BIOREMEDIATION OF PETROLEUM HYDROCARBONS IN SOIL

Activated Sludge Treatability Study

By

J. E. LA RUE-VAN ES

A Thesis
Submitted to the Faculty of Graduate Studies
in Partial Fulfilment of the Requirements
for the Degree of

MASTER OF SCIENCE

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Winnipeg, Manitoba

ISBN 0-315-85954-7

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ABSTRACT

Batch activated sludge treatability studies utilizing petroleum hydrocarbon contaminated soils (diesel oil and leaded gasoline) were conducted to determine: (1) initial indigenous biological activity in hydrocarbon-contaminated soils; (2) limiting factors of microbiological growth by investigating nutrient addition, chemical emulsifiers, and co-substrate; (3) acclimation of an indigenous population of microorganisms to utilize hydrocarbons as sole carbon source; and (4) temperature effects.

Soil samples were taken from three different contaminated sites. Four sequencing batch reactors were run from site one (southern Manitoba), three from site two (northern Manitoba), and two from site three (northern Manitoba). Substrate (diesel fuel) and nutrient were added as determined by laboratory analysis of orthophosphate, ammonia nitrogen, chemical oxygen demand (COD), and total organic carbon (TOC). Substrate was made available to the bacterial mass by experimenting with the use of four different chemical emulsifiers. All reactors were also monitored with respect to other chemical, physical, and biological parameters. Laboratory analysis followed Standard Methods.

Indigenous microorganisms capable of biotransforming hydrocarbons seem to be present in all the contaminated soil samples received from all sites. Microscopic analysis of reactors revealed no visible activity at the beginning if the study and presence of flagellated protozoa, paramecium, rotifers, and nematodes at the end of a year. Nutrient requirements (nitrogen, phosphorous) and the limiting factors in microorganisms growth have been determined for each particular site. A co-substrate was used initially to enhance bacterial mass growth. Use of an emulsifier was deemed necessary initially to make the hydrocarbons available to the microbial population. Temperature effects study (site one, temperature decreased gradually from 22 oC to 12 oC) showed a decrease in removal (TOC) and an emerging presence of filamentous bacteria. A second temperature study (site two, temperature to decrease gradually from 22 oC to 4 oC) also showed a decrease in removal.

Removal efficiencies, in terms of chemical oxygen demand, range from 50% to 90% in reactors from site one (16 months ongoing at room temperature, no waste sludge). Acclimation of indigenous microorganisms to hydrocarbons is possible and could reduce remediation time of contaminated soils.

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ABBREVIATIONS

LUST	-	Leaking Underground Storage Tank
COD	-	Chemical Oxygen Demand
TOC	-	Total Organic Carbon
MLSS	-	Mixed Liquor Suspended Solids
MLVSS	-	Mixed Liquor Volatile Suspended Solids
TKN	-	Total Kjeldahl Nitrogem
DO	-	Dissolved Oxygen
COD_i	-	Chemical Oxygen Demand, influent
COD_e	-	Chemical Oxygen Demand, effluent
L	-	litre
NH_3-N	-	ammonia nitrogen (mg/l)
Ortho P	-	orthophosphorous
Total P	-	total phosphate
ThOD	-	Theoretical Oxygen Demand
C:N:P	-	carbon: nitrogen: phosphorous
°C	-	degrees Celcius
BTEX	-	Benzene, Toluene, Ethylbenzene, Xylene
TPH	-	Total Petroleum Hydrocarbon
PAH	-	Polycyclic Aromatic Hydrocarbon
VOC	-	Volatile Organic Carbon
ppb	-	parts per billion

ACKNOWLEDGEMENTS

This thesis is dedicated to my children and their future.

Thanks Nicole, Peter, and Joelle.

I wish to thank W. Kwan of Geokwan Environmental Ltd., Manitoba Hydro, especially P. Janzen and J. Mackay, as well as T. Wingrove of UMA Engineering Ltd. for their research support.

This thesis could never have been attempted without the expert and patient technical laboratory support from J. Tingley and most especially W. Trebacz.

I have been most fortunate that Prof. A. Sparling agreed to advise me, with help from Prof. D. Shields. Thank-you Prof. J. Graham for pointing me their way and to Prof. B. Lence for encouraging me to become a grad student.

To all the graduate students in 406-C for your company.

The encouragement and interest given to me by my parents throughout my life made this thesis possible.

David, what can I say. **We made it!**

1. INTRODUCTION

An environmental problem of recent significance is the contamination of soil and groundwater by hydrocarbon spills. Petroleum contaminated soils and groundwater can result from: leaking underground storage tanks (LUST), petroleum pipeline breaks, spills of petroleum products, leaking above ground tanks, tanker spills, leaks from petroleum refineries and bulk storage facilities (Newton 1990), as well as refinery residues, coal tar sites, chemical processing sites, and wood treating sites. (Sherman and Stroo 1989) Risks of spills are also created during the production, transportation and refining of crude oil, as well as the distribution and marketing of refined products.

Bioremediation is a developing soil treatment technology that biodegrades petroleum hydrocarbons aerobically and completely to nontoxic end-products of carbon dioxide and water. It is interesting to note that in 1986, Mackay and Hoag mused that perhaps soil treatment would be the next growth industry.

1.1 Scope of the Problem

It has been estimated that 20 - 25% of all storage tanks at petroleum retail outlets in Canada are leaking or suspected to be leaking. (Environ. Sci. & Eng. 1991) In many instances, LUSTs pose a major threat to drinking water supplies as only

1 gallon of gasoline can render 1 million gallons of water unsuitable for consumption. Redevelopment of urban areas that were formerly used for industry is also an issue of great concern because much of the soil on these lands has been contaminated with petroleum hydrocarbons. (Environ. Sci. & Techn. 1991) As well as contamination of ground water, LUST can create explosion hazards from the accumulation of hydrocarbon vapours under buildings and can degrade utility lines that may come into contact with the leaking petroleum hydrocarbons. (Lingineni 1992)

The scope of the problem is large. Toronto's industrial port contains an estimated 2 million tonnes of contaminated soil which is expected to cost $160/tonne and take approximately 10 years to clean up. (Piper 1991) In Canada at present, there are approximately 200,000 UST. Of these, 70,000 are located at retail gasoline stations. The remaining 130,000 UST are used in manufacturing, transportation, commercial and agricultural industries. "If 20% of the 200,000 USTs in Canada are leaking, the total remediation cost could be many tens of billions of dollars - the same order of magnitude as the annual Canadian Federal Government Deficit." (Environ. Sci. & Eng. 1991) Bioremediation usually has lower costs associated with it than other remediation technologies due to lower mechanical and energy requirements.

1.2 Petroleum Hydrocarbons

The most common petroleum hydrocarbons contaminating soil and groundwater include: gasoline, diesel, and fuel oils. Each is a complex mixture composed of many organic chemical compounds. "Crude oil is a complex mixture made up of approximately 11 to 13% hydrogen and 84 to 87% carbon by weight. Of the 18 series of hydrocarbons identified in crude oils, paraffins, olefins, polymethylenes, acetylenes, turpenes, and benzenes are those found most often. Crude oil contains, on the average, approximately 1 % polynuclear aromatic hydrocarbons." (Custance 1992) Crude oil then is very rich in hydrocarbons which results in a very high C:N (carbon:nitrogen) ratio.

Figure 1 shows a gas chromatograph of crude oil, identifying the constituents which range from light hydrocarbons to heavy hydrocarbons. Some chemical and physical properties of diesel fuel are shown in **Table 1.**

TABLE 1: CHEMICAL/PHYSICAL PROPERTIES OF DIESEL FUEL Adapted from Custance 1992

Parameter	Value
Density (g/cm^3)	0.84 g/cm^3
Aqueous Solubility (mg/l)	0.2 g/m^3 (0.2 mg/l)
Vapour Pressure (mmHg)	0.03
Diffusion Coefficient in Air (cm^2/s)	4.63×10^{-2}
Henry's Law Constant ($atm\text{-}m^3/mol$)	4.2×10^{-2}
Log organic carbon: water partition coefficient	3.04
Biodegradation ($year^{-1}$)	1 year

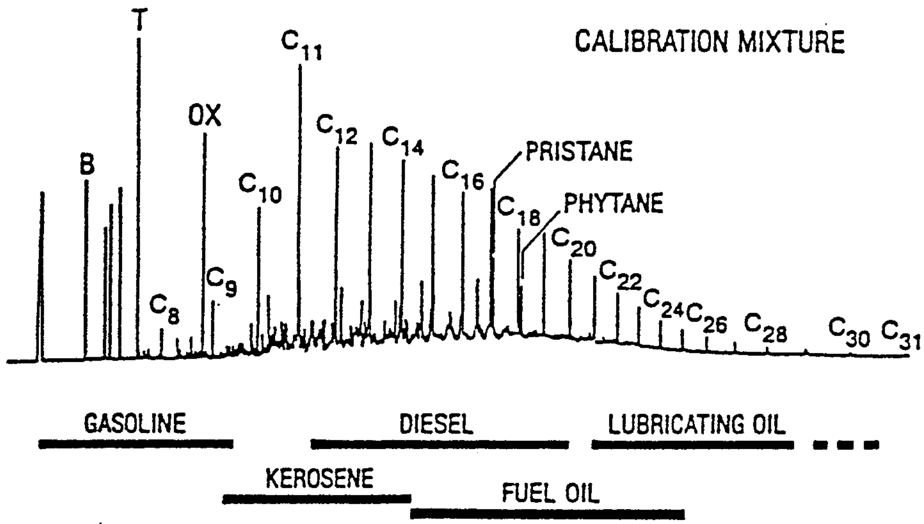


FIGURE 1: PETROLEUM HYDROCARBONS (Galaska 1990) Adapted from Senn and Johnson, 1985

In gasoline, the organic compounds generally have low solubility, low volatility, and strong adsorption characteristics. The primary gasoline constituents have monocyclic aromatic hydrocarbons which include benzene, toluene, ethylbenzene and xylene (BTEX). They are of the greatest concern because of their toxicity and mobility. "All oils and oil products differ in toxicity. In general, the lighter oils such as diesel fuel and gasoline cause the greatest short term damage, whereas heavy oils such as crude may cause acute toxic damage." (Nichols 1989)

Benzene is also a known carcinogen. Benzene has a much greater solubility in water than xylene, and can be stripped out as it is flushed with water. Xylene has the lowest solubility in water and adsorbs to clays in soil. Some of these compounds are usually contained in the vadose zone (soil area above the water table) because they are readily adsorbed to clay and the organic fractions of the soil. This makes them less mobile and thus they are not likely to make their way downward to the water table. (Newton 1990)

"The physical, chemical, and biological properties of these chemicals in a complex petroleum product has (sic) a major effect on the distribution of the compound in a soil/gas/liquid matrix." (Galaska 1990) High solubility compounds are most likely to be present in the aquifer itself. High volatility compounds are most likely present in the soil gases and the atmosphere. Therefore, on-site or in-situ biological remediation of petroleum hydrocarbon contaminated soils and groundwater must address the particular organic compound which is present.

1.3 "Typical" Spill Mechanisms

Soil consists of four phases: mineral matter (sand, clays, etc), organic matter, water and air. When petroleum hydrocarbons leak into the subsurface environment, they percolate downwards and spread laterally. A small spill may not reach the groundwater. The petroleum hydrocarbons may be held in the pores of the soil particles. A large spill may reach the ground water table and form a saturated zone above the water table.

Petroleum hydrocarbon contamination can be present in many phases including dissolved, floating and suspended. Because diesel oil and gasoline are complex mixtures, their behaviour is much more complex. Downey and Elliott (1990) found that "fuels trapped within the micropores of the soil were largely inaccessible to the nutrients and oxygen that were being provided".

Figures 2 and **3** show views of a "typical spill". The spread and adsorption rate of a fuel oil spill will depend mostly on the permeability of the soil and its water content. At the same time that the spill is spreading over the soil and absorbing into the soil, mass transfer to the air is taking place. Because of all these mechanisms, the properties of the fuel oil will change.

Figure 4 is a conceptual model showing how petroleum hydrocarbons are distributed among the soil organic matter phase, water phase, and air phase in soil. (Qiu and McFarland 1991)

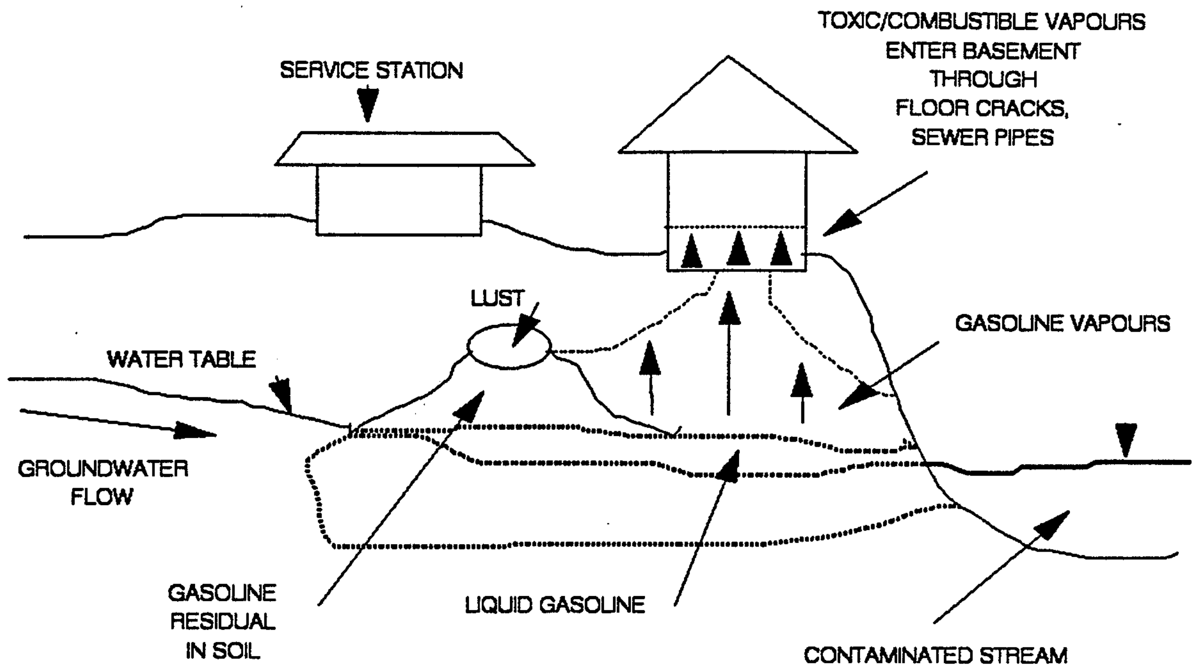


FIGURE 2: GASOLINE CONTAMINATION OF SOILS AND GROUNDWATER

(Wolowich 1991)

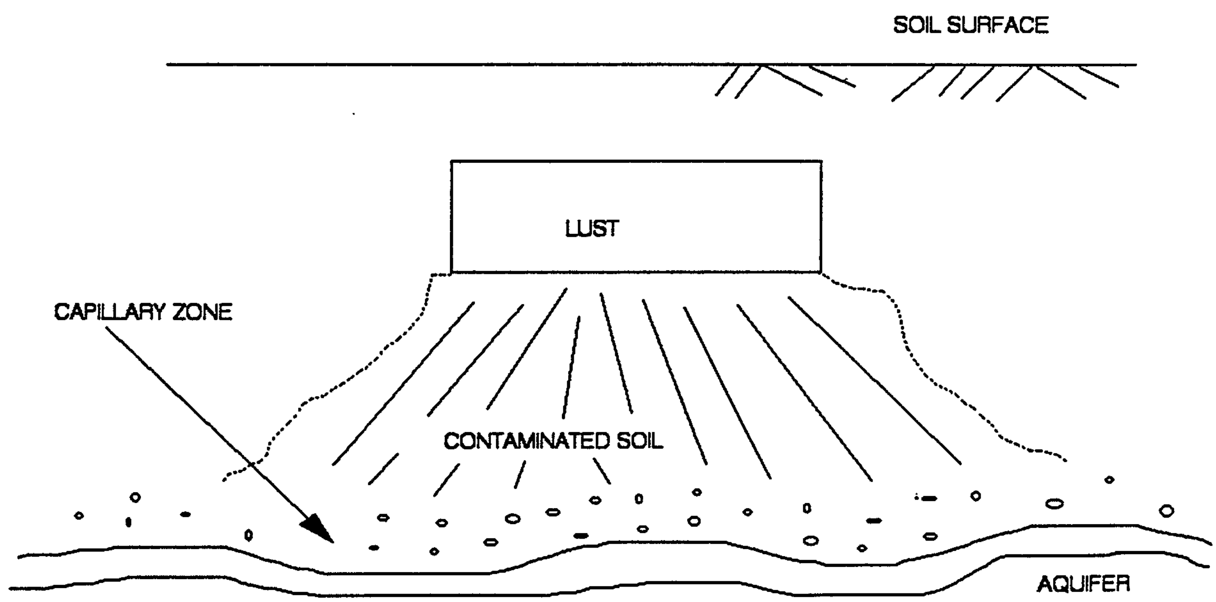
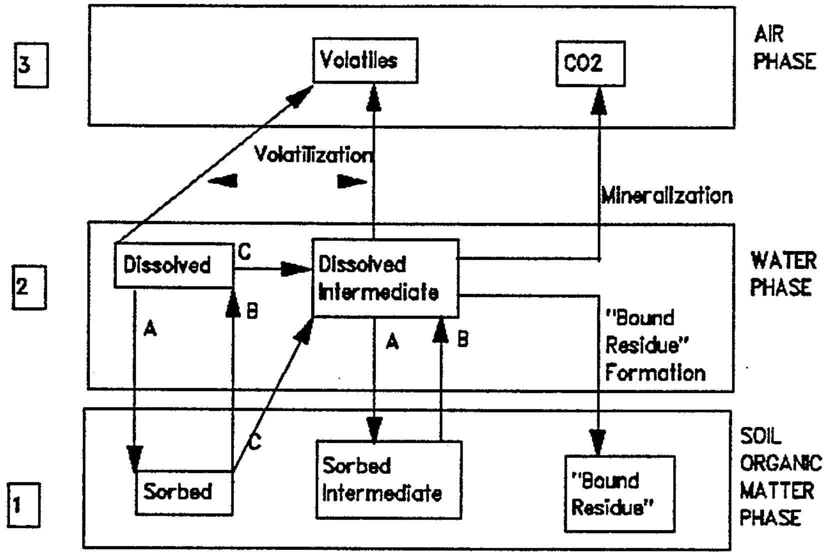


FIGURE 3: "TYPICAL" SPILL SITE



A = Adsorption
B = Desorption
C = Degradation

FIGURE 4: PETROLEUM HYDROCARBON DISTRIBUTION AMONG 3 COMPARTMENTS (Qiu & McFarland 1991)

1.4 Remediation Strategies

Remediation strategies have included: chemical fixation; soil washing; enhanced biodegradation; soil venting (soil vapour extraction); thermal treatment (low and high temperature); surface bioremediation (land farming); solidification/stabilization; asphalt incorporation; soil leaching; solvent extraction; slurry phase bioremediation; and landfill disposal. (Srivastava 1990) Soil flushing (washing) and soil excavation have been found to be very expensive and inefficient in residual contaminant removal. (Lingineni 1992)

Soil venting becomes expensive if air emissions are to be remediated as well. Thermal treatment has demonstrated excellent removal efficiencies of organics but is extremely expensive. If off-gas treatment is not provided, contaminants may only have been transferred to another phase - the atmosphere. In-situ bioremediation is a developing technology that does not transfer contaminants but renders them harmless.

1.5 Bioremediation

Biotechnology is a very old science. For millions of years organic materials have been naturally decomposing. Without this process, the earth would be buried under a blanket of leaves. For thousands of years waste has been biologically managed and the majority of the microorganisms used in this biological waste management have been extracted from soil and water.

A technology that is a viable treatment alternative and which can safely, effectively and permanently remediate petroleum contaminated soils is bioremediation. Bioremediation is a more or less viable remediation technology for contaminated soil and groundwater depending on the site characteristics which could include the presence of indigenous microorganisms and suitable site geology. Soil, contaminated with petroleum hydrocarbons, may have the capacity to detoxify, degrade, and inactivate the toxic organic chemicals.

Because of the increasing costs of current technologies, the enthusiasm of regulatory agencies to innovative technology, and the more stringent regulatory requirements, bioremediation is beginning to play an important role in soil and groundwater contamination clean-up.

1.5.1 Definition of Bioremediation

"Bioremediation is a process that uses the soil's naturally occurring microorgansms to decompose toxic or hazardous substances." (Hopper 1989) Bioremediation follows the thesis that biodegradation is a naturally occurring process in all soils. "The underlying premise of in situ bioremediation is the Ubiquity Principal which states that all types of bacteria are available at all times everywhere." (Major 1991)

"All living systems require sources of energy to develop and sustain their populations." (Torpy 1989) Microorganisms in the soil use organic compounds that contain carbon. Bioremediation works because the organic compounds in the hazardous substances can be utilized as food and energy for the microorganisms. These microorganisms, which use enzymes in the process of organic decomposition, feed on the organics (carbon) found in the soil and require oxygen and water to survive. By metabolizing the organic compounds in the soil, microorganisms derive energy and carbon which will be incorporated into new cell mass. (Mahaffey 1991, Torpy 1989) "Biotransformation refers to the conversion of a compound or its intermediates to the next product in the biochemical pathway." (McFarland et al 1991) Incorporation of certain amounts of nutrients such as nitrogen and phosphorous is necessary for microbial growth.

Microorganisms used in the degradation process may be indigenous to the site (biostimulation) or they may be especially selected (bioaugmentation). In biostimulation, the enrichment conditions are identified, quantified and then applied. In bioaugmentation, the organisms are supplied as a mass inoculum from a proprietary inoculum or by way of enrichment for the active microbes indigenous in the waste. Thus bioremediation is a microbiological process that depends on the growth and activities of a population of bacteria and other microorganisms. With the right selection of enrichment of a microbial population, it may be possible to stimulate and increase biological activity thereby degrading a contaminant.

Bioremediation then, is a biological method that can use indigenous microorganisms environmentally enriched to aerobically metabolize contaminants. The end products of this mineralization include carbon dioxide and water (which in themselves do not pose any environmental concerns) and biomass (Bouwer 1989), which provides for a "final, ecologically sound solution to toxic waste problems". (Zitrides 1990) Thus, bioremediation is a remediation technology that is considered to be a true destruction process. In this process, contaminants are permanently remediated and then require no further treatment.

In-situ bioremediation, using indigenous species, stimulates the activity of the microorganisms by the addition of nutrients and oxygen. The growth of the microorganisms will depend in part on the temperature, pH, moisture, oxygen, and nutrient levels in the soil. The optimal environmental conditions for aerobic metabolism are:

1. temperature $15^{o}C$ to $45^{o}C$, mesophilic;
2. pH 5.5 to 8.5, near neutral;
3. optimum nutrient ratio;
4. DO greater than 0.2 mg/l in soil pores; and
5. redox potential greater than 50 mV. (Andreottola 1991)

Bioremediation is limited only by the understanding of the microbial ecology and physiology of polluted sites and involves "a strong interaction between the microbial community and the physical and geochemical environment, creating a dynamic environment in which contaminants are degraded". (Major 1991)

1.5.2 In-situ, Ex-situ Bioremediation

Bioremediation as a remediation strategy, can be used in-situ or ex-situ. In-situ implies that the contaminated soil and groundwater are disturbed the least amount possible. Contamination in the soil is treated without removal from the area which had been contaminated. An advantage of in-situ biological treatment is the production of a biologically active soil. Ex-situ technologies are those where the contaminated soil is excavated. When excavated soil is removed from the site for treatment, such as in land farming, it is called off-site treatment. The "clean" soil may or may not be returned after treatment. On-site treatment can be in-situ but most commonly implies that the contaminated soil is extracted, treated at the site, and then put back. In-situ bioremediation is an appropriate method when it is impossible or too expensive to excavate contaminated soil. (Andreottola and Acaia 1991)

The in-situ bioremediation process requires a subsurface matrix that will be permeable enough to allow oxygen, nutrients and contaminant-degrading microbes to enter and travel. However, most contaminated sites have irregular geology, have been previously disturbed by construction and/or have multiple or unknown sources of contamination. (Torpy 1989)

In-situ technologies can have many advantages, such as:

- minimum intrusion to site (therefore less disruptive),
- more cost-effective (excavation and hauling are expensive),
- work well in high permeability soils (sand and gravel),
- require small above-ground surface area, and
- contaminant particulates and vapours are minimized.

In-situ technologies also have some disadvantages which may include: longer treatment times may be required; removal efficiencies and monitoring of remediation effectiveness may be difficult to obtain; and they often do not work well in low permeability material such as clay.

1.5.3 Enrichment Conditions

Since in-situ bioremediation stimulates microbiological activity in the soil which in turn causes degradation of the contaminant, it follows that microbiological activity can be stimulated by modifying any one or combination of geochemical conditions, physical conditions, nutrients, and microorganisms. Thus, environmental conditions can be optimized by supplying oxygen, nutrients, circulating water and/or increasing temperature.

One of the most important aspects in bioremediation is the carbon:nitrogen:phosphorous (C:N:P) ratio. The importance of mineral nutrient addition (N,P) for decomposition of oil has been widely recognized. (Cook and Westlake 1974) Microorganisms require carbon for growth and energy, and nitrogen for protein synthesis. In order to prosper, "bacteria require about 10 parts carbon to 1 part nitrogen". (Westlake and Cook 1973) A range of C:N:P of 100:10:1 to 100:10:5 was recomended by Torpy (1989). Thus when oil spills on soil, the carbon to nitrogen ratio becomes unbalanced and there is a nitrogen deficiency. A deficiency of phosphorous may also be aggravated by an oil spill. The rate of decomposition will be dependent on this ratio of C:N:P. What is ultimately of the most importance to biodegradation is the availability of nutrient rather than the ratio.

The limiting nutrient in bioremediation is most likely nitrogen. Rasiah (1991) reported that nitrogen amendment enhanced the carbon mineralization of an oily waste significantly and that the greatest enhancement in waste carbon mineralization occurred when the waste-C:fertilizer-N (WC:FN) ratio was in the range of 18 to 22:1. Carbon need not be supplied as it is one of the key elements of the petroleum hydrocarbon molecule. However, this carbon may not always be easily available to the active microorganisms and in this circumstance "easy" carbon is added only to the extent that the active population remains large. Typically, nutrients added to soil for enrichment include inorganic salts such as ammonium chloride, ammonium nitrate, sodium nitrate, sodium phosphate, and potassium phosphate. Trace nutrients are rarely required because they are rarely limiting in the open environment.

The rate and extent of biological degradation will therefore be a function of: (1) limitations of mass transfer; (2) lack of capable microflora; (3) complexity and variability of waste, including the nature and concentration of the waste as well as the presence of other organic substrates; (4) soil and site characteristics (including soil pH, salinity, dissolved oxygen levels, soil moisture content, soil permeability, oxidation-reduction potential, temperature); (5) nutrient availability; and (6) toxic or inhibitory compounds. (Hickman 1989, Mahaffey 1991)

The major rate-limiting steps in biodegradation have been discussed by Srivastava (et al 1990). In order to accelerate bioremediation, several strategies were explored: (1) mass transfer of waste material to microorganisms could be increased; (2) the contaminant could be made more soluble (although low solubility limits migration to groundwater, it also limits microbial degradation); (3) oxidation of PAH could occur, making them biologically more available; and (4) enrichment cultures of pollutant degrading microorganisms for aerobic environments could be developed.

In order for bioremediation to be successful, oxygen must be supplied to the microorganisms. There are many alternatives available for the supply of oxygen:

(1) air sparging (porous stone at bottom of well);

(2) injection of aerated/oxygenated water;

(3) venting (vacuum withdrawal or injection);

(4) hydrogen peroxide addition; and

(5) encapsulation of air in surfactant bubbles. (Major 1991)

Aerobic conditions could also be maintained in contaminated soil through the use of extraction and injection wells (flushing) containing oxygen saturated water or by the use of irrigation pipes. The wells could also be the vehicle for enrichment techniques such as nutrient addition.

Successful bioremediation then, will be the combined action of basic microbiological processes and sound bioprocess engineering and will require:

1. favourable environmental conditions including pH, oxygen concentration, influent organics concentration, concentration of inorganic nutrients (N in the form of ammonia and P in the form of orthophosphate) and temperature;
2. suitable microbial populations; and
3. absence of high concentrations of toxic/inhibitory chemicals.

1.5.4 Laboratory Treatability Studies

Skladany (1988) pointed out that laboratory evaluations are required for assessing the biodegradation potential of a site and could be done by placing soil in a reactor, modifying conditions and monitoring. Soil/water slurry experiments with petroleum hydrocarbon contaminated soil have been conducted by Srivastava (1990).

McFarland (et al 1991) reported the necessity of laboratory treatability studies to develop remediation strategies for contaminated soils. To evaluate soil bioremediation requires: (1) laboratory screening; (2) bench-scale testing; (3) pilot-scale testing; and (4) addressing unique design concerns related to mass transfer and the partitioning of nutrients and/or contaminants in the soil matrix.

The necessity of nutrient addition should be determined using laboratory bench-scale treatability studies. Enrichment conditions for enhancing bioremediation were discussed by Golueke and Diaz (1990) and included nutritional aspects as well as environmental factors. Nutritional aspects included: (1) setting up an enrichment culture; (2) ensuring that the concentration of nontoxic substances with easy carbon be at a minimum so that organisms capable of using the carbon in the toxic contaminant would survive and thrive; (3) identifying growth factors; and (4) nutrient availability (such as