peak at this point. Analysis of sample blanks reveals that the other peaks are trace impurities from the solvents used in extraction and class separation, concentrated by evaporation of these solvents in the gravimetric analyses. While prevalent in GC-FID analysis of aromatics to varying degrees, these interferences do not affect the gravimetric analysis.

Few compounds emerge in the chromatographic analysis of the polar class, in proportion to their prevalence in the gravimetric analysis. It is to be expected that many of these compounds will not volatilize even at the upper end of the GC-FID temperature programme, or are of sufficiently high polarity that they sorb to the chromatography column without eluting. Many of the peaks in the chromatographs shown are attributable to the surfactant applied near the end of the experiment. Since the polar and aromatic fractions offer little basis for qualitative comparison, they are shown here only for D/D'.

The phenomena seen in the chromatographic comparison of slurries D and D' was repeated in the other slurries. Removal of n-alkanes occurred in all cases, but this removal was not complete in any case. Since nutrients and oxygen were still available and conditions were non-toxic (based on DOUR data), the failure of treatment to completely remove these compounds may be attributable to a bioavailability limitation that the slurry treatment does not remedy. Partial degradation of n-alkanes in slurries E and I show that low contaminant concentration is not a hindrance well below 1000mg/kg MOG.

General

In assessing the measurement methods used, O&G measurement was again shown to very erratic. MOG, based on removal of the polars, was found to be more consistent, but reductions often do not exceed the range of statistical variability. From the literature review it is known that asphaltenes are normally colloidal particles within petroleum, kept in suspension by the NSO heterocompounds (Speight and Moscopedis, 1981, Long, 1981). It is possible that the variability seen for the extraction of polars is due to some as of yet unidentified feature of the extraction or the slurry which affects the solubility of asphaltenes in the extraction solvent. Effective quantification of all components of Oil and Grease would be preferred as this would allow accounting for transformation products such as partially oxidized compounds. GC-FID analysis clearly shows degradation of petroleum in all cases, specifically n-alkanes and other compounds presenting peaks in the saturate fraction.

While rapid reductions are observed for the saturate fraction, no reductions were observed for aromatic compounds. From the literature review it is expected that compounds in this fraction would degrade to some extent. Monitoring and maintenance of slurry conditions as well as the demonstrated degradation of saturates shows that conditions are suitable for treatment. As a possible explanation, it is presented as a hypothesis that the "ideal" conditions attempted in the slurry are not ideal for the microbes that would normally degrade the aromatic; slurry treatment itself may inhibit the growth of soil fungi.

The total reductions achieved are about the same as those achieved in solid-phase treatment of similar flare pit material (about 30%, from Visser et al, 1999). It would appear that the laboratory slurry treatment does not offer a significant advantage by removing mass-transfer limitations. Limits to the treatment of easily degradable compounds (the residual n-alkanes which were rapidly but incompletely removed) suggest that bioavailability limitations such as soil sorption or sequestration within soil particles still plays a role in limiting the extent of bioremediation achievable. Beyond these issues, the data from this experiment shows that the reductions achieved can be attributed to specific fractions of the petroleum contaminant. This reinforces findings from the literature review that the composition of the petroleum contaminant has a pronounced effect on its biodegradability. An approach to developing treatability generalizations about the composition and biodegradability to be expected at Alberta flare pit sites is detailed in the discussion, in section 3.3.6.

3.3.4 Experiment Series 4: Effect of Salinity (Test 1)

This experiment was performed as a quick check on the effect of low levels of salinity in the range created by addition of nutrient salts and NaOH/ HCl, as used in pH control. The addition of these ionic compounds is not identical across all treatments in this experimental programme, rather it occurs in response to monitoring, and is a possible source of experimental error. Slurries in this experiment were spiked with NaCl. Levels were varied arithmetically from 0% to 2.5% by mass so that if an effect were observed, a piecewise function could be developed to compensate for the effect of salinity in future experimentation.

3.3.4.1 Effect of Salinity (Test 1): Design

Table 26: Experiment Series 4, Test 1: Details

Effect of Salinity, Test1			
Run time	10 days		
Temp.	30°C		
pH	neutral		
Soil used:	D (see Table 5)		
Additives:	antifoam, nutrients, NaCl (varied)		
treatment	Salinity (g/L NaCl)	Salinity (%)	EC (dS/m)
1	0	0	0
2	5	0.5	9
3	10	1	17
4	15	1.5	26
5	20	2	34
6	25	2.5	43

The contaminant was assessed both before and after the treatment both as O&G and by gravimetric analysis of component classes.

3.4.4.2 Effect of Salinity (Test1): Results

Figure 43: DOUR Data with Varying Salinity

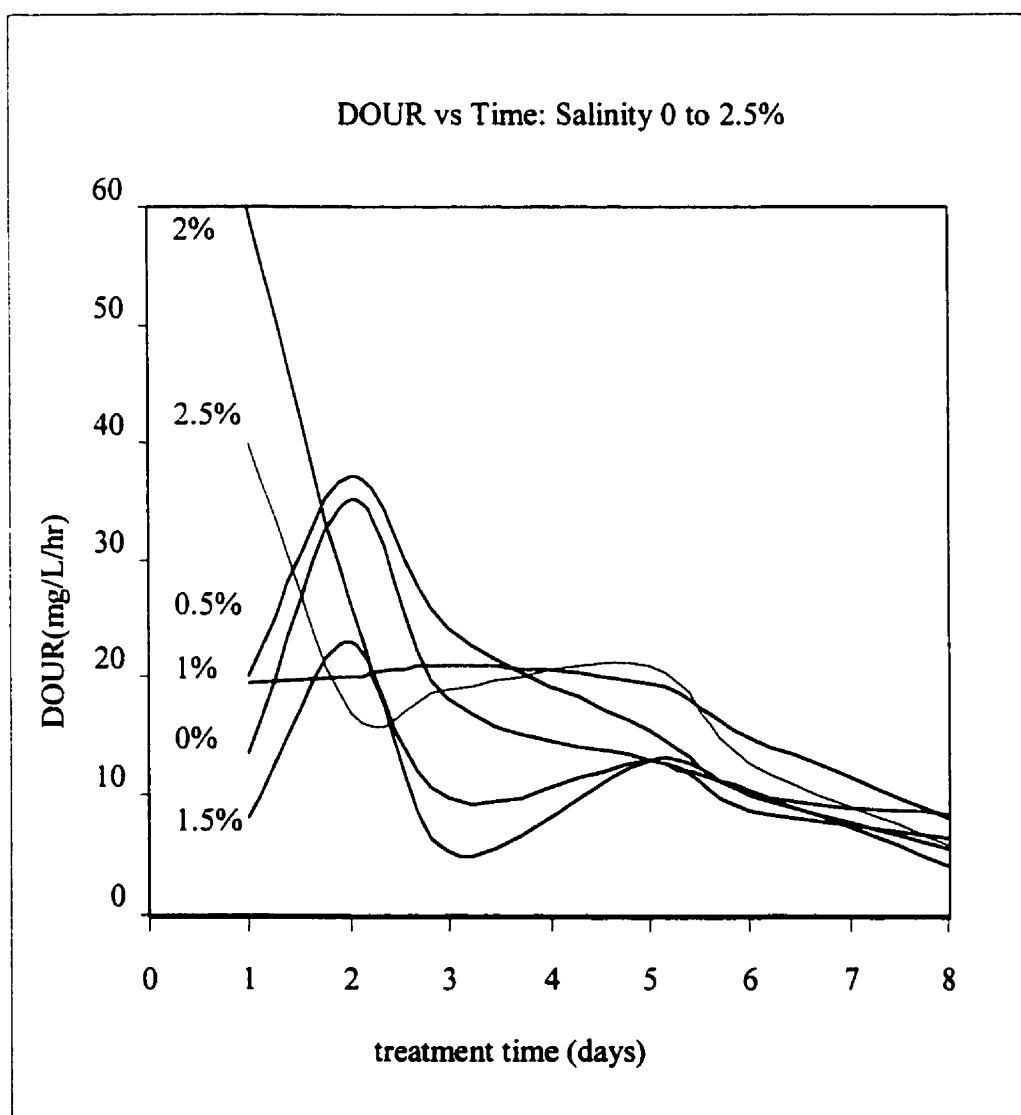
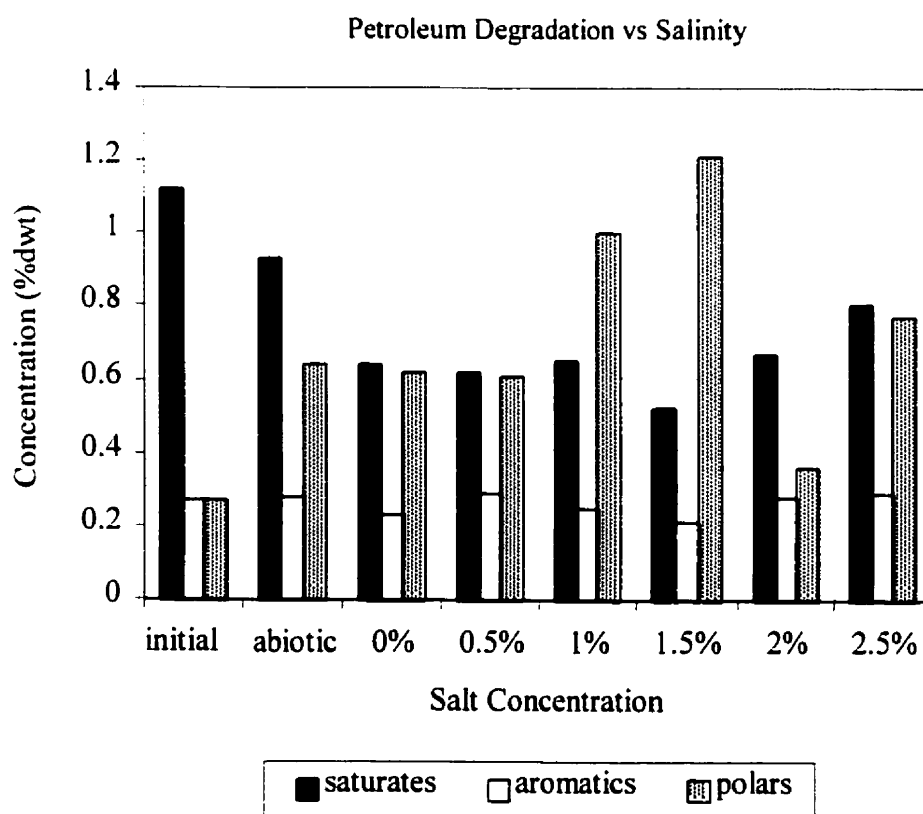


Table 27: Biodegradation at Different Levels of Salinity

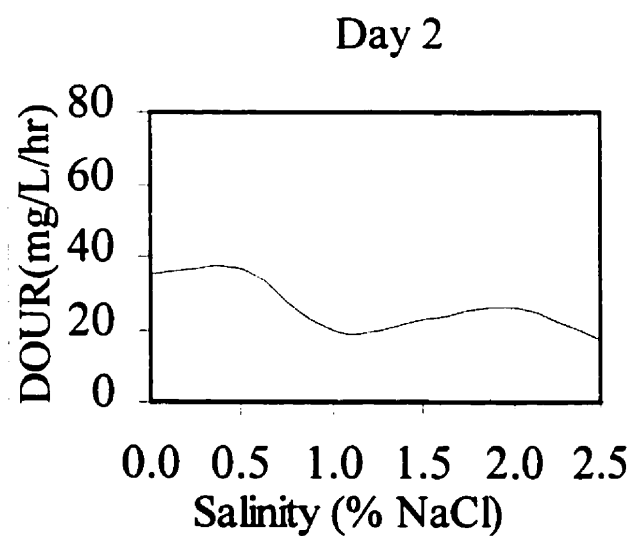
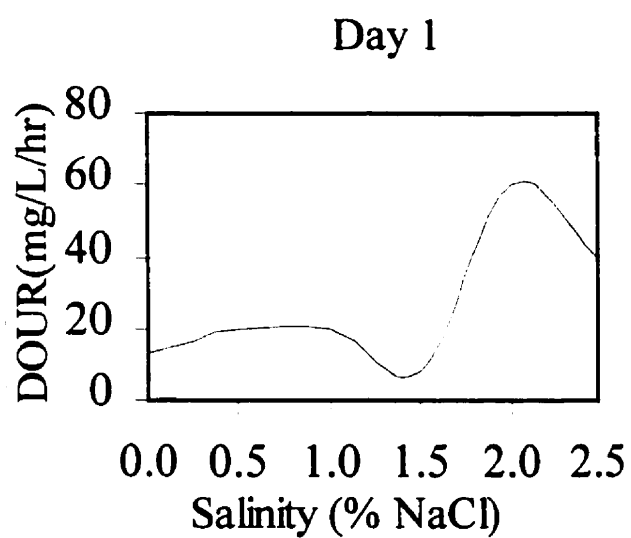
	Contaminant Levels (%dwt)							
Treatment (% Salt)	Initial	Abiotic	0%	0.5%	1%	1.5%	2%	2.5%
Saturates	1.12	0.93	0.64	0.62	0.65	0.52	0.67	0.8
Aromatics	0.27	0.28	0.23	0.29	0.25	0.21	0.28	0.29
Polars	0.27	0.64	0.62	0.61	1.00	1.21	0.36	0.77

Figure 44: Biodegradation at Different Levels of Salinity

3.4.4.3 Effect of Salinity (Test1): Interpretation

From the results, it is readily apparent that biological activity is proceeding rapidly in each treatment. DOUR is high in all samples when compared to DOUR from other experiments. Considering degradation by class, within a week the saturates have degraded with respect to the initial concentration as well as the abiotic control. Aromatics remain stable. The polar fraction (aphaltenes and NSO's), is highly variable, in many cases increasing with respect to the initial concentration.

No relation between salinity and DOUR or contaminant reduction is readily apparent. As illustrated in Figure 49, the relation of DOUR to salinity changes from day to day.

Figure 45: Relation of DOUR to Salinity on Two Days

A rough comparison of the amount of DOUR that is attributable to contaminant reduction can be achieved by comparing the amount of oxygen consumed in the slurry to the measured

contaminant reductions. Table 28 lists a cumulative oxygen uptake for each slurry in this experiment, estimated by multiplying average DOUR by time for the seven days DOUR was measured, given a slurry volume of 1L. The equivalent mass of hydrocarbons (n-CH₂) to which this would correspond (if all oxygen was used for the mineralization of hydrocarbons) is calculated from the stoichiometric relation:



The actual mass of hydrocarbons degraded, based on the percentage reductions in Mineral O&G with respect to the initial concentration over 10 days and using a 100g mass of soil, is presented for comparison.

Table 28: Comparing Oxygen Uptake to Hydrocarbons Degraded

Salinity (%NaCl)	For 10 days of treatment		
	Cumulative O ₂ (g)	Equivalent n-(CH ₂) (g)	Actual n-(CH ₂) (g)
0	2.4	0.7	0.48
0.5	2.9	0.85	0.5
1.0	2.7	0.79	0.47
1.5	1.7	0.50	0.60
2.0	3.1	0.91	0.45
2.5	2.9	0.85	0.32

By this estimate, the total oxygen consumed by the slurry is over twice that required to mineralize the MOG in the range of measurement for most of these slurries. This is expected, as it is acknowledged that DOUR measures all aerobic activity in the slurry, including the degradation of volatile hydrocarbons not detected by the MOG measurement. It would be advantageous to develop a monitoring method more directly linked to the degradation of the non-volatile petroleum contaminant. This is approached in the second part of this experiment series, Effect of Salinity Test 2.

3.3.5 Experiment Series 4: Effect of Salinity (Test 2)

This second test concerning salinity was conducted over a broader range of salt concentrations to establish a level at which inhibition of petroleum degradation is incurred. Monitoring focused on the degradation of alkanes, which in previous experiments were observed to degrade rapidly at the beginning of the slurry treatment. The collection of data focused on measurement of the ratio of heptadecane (n-C17) to pristane (an easily identifiable branched saturate which degrades more slowly) as a means of comparing the rate of biodegradation. This was achieved by direct GC-FID analysis of DCM extracts. The details of this procedure are presented in Appendix 9. Soil D was selected for this test because its high n-alkane content would facilitate readings.

3.3.5.1 Effect of Salinity (Test 2): Design

Table 29: Experiment Series 4, Test 2: Details

EFFECT OF SALINITY, TEST2			
Run time	7 days		
Temp.	30°C		
pH	neutral		
Soil used:	D (see Table 5)		
Additives:	antifoam, nutrients, NaCl (varied)		
Treatment	Salinity (g/L NaCl)	Salinity (%)	EC (dS/m)
1	0	0	0
2	10	1	17
3	50	5	86
4	100	10	172
5	150	15	259
6	200	20	345

For this experiment, GC-FID analysis was performed directly on the soxhlet extract of the soil contamination over the course of the seven day experiment. From the chromatograph, the areas of the n-C17 peak and the pristane peak were converted to a dimensionless ratio.

3.3.5.2 Effect of Salinity (Test 2): Results

Table 30: n-C17/Pristane Ratio Over 6 Day Treatment

NaCl (%)	Day of treatment						
	initial	1	2	3	4	5	6
0	0.66	0.69	0.29	0.22	0.32	--	0.37
1	"	0.69	0.50	0.38	0.39	--	0.36
5	"	0.75	0.67	0.67	--	0.67	--
10	"	0.75	0.96	0.70	--	0.72	--
15	"	0.85	0.8	0.70	--	0.94	--
20	"	0.73	0.67	0.70	--	--	--

3.3.5.3 Effect of Salinity (Test 2): Interpretation

Examining the data in Table 30, it is clear that only the n-C17 to pristane ratio decreases for the first two treatment alone. Figure 46 through Figure 54 illustrate this with a comparison of best-fit lines for the data of each treatment.

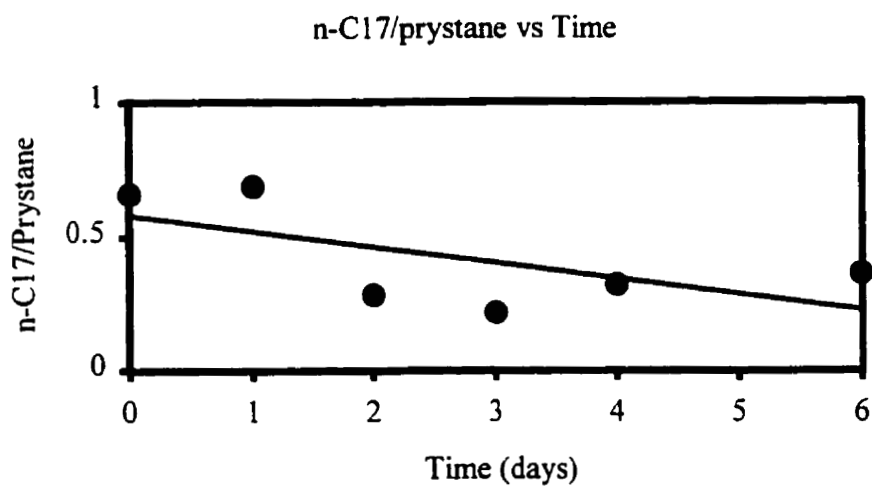
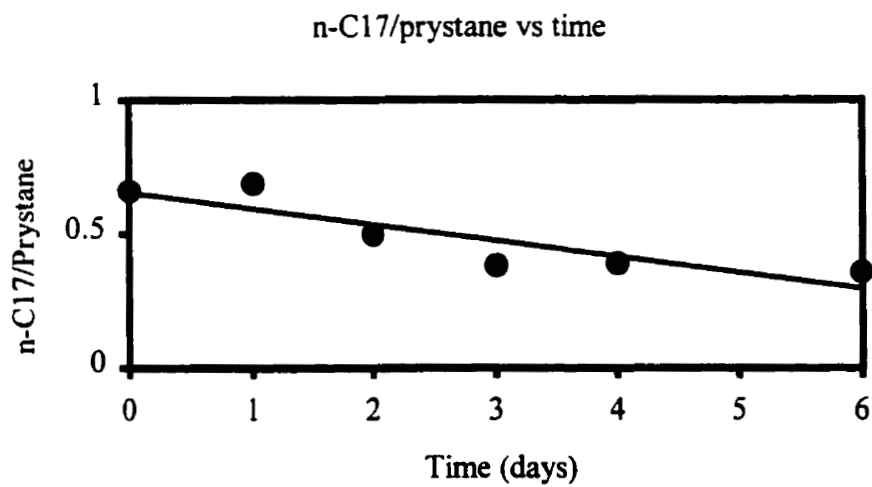
Figure 46: Graph of n-C17/Prystane vs Time: 0% Salt**Figure 47: Graph of n-C17/Prystane vs Time: 0.1% Salt**

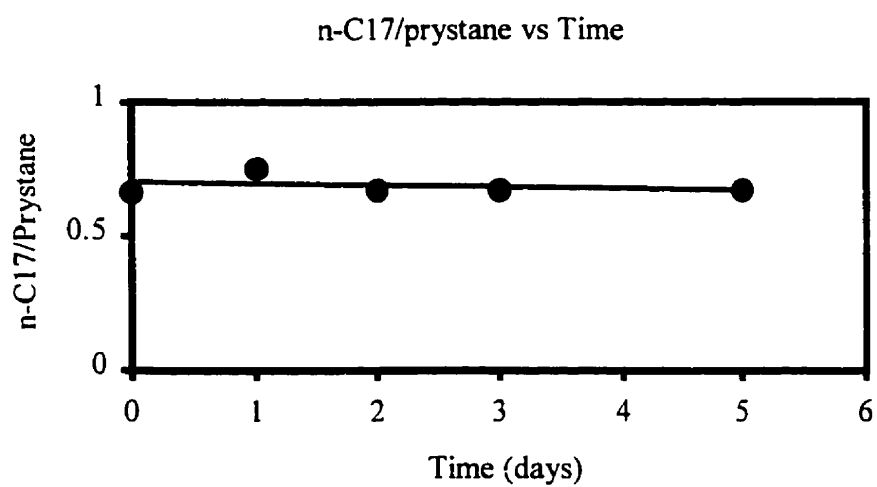
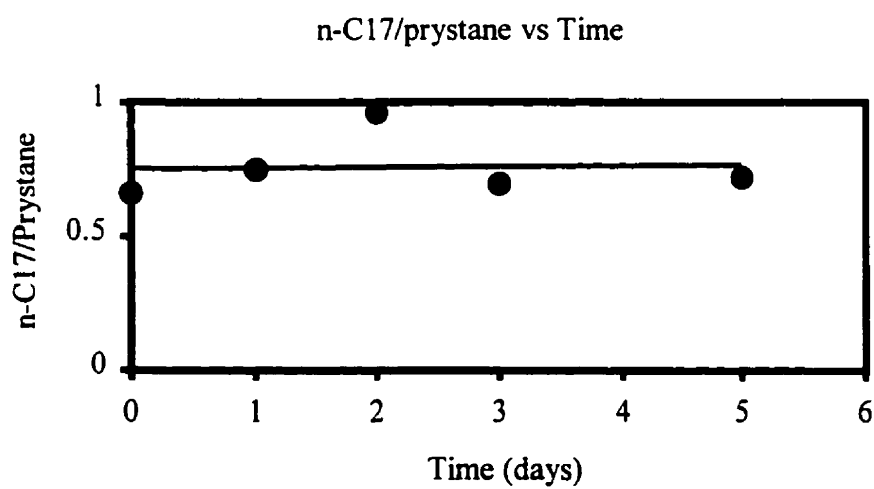
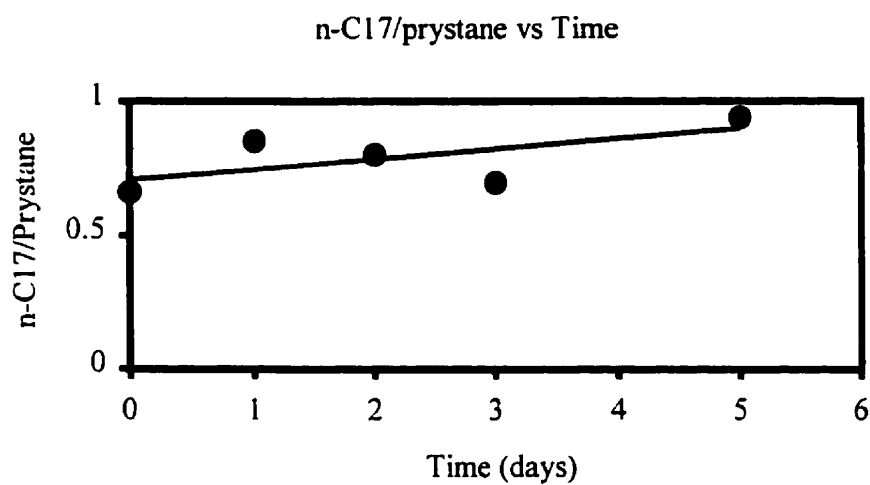
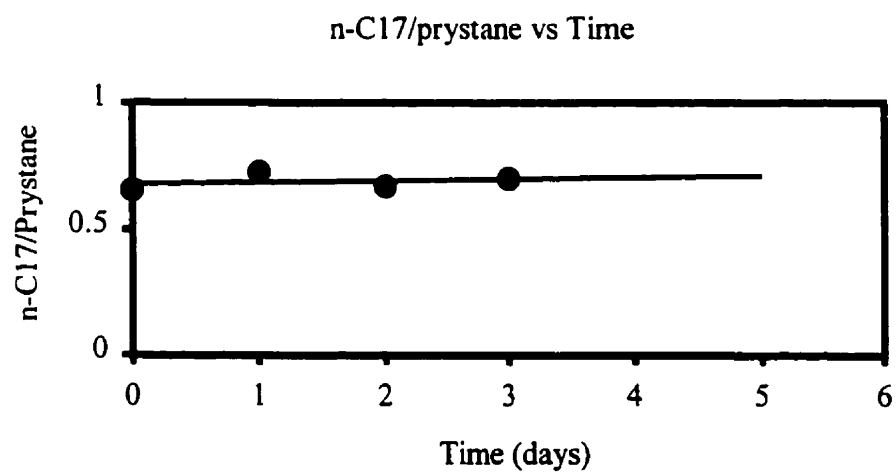
Figure 48: Graph of n-C17/Prystane vs Time: 0.5% Salt**Figure 53: Graph of n-C17/Prystane vs Time: 1% Salt**

Figure 54: Graph of n-C17/Prystane vs Time: 1.5% Salt**Figure 54: Graph of n-C17/Prystane vs Time: 2.0% Salt**

Comparing the rate of degradation between these treatments, and the findings of Salinity test 1, inhibition occurs between 40 and 80 dS/m, which is a slightly higher range than results

reported in the literature (Macmillen (1994) and Danielson (1994) reported inhibition at 40 dS/m and 36 dS/m, respectively). The 1 week time frame of the test cannot take into account an acclimation phase for the microbes involved; it is possible that bacteria may tolerate even higher salinity if given sufficient adjustment time. Experiments conducted on soils from aged sites of high salinity would be valuable in testing this possibility. The short term test is also subject to fluctuations that would be inconsequential over a longer term, increasing the possibility of error. This could be overcome by generating a large number of replicate analyses.

The method used to quantify the rate of biodegradation (using the n-C17/Prystane ratio) is simpler than measurements based on the mass of contaminant, requiring less time, effort, and chemical supplies to extract and quantify the contaminant. It is also preferable to the method of quantifying activity by dissolved oxygen uptake rate (DOUR), as oxygen consumption in the slurry is not necessarily restricted to petroleum degrading organisms, although DOUR offers real time results.

A limitation of this method is that it requires significant amounts of n-C17 and prystane in the contamination to perform the comparison. This will not be the case in many weathered or heavy oil sites. Furthermore this method is limited to short term tests in the slurry reactor, since these compounds degrade quickly. It is based on the premise that n-C17 degrades faster than prystane, but under slurry conditions both have been shown to degrade rapidly and spurious results can be expected when the denominator of the ratio (prystane) approaches zero (this effect is demonstrated by the 0g/l Na Cl treatment, where the ratio increase after the third day as prystane is degraded. Indexing biodegradation to the highly recalcitrant compound (such as the hopane class of aromatic steroids, (Prince, 1995)) would be a useful method.

The benefit of short-term testing is that it allows for the rapid screening of numerous parameters in short order, and this experiment demonstrates that the slurry reactor can be

used as a useful tool to study the many factors affecting bioremediation of flare pit wastes. The findings of such testing should be backed up with long-term studies, as it is expected that any findings based on the initial phase of rapid degradation demonstrated in the slurry reactor are limited to the subset of the microbial community which degrades the most easily degraded fraction of the petroleum contamination. These findings can be applied to other petroleum degrading organisms only with uncertainty.

3.3.6 Experiment Series 4: Effect of Salinity (Test 3)

This third test concerning salinity was conducted as a comparison between sodium and calcium salinity at the same levels of ionic strength, as the beginnings of an approach to take into account the effects of different types of salinity on the bioremediation process.

The same method of analysis by GC-FID chromatograms was applied as in Test 2. Soil D was spiked with petroleum with pronounced alkane peaks, to increase the amount of petroleum available for analysis.

3.3.6.1 Effect of Salinity (Test 3): Design

Table 31: Experiment Series 4, Test 3: Details

Effect of Salinity, Test 3				
Run time	5 days			
Temp.	30°C			
Ph	Neutral			
Soil used:	D (see Table 5)			
Additives:	antifoam, nutrients, petroleum (3g/kg), NaCl or CaCl ₂ (varied)			
Treatment	(% NaCl)	(% CaCl ₂)	meq/L	EC (dS/m)
1	0	0	0	0
2	1.5	0	250	25
3	2.9	0	500	50
4	4.4	0	750	75
5	5.8	0	1000	100
7	0	1.4	250	25
8	0	2.8	500	50
9	0	4.1	750	75
10	0	5.5	1000	100

As in Test 2, GC-FID analysis was performed directly on the soxhlet extract of the soil contamination over the course of the 7 day experiment. From the chromatograph, the areas of the n-C17 peak and the pristane peak were converted to a dimensionless ratio.

3.3.6.2 Effect of Salinity (Test 3): Results

Table 32: n-C17/Prystane ratio over 5 day treatment

	(% NaCl)	(% CaCl ₂)	Day of treatment					
			Initial	1	2	3	4	5
1	0	0	1.25	1.20	0.87	0.91	0.90	0.80
2	1.5	0	---	---	---	---	---	---
3	2.9	0	1.34	1.22	0.98	1.05	1.05	1.07
4	4.4	0	---	---	---	---	---	---
5	5.8	0	1.23	1.27	1.18	0.91	0.93	1.01
7	0	1.4	1.29	1.16	1.13	1.16	1.10	0.94
8	0	2.8	---	---	---	---	---	---
9	0	4.1	1.26	1.13	1.18	0.91	0.93	1.02
10	0	5.5	---	---	---	---	---	---

3.3.6.3 Effect of Salinity (Test 3): Interpretation

This experiment didn't work as intended; microbial degradation did not proceed in the same manner as observed for other tests, with rapid degradation of n-alkanes. At the time of this experiment investigation of the effects of petroleum biodegradability and salinity, based on available site data, was being undertaken. This work is reported in the discussion that follows). From section 4.3.5 it was concluded that while differences in salinity may have an effect on bioremediation the number of sites for which it is a consideration is small. Because of this, Test 3 was suspended.

4.0 DISCUSSION

4.1 General

Analytical methods are challenged when approaching 1000 mg/kg MOG. The O&G method was not very sensitive for cases of low petroleum content (approaching 1000 mg/kg). Without gas chromatograph analysis it was difficult to conclude any degradation was occurring given that the measured reductions were well within the range of measurement error. Based on the results of the various experiments undertaken, it is believed that the variability of the O&G measurement can be attributed to the polar fraction of the petroleum. In Experimental Series 1, where a pronounced increase in measured values was observed, it is likely that slurry water also contributed to the measured increase, since the highest of the measurement taken in that experiment was about 30% dwt, a level of contamination that would be clear to visual examination if it were entirely petroleum. While water is not normally highly soluble in DCM and filtration of the extract through anhydrous sodium sulfate is undertaken to remove any small amounts of water normally dissolved, the addition of large amounts of surfactant in this experimental series may have altered the solubility of water in the DCM and changed its properties with respect to adsorption with the anhydrous sodium sulfate. In later experiments with less or no surfactant, the variability is much less pronounced.

It is also noted that multiple extractions undertaken on individual soil samples produced measurements with less variation than that observed over time, such as the fluctuations observed in Experimental Series 3.

O&G variability of the type encountered in later experiments may be attributable to smaller amounts of water not properly removed, or polar compounds of inconsistent solubility in the DCM. From the literature review it is known that the solubility of asphaltenes in petroleum

is a function of the concentration of other classes (Speight and Moscopedis, 1981; Long, 1991), and the same effect may affect the efficiency of the extraction. A more dependable method may involve a combination of solvents with different polarities such as a DCM/methanol mixture, or extractions made with the separate solvents in sequence.

For the experimentation in this project the O&G method was initially chosen because of its relative simplicity and similarity to the MOG method, which is the basis for regulation. When the dramatic increases seen in Experimental Series 1 were overcome, the variability of the O&G measurement still made it difficult to infer trends in biodegradation over time, since the observed degradation (based on MOG) was quite modest. Component class analysis, together with GC-FID, proved the most useful tool for explaining the observed trends. Consideration of petroleum contamination from that perspective allowed application of experimental findings to flare pit sites as a whole, by making generalizations about the biodegradability of each component class, and the expected composition of the original petroleum contamination.

The extent to which a particular flare pit petroleum contaminant may be degraded is to a large extent a function of the extent to which it has already been biodegraded by the time characterization measurements are made, whether over geologic time as in the case of heavy oils, or over recent years in an exposed pit. A GC-FID chromatogram provides indication as to the extent to which biodegradation has occurred, based on comparison of the n-alkane peaks to the size of the UCM hump, composed of more recalcitrant compounds. Such a method might be used to assess the potential biodegradability of a particular flare pit soil. The qualitative interpretation required is more dependent on the experience and confidence of the interpreter to predict the results accurately, than an actual treatability test.

When it was quantified, biodegradation was shown to proceed rapidly. The long treatment times applied in most of these experiments are not required if further degradation is not going to occur. Tests based on rate of degradation occurring in the first week of treatment are more

capable of addressing many parameters in combination. In general, the lab analysis need not be as cumbersome as that required for the experiments conducted here, which were based on the extent of biodegradation of extended length to ensure that an endpoint had been reached.

Experimentation confirms that salinity does not have a pronounced inhibitory effect on bioremediation until very high levels (~40 dS/m), levels which will require separate remediation for salinity to meet regulatory requirements as they are detrimental to plant life. While substantial metal concentration characterization is available for many sites, their effect on treatability was not addressed experimentally since the literature review indicated that the toxicity of metals depends on many site factors in addition to concentration. When an organized approach to addressing these many factors is developed, the slurry reactor might be used to study their effect.

An estimate of the number of sites that are biologically treatable is developed here by relating the findings of the lab study of hydrocarbon degradation by class composition to the composition data for the oil producing formations of the Western Canadian Sedimentary Basin as presented in Deroo *et al.* (1977). Biodegradation results from the literature (Huesemann, 1995) are applied to each component class, to arrive at a reasonable best-case for total biodegradation. For each of the four types of oil discussed in that report, the fraction expected to remain after treatment is calculated.

Assessing the general treatability of flare pit sites is difficult due to the variability of contamination. Collection of samples according to categorization developed through the database would offer some guidance in this matter, though several samples would be required of each category to account for variability within. The cost of sample acquisition limited this approach. In this experimental programme, treatability is assessed by considering contaminant parameters prevalent in site characterization, and determining how they affect treatability. An overall treatability estimate is developed from the expected treatabilities of

entries in the database, based on the knowledge developed. Analysis of the data revealed petroleum, salinity, and metals to be the contaminants with the most significant entries. While analysis of the effects of metals on treatability was too broad an issue to be included within the scope of this project, experimentation was conducted on the effect of petroleum and salinity. These findings enter into the treatability estimate.

Laboratory experimentation confirmed that component class composition affects achievable biodegradation in absence of other interferences; since such composition information is lacking in the database, it was sought from geological studies of the composition of Alberta oils. Reasonable best-case estimates for biodegradation were developed for saturates, aromatics, and resins/asphaltenes, and these were applied to the geological data to yield estimates of the biodegradability to be expected for Alberta oils. These biodegradabilities were applied to individual database entries, and the treatability of each site was assessed.

These reduction figures assume that the hydrocarbons present in the flare pit are of the same makeup as those freshly produced. However, burning the hydrocarbons will preferentially remove hydrocarbons of lower ignition points (generally, those of fewer carbons and lower molecular weight), leaving less substrate for growth, altering the remaining petroleum chemically and physically, and sterilizing the burnt soil. The history of burning for these sites is not generally known. A pit will also have lesser potential for bioremediation if natural microbial activity has already removed the easily degradable fractions of the oil prior to measurement of the petroleum present. Indications are that this will frequently be the case; n-C17/ prystane ratios and general hydrocarbon boiling point profiles for soils tested in this project resembled those of oils that have already undergone substantial degradation (Tissot and Welte, 1978). As noted, other factors such as salinity, metals, sterilants, and soil sorption will affect the practical extent of treatment. As such, the estimate developed herein which considers flare pits simply as crude oil spilled on soil is intended simply as an initial "best-case" scenario. A conservative assumption used in the development of this estimate is that all petroleum concentrations in the database correspond to non-volatile material.

The laboratory slurry treatment failed to biodegrade petroleum to the same extent as similar experiments reported in the literature. It is hypothesized that the slurry treatment itself may impose a limitation by inhibiting the development of filamentous fungi (known to degrade polynuclear aromatics). This requires development of an experimental framework to assess the growth and development of the microbial consortia, which is proceeding along with solid-phase testing in the next phase of laboratory testing conducted by others.

4.2 Estimating the General Biodegradability of Alberta Oil

4.2.1 Composition Data

The data presented in GSC 262 (Deroo *et al.*, 1977) is used in this estimate to represent the composition of Alberta crude oils. This study was undertaken to develop a theory for the formation and maturation of petroleum and gas in the Alberta part of the western Canadian Sedimentary Basin. Data from 106 conventional oils, 63 heavy oil extracts, and 189 sedimentary rock samples was collected. Component class analysis for these samples is presented in the form of ternary diagrams; plots of oil samples on triangular graphs where the percentage of each of three components is given. An example of such a data plot can be seen in Figure 49, for the Group 2 oil samples. To apply this data in calculations to estimate the biodegradability of such oil, these distributions of points were simplified to a central tendency (from the centroid of the data points), as well as a range of variability (by graphically representing the scattered points as a polygon, and using the vertices for calculation). The method used was as follows:

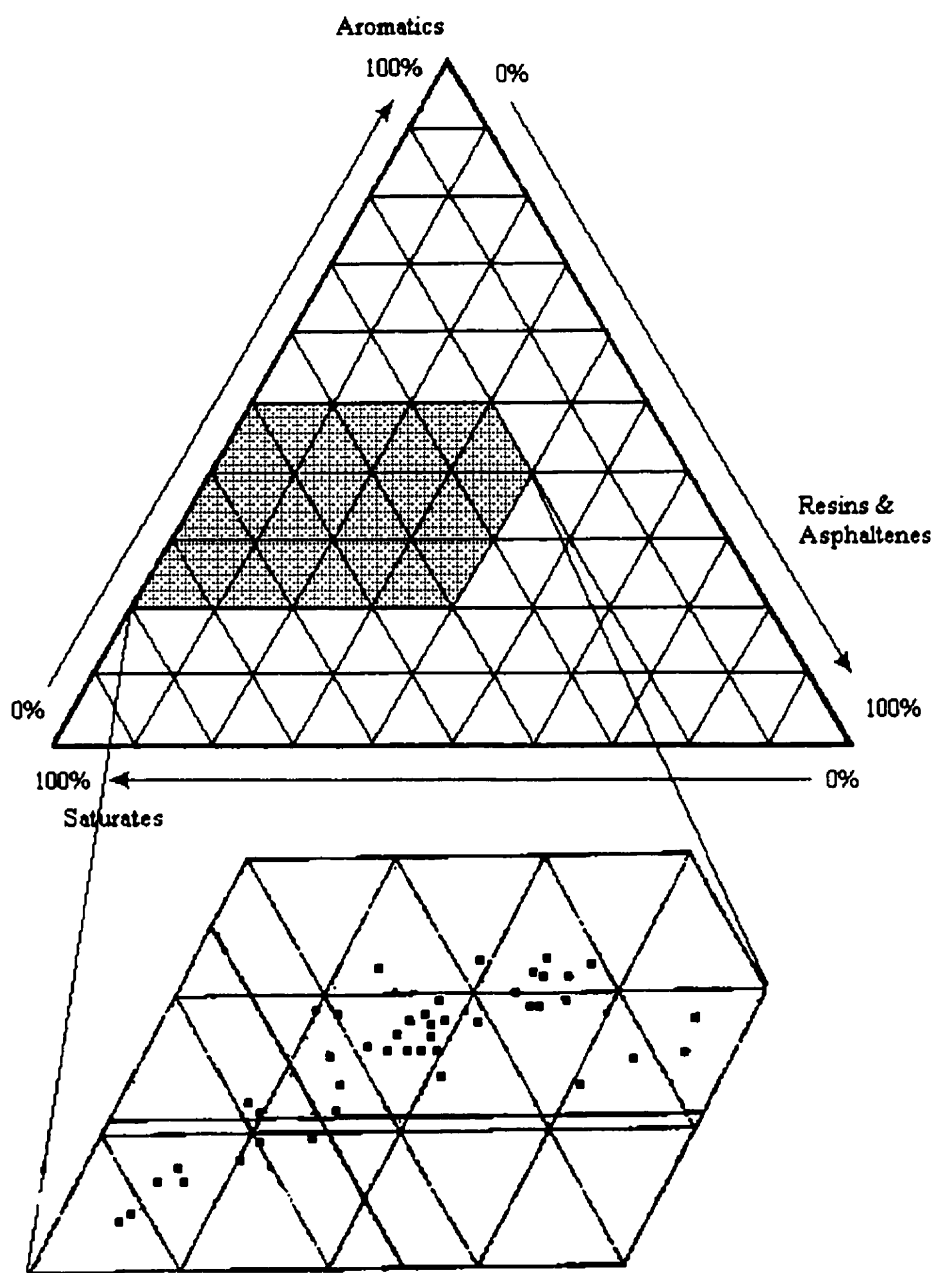
1. For a given ternary diagram, a polygon was sketched which encloses the bulk of the sample points. The polygon for the group 2 oils is illustrated in Figure 50.
2. Some outliers were excluded from the polygon. The number of samples included divided

by the total number yields a level of confidence of the estimate developed by this method; the percentage of samples falling within the polygon.

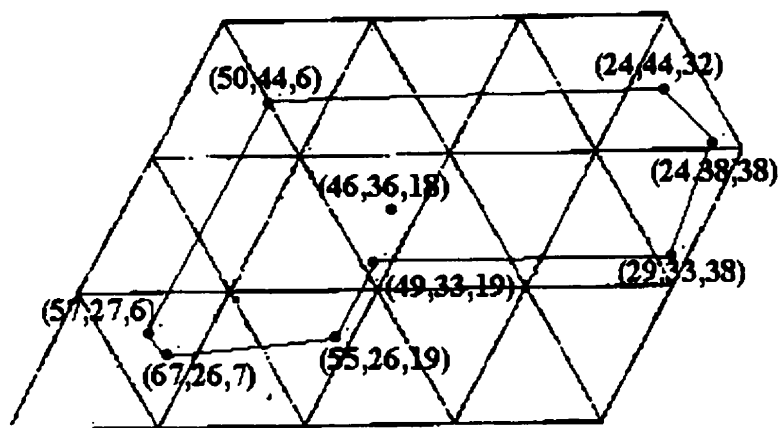
3. The corner points of this polygon are taken as extrema and will be used to develop a measure of the variability of the composition of the oil in a given group, as a confidence interval.

4. The centroid of the sample distribution is calculated by locating the mean of all points along each axis, giving each sample point equal weight. This centroid is used as the central tendency of the estimate.

Figure 49: Ternary Diagram for Type 2 Oils, (Deroo *et al.*, 1977)



(area enlarged)

Figure 50: Polygon for Simplified Analysis**Table 33: Gross Composition Boiling Above 210°C**

(Alkanes/Aromatic/Asphaltenes&Resins) Fractions as percentage				
Group	Centroid	Extrema		Confidence
		(74, 22, 4)	(73, 21, 6)	
1	(67, 27, 9)	(63, 21, 16)	(56, 28, 15)	15/16 (94%)
		(56, 31, 13)	(64, 32, 4)	
		(67, 26, 7)	(55, 26, 19)	
2	(46,36,18)	(49, 33,19)	(29, 33, 38)	48/51 (94%)
		(24, 44, 32)	(50, 44, 6)	
		(67, 27, 6)		
		(75, 21, 4)	(59, 21, 20)	
3	(60,28,12)	(60, 28, 12)	(50, 30, 20)	37/39 (95%)
		(50, 36, 14)	(58, 36, 6)	
		(15, 19, 66)	(4, 30, 66)	
HEAVY	(23,29,47)	(4, 40, 56)	(33, 40, 27)	71/77 (92%)
		(48, 25, 27)	(48, 19, 33)	

Table 34: Cyclic, Acyclic, and Aromatic Hydrocarbons Boiling Above 210°C

(Cyclic, Acyclic, and Aromatic Hydrocarbons) Fractions as percentage				
Group	Centroid	Extrema		Confidence
1	(33, 34, 31)	(40, 38, 22)	(30, 38, 32)	15/16 (94%)
		(30, 33, 37)	(40, 33, 27)	
2	(22, 34, 44)	(35, 41, 24)	(30, 46, 24)	50/51 (98%)
		(24, 46, 30)	(7, 33, 60)	
3	(31, 38, 31)	(38, 42, 20)	(26, 41, 33)	37/39 (95%)
		(28, 30, 42)	(37, 33, 30)	
	(27, 35, 38)	(41, 17, 42)	(25, 28, 47)	75/77 (97%)
HEAVY		(48, 25, 27)	(48, 19, 33)	
		(30, 48, 22)	(40, 30, 30)	

(data adapted from Deroo *et al.*, 1977)

Data in the GSC report on the composition of each oil's saturates is also utilized in some calculations in this analysis. Since the distribution of these datapoints was closely packed, only a centroid is used to simplify calculation:

Table 35: Saturates : Percentage Proportion by Specified Fractions

Group	Centroid	Fractions
1	(53, 27, 20)	acyclic/ 1-2 rings/ 3+ rings
2	(39, 32, 29)	“ “ “
3	(50, 31, 19)	“ “ “
Heavy	(40, 33, 27)	2 rings/ 3 rings/ 4+ rings

(data adapted from Deroo *et al.*, 1977)

4.2.2 Biodegradability of Component Classes

These generalizations on the chemical composition of Alberta's oils are used to estimate their biodegradability. Reductions reported in the literature are used to establish a reasonable "best case" of biodegradability to be expected for each component class, as discussed below. The results of the slurry treatment fall short of values reported in the literature, and as such a best-case estimate cannot be developed from them. However, they are similar to other reductions found specifically for flare pits (Visser *et al.*, 1999).

4.2.2.1 Saturates

For the saturate class, the reductions reported by Huesemann and associates are employed (from Huesemann *et al.*, 1995). The Field Desorption Mass Spectrometry (FDMS) analysis utilized in that report offers the ability to crudely account for different subclasses of saturates. The FDMS analysis quantifies compounds both by carbon number (n) as well as the hydrogen deficiency of the compound (z), according to the general molecular formula C_nH_{2n+z} . This allows inferences to be made as to the approximate ring structure of compounds detected; cyclic formations involve more carbon-carbon bonds, and consequently a greater deficiency of hydrogen.

Table 36 presents the reported biodegradation (converted to "percent biodegraded" from the "percent remaining" as reported in Huesmann *et al.*, (1995)). The saturate fraction is subdivided by z-number, and carbon-number intervals. The figures represent average results from seven oils after solid-phase treatment:

Table 36: Biodegradation Achieved for Saturates (%)

carbon group	z-number						
	2	0	-2	-4	-6	-8	-10
C13-C16	100	100	100	100	100	86	90
C17-C20	99	97	98	74	58	62	81
C21-C24	94	85	72	38	11	25	75
C25-C28	86	90	68	30	0	1	53
C29-C32	82	86	65	0	0	0	46
C33-C44	82	86	65	0	0	0	41
>C44	74	(independent of z-number)					

(this table from Huesemann *et al.*, 1995)

A possible error in this method of quantification is the transformation of a compound from one category to another, such as a ring compound broken into a branched formation, without complete degradation. Furthermore, aging will have an effect within these categories if more readily degradable compounds within that category were already removed. Despite errors and biases, considering biodegradability through these categories offered a means of comparing for the effect of differences in the makeup of the saturate fraction, mainly the easily-degradable acyclic compounds ($z=2$).

The makeup of the saturates for each oil group from Table 35 were used in the calculations that follow, to estimate the extent of biodegradability achievable for the saturates of each group. The following z-number relations were applied:

Acyclic compounds (n-alkanes, isoprenoids): $z=2$

1-2 ring saturates: $z=0, -2$

2 ring saturates: $z=-2$

3 ring saturates: $z=-4$

3+ ring saturates: $z= -4$ to -10

4+ ring saturates: $z= -6$ to -10

For each group of compounds indicated, an average was taken of the categories C17 and higher for the applicable z-numbers.

example: Acyclic compounds , $z=2$,
(the average of the $z=2$ column for C17 and above)

$$(99 + 94 + 86 + 82 + 82 + 74) / 6 = 88\%$$

By considering C17 and above, this calculation pertains strictly to the non-volatile fraction of petroleum. The non-weighted average assumed an equal distribution amongst carbon number groups, resulting in a crude estimate which would benefit from more information on the distributions to be expected between these ranges; a weighted average would improve accuracy, but this information is limited in the GSC data.

Results are prepared in this manner for the components of concern in Table 35. For Components such as saturates with 3+ rings, the average was calculated over multiple columns in the table.

Acyclic compounds: 88% degradability

1-2 rings: 80%

2 rings: 74%

3 rings: 36%

3+ rings: 34%

4+ rings: 36%

These figures are applied to the proportions presented in Table 35 to calculate the expected

degradability for the saturate fraction of each oil group:

For example, Group 1

53% acyclics (86% degradable): $53\% \times 88\% = 46\%$

27% 1-2 rings (80% degradable): $27\% \times 80\% = 22\%$

20% 3+ rings (34% degradable): $20\% \times 34\% = \underline{7\%}$

total: 75% degradable.

The results for each group:

Group 1: 75%

Group 2: 70%

Group 3: 75%

Heavy: 51%.

4.2.2.2 Aromatics

While Huesemann and associates offer a similar analysis on the aromatic fraction of the oils they tested, the geological data for Alberta oils does not offer any insight as to the composition of aromatics by different subclasses, as it does for saturates. The wide range of reductions achieved between different subclasses shows that further data characterizing the aromatic fraction of Alberta oils or flare pit contaminants could improve the accuracy of this estimate.

Table 37: Biodegradation Achieved for Aromatics(%)

z-number							
carbon group	2	0	-2	-4	-6	-8	-10
C13-C16	96	96	98	100	100	100	100
C17-C20	75	80	65	80	81	88	76
C21-C24	43	43	26	28	72	61	42
C25-C28	38	37	26	21	55	60	43
C29-C32	43	42	29	31	70	67	50
C33-C44	43	42	29	32	70	67	61
>C44	38	(independent of z-number)					

(this table from Huesemann *et al.*, 1995)

Average of all categories above C17: 50%

This compares closely with the findings of Johnson and Danielson, who reported 41% reductions for PAHs and polycyclic aromatic sulfur heterocycles combined over 346 days of biopile treatment (Johnson and Danielson, 1994). As in the case of the calculations for the saturate fraction, a weighted average based on some understanding of the proportions to be expected would improve the accuracy of this estimate.

It might be reasonable to take the biodegradation of 86% reported by McMillen and associates, (1992) as a best case estimate for the aromatic class (McMillen *et al.*, 1992). The reduction achieved in this experiment was the best of all the studies examined, and unlike the saturate fraction, no information could be found to suggest that differences in the composition of the aromatic fraction may occur between different oils in ways that could be applied to the estimation of biodegradability. While this experiment was performed on a crude oil topped for several days at 40°C to minimize the effect of volatilization, an

examination of the GC data presented in that report suggests that significant losses are quantified in the volatile and semi-volatile range (<C17). For this treatability estimate, it was decided to use the 50% biodegradability estimated above, as it is more consistent with the ranking of Raymond and associates placing the biodegradability of non-volatile aromatics and PAH as comparable to or less than that of heavy saturates (Raymond *et al.*, 1990).

4.2.2.3 Resins and Asphaltenes

Reported biodegradation values for resins and asphaltenes vary widely. Conventional wisdom holds that they are the most recalcitrant fractions of oil. Resins are more readily degradable than asphaltenes, but the geological data groups them together so no distinction can be made in this estimate. They are highly variable in extraction for measurement. A conservative biodegradability of 0% is used in this estimate. A better understanding of the degradability of these classes as well as their respective proportions as separate classes would benefit the treatability estimate.

4.2.3 Degradabilities of Oil Based on Composition

Biodegradability of O&G as well as MOG (Saturates and Aromatics) are calculated separately to contrast the effect that the poorly understood resin/ asphaltene fraction has on the estimate.

Saturates: Group 1	75%
Group 2	70%
Group 3	75%
Heavy	51%
Aromatics	50%
Resins/Asphaltenes	0%

For Hydrocarbons (Saturated and Aromatics classes together), an example calculation is presented here:

For group 1:

percentage proportions (Acyclic/Cyclic/Aromatic)

for the centroid: (35,34,31)

Acyclic and Cyclic proportions of Group 1 were taken into account in calculation of saturate biodegradability above:

Saturates:	$75\% \times (35\% + 34\%) =$	52%
Aromatics:	$51\% \times 31\% =$	<u>16%</u>
Total:		68%

Similarly for the extrema:

(%Acyclic/Cyclic/Aromatic) Biodegradability

(40,38,22)	70%
(30,38,32)	67%
(30,33,37)	66%
(40,33,27)	68%.

in this manner the value of 68% is taken for the central tendency of this distribution, while 66% and 70% are taken as lower and upper values.

Table 38: Estimated Biodegradability of Alberta Oils: Hydrocarbons

Group	lower	central	upper
1	66	68	70
2	58	61	64
3	65	67	70
heavy	51	51	51

Table 39: Estimated Biodegradability of Alberta Oils: Complete Oil

Group	lower	central	upper
1	56	64	66
2	39	50	60
3	52	59	67
heavy	17	26	37

(Calculated from proportions of Alkanes/Aromatic/Asphaltenes&Resins):

These figures in Table 36 and Table 37 may be compared to an alternative method for predicting the biodegradability of crude oil based on oil density, presented by McMillen and associates, based on a comparison of several oils (McMillen *et al.*, 1994):

$$\% \text{ biodegradation} = (\text{API gravity})^2 * 0.03358$$

The experiment was conducted on topped crude oils so the results correspond to the non-volatile fraction. Based on oil density, this formula circumvents component class analysis and predicts biodegradability to be achieved in 4 weeks of treatment in soil. Sample figures for Alberta oils using API gravity data from McCrossan and Glaister (1966) are presented here for comparison:

Table 40: Biodegradabilities Based on °API Gravity

Formation	group (as above)	API gravity (°) (interval with mean in bold)	percentage degraded (interval with mean in bold)
belly river	1	35-48	41-77
cardium	1	34- 38 -43	38- 48 -62
viking	1	31- 37 -43	32- 46 -62
manville	2/ heavy	14- 22 -38	6- 16 -48
carboniferous	2	18-37-39	11- 46 -51
winterburn	3	30- 36 -44	30- 44 -65
woodbend	3	25- 36 -44	21- 44 -65
beaverhill	3	37- 39 -45	46- 51 -68

(oil gravity data from McCrossan and Glaister, 1966)

(percentage degrades calculated by method presented in Macmillen *et al.*, 1994)

Comparing these results to those achieved from the petrological data (Deroo *et al.*, 1977) and component-class biodegradability (Huesemann *et al.*, 1995), the results for the highest biodegradability to be achieved for each oil are similar, but in general this method presents broader intervals and lower reductions. The difference may be in part due to the longer (one year) treatment applied by Heusmann and associates' component-class experiment, compared with 4-month treatment applied in Macmillen and associates' oil-density study. However, a density-related method of this type has great potential for site-specific estimates since it is based on a measurement common to oil production.

4.3 Estimate of Proportion of Sites Treatable Using Database:

4.3.1 Treatability Estimate 1: Preliminary

The method and terminology of obtaining percentage estimates of treatable sites is illustrated in Figure 54, using a broad interval of possible biodegradabilities for clarity. Based on reductions achieved in literature, the solid biodegradability of petroleum has been demonstrated to be anywhere between 30% and 92.1% (Danielson, 1995; McMillen, 1992). If it is assumed that all sites have a biodegradability in this range, any site with a concentration of 1429 mg/kg (corresponding to 30% biodegradability) or lower can be treated to a regulatory criteria of 1000 mg/kg;

$$\text{e.g. } 1429 \times (1-0.3) = 1000$$

Sites with petroleum concentrations 1429 mg/kg and lower are deemed *treatable with certainty*, with the exception of sites with concentrations below 1000 mg/kg, which are said to need *no treatment*. The upper limit of treatment, at 92.1%, corresponds to a concentration of 12658 mg/kg. Sites above this concentration are deemed *untreatable*, while those in the interval between 1429 mg/kg and 12658 mg/kg are deemed *uncertain*.

These figures represent an estimate of the type that might be made at the advent of experimentation, when it was not clear what factors controlled the differences between the 30% and the 92.1%. The finding that the composition of the petroleum contaminant would greatly limit biodegradation regardless of other factors is developed in the revised estimates that follow.

Sorting entries from the database for these figures, 485 sites have entries for petroleum

concentration:

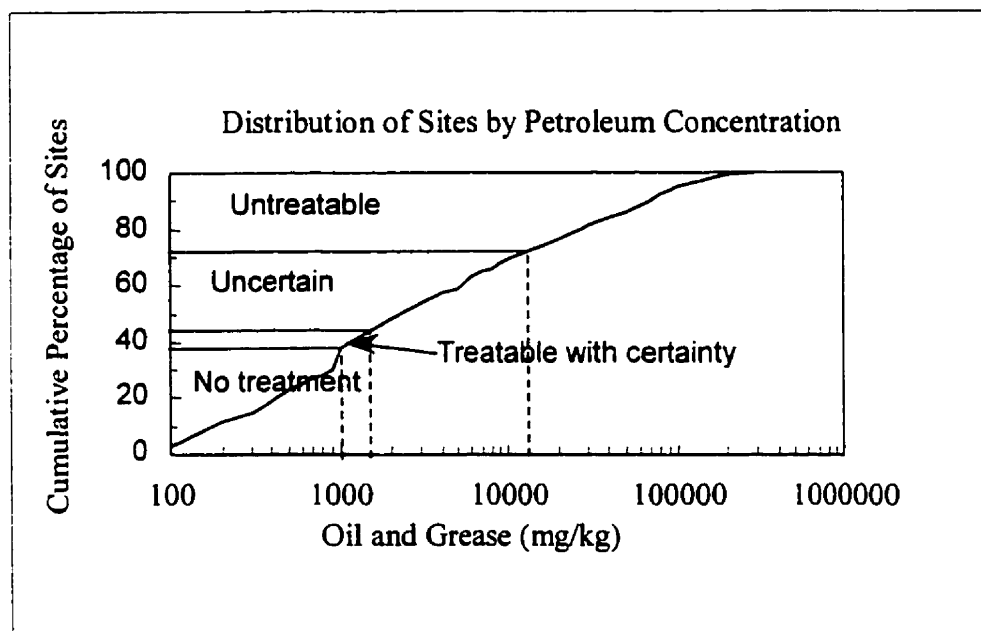
in the interval $0 < x < 1000$ mg/kg, 182 sites need no treatment (38%)

in the interval $1000 < x < 1429$ mg/kg, 25 sites are treatable with certainty (5%)

in the interval $1428 < x < 12658$ mg/kg, 142 sites uncertain (29%)

and for $12658 < x$, 136 sites untreatable (28%).

Figure 51: Treatability Estimate: Wide Preliminary Interval



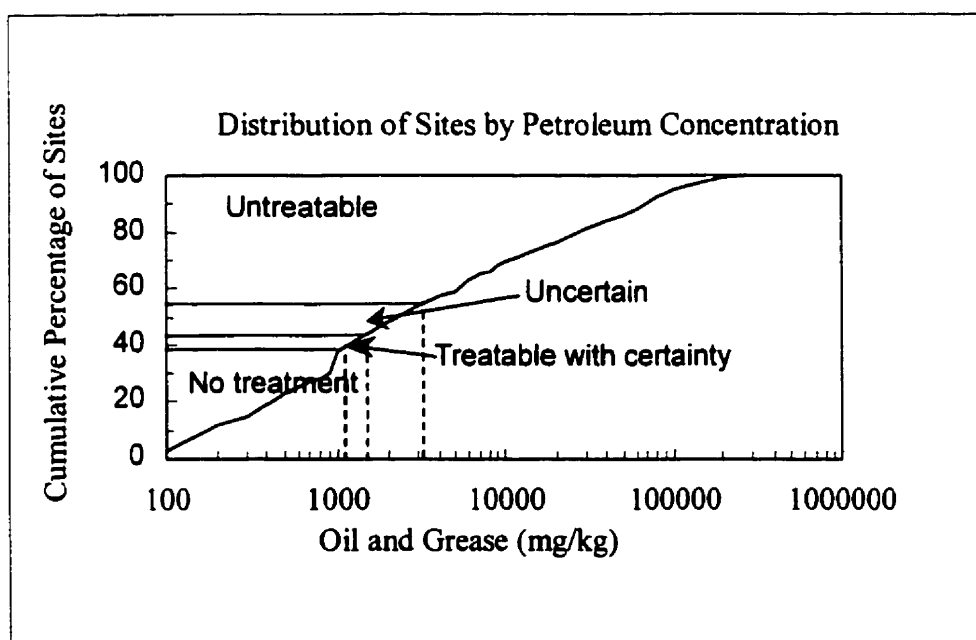
4.3.2 Treatability Estimate 2: Effect of Oil Composition

Laboratory investigation confirmed that component class composition was very important, and estimate 2 was developed from degradabilities predicted for oil compositions encountered in Alberta (as calculated in section 4.2), as a means of approximating the contamination to be found in flare pit sites. Biodegradability ranged from 17% to 67%, corresponding to concentration bounds of 1205 to 3030 mg/kg.

Sorting entries from the database for these figures, 485 sites have entries for petroleum concentration:

in the interval $0 < x < 1000$ mg/kg, 182 sites need no treatment (38%)
 in the interval $1000 < x < 1205$ mg/kg, 13 sites are treatable with certainty (3%)
 in the interval $1205 < x < 3030$ mg/kg, 54 sites uncertain (11%)
 and for $3030 < x$, 236 sites untreatable (28%).

Figure 52: Treatability Estimate: Effect of Oil Composition



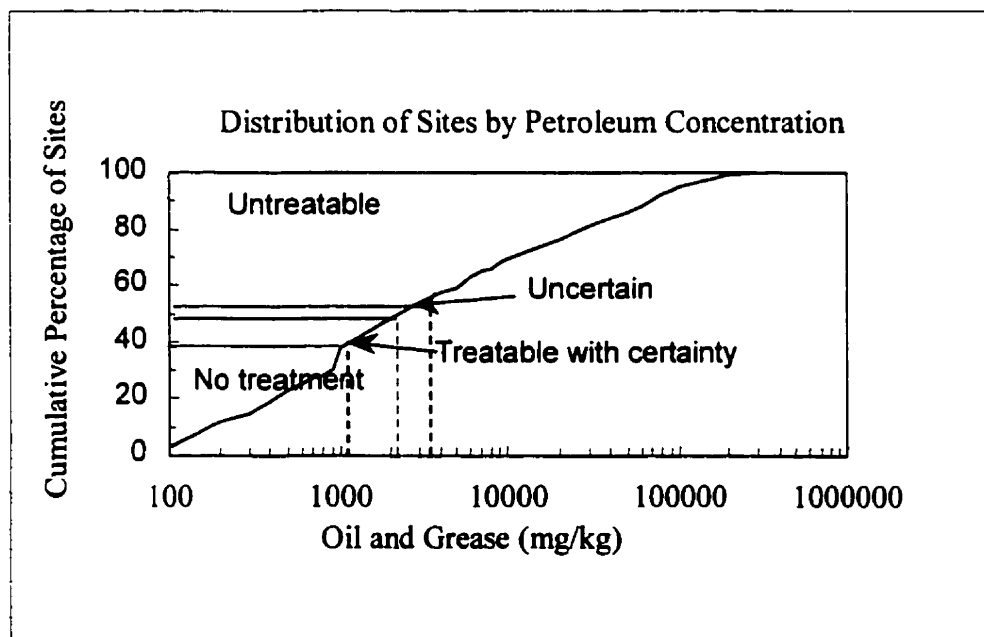
4.3.3 Treatability Estimate 3: Effect of Hydrocarbon Composition

Estimate 3 considers the biodegradabilities estimates developed for hydrocarbons alone (as calculated in section 4.2), and presents a result more closely related to the Mineral Oil and Grease measurement method upon which regulation is based. Biodegradability ranged from 51% to 70%, corresponding to concentration bounds of 2041 to 3333 mg/kg.

Sorting entries from the database for these figures, 485 sites have entries for petroleum concentration:

in the interval $0 < x < 1000 \text{ mg/kg}$, 182 sites need no treatment (38%)
 in the interval $1000 < x < 2041 \text{ mg/kg}$, 51 sites are treatable with certainty (11%)
 in the interval $1205 < x < 3333 \text{ mg/kg}$, 16 sites uncertain (3%)
 and for $3333 < x$, 236 sites untreatable (49%).

Figure 53: Treatability Estimate: Effect of Oil Composition



4.3.4 Treatability Estimate 4: Accounting for Heavy and Conventional Oils

Estimate 4 narrows the interval of uncertainty by dividing the samples by location into conventional (Groups 1,2,3) and heavy oils by site, according to Figure 6. For this estimate, proportions for heavy and conventional oils are presented separately, then in combination.

Heavy Oils:

Biodegradability ranged from 17 to 37% for heavy oils, corresponding to concentration bounds of 1205 and 1587 mg/kg in the calculation. Sorting entries from the database for these figures, 149 sites within the heavy oil area have entries for petroleum concentration:

in the interval	$0 < x < 1000 \text{ mg/kg}$,	65 sites need no treatment
in the interval	$1000 < x < 1205 \text{ mg/kg}$,	5 sites are treatable with certainty
in the interval	$1205 < x < 1587 \text{ mg/kg}$,	4 sites uncertain
and for	$1587 < x$,	75 sites untreatable.

Conventional Oils:

Biodegradability ranged from 36 to 67% for conventional oils, for concentration bounds of 1563 to 2857 mg/kg. Sorting entries from the database for these figures, 309 sites in the conventional oil area have entries for petroleum concentration:

in the interval	$0 < x < 1000 \text{ mg/kg}$,	113 sites need no treatment
in the interval	$1000 < x < 1563 \text{ mg/kg}$,	20 sites are treatable with certainty
in the interval	$1563 < x < 2857 \text{ mg/kg}$,	24 sites uncertain
and for	$2857 < x$,	152 sites untreatable.

Combining these results, of 458 sites with both location and petroleum data:

178 need no treatment (39%)
25 are treatable with certainty (5%)
38 are uncertain (6%)
227 are not treatable (50%)

4.3.5 Considering the Effects of Salinity and Metals

An estimate of the overall effect of salinity may be obtained by enumerating the sites in estimate 3 that would be inhibited by high salinity, taking EC >40 to be inhibitory (based on literature and lab findings).

From 437 entries with petroleum and salinity data,

- 3 sites in the “no treatment” interval would be inhibited (>1%), as would
- 0 sites in the “certain” interval (>1%),
- 1 site in the “uncertain” interval (>1%), and
- 18 sites in the “untreatable” interval (4%).

Based on these findings, inhibition due to salinity is not expected to be a prominent concern for the bioremediation of these sites

A similar estimate for the inhibitory effect of metals cannot be made since inhibitory metals concentrations have not been firmly established. However, a rough estimate may be performed by considering sites whose metal concentrations exceed WCR limits as inhibitory. These regulatory limits are not connected to microbial activity, but signify a very high concentration, so this estimate assumes that bioremediation would proceed at fairly high metals concentrations, as is the case with salinity. The number of sites where a metal concentration exceeds this high regulatory limit were enumerated. This estimate shows a much reduced sample sizes due to availability of data.

From 174 sites with petroleum and metal concentration data,

- 15 sites in the “no treatment” interval would be inhibited, (9%) as would
- 5 sites in the “certain” interval (3%),
- 3 site in the “uncertain” interval (2%), and
- 84 sites in the “untreatable” interval (48%).

Metal contamination is prevalent, when considered as a proportion of the sites with data. The value of more precisely defining the effect of metals would increase if the interval of sites for which bioremediation could be considered (the “certain/uncertain” interval) were broadened.

4.3.6 General Discussion of Treatability Estimates

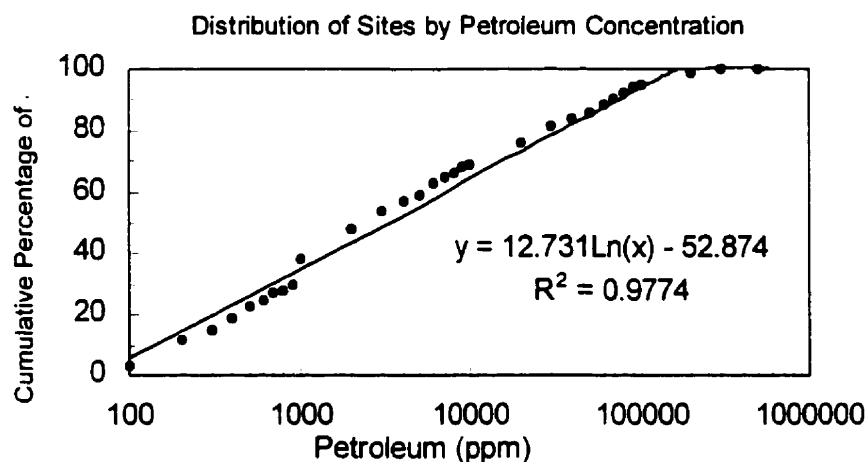
Given the uncertainty involved in the development of these estimates, the precision of these figures can not realistically be taken to a single percentage point. There is a degree of imprecision in the methods and results used to estimate the percentage degradability of different components of petroleum, and while the calculation of the estimate accounts for the variability of petroleum composition by representing it as a range of composition, there is the possibility of error in the assumption that the composition of flare pit petroleum will resemble that of crude oil. The effect of weathering tends to make oil less degradable. This estimate is also subject to any errors and biases present in the database for the parameters used.

The number of sites revealed to be untreatable is striking. The narrow breadth of the interval of treatable or uncertain soils can be understood by considering the roughly logarithmic distribution of sites by petroleum concentration, shown in Figure 54. The data points fit a trendline of

$$y = 12.31 \ln(x) - 52.84$$

with an R^2 coefficient of 0.9774 (these values calculated by the spreadsheet program used to prepare the figure).

Figure 54: Trendline of Distribution of Sites by Petroleum Concentration



By the slope of this trendline, an interval on the horizontal axis that could be achieved by 50% biodegradability (say, from 2000 to 4000ppm, or any value and its double) corresponds to a vertical interval of about 9%;

$$\begin{aligned}
 \Delta y &= 12.731 \Delta (\ln(x)) \\
 &= 12.731 (\ln (x_2) - \ln(x_1)) \\
 &= 12.731 (\ln (x_2/x_1)) \\
 &= 12.731 \ln (2) \\
 &= 8.82\%
 \end{aligned}$$

An increase of the biodegradability to 75% would increase the proportion of sites by a further 9%, and so on. If the concentration of a flare pit contamination could be reduced by 90%, representing one decade on the horizontal axis, about 30% of the sites would be treatable. A further horizontal decade (99% biodegradability) would allow about 60% of the sites to be treated. Substantial increases in the extent of biodegradability achievable are required to

bring a large amount of sites into the range where bioremediation is applicable.

Considering site contamination as a logarithmic distribution, if the regulatory limit were to be increased from 1000 mg/kg, then the number of sites requiring no treatment would increase, the number of sites not treatable would decrease, but the number of sites for which bioremediation is applicable would remain the same.

5.0 CONCLUSION AND RECOMMENDATIONS

5.1 The Slurry Treatment

While biodegradation in the slurry proceeded quickly, the treatment did not increase the extent of biodegradation achievable. After over 6 weeks of treatment it did not match reductions found in the literature. To understand this effect, a framework for analysis was applied whereby the degradation of petroleum was considered in terms of groups of similar compounds. This was achieved by component-class separation of petroleum into saturates, aromatics, and polar compounds, applied together with gas chromatograph analysis, allowing generalizations to be made on the basis of similarities in chemical structures and molecular weight.

This framework helps explain observed degradation phenomena such as the high initial rate of degradation and the eventual limits of treatment. Rapid reductions were observed for the n-alkane and isoprenoid portion of the saturate fraction, and studies based on the rate of this reduction can be conducted in the span of about a week. It is important to note such tests would not be able to take into account changes in the microbial population over time, such as due to acclimatization. It is suitable as a research tool, but findings should be followed up with long-term testing. The slurry treatment falls short in the degradation of multi-ring cyclic alkanes and aromatics when compared to results demonstrated in the literature. It is hypothesized that microbes specializing in the degradation of these compounds (e.g. soil

fungi) are inhibited by slurry treatment. Another possibility is that these fractions are already extensively biodegraded prior to testing.

5.2 Treatability Estimate

The primary objective of this project was to develop an estimate of the number of Alberta flare pit sites that are feasibly treatable using bioremediation. This objective was approached through a programme of lab testing using bench-scale slurry treatment to control the large number of variables involved. Experimentation showed that composition of the petroleum limited biodegradation regardless of the removal of other limiting factors. This has often been shown to be the case with tests of individual flare pit soils. A general treatability estimate was developed by applying biodegradabilities of different component classes (saturates, aromatics, polar compounds and asphaltenes, as established in the literature) to data on the composition of Alberta crude oils.

By the estimate developed, the prospects for bioremediation are quite slim: the number of sites with oil content too high for treatment to 1000 mg/kg is about half, one third of sites have oil contents low enough that treatment isn't necessary, and roughly an eighth of the sites fall into a region where treatment to 1000 mg/kg is required and believed possible. The calculation was based on the generally optimistic assumption that the composition of flare pit petroleum contamination would resemble Alberta crude oil; weathering, burning, and biodegradation prior to characterization will all result in a contaminant with less biodegradation potential. Furthermore, this estimate represents biodegradation potential free of other limiting factors. It is however based on a conservative consideration of the non-volatile fraction of petroleum alone, and an assumption that all petroleum measurements in the database refer to this fraction.

Concerning the effect of other site contaminants on bioremediation, modified estimates considering salinity and metal contamination differ little from the general estimate, due to the narrow interval of treatable sites that would be affected by these factors.

5.3: Recommendations

5.3.1 Treatability from a Contaminant Mass Reduction Perspective

Given the large number of Alberta flare pit sites limited by petroleum biodegradability, there would be much benefit to developing new methods to degrade the recalcitrant components; polars and asphaltenes, cyclic saturates and aromatics. Analysis of the database shows that there is not much value in considering the effect of other contaminants on biodegradation given the narrow interval of treatable sites. The results in the lab and the results applied in the development of the estimate were based on a fairly narrow range of operating parameters, found in the past to be optimal for the degradation of petroleum as a whole. Experimental exploration of a broad array of operating parameters may reveal that different operating conditions are ideal for the degradation of different components of petroleum, if different microbial populations are responsible. If this is the case, treatment conditions may be found to increase the extent of biodegradation achievable.

Recommendations:

- Experimentation should proceed with a more comprehensive protocol to characterize the microbial population that is causing degradation, and that studies be conducted on solid phase treatments to test the hypothesis that the slurry treatment is limiting the degradation of aromatics.
- Research should be directed at fractions thought to be recalcitrant, to test the possibility that under certain conditions they will degrade.
- If the range of treatable sites can be expanded such that other factors become significant concerns, investigate those factors.

5.3.2 Treatability from an Ecotoxicity Perspective

There is a general trend in current discussion on the flare pit issue towards replacing the

Alberta Tier I 1000 mg/kg MOG contaminant concentration criteria with an endpoint based on ecological toxicity testing. While this is expected to be more closely related to actual hazard than is mass concentration, consideration of ecotoxicity introduces an even broader range of variables. Research in this area is directed at defining suitable criteria, and studying how treatment affects toxicity. The development of protocol to consider individual components of petroleum will be beneficial if ecological endpoint criteria are developed, and identifiable high-risk compounds would be suitable subjects for study. This would require developing means of accounting for transformations between categories.

Recommendations:

- Investigate the possibility of understanding ecotoxicity in terms of fractions and classes of petroleum separately considered, to relate results to mass reduction findings.
- Direct research at degrading components of contamination shown to be the most toxic.
- Reassess the overall applicability of bioremediation, given ecological endpoint criteria as those criteria develop.

5.3.3 General Site Management

The focus of this research project was on the non-volatile fraction of petroleum in terms of the existing cleanup criteria, and only a few possibilities exist to expand the prospects for biological treatment beyond a fraction of the sites. The development of ecotoxological criteria may change that, but assessment may reveal that mixing an otherwise immobile contaminant in the same media as receptor organisms increases the hazard it presents.

A simple containment method such as backfilling should present little threat to groundwater or organisms at the surface, and may be a more suitable alternative than bioremediation for non-volatile petroleum. For such a site treatment the issue of more mobile contaminants such as metals, salt, and the volatile fraction of petroleum must be addressed, and research

may indicate that this remediation alternative is also applicable only to a handful of sites. The database makes it clear that there is no one dominant category of contamination. The database and categorization should be further developed as a site management tool to assess the applicability of other treatments and combinations of treatments, and coordinate several remediation possibilities.

Recommendations:

- Assess the applicability of other remediation methods, based on the characterization data available.
- Assess the applicability of different combinations of treatments.

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