THE EFFECTS OF FREEZING AND THAWING ON THE BIOREMEDIATION OF A DIESEL FUEL CONTAMINATED SOIL

BY

TOM SARAUSKAS

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

MASTER OF SCIENCE

Department of Civil Engineering
University of Manitoba
Winnipeg, Manitoba

THE UNIVERSITY OF MANITOBA

FACULTY OF GRADUATE STUDIES

*****

COPYRIGHT PERMISSION PAGE

THE EFFECTS OF FREEZING AND THAWING ON THE BIOREMEDIATION OF A DIESEL FUEL CONTAMINATED SOIL

BY

TOM SARAUSKAS

A Thesis/Practicum submitted to the Faculty of Graduate Studies of The University of Manitoba in partial fulfillment of the requirements of the degree of MASTER OF SCIENCE

Abstract

Studies have found that under constant environmental conditions, the rate of biodegradation of petroleum hydrocarbons decreases with time and may become negligible after a period. This decrease in the availability of hydrocarbons for biodegradation can be attributed to the diffusion of the hydrocarbons into soil micropores, the partitioning of the hydrocarbons into soil organic matter, strong surface adsorption or a combination of these processes. Studies have also shown that naturally occurring freeze-thaw cycles act to disrupt soil aggregates to physically change the soil's structure. This study investigated the effects of freeze-thaw cycles on the biodegradation rates of hydrocarbon contaminated soils. A diesel fuel contaminated soil was bioremediated in bench-scale reactors until respiration monitoring indicated a decrease in microbial activity. Designated reactors were then subject to 1, 3, 6 and 9 freeze-thaw cycles. The results indicated an increase in the microbial activity in the freeze-thaw treated reactors, while the microbial activity in the control reactors decreased over the same period of time. The results also indicated that microbial activity increased with increasing numbers of freeze-thaw cycles.

Acknowledgments

I would like to acknowledge Mr. Errol McComb and Mr. Paul Janzen, of Manitoba Hydro, without who's help, this study would never have been possible. I would also like to acknowledge the countless hours of assistance provided by Judy Tingley of the Environmental Engineering Lab.

I will take this opportunity to thank my most bestest of friends, Christine Turenne, who's constant encouragement and support kept me in the highest of spirits during the darkest periods of this Masters Thesis. Thank-you Tanya Rideout for all those late-night typing sessions; without your help, I would never have gotten through the last stretch.

My sincerest thanks goes out to Ms. Norma Lindsay of the Department of Civil Engineering. Her infinite wisdom on life and love helped to steer me down the right path, time and time again. And she even found time to occasionally save me from my self-inflicted administrative woes.

And lastly, I would like to acknowledge and thank my advisor, Dr. Jan Oleszkiewicz, for supporting me and my ideas in spite of my unconventional approach to everything. Thank you for giving me enough rope, even after I began to tie a noose with it.

Table of Contents

List of Tables

List of Figures

1.0 Introduction

The purpose of this study is to investigate the effects of freezing and thawing on the bioremediation of a diesel fuel contaminated soil. It was hypothesized that successive freeze-thaw cycles would act to increase the bioavailability of sequestered contaminant concentrations to soil microorganisms, and thereby increase the rate of biodegradation. The study was conducted on a contaminated soil sample obtained from a diesel fuel impacted site. The contaminated soil was bioremediated in bench-scale bioreactors, and was subjected to various freeze-thaw cycles. The final contaminant concentrations of the reactors subjected to different freeze-thaw treatments were compared.

1.1 Objectives

This purpose of this study was to determine the following:

1. Would the soil sample's bacteria biodegrade the contaminant significantly to remediate the soil ?

2. Would freeze-thaw treatments affect the degradation rate of the contaminated soil samples ?

3. Would multiple freeze-thaw cycles effect the degradation rates significantly over a single freeze-thaw cycle ?

2.0 Literature Review

2.1 Bioremediation

Bioremediation has been defined as the use of biological agents to degrade or render various types of hazardous waste to a non-hazardous or less hazardous state [1]. Bioremediation involves the use of microorganisms and their biodegradative capacity to remove pollutants. The byproducts of effective bioremediation, such as water and carbon dioxide, are nontoxic and can be accommodated without harm to the environment and living organisms [2].

Bioremediation has grown from an unknown technology to one of the major treatment technologies considered for source control at Superfund sites [3]. The basis for this growth is bioremediation's low cost as compared to other technologies such as incineration and containment. Bioremediation also attracts interest because it destroys most organic wastes, thereby eliminating the aforementioned health and ecological effects as well as future environmental liabilities.

Almost all organic compounds are biodegraded given the proper circumstances and time. However, a range of physical, chemical and biochemical conditions can interfere with bioremediation [4]. Talley and Sleeper [5] define three scales involved in bioremediation, with each level possessing a limiting process:

Microscale (10^{-6} to 10^{-5} m) = Can the bacteria eat the contaminant ?

Mesoscale (10^{-5} to 10^{-2} m) = Can the bacteria get to the contamination ?

Macroscale (10^{-2} to 10^{2} m) = Are the conditions optimal for the bacteria to work ?

The microscale represents the level at which chemical/microbial species and reactions can be characterized independently of any transport phenomena, and occurs at microbial cell level. The mesoscale is the level at which transport phenomena and system geometry become apparent, and occur at the pore channel, soil particle or microbial aggregate level. The macroscale is the scale at which advective or mixing phenomena occur, and can be considered the remediation area. Factors involved at the macroscale level include, although are not limited to, nutrient concentrations, moisture content and temperature.

Successful biodegradation can only occur if the conditions are met at all three bioremediation scales. At the microscale level, a proper mix of bacterial species must exist that can degrade the contaminant present. At the mesoscale level, the contaminant must be available to these bacteria for degradation. At the macroscale level, proper environmental conditions, including moisture, nutrient concentrations and temperature, must exist.

The limiting processes involved in bioremediation at the macroscale can be controlled. The moisture content of soils can be increased or decreased. Nutrient concentrations can be adjusted by the application of chemical fertilizers. Temperature, particularly in ex-situ

remediation, can be regulated by the implementation of heat exchangers. The presence of bacterial species that can degrade a contaminant or mixture of contaminants is the limiting process at the micoscale level. Blackburn and Halker [6] reviewed research spanning many years that supported the view that petroleum hydrocarbons are amenable to microbial degradation. Furthermore, they found that these organisms possessing this potential are found at least in small numbers in many environments. The ability of organisms to grow on petroleum hydrocarbons is due these compounds' similarities in chemical bonds found in natural microbial substrates such as lignin, tannins, waxes, and resins [7]. Studies reviewed [8-11] indicated that for a diesel contaminant, indigenous bacterial species possess the ability to successfully degrade diesel fuel compounds.

Given that bacterial species which can degrade a contaminant exist at the microscale level, and that the environmental conditions conducive to biodegradation can be controlled at the macroscale level, many compounds that would normally be quickly destroyed by microorganisms apparently are not easily degraded, and persist in polluted soils and subsoils. These persistent compounds may not be degraded because they are not readily available to microorganisms at the mesoscale level. These compounds may become sorbed or bound in some way to the soil particles, or be present in a physically inaccessible state that prevents microorganisms with biodegradative enzymes from carrying out a rapid transformation of the contaminant [12].

Even in situations where a hydrocarbon contaminant is initially readily and easily biodegraded, Blackburn and Halker [13] found that under constant environmental conditions, the rate of biodegradation of petroleum hydrocarbons decreases with time and may become negligible after a period. Tabak and Govind [14] conducted an extensive literature review of research studies conducted on bioremediation of chemicals in soil. Their review indicated that:

> 1) chemicals are biodegraded by indigenous soil microbiota to a "plateau" concentration, i.e. a concentration which no longer decreases, or decreases very slowly with continued treatment
>
> 2) reduction below the plateau concentration is limited by the "availability" of hydrocarbons to the microorganisms

In general, the bioavailability of the target compounds became the limiting factor to biodegradation in the above cases.

2.2 Bioavailability

Bioavailability has been generally defined as the availability of a chemical to biological transformation, and is determined by the extent to which a chemical is exposed to an organism [15]. The bioavailability of compounds can be affected by chemical aging [16]. Chemical aging involves the diffusion of the contaminant into soil micropores, the partitioning into soil organic matter, strong surface adsorption or a combination of these processes. When any of these chemical aging processes occur, the contaminant compounds become unavailable to bacteria to degrade.

Zhang *et al.* [17] found, after extensively reviewing experimental evidence, that microorganisms are most effective in utilizing "freely" dissolved organic chemicals. The free bulk water concentration of organic substrate determines its rate of uptake and consequently its bioavailability. They concluded that transfer of the contaminant to the aqueous phase is first required for biodegradation to occur. However, sorption of organic contaminants tends to prevent the direct contact between microorganisms and contaminants, which is necessary for biodegradation to occur. Sorption reduces the aqueous concentration of organic contaminants and therefore lowers their rate of transformation. As well, slow desorption rates may reduce the effectiveness of biodegradation by limiting the flux of contaminants to the aqueous phase. Slow desorption can completely control the apparent rate of biodegradation, leading to a situation under which essentially all of the remaining organic compound reside in biologically inaccessible areas [18].

The vast majority of bacterial population exists on the external surfaces of solid grains and aggregate particles. Jones *et al.* [19] conducted a study on diffusion and reactions within porous packing material and found that for a highly porous diatomaceous earth pellets (30% intraparticle porosity), that 90% of the bacteria were observed in the outer 5% volume of the pellets. They further observed that the internal porosity of sediments and aquifer materials is usually much lower, often around 1% to 5%, and that the physical exclusion of bacteria will be greater for such low-porosity natural materials. This was further confirmed by Zhang *et al.* [20] after analyzing aquifer material from the Borden

Aquifer, Ontario, Canada. Analysis of one of the coarser sand sizes indicated that that roughly 50% of the intraparticle pore volume resides in pores that are less than 0.1 μm in diameter. Pores with diameters larger than 1 μm comprised about 12% of the total pore space, and only about 5% of the pore volume was attributed to pores larger than 2 μm. They went on to state that considering most indigenous bacteria range in size from 0.5 to 1.0 μm, that these bacteria will be physically excluded from most of the interparticle pores of these grains. Zhang *et al.* also stated that the mean diameter of intraparticle pores occupied by bacteria has been estimated to be typically larger than 2 μm, and this is likely to be larger than the intraparticle pore space of many natural sorbent solids. As a result, they concluded, that organic chemicals sorbed into natural minerals may commonly be unavailable for direct microbial degradation.

2.2.1 Bioavailability Studies

If a contaminant becomes sorbed into these microscopic particulate pores, the contaminant compounds will become unavailable to bacteria for degradation. The following examples cited from literature present examples of the above state scenario.

Steinberg *et al.* [21] studied the persistence of 1,2 dibromoethane (EDB) in agricultural topsoils. They found that EDB could be found in these soils up to 19 years after its last known application. The residual EDB was found to be highly resistant to both mobilization and microbial degradation in contrast with freshly applied EDB and they

concluded that the compound was present in soil micropore sites that were sterically inaccessible to bacteria.

In a similar study, Hatzinger and Alexander [22] discovered that the extent of degradation and mineralization of phenanthrene and 4-nitrophenol in soils decreased significantly with aging time. They concluded that these reductions in mineralization suggest that the concentration of the contaminant available to the bacterium that degrades it was declining due to entrapment in the soil structure.

Kelsey *et al.* [23] conducted a study to determine the feasibility of devising a chemical assay to predict the bioavailability of organic compounds that become sequestered in soil. Mild extractants were used to predict the bioavailability of select compounds (phenanthrene and atrazine) in soil over time. These results were compared to actual degradation by earthworms and bacteria. A vigorous extraction of phenanthrene revealed no disappearance even as the compound became less available to the test organisms.

In a similar study, Kelsey and Alexander [24] compared the amount of a contaminant (atrazine, phenanthrene and naphthalene) removed from freshly inoculated and aged soils by earthworms and bacteria, and compared them to the amount that could be recovered through a vigorous extraction method. Results indicated that persistent (contaminant) compounds undergo some type of slow sequestration in soil, a sequestration that resulted

in a diminution in the quality of some organic chemicals that are available to earthworms and bacteria.

Fu *et al.* [25] conducted a study on the desorption and biodegradation of sorbed styrene in soil. They found that styrene freshly added to a soil was extensively mineralized by microbial degradation. However, if the styrene was present in the soil for increasingly long periods in the absence of microbial activity, the extent of biodegradation by subsequently added microorganisms became progressively lower until <3% was mineralized in soils where the chemical was present for four months. They concluded that a sorbed molecule is less readily metabolized by microorganisms than the same molecule present in the aqueous phase. They also hypothesized that this sorption may result in the persistence in nature of an organic molecule that is readily metabolized if present in a nonsorbed form. During this period of persistence, abiotic changes may occur that make the chemical increasingly less available for microbial use.

Bosma *et al.* [26] presented a generic mathematical concept for the bioavailability of a contaminant in a soil environment. They postulated that biotransformation is controlled by the biochemical activity of microorganisms and the mass transfer of a chemical to microorganisms. Their mathematical concept took both of these aspects into account. A critical analysis of bioremediation data using their concept revealed that the intrinsic microbial activities limited bioremediation in only a few cases. In most cases, mass transfer limitations prevented the full exploitation of the microbial degradative potential.

They concluded that technical measures are needed to change the physical structure of the contaminated material, which would enhance the bioavailability of the pollutants.

2.2.2 Increasing Bioavailability

Technical measures were employed in the above mentioned studies to physically change the structure of contaminated materials, in attempts to expose the sequestered and sorbed contaminants, and thereby enhance the bioavailability of pollutants. The technical measures employed are summarized following.

Both Hartzinger and Alexander [22] and Steinberg *et al.* [21] hypothesized that breaking up and converting soil aggregates to primary particles would make previously inaccessible compounds available to bacteria for degradation. Hartzinger and Alexander [22] used sonic disruption to break up soil aggregates and found the rate of phenanthrene mineralization increased by 4 times. Steinberg *et al.* [21] found that mechanical breakup of the soil particles in a ball mill resulted in a 20-fold increased release of aged EDB over that released from the unpulverized material.

Both Kelsey *et al.* [23] and Kelsey and Alexander [24] found that vigorous extractions of contaminants were ineffective for predicting bioavailability because the vigorous extraction processes appreciably over estimated the quantity of the contaminant that was actually bioavailable.

Rasiah *et al.* [27] exposed soils from an oil-waste land-treatment farms to varying levels of sonication and then allowed these soils to biodegrade. They found increased levels of compound mineralization that correlated with increased levels of sonic energy applied before bioremediation. They concluded that the increase in the bioavailability of the contaminant was due to aggregation caused by the sonic treatments.

Gregorich *et al.* [28] studied carbon mineralization in soil fractions after various amounts of aggregate disruption. They subjected aggregates (1-2 mm) to shaking, increasing intensities of ultrasonification, and then physically separated the resultant soils into sand-, silt- and clay-size fractions. All of the size fractions showed a large increase in the amount of readily decomposable C when the utrasonic energy input was increased, and disruption of microaggregates occurred. The data suggested that some readily decomposable organic matter was sequestered within microaggregates and protected from microbial attack.

Wang *et al.* [29] studied the effects that freeze-thaw would have on the loss of soluble organic carbon from soils. They found that increased leaching of SOC from the freeze-thaw treated soils was attributed to additional sources of SOC being released by the freeze-thaw process. They concluded that the shrinking and expanding of soil organic matter under freeze-thaw conditions enhanced fragmentation and surface exposure of the soil's organic matter. They also concluded that this in turn may facilitate the subsequent biochemical and biological depolymerization of the fragmented materials.

Of all the above techniques employed to physically alter the structure of soil to increase the bioavailability of sequestered contaminants, the action of freeze-thaw was the least technologically and physically intensive method. As well, because it occurs naturally, employing freeze-thaw cycles to enhance the bioavailability of inaccessible contaminants may prove to be the most economically feasible method utilized.

2.3 Freeze-Thaw

As demonstrated in the previous section, physically changing the structure of contaminated soils, and thereby exposing compounds that may be sequestered and sorbed within soil micropores, increased the bioavailability of hydrocarbon contaminants.

2.3.1 Freeze-Thaw and Aggregate Stability

Aggregate stability has been defined as the measure of a soil aggregate's resistance to breakdown [32]. The disruption of soil aggregates occurs naturally during seasonal freeze/thaw cycles. As water freezes in the small crevices and micropores of soil, it draws moisture from the surrounding soil causing ice crystals to grow. This crystal growth tends to exert pressure on and break up soil clods and aggregates [30]. This process is demonstrated schematically in Figure 2.1 below.

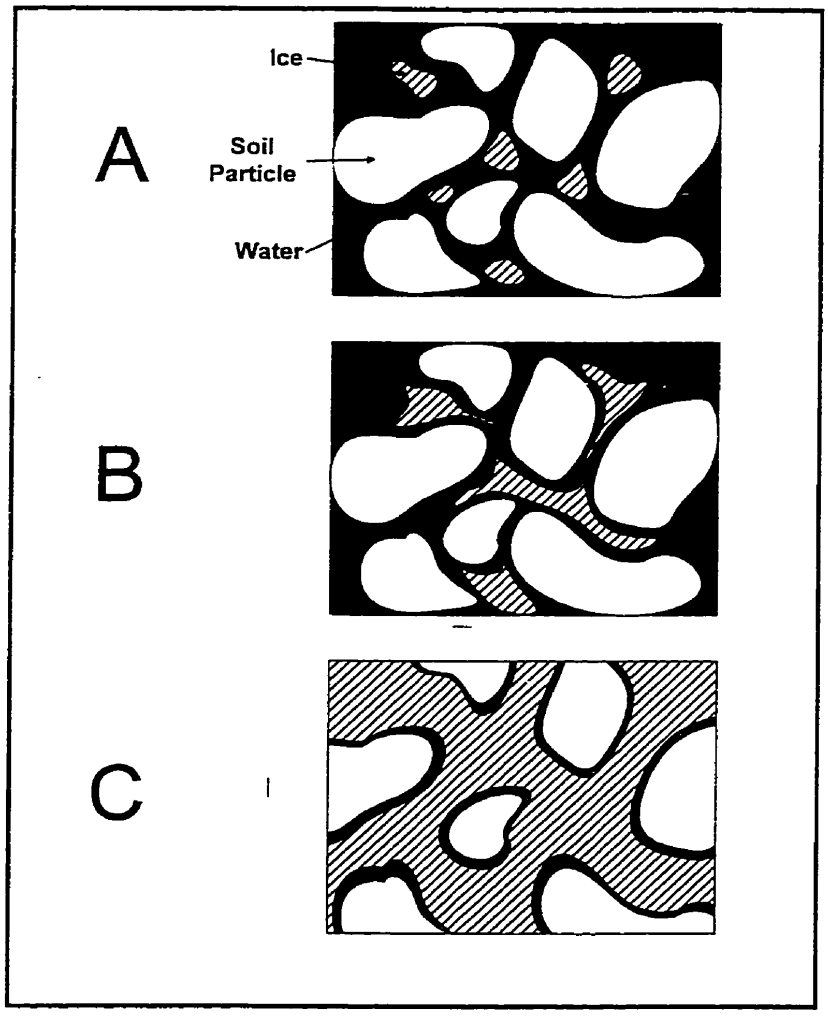

Figure 2.1. Schematic representation of the relationship between ice formation and unfrozen water films. As the amount of ice present increases from A to C, soil pore enlargement may occur, resulting in the soil particles being pushed apart and the break-up of the continuous liquid films. [31]

Studies have shown that repeated stress on soil aggregates by freeze-thaw cycles tended to degrade aggregate stability and reduce their size distribution.

Edwards [33], while studying the effects of freezing and thawing on aggregate stability and size distribution of Prince Edward Island soils, found that the largest size fraction of soil particles (4.75 mm to 9.5 mm) decreased from 58% to 35% while the smallest size fraction (< 0.5 mm) increased from 12% to 45%.

Sillanpaa and Webber [34] who studied the effects of freezing-thawing and wetting-drying cycles on soil aggregation, found a 20% to 42% decrease in the mean-weight diameter of soil aggregates after 5 cycles of freeze/thaw.

Eigenbrod [35] found that cyclic freezing and thawing caused increased permeability proportional to the number of freeze-thaw cycles that a fine grained soils were exposed to. The increase in permeability was attributed to the development of fissures and joints within the soil matrix.

Vaz *et al.* [36] found aggregate stability to decrease after freezing, increasing a soil's susceptibility to disaggregation. They further postulated that the disaggregation of a soil as a result of freeze-thaw can result in "fresh" reactive surfaces becoming exposed, and in turn, cause an increase in nutrient availability to bacteria.

2.3.2 Freeze-Thaw Effects on the Chemical and Biological Properties of Soil

As mentioned above, Vaz *et al.* hypothesized that soil freeze-thaw cycles increased the availability of soil nutrients to bacteria by exposing previously inaccessible reactive surfaces. Other studies have found that the chemical and biological properties of soils are affected in conjunction with the soil's physical properties when subjected to freeze-thaw. The results of some of these studies are summarized following.

Edwards and Cresser [31] conducted an extensive literature review on the effects that freezing had on the chemical and biological properties of soil, and found that the structural disintegration of soil associated with the volumetric changes of water upon freezing, could result in the exposure of fresh reactive surfaces within a soil matrix. They further found that these results would not be limited to mineral soils, but that physical disruption of soil organic matter also occurred.

Christensen and Christensen [37] studied the effects of freeze-thaw cycles on organic matter availability for denitrification in different soil fractions. They found that freeze-thaw treatments increased the soil organic matter concentrations available for denitrification and that for whole soil and aggregate (sandy and silty) soils, the freeze-thaw process disintegrated these soil aggregates.

As aggregate stability is decreased by freeze-thaw processes, the overall reactive surface areas exposed to soil bacteria increases. This process may expose contaminant

compounds that had previously been unavailable to bacterial degradation due to the compounds being sequestered and sorbed within soil micropores. Freeze-thaw may also have other positive effects on bioremediation related factors and processes such as nutrient availability and microbial activity.

Edward and Cresser's [31] review of soils literature revealed that freezing and thawing increased nutrient availability in soils. They found that numerous freeze-thaw cycles caused increased concentrations of extractable ammonium and phosphorous. They also found that the greatest effect of freezing on nutrient availability were associated with highly organic soils.

Mack [38] found that the effect of a single freezing and thawing of soil increased bacterial activity and the bacterial mineralization of nitrogen. Vaz *et al.* [36] found that freezing significantly increased the total dissolved and soluble phosphorous fractions in soil. They concluded that the substantial increase in soluble phosphorous observed for the organic soil suggested that the physical disruption of biological components were important.

2.3.3 Freeze-Thaw and Microbial Activity

Freezing and thawing also effects microbial activity. Freezing and thawing has been noted to cause large flushes in respiration measured by CO_2 production or O_2 uptake [31]. This burst of activity is related to the presence of readily available nutrients and soluble

carbon compounds released by the freeze-thaw process. Some of these readily available nutrients and soluble carbon are attributed to intercellular solutes which leak out of lethally damaged cells and serve as metabolic substrates for cells not damaged [39].

Morley *et al.* [40] studied the effects of freeze-thaw stresses on bacterial populations in soil microcosms and found that freezing caused a proportion of the bacterial population (40% to 60%) to be killed. They estimated that the death and lysis of 40% of a soil's bacterial population can produce up to 9 μg of mineralized nitrogen per g of soil. They concluded that these bioavailable, mineralized nutrients would be available for microbial uptake in the spring, and could conceivably prime spring microbial activity.

Skogland *et al.* [41] found an increase in O_2 uptake and CO_2 evolution after freeze-thaw and concluded that there was a positive correlation between the killing effect of the freeze-thaw treatment of soil and the respiratory increase per surviving bacterium. This increase in microbial activity was attributed to the uptake of leaked cellular material from lysed cells by the surviving bacteria.

2.4 Literature Review Summary

Naturally occurring freeze-thaw cycles disrupt soil aggregates, physically changing the structure of soils. Freezing and thawing have also been shown to increase the nutrient availability in soils. Bacterial activity has been documented to increase after freeze-thaw

cycles. All of these effects of freezing and thawing should combine to increase the bioavailability of sorbed and sequester contaminant compounds, as well as to increase the activity of the soil bacteria required to degrade these compounds. By increasing contaminant bioavailability and soil microbial activity, the biodegradation rates of freeze-thaw treated soils should also increase.

3.0 Method and Apparatus

3.1 General

The study utilized bench scale bioreactors to biologically treat a diesel fuel contaminated soil. A series of reactors, including an abiotic control, were run in triplicate. The contaminated soil sample was analyzed for it's physical and chemical characteristics. The soil was amended with nutrients and was brought up to optimum moisture levels. Samples were placed into the reactors, and the initial concentration of Total Extractable Hydrocarbons (TEH) was determined for each reactor. After the reactors were set up, the progress of degradation by the bacteria was monitored through head-space analysis of oxygen utilization and carbon dioxide production for 229 days. These respiration results were used to estimate the concentration of contaminants remaining in the reactors. The bacterial activity was monitored until it was determined that rate of degradation had leveled off. This decrease in activity suggested that the contaminant compounds had become unavailable to bacterial degradation, and that the soil microorganisms had shifted to endogenous respiration. At this point, designated reactors were subjected to various freeze-thaw cycle treatments to disrupt soil aggregates in attempts to expose previously unavailable concentrations of contaminants, as well as to stimulated microbial activity. After observing the response of the reactors to the freeze-thaw treatments, the reactors were opened up and the final TEH concentrations were determined. The data was

analyzed, and the results were compared for the effects of different freeze-thaw treatments on the degradation of the hydrocarbons.

A detailed description of the specific methods and apparatus employed are presented in the following sub-sections.

3.2 Soil Characterization

Contaminated soil was obtained from a diesel impacted Manitoba Hydro site in Churchill, Manitoba. Twenty samples, in the form of bagged auger cuttings (grab samples), were acquired from a drilling program conducted on the site in August 1995. These bagged samples were all obtained within the first meter of overburden, characterized in the drilling logs as a silty-sand deposit [42].

The 20 samples were consolidated into one large sample. The consolidate sample was then passed through three sizes of screens to remove larger than sand-sized particles. The screens utilized are presented in Table 3.1.

Table 3.1: Screens Utilized to Partition Consolidated Soil Sample

Pass	Screen Size	Material Removed
1	1/2 inch	pebble-sized aggregates
2	3/8 inch	coarse-gravel aggregates
3	No. 4	fine-gravel aggregates

Once all aggregates larger than coarse-sand were removed, the consolidated sample was passed through a batch splitter 5 times to ensure that the final sample was well mixed. The consolidated sample's physical and chemical properties were analyzed. From the results, optimal moisture content and nutrient concentrations could be determined to ensure a successful onset of hydrocarbon degradation. All of the soil characterization analyses were conducted on triplicate samples. The initial soil characterization analysis that were conducted on the consolidated sample are summarized in Table 3.2 below.

Table 3.2: Initial Soil Characterization Analysis of Soil Sample's Physical and Chemical Parameters

Analysis	Method
Soil pH	water extraction, pH meter
Volatile Organic Carbon	EPA 8240/8260
Moisture Content	oven drying at 105 °C
Bioavailable Phosphorous	SM 4500-P D [1]
Bioavailable Nitrogen	SM 4500-NO_3^- F [2]
Soil Texture	ASTM D-2487
Soil Porosity	
Soil Density	Drying Oven and Scale
Total Extractable Hydrocarbons (TEH)	Hexane extraction, GC analysis

[1] Soil extracted with 0.5M $NaHCO_3$
[2] Soil extracted with 2M KCL

The results of the soil characterization analysis is presented in section 4.1.

All of the above analyses were conducted at the University of Manitoba Department of Civil Engineering, Environmental Engineering laboratory. A summary of the in-house analytical equipment utilized to conduct these soil characterization analyses is presented in Table 3.3.

Table 3.3: Summary of Soil Characterization Analytical Equipment

Analysis	Analytical Equipment
Soil pH	pH meter
Volatile Organic Carbon	Muffle Furnace
Moisture Content	Drying Oven and Scale
Bioavailable Phosphorous	Technicon colorimetric auto analyzer
Bioavailable Nitrogen	Technicon colorimetric auto analyzer
Soil Texture	Dry Sieve
Soil Porosity	
Soil Density	Drying Oven and Scale
Total Extractable Hydrocarbons (TEH)	HP 5890 Gas Chromatograph (FID), HP 1 Capillary Column

3.3 *Soil Preparation and Amendments*

Based on the initial soil characterization, the optimal soil pH, soil moisture content and nutrient concentrations required for successful bioremediation were determined.

3.3.1 Soil pH

While microorganisms will often grow over a wide range of pH, drastic variations in pH can affect microorganisms by disrupting plasma membranes, or by inhibiting the activity of enzymes and membrane transport proteins [43]. Rowell *et al.* [44] found that the pH requirements for hydrocarbon degradation fall within the range of 6.0 to 8.5. They recommended that soil pH adjustment is only required when the value falls outside of that range. Riser-Roberts [45] suggests that the optimum pH for rapid decomposition of hydrocarbon wastes and residues is usually in the range of 6.5 to 8.5. Cookson [46] reported that hydrocarbon degradation proceeded quicker at pH's above 7 than below, when the pH falls within the optimum range of 6.0 to 8.0.

3.3.2 Soil Moisture Amendment

As bioremediation of aliphatic hydrocarbons is most efficient through aerobic processes [47], soil moisture content must be controlled to provide a sufficient amount of intra-particle water for bacterial activity, while not eliminating the pore-space air required for aerobic respiration. Riser-Roberts [48] suggested that aerobic degradation of petroleum hydrocarbons in soil is commonly greatest at 50 to 70 percent of the soil (water-holding) field capacity. Cookson [49] reported that a moisture content of 80 percent of the soil's field capacity (approximately 15 percent moisture by weight) was optimum for bioremediation in soil. Calabrese and Kostecki [69] also found that the majority of bioremediation studies indicated that generally for soils, the optimum moisture content is within 50 to 70 percent of the water-holding capacity, or approximately 15 to 20 percent

by dry weight. De-ionized water, produced in the Environmental Engineering Laboratory, was used to adjust the soil sample's moisture content to 15 percent by weight.

3.3.3 Nutrient Amendments

The initial concentration of Total Extractable Hydrocarbons (TEH) in the composite soil sample was determined during the initial soil characterization, as described previously. From these contaminant concentration values, the required concentrations of nitrogen and phosphorous amendments for successful bioremediation were determined. The reported optimal carbon to nitrogen to phosphorous ratios (C:N:P) for biodegradation is 100:10:1 [50-52]. Lab-grade Potassium Nitrate (KNO_3) and Potassium Phosphate Monobasic (KH_2PO_4) were utilized for the nitrogen and phosphorous amendments respectively. The required concentrations of KNO_3 and KH_2PO_4 were completely dissolved into the de-ionized water intended for the soil moisture correction and the solution was applied to the soil samples.

Although commercial agricultural fertilizers, such as Urea (46-0-0), Ammonium Nitrate (34-0-0), Ammonium Phosphate (11-55-0), and Mono-Ammonium Phosphate (11-52-0), are recommended in the literature [50], lab-grade Potassium Nitrate and Potassium Phosphate Monobasic were chosen for their absence of ammonium and filler materials. The application of ammonium salts or urea as nitrogen sources may cause ammonia toxicity, and thereby reduce microbial activity [51]. As well, nitrate is reported to be a better form of nitrogen for hydrocarbon decomposition [51]. The commercial fertilizers

mentioned also contain filler materials of unknown composition. These filler materials may consist of carbon-based compounds. A concern arises that any carbon-based filler materials may inhibit the biodegradation of the hydrocarbon contaminants due to Diauxie effect. The Diauxie effect [53] occurs when a compound cannot be degraded in the presence of another compound. The metabolic pathways of degradation are not altered, but the enzymes necessary for metabolic attack of a particular hydrocarbon may not be produced when a preferred substrate is present. A previous study conducted at the University of Manitoba [11] suggested that the utilization of urea as a nutrient source caused a diauxie effect in the fertilizer amended samples. It was hypothesized that the hydrocarbon-degrading bacterial attacked the urea carbon source prior to the contaminant compounds. This theory was supported by high CO_2 production coupled with low hydrocarbon degradation rates in the urea-amended samples and the opposite trend in non-amended samples.

3.4 Bioreactor Study

3.4.1 Bench-Scale Protocol

A bench-scale protocol developed by the Site Remediation Division of the Wastewater Technology Center [54] with funding from the Development and Demonstration of Site Remediation Technology (DESRT) Program of Environment Canada, was used for this study. The protocol was developed through discussions by an expert review committee of existing bioremediation bench-scale protocols presented in literature. The protocol

was reported to have yielded good quality results for a number of different contaminants (volatile and non-volatile), contaminant concentrations, and soil types [55].

The method calls for the monitoring of microbiological activity within bioreactors containing various configurations of contaminated soil. The various reactor configurations recommended included the utilization of "treatment" reactors, "treatment control" reactors, and "abiotic control" reactors. Microbiological activity is monitored through the measurement of respiration by-products, namely CO_2. These by-products can be correlated to an equivalent degradation of contaminant through a mass-balance equation. As both the initial and final concentrations of the contaminant are determined through other analytical methods, the CO_2 production data and estimated contaminant degradation can be compared to the directly measured concentrations. From these results, biodegradation rates can be estimated and the feasibility of utilizing bioremediation to treat the specific contaminant can be assessed.

The method utilized for monitoring bacterial activity for this study has been modified from the protocol. A detailed explanation is provided in Section 3.4.3 bellow.

3.4.2 Mass Balance

The microbial activity within the bioreactors were measured by monitoring the concentration of Oxygen (O_2) and Carbon Dioxide (CO_2) within the reactor headspace. As the bacteria degrade the hydrocarbon contaminant through aerobic respiration, the

concentration of CO_2 within the reactor headspace will increase, and inversely, the concentration of O_2 will decrease. This decrease in headspace O_2 and increase of CO_2 can be correlated to a decrease in the concentration of hydrocarbons within the soil through a simple mass balance prescribed by Cookson [57] and Saberiyan *et al.* [58]. Given an amount of contaminant per mass of soil, the total quality of reactants can be calculated from a balance reaction. This is conducted by the balancing of a redox equation. The development of stoichiometric equations for the breakdown of the organic compounds must include the organic species being oxidized, the electron acceptor, and the major nutrients utilized for cell growth.

Three half reactions are provided in the references [57-58]: An Electron Donor Half Reaction (H_D), an Electron Acceptor Half Reaction (H_A), and a Microbial Cell Synthesis Equation (C_S). These half reactions are provided below.

$$H_D: \quad \frac{1}{Z}(C_aH_bO_cN_d) + \frac{2a-c}{Z}(H_2O) = \frac{a}{Z}(CO_2) + \frac{d}{Z}(NH_3) + H^+ + e^- \qquad (3.1)$$

$$where \quad Z = 4a + b - 2c - 3d$$

$$H_A: \quad \frac{1}{4}O_2 + H^+ + e^- = \frac{1}{2}H_2O \qquad (3.2)$$

For Oxygen as the Electron Acceptor

$$C_S: \quad \frac{5}{28}CO_2 + \frac{1}{28}NO_3^- + \frac{29}{28}H^+ + e^- = \frac{1}{28}C_5H_7O_2N + \frac{11}{28}H_2O \qquad (3.3)$$

For Nitrate as the Nitrogen Source

The term "$C_5H_7O_2N$" in equation 3.3 represents the approximate composition of cellular structure from microbial growth resulting from degradation of the contaminant [57].

The overall reaction can be given in general terms by:

$$H_D + f_e H_A + f_s C_s \tag{3.4}$$

f_e = fraction of organic oxidized for energy

f_s = fraction of organic associated with conversion to microbial cells

where:

$$f_e + f_s = 1 \tag{3.5}$$

For aerobic reactions, the range of values for f_s is 0.12 - 0.60, with the mean being 0.50 [57]. For this study, f_s was chosen as 0.50.

The estimated average carbon chain length for Diesel Fuel is $C_{14}H_{24}$ [58]. When substituting in this value into the electron donor half reaction (3.1), the following results:

$$H_D: \quad \frac{1}{80}(C_{14}H_{24}) + \frac{28}{80}(H_2O) = \frac{14}{80}(CO_2) + H^+ + e^- \tag{3.6}$$

When applying equation 3.4 to the equations 3.1, 3.2, 3.3, and 3.5, and collecting all the terms, one obtains:

$$C_{14}H_{24} + 10O_2 + \frac{10}{7}NO_3^- + \frac{10}{7}H^+ = \frac{10}{7}C_5H_7O_2N + \frac{48}{7}CO_2 + \frac{54}{7}H_2O \quad (3.7)$$

In summary, for every mole of hydrocarbon ($C_{14}H_{24}$) degraded:

- 10 moles of O_2 are utilized
- 1.43 moles of NO_3^- are utilized
- 6.86 moles of CO_2 are produced
- 1.43 moles of Microbial mass are produced
- 7.71 moles of H_2O are produced

The molar relation,

$$C_{14}H_{24}:O_2:CO_2 = 1:10:6.86 \quad (3.8)$$

was used to estimate the degradation rate of hydrocarbons through the monitoring of the bioreactor's products and by-products of respiration.

3.4.3 Respiration Monitoring

After the contaminated soils were placed into the reactors, samples from each reactor were analyzed for their initial Total Extractable Hydrocarbon (TEH) concentration. The reactors were then sealed up, and the concentration of O_2 and CO_2 in the reactor's headspace were monitored for 229 days. Initially, the headspace gas concentrations were

monitored daily. As the rate of O_2 utilization and CO_2 production started to decrease, headspace monitoring decrease to by-weekly and then weekly sessions.

The headspace gas concentrations were determined utilizing a Gow Mac Gas Chromatograph (GC) utilizing a Series 550 Thermal Conductivity Detector. The GC was calibrated by sampling and injecting prepared concentrations of O_2 from sealed serum bottles. After calibration, samples of the reactor's headspace were withdrawn with a syringe through the reactor's sampling port, and injected into the GC. The results, expressed as "percent O_2", were recorded. After all the reactors were sampled for their headspace O_2 concentrations, the GC was re-calibrated for CO_2 and the sampling procedure was repeated.

The headspace O_2 and CO_2 concentrations converted from percent values to molar concentrations utilizing the Ideal Gas Law. The equations utilized were as follows:

$$Moles\ O_2 = \frac{P * V_{O_2}}{R * T} \tag{3.9}$$

P	=	*Headspace Pressure (atmospheres)*
V_{O_2}	=	*Volume of Headspace Oxygen (litres)*
	=	*Volume of Air * % O_2*
R	=	*Ideal Gas Constant*
	=	*0.08206 (atm*L / °K * moles)*
T	=	*Temperature (Kelvin)*

$$Moles\,CO_2 = \frac{P * V_{CO_2}}{R * T} \tag{3.10}$$

P	=	*Headspace Pressure (atmospheres)*
V_{CO_2}	=	*Volume of Headspace Carbon Dioxide (litres)*
	=	*Volume of Air * % CO_2*
R	=	*Ideal Gas Constant*
	=	*0.08206 (atm*L / °K * moles)*
T	=	*Temperature (Kelvin)*

Ambient temperatures were recorded at the time of sampling. Headspace pressures were assumed to be ambient (1 atm) or near ambient. It was also assumed that the soil pore space did not contribute to the total headspace volume.

The cumulative O_2 utilized and CO_2 produced were recorded, and utilizing equation 3.7, the cumulative degradation of $C_{14}H_{24}$ was estimated.

When it was observed that the oxygen concentration within the reactors had dropped below approximately 5 %, the headspace gas was exchanged as prescribed in the protocol [59]. The bioreactors were opened and headspace gas was allowed to be exchanged for ambient air. The reactors were then re-sealed and the headspaces were then sampled and analyzed on the GC to ensure that the O_2 within was at approximately 21 %. The O_2 concentrations within the abiotic reactors never decreased below 5 %, and were therefore never opened and exposed to ambient air.

The reactors were monitored for the duration of 229 days, at which time the bioreactors were opened-up, and the bioremediated soils were sampled and analyzed for their final TEH concentrations.

3.4.4 Freeze-Thaw Cycles

The by-products of microbial respiration within the reactors were monitored until it was determined that rate of O_2 utilization and CO_2 production had leveled off. This decrease in activity suggested that the contaminant compounds had become unavailable to the soil bacteria, and that aerobic degradation of the target compounds was no longer occurring. At this point, designated reactors were subjected to various freeze-thaw cycle treatments to disrupt soil aggregates in an attempt to expose previously unavailable concentrations of contaminants, as well as to stimulate microbial activity.

A different number of freeze-thaw treatments were applied to the four groups of treatment reactors. One, three, six and nine freeze-thaw cycles were applied in attempts to re-stimulate biodegradation. The treatment program is summarized in table 3.4 below.

Table 3.4: Freeze-Thaw Treatments and Associated Reactor Groups

Reactor Group Designation	Number of Reactors	Number of Freeze-Thaw Treatments Applied
F/T 1	3	1
F/T 3	3	3
F/T 6	3	6
F/T 9	3	9

The complete freeze-thaw treatment program required nine days to complete. The program was arranged so that all of the reactors began their freeze-thaw cycles on day one, and they all were completed by day ten. Table 3.5 summarizes the events of the nine-day freeze-thaw treatment program.

Table 3.5: Summary of Events for Freeze-Thaw Treatment Program

		Nine-Day Freeze-Thaw Treatment Period								
Reactor Designation	Freeze-Thaw Cycles	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9
F/T 1	1									√
F/T 3	3	√				√				√
F/T 6	6	√		√	√		√	√		√
F/T 9	9	√	√	√	√	√	√	√	√	√

√ - Denotes one Freeze-Thaw Cycle

All the reactors were placed in a deep freezer at -20°C. To achieve complete freezing of the soil samples, the treatment reactors were placed in a deep-freezer for a minimum of 12 hours. For the thawing-phase of the freeze-thaw treatment, the reactors were allowed

to thaw for approximately 12 hours. To ensure that complete freezing and thawing occurred, freeze-thaw monitoring reactors were utilized. These three reactors employed the same configuration as the study's treatment reactors, less the gas-sampling ports. Five hundred gram soil samples from the original Hydro composite sample were employed in the freeze-thaw monitoring reactors. These samples were amended to the same moisture content and nutrient concentrations as the as the test reactor samples. To ensure complete freezing and thawing, the monitoring reactors were place into the deep-freezer with the treatment reactors. Before a group of treatment reactors were removed for thawing, the monitoring reactors were physically checked to ensure that the soil matrix was completely frozen. As well, before the treatment reactors were again placed back into the freezer for the next freezing cycle, the monitoring reactors were checked to ensure that the soil matrix had completely thawed.

3.4.5 Bioreactors

The bioreactors were constructed from 2 litre glass Mason™ canning jars, fitted with Teflon-lined screw-top lids. A schematic drawing of the bioreactor construction is provided in Figure 3.1 below.

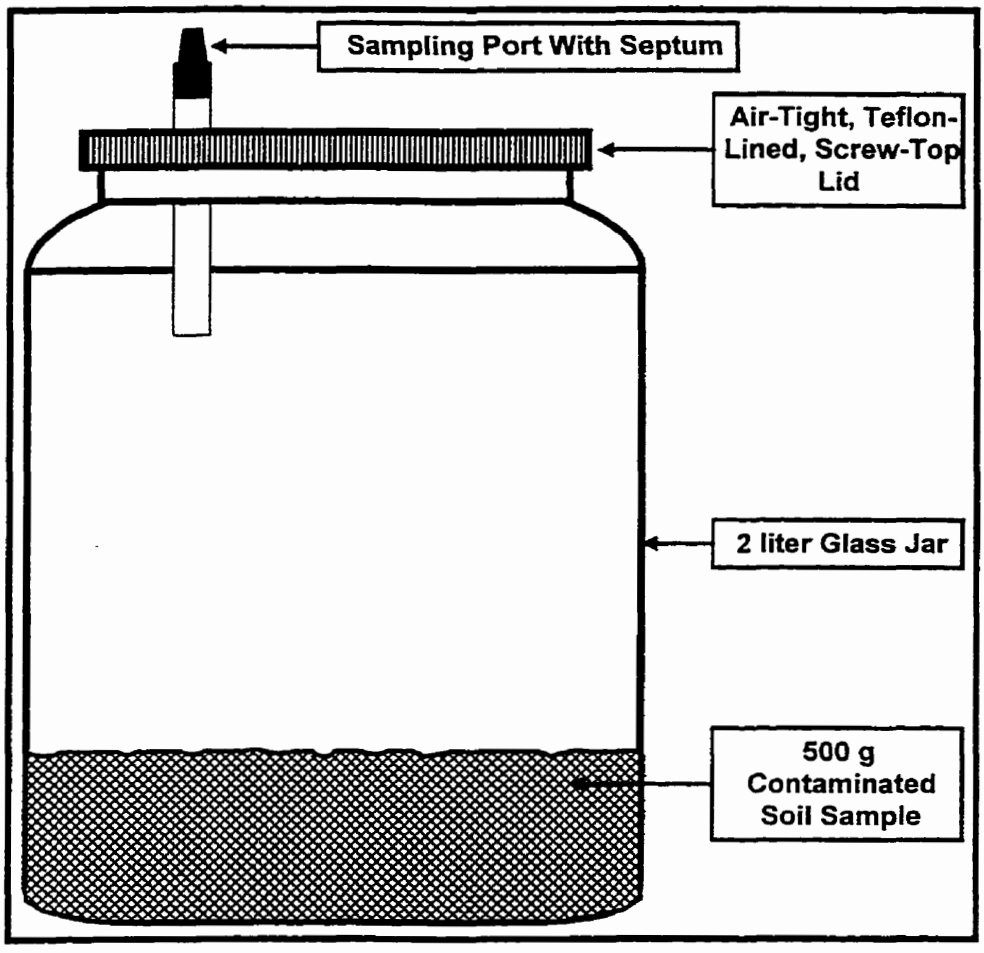

Figure 3.1: Schematic Representation of the Bioreactors Utilized in this Study.

Within the reactors were placed 500 g of contaminated soil, which occupied approximately 400 ml of the reactor's 2100 ml volume. The soil had been amended with nutrients and moisture, as described in Section 3.3 above. The reactors included treatment reactors, treatment controls and, abiotic controls. All reactor configurations were run in triplicate. Table 3.6 provides a break-down of the reactor configurations.

Table 3.6: Break-Down of Bioreactor Configurations

Reactor Designation	Reactor Description	Number of Reactors
ABIOTIC	Abiotic Control	3
F/T CNTL	Freeze-Thaw (Treatment) Control	3
F/T 1	1 Freeze-Thaw Treatment	3
F/T 3	3 Freeze-Thaw Treatments	3
F/T 6	6 Freeze-Thaw Treatments	3
F/T 9	9 Freeze-Thaw Treatments	3

Abiotic Control was provided by the addition of 0.2% (120 mg/kg_{soil}) of Mercuric Chloride ($HgCl_2$) in solution to the 500 g soil sample [56]. No nutrient amendments were provided to the Abiotic Control Reactors.

4.0 Results

4.1 Soil Characterization

The consolidated soil sample composed of bagged auger cuttings from a diesel fuel-impacted site in Churchill, Manitoba were characterized for it's physical and chemical properties, as described in Section 3.2. The results of this characterization are summarized in Table 4.1 below.

Table 4.1: Results of Initial Soil Characterization Analysis

Analysis	Results[1]
Soil pH	8.13
Volatile Organic Carbon	1.19×10^{-2} g VOC / g Soil
Moisture Content	8.94×10^{-2} g H_2O / g Soil
Bioavailable Phosphorous	0.333 mg PO_4-P / kg Soil
Bioavailable Nitrogen	3.67 mg NO_3-N / kg Soil
Soil Texture	SW-SM [2]
Soil Porosity	38 %
Soil Density	1426 kg / m^3
Total Extractable Hydrocarbons (TEH)	3880 mg TEH / kg Soil [3]

[1] Results are the average of three analysis
[2] See table 3.2 below
[3] per kg of dry soil

The soil pH fell within the accepted range of 6.5 to 8.5 for bioremediation [46], and no pH adjustment of the soil sample was necessary.

The consolidated soil sample's moisture content was determined to be approximately 10% by weight. This was lower than the recommended concentration of 15% by weight [49] for successful bioremediation. The soil's moisture content required the addition of approximately 61 g of deionized water (61 ml) per kg of soil to bring the consolidated sample up to the optimal moisture content of 15% by weight.

The Volatile Organic Carbon (VOC) content of a soil can result from two sources [60]:

1. Human Activities (the application of fertilizers, petroleum spills, etc.); or
2. Soil Biota (soil vegetation, flora and fauna).

Typical VOC values range from 1% to 8% for topsoils, and 0.1% to 1.0% for subsoils [60]. The results of the soil characterization indicate that the VOC content of the consolidated soil sample is approximately 1.2% by weight. Although this concentration is within expected range for topsoils, it is on the lower end of the range. As partitioning of contaminant compounds into soil organic matter affects it's bioavailability [16], the effect of freeze-thaw treatments to increase the availability contaminants sequestered within the sample's soil organic matter [29] may be minimal.

The soil sample's texture is classified as SW-SM: well-graded sand with silt [61]. The results of the sieve analysis is provided in Table 4.2 below.

Table 4.2: Sieve Analysis Results for Composite Soil Sample

Sieve Size	Approximate Diameter (mm)	% Retained (by weight)	Soil Classification
1/2	12.7	0	Fine Gravel
No. 4	4.75	10.8	Coarse Sand
No. 8	2.36	12.9	Coarse Sand
No. 10	2.0	2.4	Medium Sand
No. 16	1.18	10.4	Medium Sand
No. 20	0.85	7.5	Medium Sand
No. 30	0.6	10.7	Medium Sand
No. 40	0.425	15.1	Fine Sand
No. 60	0.25	15.4	Fine Sand
No. 100	0.15	4.0	Fine Sand
No. 200	0.075	3.6	Fine Sand
< No. 200	< 0.75	7.2	Silts and Clays

The composition of the composite soil sample is approximately 24% coarse sand (particle diameter 4.75 mm to 2.0 mm), 31% medium sand (particle diameter 2.0 mm to 0.425 mm), 38 % fine sand (particle diameter 0.425 mm to 0.075 mm), and 7% silts (particle diameter > 0.075 mm). A summary of the composite soil sample's texture is provided in Table 4.3.

Table 4.3: Soil Composition by Particle-Size Percentages

Soil Type	Percent (by weight)	Particle Diameter (mm)
Fine Gravel	0	>4.75
Coarse Sand	23.69	4.75 - 2.0
Medium Sand	31.07	2.0 - 0.425
Fine Sand	38.22	0.425 - 0.075
Silts and Clays	7.02	< 0.075
Total	100	

The composite soil sample's density was found to approximately 1,426 kg/m^3. This value falls with in the range of 1,300 kg/m^3 to 1,800 kg/m^3 typically found in coarse-textured surface soils [62]. As well, the composite soil sample's porosity of 38% was found to fall within the typical range of 26% to 53% for fine sands [63]. Soil porosity is an important soil characteristic for the bioremediation of diesel contaminated soils. As hydrocarbons are biodegraded through aerobic respiration, a sufficient amount of pore space is required to ensure that adequate air is available for the microbial respiration. A porosity of 38% is sufficient to ensure that aerobic biodegradation of the contaminant will occur.

The initial concentration of Total Extractable Hydrocarbons (TEH) found in the composite soil sample was 3,880 mg TEH/ kg Soil. These TEHs comprise approximately 0.4% of the soil by weight. For successful biodegradation to occur, the C:N:P ratio of

100:10:1 needed to be achieved. This required nutrient concentrations of 396 mg N / kg Soil and 88 mg P / kg Soil. The initial analysis of bioavailable nitrogen and phosphorous indicated that the composite soil sample possessed 3.67 mg NO_3-N / kg Soil and 0.333 mg PO_4-P / kg Soil. Stoichiometric analysis of the bioavailable N and P indicated that 2852 mg KNO_3 / kg Soil and 385 mg KH_2PO_4 / kg Soil would be required to meet the C:N:P ratio of 100:10:1 for successful bioremediation. The composite soil sample was amended with the required concentrations KNO_3 and KH_2PO_4 as described in Section 3.3.3. Table 4.4 summarizes the nutrient requirements and applied amendments.

Table 4.4: Required Nutrient Concentrations and Applied Amendments for Successful Bioremediation of the Composite Soil Sample

	Nitrogen	Phosphorous
Nutrient Requirement for C:N:P of 100:10:1	396 mg N / kg Soil	88 mg P / kg Soil
Bioavailable Nutrients	3.67 mg NO_3-N / kg Soil	0.333 mg PO_4-P / kg Soil
Required Nutrients Amendments	1750 mg NO_3-N / kg Soil	270 mg PO_4-P / kg Soil
Nutrient Amendments Added	2852 mg KNO_3 / kg Soil	385 mg KH_2PO_4 / kg Soil

4.2 Respiration Monitoring

The initial concentration of TEH as $C_{14}H_{24}$ for each reactor sample was determined prior to the reactors being sealed to begin the study. The results of the initial contaminant concentrations in the reactors are presented in Table 4.5 below.

Table 4.5: Initial TEH Concentrations in Reactor Soil Samples

Reactor Group Designation	Initial Concentration of TEH[1] (mg_{TEH} / kg_{soil})
ABIOTIC	3790.71
F/T 1	3394.70
F/T 3	3523.48
F/T 6	3640.04
F/T 9	3053.10
F/T CNTL	3414.41

[1] Values are an average of triplicate reactor samples

All of the initial TEH concentrations are lower than the composite soil sample's concentration. The nutrient amendments of 2852 mg KNO_3 / kg_{soil} and 385 mg KH_2PO_4 / kg_{soil} were sufficient, and in fact exceeded, the C:N:P ratio of 100:10:1.

A total of 48 data points were collected on the reactor's headspace O_2 and CO_2 concentrations over the duration of the 229 day study. The data was tabulated as

cumulative moles of O_2 utilized and cumulative moles of CO_2 produced. The cumulative utilization of O_2 and production of CO_2 are presented graphically Figures 4.1 and 4.2.

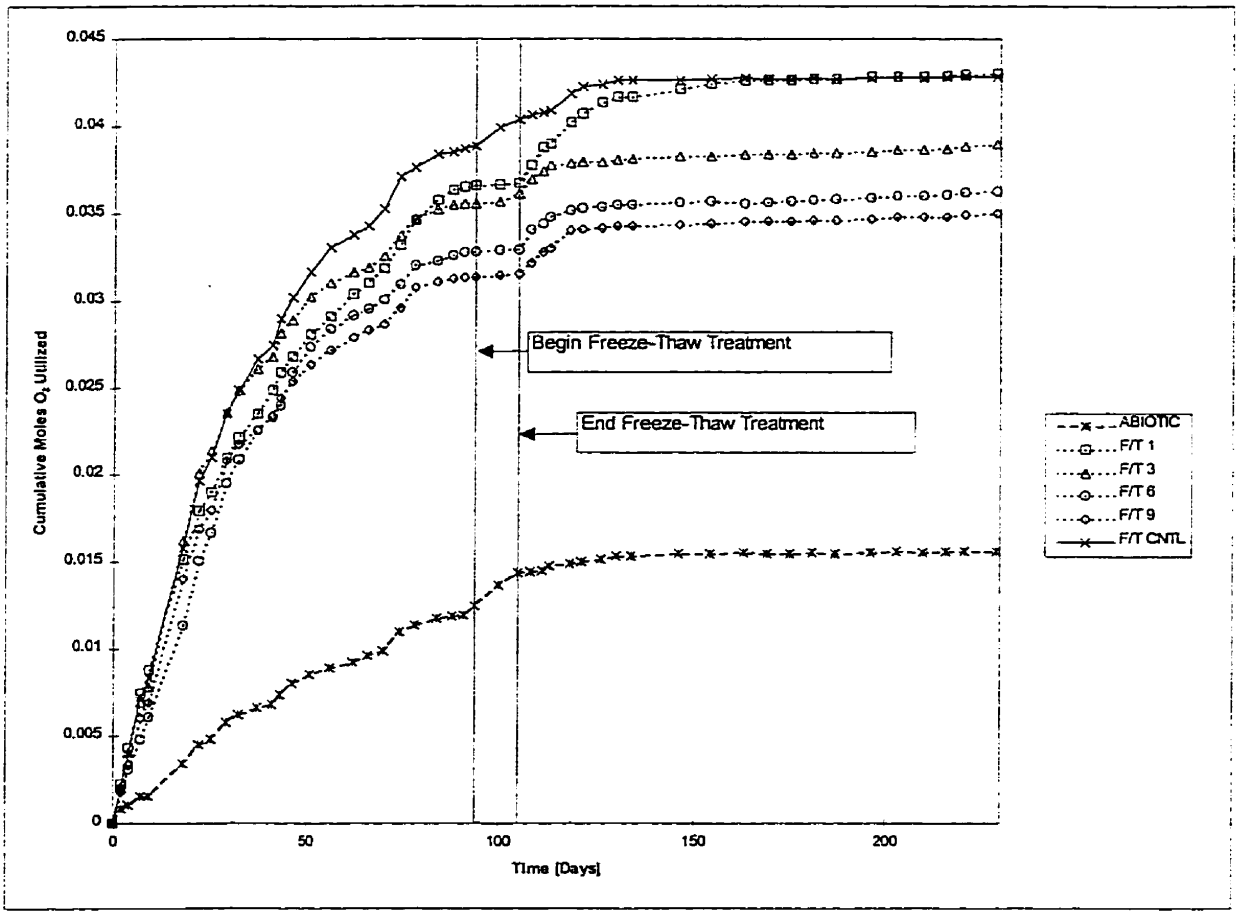

Figure 4.1: Cumulative Utilization of Reactor Headspace O_2 . The concentration of O_2 in the reactor headspace was monitored for 229 days. When the microbial activity appeared to have leveled-off at Day 94, the Freeze-Thaw Reactors (F/T1, F/T 9, F/T 6, F/T 9) were subjected to a 9-day freeze-thaw treatment. The O_2 utilization in the Freeze-Thaw reactors appeared to increase for approximately 12 days after the Freeze-Thaw Treatment, with the exception of F/T 1, which displayed elevated rates of O_2 utilization for approximately 30 days. Each data point represents an average value from 3 reactors.

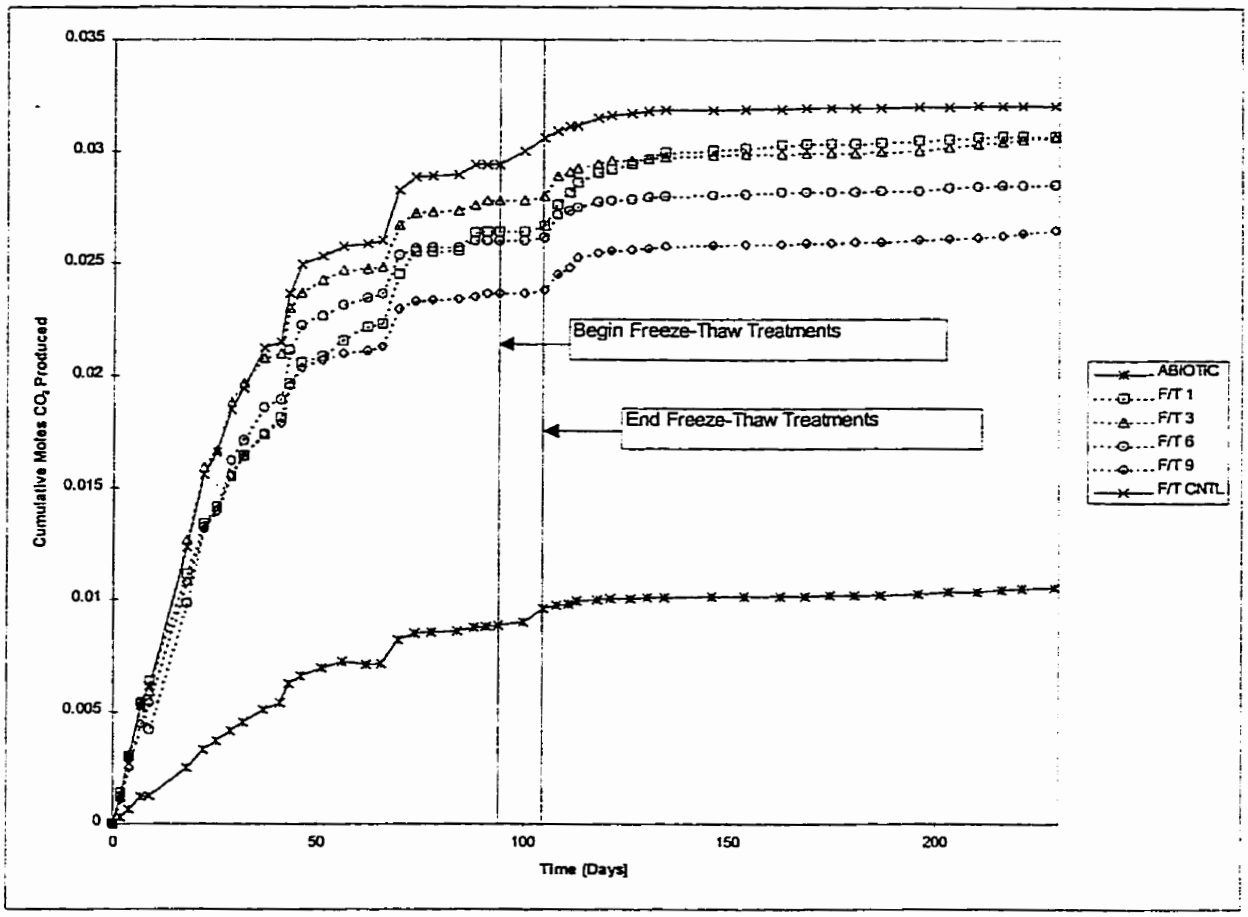

Figure 4.2: Cumulative Production of Reactor Headspace CO_2 . The concentration of CO_2 in the reactor headspace was monitored for 229 days. When the microbial activity appeared to have leveled-off at Day 94, the Freeze-Thaw Reactors (F/T1, F/T 9, F/T 6, F/T 9) were subjected to a 9-day freeze-thaw treatment. The CO_2 production in the Freeze-Thaw reactors appeared to increase for approximately 12 days after the Freeze-Thaw Treatment, with the exception of F/T 1, which appeared to display elevated CO_2 production for approximately 30 days. Each data point represents an average value from 3 reactors.

The utilization rate of O_2 and the production rate of CO_2 were initially high in all reactors, except the abiotic control reactor. These rates leveled-off at approximately day 80. The nine-day freeze-thaw treatment described in Section 3.4.4 was applied to the Freeze-Thaw Reactors (F/T1, F/T 9, F/T 6, F/T 9) at Day 94. As can be seen in Figures

4.1 and 4.2, the utilization of O_2 and the production of CO_2 in the Freeze-Thaw Reactors appeared to increase for approximately 12 days after the freeze-thaw treatment ended on day 105. The exception to this was reactor F/T 1, which appeared to display elevated O_2 utilization and CO_2 production for approximately 30 days after the completion of the freeze-thaw treatment.

The results of the Abiotic Control reactors were unexpected. From Figures 4.1 and 4.2, it appears that the Abiotic Control reactors (ABIOTIC) were utilizing O_2 as well as producing CO_2 . This would indicate that the Mercuric Chloride treatment utilized to sterilize these reactors was ineffective. The utilization of O_2 and production of CO_2 may also indicate an abiotic transformation of the hydrocarbon, such as abiotic oxidation.

With the initial concentration of TEH as $C_{14}H_{30}$ in each reactor, the relationship presented in equation 3.8 in Section 3.4.2 can be employed to estimate the concentration of $C_{14}H_{30}$ in each reactor from the O_2 utilization and CO_2 production data. This estimation of the $C_{14}H_{30}$ concentration in the reactors is provided graphically in Figures 4.3 and 4.4.

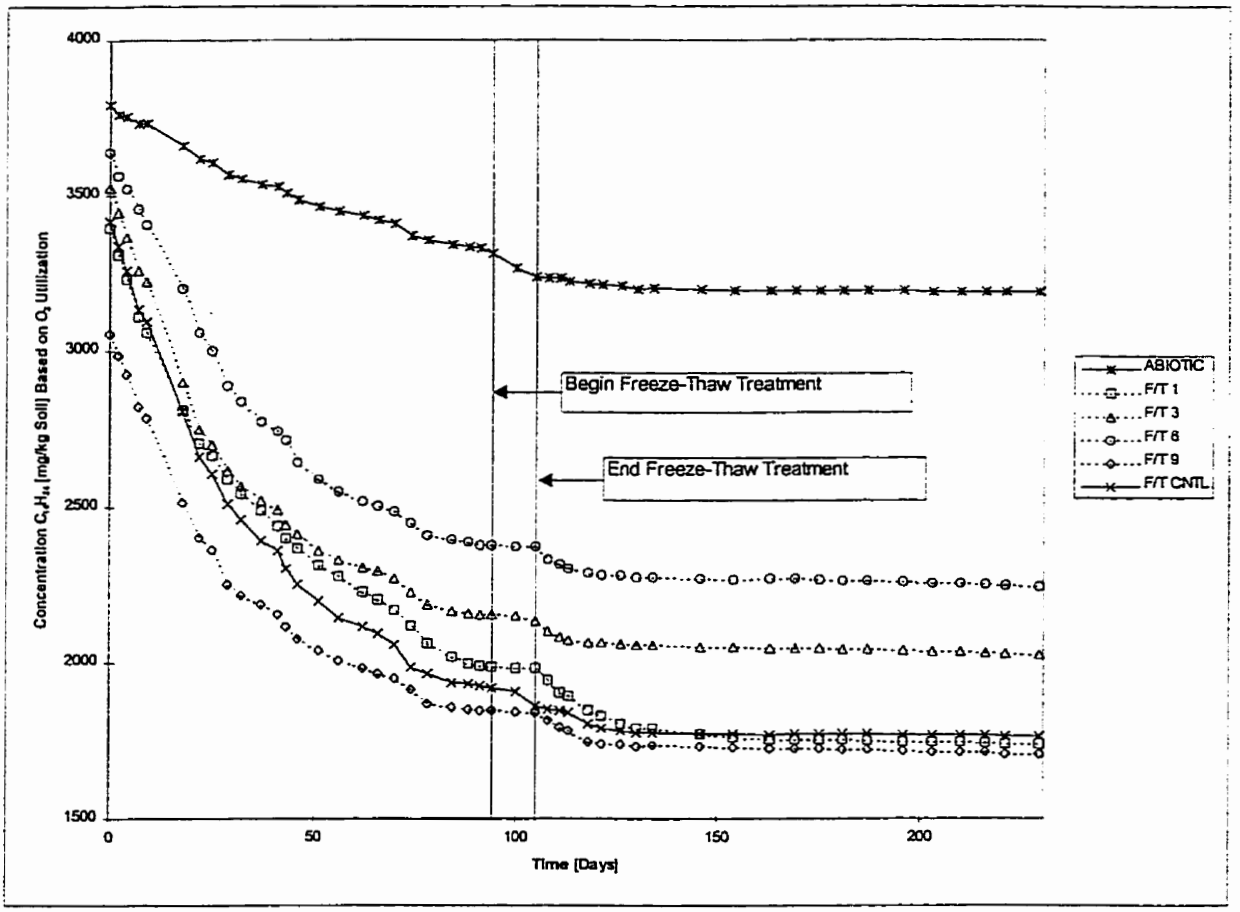

Figure 4.3: Estimation of Reactor $C_{14}H_{24}$ Concentration Based on Cumulative O_2 Utilization. Based on the stoicheometric relationship of 1 mole of $C_{14}H_{24}$ is degraded for every 10 moles of O_2 utilized, the concentration of $C_{14}H_{24}$ in the reactors' soil samples was estimated from the cumulative O_2 utilization data. Just as the O_2 utilization rate increased after freeze-thaw treatment, the Freeze-Thaw reactors displayed increased hydrocarbon degradation rates after the freeze-thaw treatments were applied. Each data point represents an average value from 3 reactors.

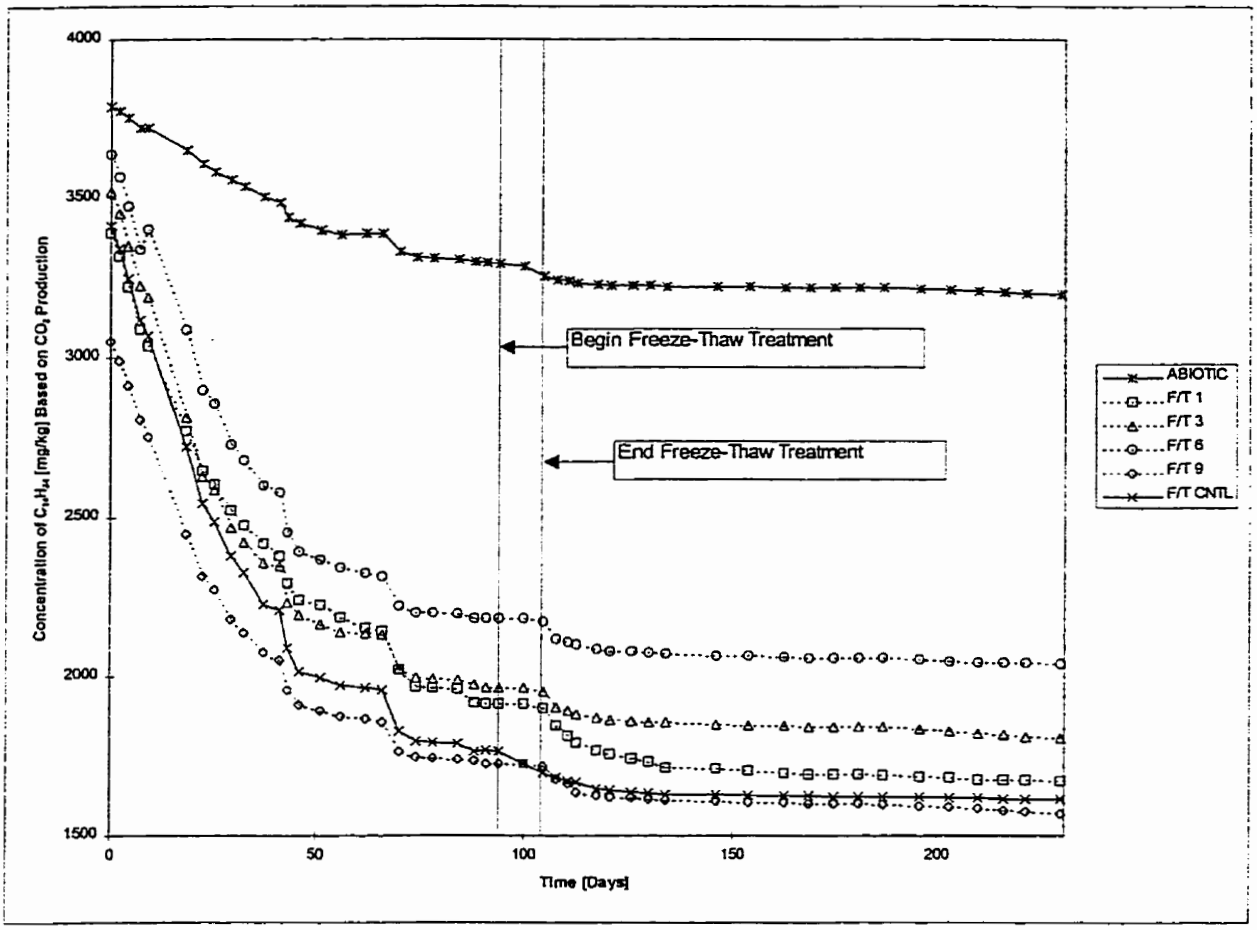

Figure 4.4: Estimation of Reactor $C_{14}H_{24}$ Concentration Based on Cumulative CO_2 Production. Based on the stoicheometric relationship of 1 mole of $C_{14}H_{24}$ is degraded for every 6.86 moles of CO_2 produced, the concentration of $C_{14}H_{24}$ in the reactors' soil samples was estimated from the cumulative CO_2 production data. Just as the CO_2 production rate increased after freeze-thaw treatment, the Freeze-Thaw reactors displayed increased hydrocarbon degradation rates after the freeze-thaw treatments were applied. Each data point represents an average value from 3 reactors.

To aid in the direct comparison of the reactors' hydrocarbon degradation over time, the $C_{14}H_{24}$ concentrations presented in Figures 4.3 and 4.4 were divided by their initial $C_{14}H_{24}$ concentrations (Co) presented in Table 4.5. The concentrations of $C_{14}H_{24}$ as C/Co over the duration of this study are presented Figures 4.5 and 4.6 .

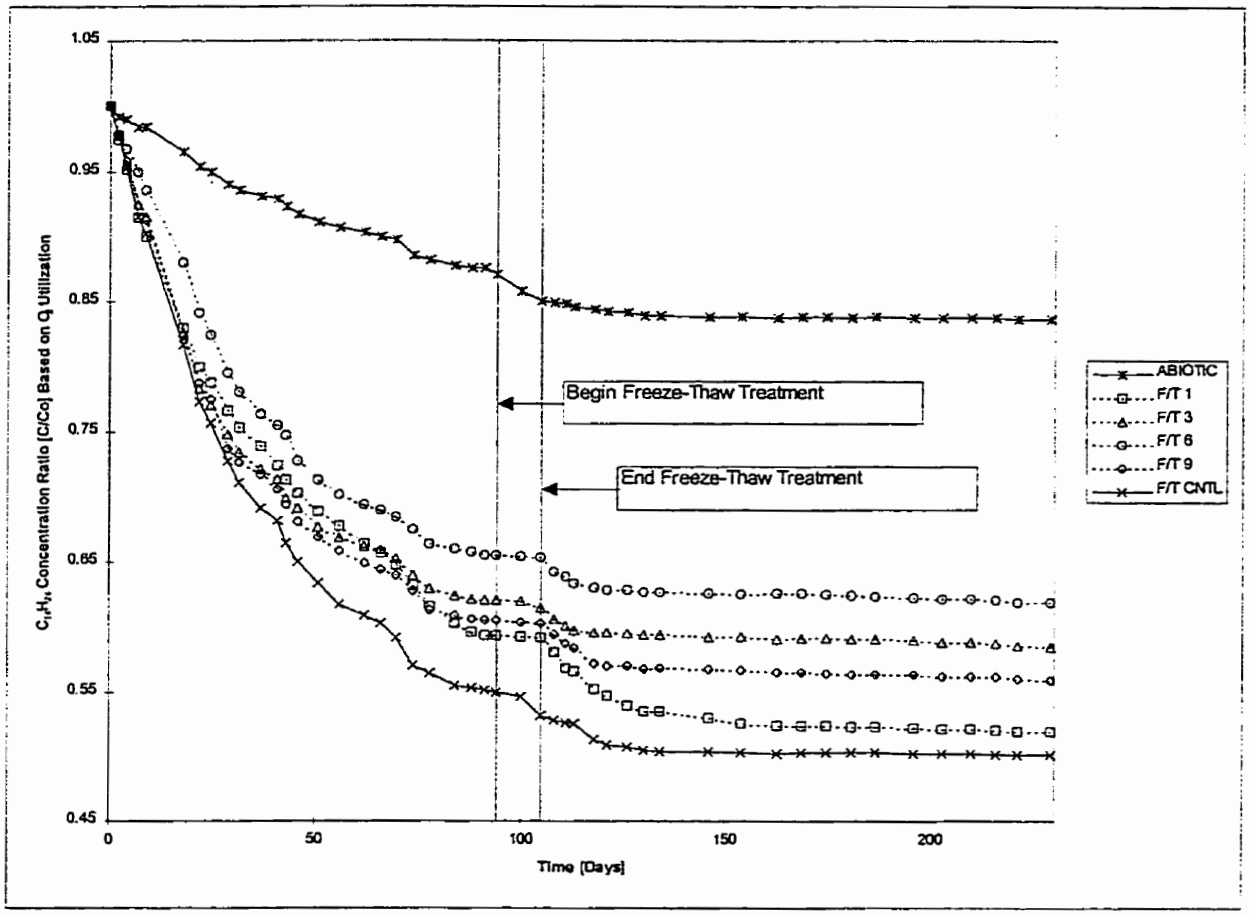

Figure 4.5: Estimated Reactor $C_{14}H_{24}$ Concentration Ratio (C/Co) Based on Cumulative O_2 Utilization. The data point values are obtained by dividing the estimated concentration of $C_{14}H_{24}$ based on O_2 utilization by the initial reactor concentration of TEH. Each data point represents an average value from 3 reactors.

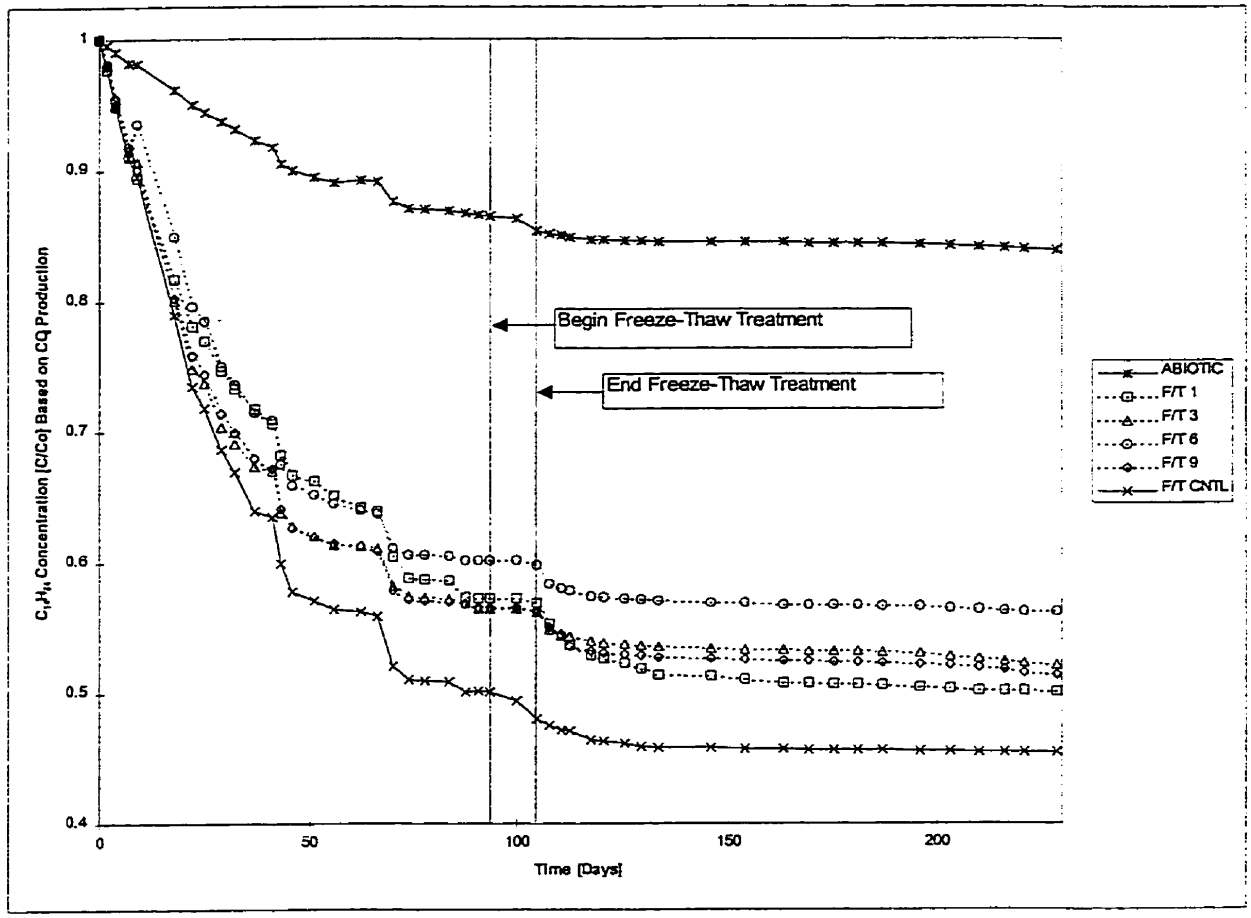

Figure 4.6: Estimated Reactor $C_{14}H_{24}$ Concentration Ratio (C/Co) Based on Cumulative CO_2 Production. The data point values are obtained by dividing the estimated concentration of $C_{14}H_{24}$ based on CO_2 production by the initial reactor concentration of TEH. Each data point represents an average value from 3 reactors.

From Figure 4.5 and 4.6, one can observe that the greatest $C_{14}H_{24}$ degradation rate was achieved by the freeze-thaw treatment control reactor, F/T CNTL.

Based on the O_2 utilization data (Figure 4.5), reactors F/T 1, F/T 3 and F/T 9 appeared to have similar degradation rates up to approximately day 80. At this point, the degradation rates for reactors F/T 3 and F/T 9 appear to begin leveling-out before reactors F/T 1. The application of the freeze-thaw treatment between days 94 and 105 appeared to have the greatest effect on the degradation rate of reactors F/T 1. The freeze-thaw treatment had the second greatest effect on reactors F/T 9. The freeze-thaw treatments appeared to have minor effect on the degradation rates of reactors F/T 3 and F/T 6.

The results based on the CO_2 production data (Figure 4.6) differ from the O_2 utilization results. Reactors F/T 1 and F/T 6, and reactors F/T 3 and F/T 9 initially appear to have the same degradation rates, with the latter pair slightly out-performing the former. At approximately day 75, the degradation rates for reactors F/T 3, F/T 6 and F/T 9 appear to begin leveling-out, with the degradation rate for reactors F/T 1 leveling out at approximately day 88. At this point, reactors F/T 1, F/T 3 and F/T 9 appear to have similar $C_{14}H_{24}$ concentrations. The application of the freeze-thaw treatment between days 94 and 105 appeared to have equal effect on the degradation rates of all the freeze-thaw treatment reactors. The duration of increased degradation rate from the freeze-thaw treatment appears to be equal in reactors F/T 3, F/T 6 and F/T 9, however, reactors F/T 1 appears to benefit for a longer period of time from the treatment.

As described above, the trends found in Figures 4.3 to 4.6 appear all to be similar for all study reactors. Four periods with different degradation rates can be observed. The first period occurs at initial reactor start-up, between days Zero and 78. During this period, the most significant $C_{14}H_{24}$ degradation occurs. The second degradation rate period occurs between days 78 and 105. During this period, the degradation rates of all study reactors begin to level-off. During the last 10 days of this period, the freeze-thaw treatment is applied to the freeze-thaw treatment reactors. The third degradation rate period occurs immediately after the completion of the freeze thaw treatments, beginning at day 105 and continuing to day 134. At this point, all 4 freeze-thaw treatment reactors demonstrate increases in their $C_{14}H_{24}$ degradation rates. The final degradation rate period occurs between days 134 and 229. During this period, all the study's reactors demonstrate nearly zero $C_{14}H_{24}$ degradation rates. This degradation rate trend is schematically displayed in Figure 4.7.

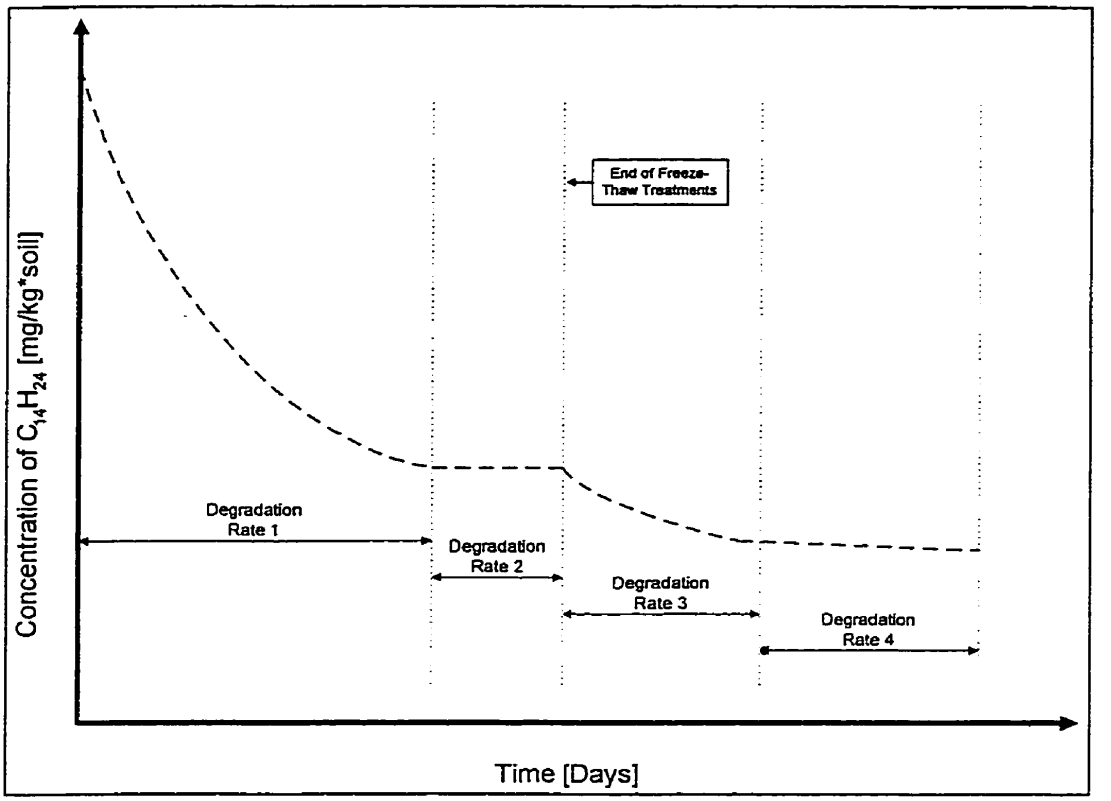

Figure 4.7: Schematic Representation of the Different Degradation Rate Periods Occurring During the Duration of the Reactor Respiration Monitoring. The Initial degradation rates occur for approximately 78 days. During the second period (days 78 to 105), degradation rates approach zero in all reactors. It is during this period that the Freeze-Thaw treatment is applied to the Freeze-Thaw Treatment reactors. The third period occurs between days 105 and 134. During this period, the Freeze-Thaw Treatment reactors experience an increase in $C_{14}H_{24}$ degradation. During the final period, the degradation rates of all the reactors again approach zero.

Four degradation rate periods were identified for the study reactors from the $C_{14}H_{24}$ concentration data based on both O_2 utilization and CO_2 production. These values are presented in Table 4.6.

Table 4.6: Reactor Degradation Rates for the Four Degradation Periods

	Reactor Degradation Rate (mg $C_{14}H_{24}$ / kg_{soil}*day)					
	ABIOTIC	**F/T 1**	**F/T 3**	**F/T 6**	**F/T 9**	**F/T CNTL**
Degradation Rates Based on O_2 Utilization Data						
Period 1	5.60	17.08	17.12	15.79	15.16	18.56
Period 2	1.48	1.02	0.72	0.46	0.37	1.37
Period 3	0.49	2.45	0.97	1.26	1.37	1.10
Period 4	0.13	0.67	0.41	0.38	0.35	0.11
Degradation Rates Based on CO_2 Production Data						
Period 1	6.16	18.35	19.63	18.48	16.79	20.79
Period 2	0.73	0.80	0.49	0.34	0.32	1.22
Period 3	0.38	2.39	1.26	1.31	1.39	0.88
Period 4	0.33	0.54	0.65	0.40	0.54	0.17

The data from Table 4.6 is presented graphically in Figure 4.8, for the values based on O_2 utilization, and in Figure 4.9, for the values based on CO_2 production.

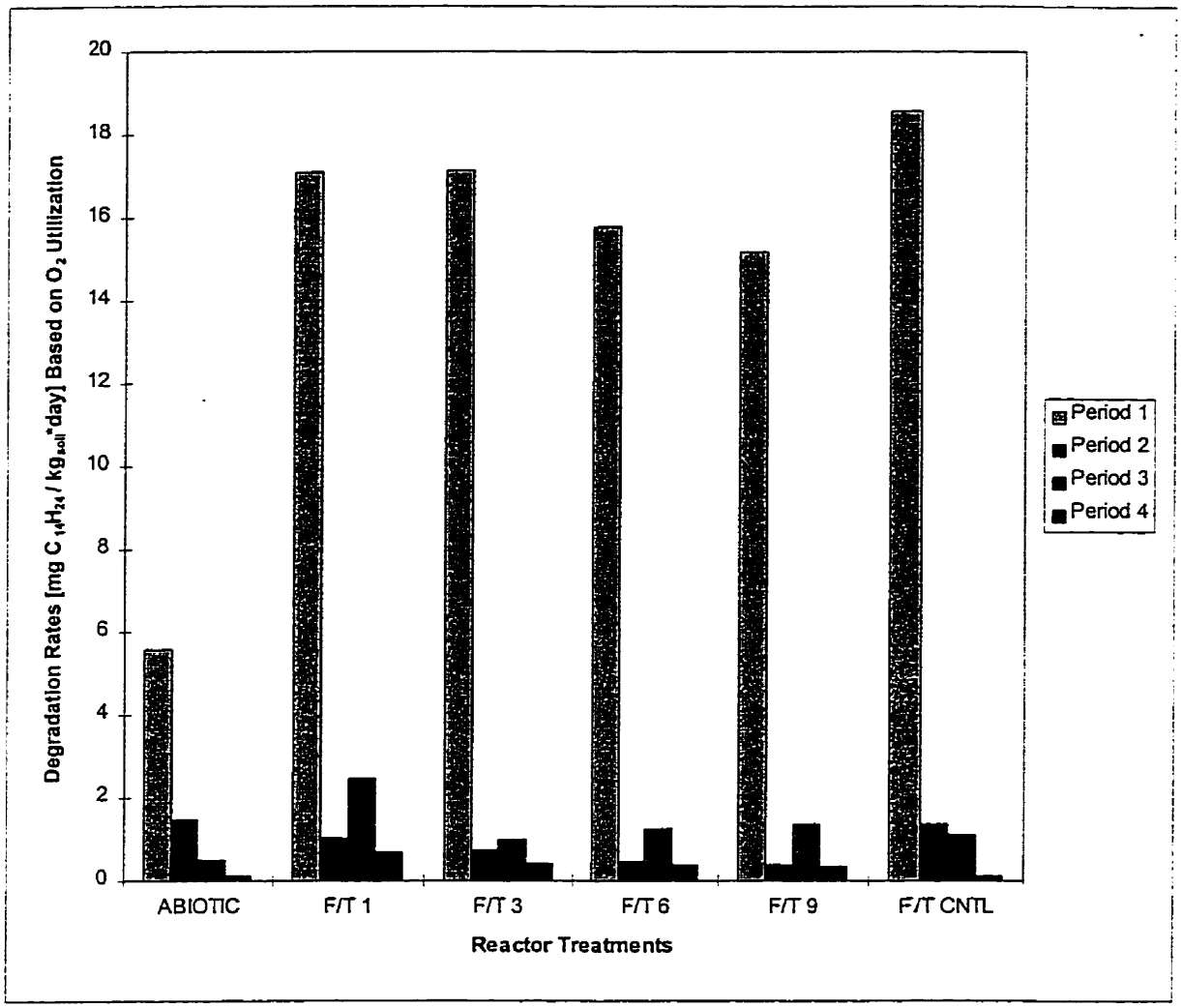

Figure 4.8: Reactor Degradation Rates for the Four Degradation Periods Based on O_2 Utilization Data. During the initial degradation period (Period 1), all of the reactors exhibited relatively high degradation rates. With the exception of the ABIOTIC reactors, the degradation rates decreased to approximately 5% of the initial rates during Period 2. The freeze-thaw treatments were applied to the Freeze-Thaw Treatment reactors (F/T 1, F/T 3, F/T 6 and F/T 9) near the end of Period 2. During Period 3, degradation rates continued to decrease in the ABIOTIC and F/T CNTL reactors, but the Freeze-Thaw Treatment reactors demonstrated an increase in their degradation rates. All of the reactors displayed decreases in their degradation rates during the final degradation period, Period 4.

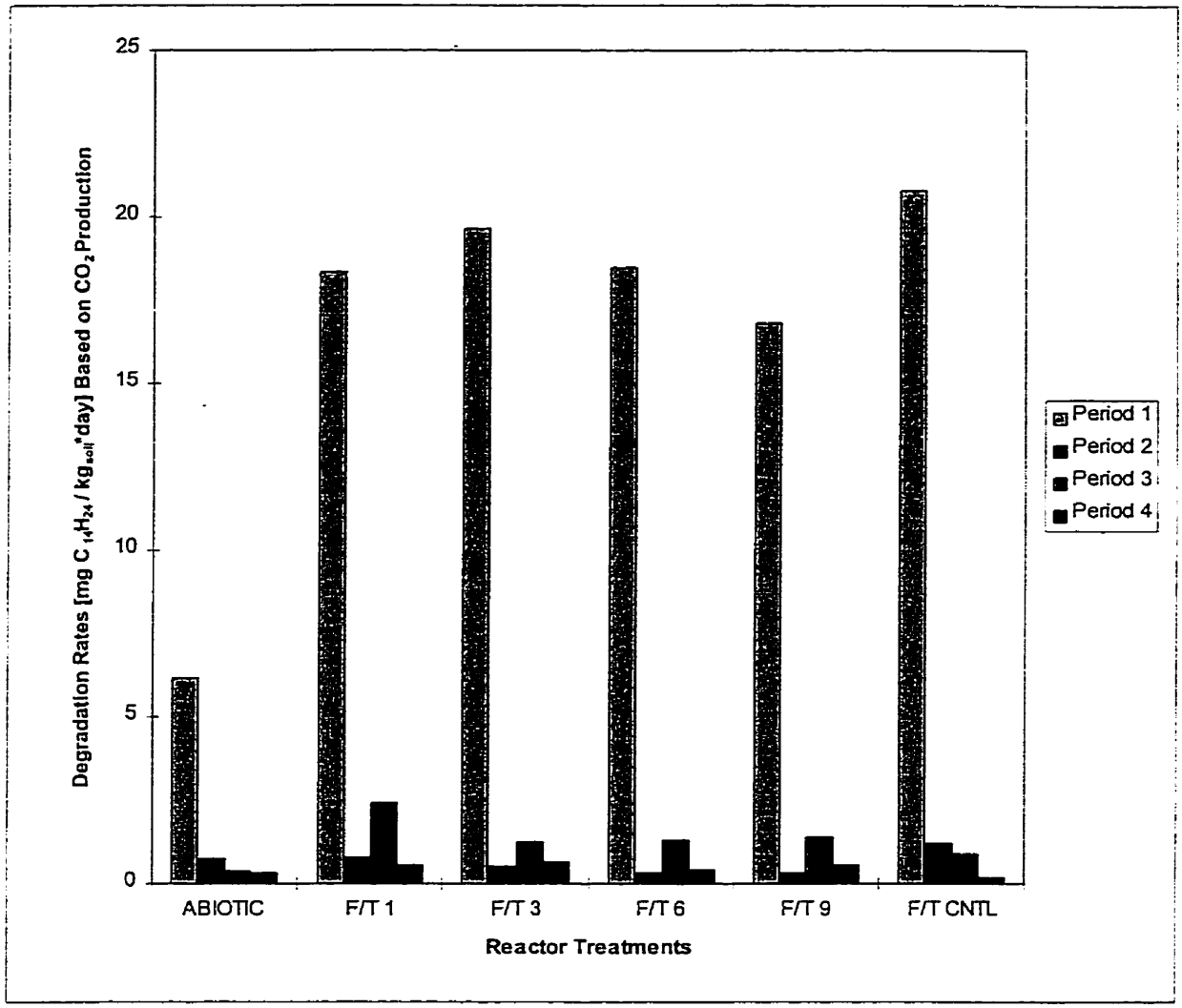

Figure 4.9: Reactor Degradation Rates for TEH Four Degradation Periods Based on CO_2 production Data. During the initial degradation period (Period 1), all of the reactors exhibited relatively high degradation rates. With the exception of the ABIOTIC reactors, the degradation rates decreased to approximately 3% of the initial rates during Period 2. The freeze-thaw treatments were applied to the Freeze-Thaw Treatment reactors (F/T 1, F/T 3, F/T 6 and F/T 9) near the end of Period 2. During Period 3, degradation rates continued to decrease in the ABIOTIC and F/T CNTL reactors, but the Freeze-Thaw Treatment reactors demonstrated an increase in their degradation rates. All of the reactors displayed decreases in their degradation rates during the final degradation period, Period 4.

During the initial degradation period (Period 1), all of the reactors exhibited relatively high degradation rates. Degradation rate averages between 5.60 to 18.56 mg $C_{14}H_{24}$ / kg_{soil}*day based on O_2 utilization, and 6.16 to 20.79 56 mg $C_{14}H_{24}$ / kg_{soil}*day based on CO_2 production, were observed during Period 1. During Period 2, the degradation rates decreased to approximately 5% of the initial rates based on O_2 Utilization, and to approximately 3% of the initial rates based on CO_2 production, with the exception of the ABIOTIC reactors. The freeze-thaw treatments were applied to the Freeze-Thaw Treatment reactors (F/T 1, F/T 3, F/T 6 and F/T 9) near the end of Period 2. This resulted in the Freeze-Thaw Treatment reactors demonstrating an increase in their degradation rates during Period 3, while the degradation rates continued to decrease in the ABIOTIC and F/T CNTL. All of the reactors displayed decreases in their degradation rates during the final degradation period, for both the rates based on O_2 utilization and CO_2 production. These rates are very low, indicating limited microbial activity.

A summary of the degradation rate changes over the 4 degradation rate periods is presented in Table 4.7 and 4.8 below.

Table 4.7: Summary of the Changes in Degradation Rates Between the Four Degradation Rate Periods Based on O_2 Utilization

	Change in Degradation Rate [Δ %]		
Reactor	**Rate 1 - Rate 2**	**Rate 2 - Rate 3**	**Rate 3 - Rate 4**
ABIOTIC	-73.51	-66.86	-73.48
F/T 1	-94.00	139.22	-72.84
F/T 3	-95.81	34.69	-57.97
F/T 6	-97.07	171.91	-69.93
F/T 9	-97.56	270.22	-74.42
F/T CNTL	-92.63	-19.92	-90.15

Table 4.8: Summary of the Changes in Degradation Rates between the Four Degradation Rate Periods Based on CO_2 Production

	Change in Degradation Rate [Δ %]		
Reactor	**Rate 1 - Rate 2**	**Rate 2 - Rate 3**	**Rate 3 - Rate 4**
ABIOTIC	-88.13	-47.83	-14.49
F/T 1	-95.64	199.28	-77.53
F/T 3	-97.49	154.49	-48.37
F/T 6	-98.14	279.99	-69.46
F/T 9	-98.10	334.74	-61.22
F/T CNTL	-94.11	-28.53	-80.26

As can be observed from Tables 4.7 and 4.8, the Freeze-Thaw Treatment reactors experienced significant increases in the rates of degradation of $C_{14}H_{24}$ after the application of the freeze-thaw treatment. The reactors subjected to 9 freeze-thaw cycles demonstrated the largest increases in degradation rates, with a 270% observed rate increase based on O_2 utilization and a 335% observed rate increase based on CO_2 production. The reactors subjected to 6 freeze-thaw cycles experienced a 172% rate increase based on O_2 utilization and a 280% rate increase based on CO_2 production. A 139% increase and a 199% increase base on O_2 utilization and CO_2 production respectively, was observed in the reactors subjected to 1 freeze-thaw cycle. The smallest increase in post freeze-thaw treatment degradation rate was demonstrated by the reactors subjected to 3 freeze-thaw cycles. The observed degradation rate increases were 35% and 154% base on O_2 utilization and CO_2 production respectively.

The $C_{14}H_{24}$ degradation rates observed in Period 4 may be misleading. As these rates are estimated from the concentration of microbial respiration bi-products within the reactor headspace, the low rates of O_2 utilization and CO_2 production may indicated a change in microbial activity. The bacteria may have degraded all readily-available substrates during previous degradation periods, and may have shifted to a cannibalistic endogenous respiration. Therefore, the utilization of O_2 and production of CO_2 during Period 4 may not be truly estimating microbial aerobic-degradation of hydrocarbons, but microbial utilization of leaked intercellular solutes from leathally damaged cells.

4.3 Final Reactor Concentrations

After the respiration study was completed, the reactors were opened up and the soil samples were analyzed for their final TEH concentrations. The degradation rate achieved in the reactors can be determined by dividing the concentration of TEH degraded by the duration of the study, 229 days. The reactors' final TEH concentration and the observed degradation rate are provided in Table 4.9 below.

Table 4.9: Final Reactor TEH Concentrations and Degradation Rates

Reactor Group Designation	Initial Concentration of TEH[1] (mg_{TEH} / kg_{soil})	Final Concentration of TEH[1] (mg_{TEH} / kg_{soil})	Concentration of TEH Removed[1] (mg_{TEH} / kg_{soil})	Degradation Rate (mg_{TEH} / kg_{soil}*day)
ABIOTIC	3790.71	57.59	3733.12	16.30
F/T 1	3394.70	31.96	3362.74	14.68
F/T 3	3523.48	25.64	3497.84	15.27
F/T 6	3640.04	50.97	3589.07	15.67
F/T 9	3053.10	55.02	2998.08	13.09
F/T CNTL	3414.41	35.46	3378.95	14.76

[1] Values are an average of triplicate reactor samples

Based on the change in TEH concentrations ($TEH_{INITIAL}$ - TEH_{FINAL}), all the reactors degraded in excess of 98% of their original hydrocarbon contamination. Specifically, the individual groups of reactors achieved the following removal efficiencies:

ABIOTIC = 98.48 %

F/T 1 = 99.06 %

F/T 3 = 99.24 %

F/T 6 = 98.60 %

F/T 9 = 98.20 %

F/T CNTL = 98.96 %

The range of calculated degradation rates from initial and final reactor TEH concentrations are slightly less than the estimated rates based on O_2 utilization and CO_2 production for the first "degradation period" presented in Table 4.6 previously. However, the estimated rates are based on a 78 day time period, while the actual degradation rates are applied to the entire 229 days of the study. As discussed in Section 4.2, the low rates of O_2 utilization and CO_2 production observed during the final 95 days of the study (degradation Period 4), indicated decreased microbial activity, during which time no hydrocarbon degradation was occurring.

A "corrected" degradation rate can be estimated by assuming that all the hydrocarbons available to microbial degradation had been utilized by the end of the third degradation period (day 134). This "corrected" degradation rate can be calculated by dividing the mass of TEH degraded in the reactors by the 134 day period occurring over the first three degradation periods. This "corrected" degradation rates are presented in Table 4.10.

Table 4.10: Corrected TEH Degradation Rates Based on a 134 Day Period

Reactor Group Designation	Concentration of TEH Removed[1] (mg_{TEH} / kg_{soil})	Degradation Rate[2] (mg_{TEH} / kg_{soil}*day)	"Corrected" Degradation Rate[3] (mg_{TEH} / kg_{soil}*day)
ABIOTIC	3733.12	16.30	27.86
F/T 1	3362.74	14.68	25.1
F/T 3	3497.84	15.27	26.1
F/T 6	3589.07	15.67	26.78
F/T 9	2998.08	13.09	22.37
F/T CNTL	3378.95	14.76	25.22

[1] Values are an average of triplicate reactor samples
[2] Based on a 229 day degradation period
[3] Based on a 134 day degradation period

The final reactor concentrations of extracted TEH are compared to the final $C_{14}H_{24}$ concentrations estimated from O_2 utilization and CO_2 production rates in Table 4.11.

Table 4.11: Comparison of Final Reactor TEH Concentrations with Estimated $C_{14}H_{24}$ Based on O_2 Utilization and CO_2 Production Rates

Reactor Group Designation	Final Concentration of TEH[1] (mg_{TEH} / kg_{soil})	Estimated Final Concentration of $C_{14}H_{24}$ Based on O_2 Utilization[1] (mg_{C14H24} / kg_{soil})	Estimated Final Concentration of $C_{14}H_{24}$ Based on CO_2 Production[1] (mg_{C14H24} / kg_{soil})
ABIOTIC	57.59	3189.81	3197.76
F/T 1	31.96	1739.61	1672.20
F/T 3	25.64	2025.33	1805.26
F/T 6	50.97	2244.69	2038.57
F/T 9	55.02	1707.51	1567.71
F/T CNTL	35.46	1766.15	1615.20

[1] Values are an average of triplicate reactor samples

As can bee seen from Table 4.11, the final concentrations of $C_{14}H_{24}$ based on O_2 utilization and CO_2 production rates are substantially larger than final reactor concentrations determined through the soil extraction and GC analysis. The estimated values range from 28.5 to 79 times higher than the actual values.

5.0 Discussion

5.1 Degradation Rates

The final TEH values presented in Table 4.9 previously, indicated that the passive aeration method of biological treatment of the contaminated soil achieved removal efficiencies in excess of 98%. One can conclude that the contaminated soil was successfully bioremediated through passive aeration with the nutrient and moisture amendments used.

The reactors' observed contaminant degradation rates presented in Sections 4.2 and 4.3 are compared to rates reported in literature [64-65] in Table 5.1.

Table 5.1: Study Degradation Rates Compared to Reported Rates

Reactor Group Designation	Actual Degradation Rate (mg_{TEH} / kg_{soil}*day)	Corrected Degradation Rate (mg_{TEH} / kg_{soil}*day)	Degradation Rate Base on O_2 Utilization[2] (mg $C_{14}H_{24}$ / kg_{soil}*day)	Degradation Rate Base on CO_2 Production[2] (mg $C_{14}H_{24}$ / kg_{soil}*day)	Reported Degradation Rates (mg_{Diesel} / kg_{soil}*day)[3]
ABIOTIC	16.30	27.86	5.60	6.16	
F/T 1	14.68	25.10	17.08	18.35	0.4 - 19 [64]
F/T 3	15.27	26.10	17.12	19.63	8 [64]
F/T 6	15.67	26.78	15.79	18.48	7.8 - 40 [65]
F/T 9	13.09	22.37	15.16	16.79	6.6 - 69 [66]
F/T CNTL	14.76	25.22	18.56	20.79	

[1] values are an average of triplicate reactor samples
[2] rates from respiration monitoring degradation Period 1
[3] the estimate for diesel fuel varies between studies

The contaminant degradation rates observed during this study are comparable to the rates reported in literature[64-66]. The degradation rates compare particularly with the values reported by Davis *et al.* [65-66].

The degradation rates presented in the literature are based on in-situ remediation studies which employed a forced aeration, or bioventing, method of delivering oxygen to the soil microbes. The in-situ respiration tests consisted of ventilating the contaminated soil of the unsaturated zone with air and monitoring the depletion O_2 and/or the production of CO_2 over time after the air is turned off [64-66]. In attempts to make a fair comparison of the degradation rates achieved in this study with those found in the literature, only the degradation rates based on O_2 utilization and CO_2 production from the first degradation period are presented. The subsequent degradation periods (periods 2 to 4), exhibited reduced rates. These lower rates may have occurred due to the reduced concentration of readily available contaminants to soil microbes, conditions that were not achieved in the literature studies.

The bioreactors achieved degradation rates within ranges that have been previously observed in actual in-situ remediation studies. The observed degradation rates, both actual (based on the change in TEH concentrations) and estimated (based on headspace O_2 and CO_2 concentrations), are comparable. These results suggest that the method employed for correlating measured biological activity to contaminant degradation may be employed to track site remediation efforts. Soil gas concentrations can be monitored

until observed results indicate a decrease in O_2 utilization or CO_2 production, that would suggest a decrease in microbial activity. A decrease in biological activity may indicate environmental conditions inappropriate for further biodegradation of the contamination. Simple lab analysis of the soil for contaminant and nutrient concentrations, and moisture levels will indicate the causes for the decrease in biological activity. Appropriate actions can be implemented to increase biological activity, should further remediation be required.

The results of this study suggest that field implementation of soil nutrient and moisture amendments, in conjunction with a passive aeration treatment, such as land farming or biopiles, should effectively remediate the site from which the original contaminated soils were obtained. As well, the method employed for correlating measured biological activity to contaminant degradation may be employed to track site remediation efforts.

As can be seen from the table above, the Abiotic Control Reactors (ABIOTIC) achieved estimated degradation rates of 5.60 to 6.16 mg_{C14H24} / kg_{soil}*day and actual rates of 16.30 to 27.86 mg_{C14H24} / kg_{soil}*day. These results indicate that the application of 120 mg/kg_{soil} of Mercuric Chloride was ineffective at killing off all the soil bacteria. However, the lower rates of O_2 utilization and CO_2 production observed from the Abiotic Control Reactors indicate that the Mercuric Chloride dosage did inhibit microbial activity to a certain degree.

5.2 Effects of Freeze-Thaw Treatments

From the estimated $C_{14}H_{24}$ concentration data summarized in Tables 4.6, 4.7, and 4.8, it appears that the freeze-thaw treatments applied to the bioreactors produced positive effects on the hydrocarbon degradation rates. The estimated, post freeze-thaw treatment degradation rates, increased 35% to 270% from the pre-freeze-thaw rates in the data based on O_2 utilization, and 155% to 335% increases were observed in the data based on CO_2 production. Degradation rates were observed to decrease in the non freeze-thaw treated bioreactors (ABIOTIC and F/T CNTL) during the same period. The data also suggests that the degradation rates generally increase with the number of freeze-thaw cycles applied. The degree of degradation rate increases after freeze-thaw treatment, based on both O_2 utilization and CO_2 production were:

$$\text{F/T } 9_{\text{Rate Change}} > \text{F/T } 6_{\text{Rate Change}} > \text{F/T } 1_{\text{Rate Change}} > \text{F/T } 3_{\text{Rate Change}}$$

However, $C_{14}H_{24}$ degradation was estimated from the measurement of the concentration of microbial respiration by-products in the reactor headspace. Therefore, the respiration data only directly measured the microbial activity in the reactors, and not the concentration of $C_{14}H_{24}$ in the reactors' soils.

As mentioned in Section 2.3, freezing and thawing has been noted to cause large flushes in microbial respiration measured in O_2 uptake and CO_2 production [31]. This burst of

activity has been related to the presence of readily available nutrients and soluble carbon compounds released by the freeze-thaw process. Some of these readily available nutrients and soluble carbon are attributed to intercellular solutes which leak out of lethally damaged cells and serve as metabolic substrates for cells not damaged [39].

Therefore, the increased rates of O_2 uptake and CO_2 production after the freeze-thaw treatments may actually have measured the microbial degradation of leaked intercellular solutes, and not the degradation of $C_{14}H_{24}$. The presence of easily degraded intercellular solutes may cause a diauxie effect, inhibiting hydrocarbon degradation [53]. The soil bacteria may have ceased producing the enzymes necessary for hydrocarbon degradation, in favor of the enzymes required to utilize the easier degradable intercellular materials. As each freeze-thaw cycle can destroy up to 60% of the soil's bacteria population [40], the concentration of leaked intercellular material would increase with the number of freeze-thaw cycles. With increasing concentrations of easily-degraded intercellular material, the post freeze-thaw microbial activity should increase with the number of freeze-thaw cycles. As mentioned previously, this trend was observed.

The theory that increased freeze-thaw cycles causes increased microbial activity due to degradation of leaked intercellular solutes, and not the degradation of hydrocarbons, is supported by the bioreactor hydrocarbon removal efficiencies. The observed bioreactor removal efficiencies were opposite to the observed increases in post freeze-thaw degradation rate changes:

$$F/T\ 3_{Removal\ Efficiency} > F/T\ 1_{Removal\ Efficiency} > F/T\ 6_{Removal\ Efficiency} > F/T\ 9_{Removal\ Efficiency}$$

With more readily degradable intercellular material present in the soil matrix, the soil bacteria will concentrate more energy in producing non-hydrocarbon degrading enzymes, and ignoring the target compounds. Therefore, more hydrocarbons would be degraded in the bioreactors with lower concentrations of leaked intercellular materials (caused by less freeze-thaw cycles) during the same time period.

Although the removal efficiencies observed only varied slightly between the Freeze-Thaw Treatment reactors (98.20% to 99.24%), the trend is exactly opposite of the observed increases in post freeze-thaw degradation rates, and supports the theory that these rate changes are due to the bacterial degradation of leaked intercellular materials.

The effect of freeze-thaw on releasing sequestered and sorbed contaminant compounds within soil micropores may have had limited effect on the study's soil. The action of freeze-thaw was intended to disrupt the physical structure of a soil, thereby exposing new soil particle surfaces and any sorbed hydrocarbons to microbial attack. As determined through soil characterization, the study's soil sample was classified as a well graded sand with silt. Approximately 45% of the soil particles were determined to be a fine sand and smaller particles. Sillanpaa and Webber [34] found that freeze-thaw did effect the mean weight diameter of larger aggregates, but had little effect on the soil fraction with diameters less than 0.25 mm. With approximately 45% of the sample's material being in

the fine sand size distribution, and approximately 30% of the material having diameters equal to or less than 0.25mm, the over all affect that the freeze-thaw treatment had on disrupting the soil's physical structure was limited.

5.3 Estimated and Measured Final Contaminant Concentrations

Table 4.11 compares the results of the final hydrocarbon concentrations in the bioreactors. The estimated final concentrations of $C_{14}H_{24}$ based on reactor O_2 utilization and CO_2 production are compared to the actual concentration determined by soil extraction with hexane and GC analysis. The estimated final concentrations are an average of 50 times higher than those determined by the extraction and GC analysis. Three items may contribute to this discrepancy.

5.3.1 Compounds Not Detected By The TEH Method

The TEH method employed utilized a gas chromatograph to detect hexane-extracted hydrocarbon chains within a C_{10} to C_{19} range. Diesel fuel normally consists of hydrocarbon compounds within this C_{10} to C_{19} range [67]. However, as the soil bacteria metabolize these compounds through aerobic degradation, smaller hydrocarbon chains are formed. Some of these chains may be smaller than the C_{10} to C_{19} detection range of the TEH method employed.

Soil bacteria aerobically attack the hydrocarbon chains in one of two ways: terminal oxidation and subterminal oxidation. These forms of attack are demonstrated in figures 5.1 and 5.2 below.

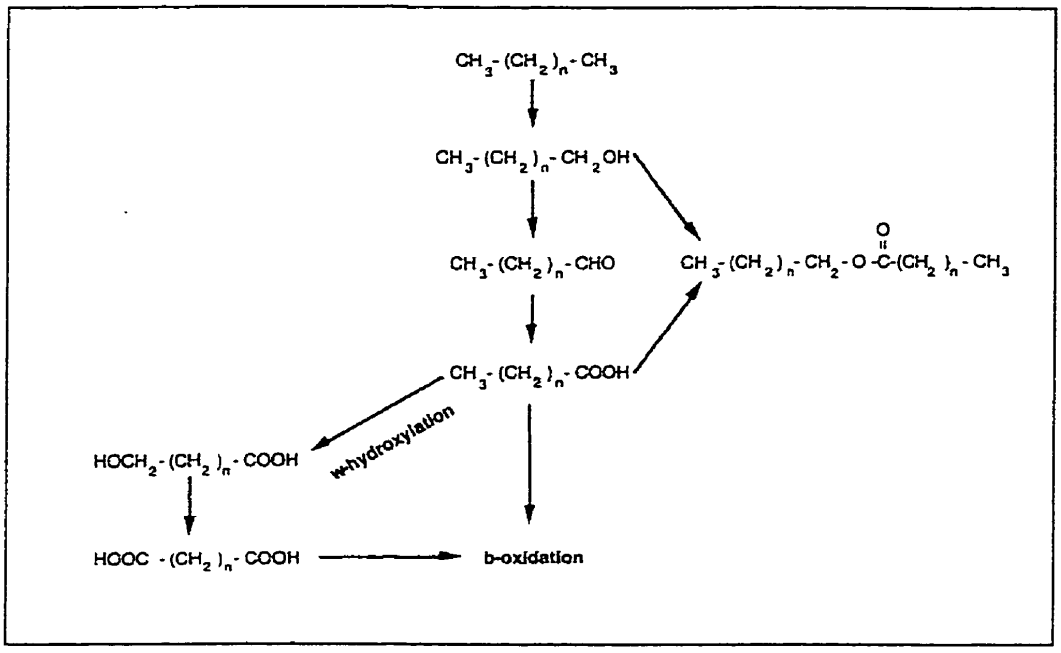

Figure 5.1: Degradation of n-alkanes by Oxidation of the Terminal Methyl Group. The alkane is first oxidized to an alcohol, and then to a corresponding fatty acid. The formed carboxyl groups are then removed by b-oxidation, resulting in a smaller alkane chain. [68]

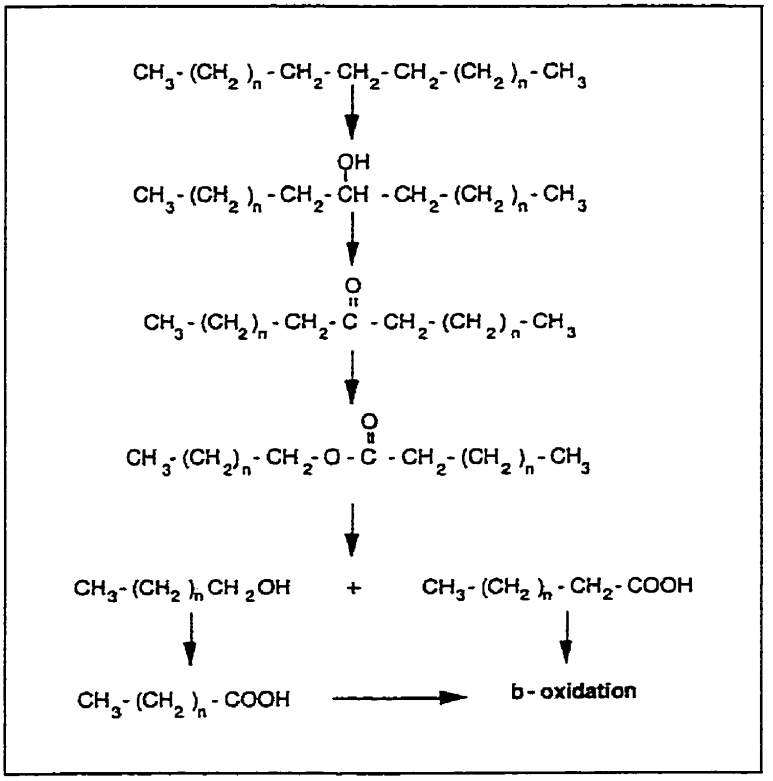

Figure 5.2: Degradation of a Aliphatic Hydrocarbon by Subterminal Oxidation. Through the oxidation of a subterminal methyl group, the hydrocarbon chain is split into two smaller chains, with terminal carboxyl groups. These carboxyl groups are then removed through b-oxidation. [68]

If the majority of the contaminant compounds readily available to the soil bacteria were degraded to smaller hydrocarbon chains, the intermediate compounds may not have been "long enough" to have been detected by the TEH method. The results of the final hydrocarbon concentration analysis may not have accurately reflected the actual concentration of contaminants in the soil. The actual concentration of hydrocarbons

remaining in the soil may have been higher, and may have actually been at the same levels estimated by the monitoring of respiration by-products.

This may also offer reasons for the decrease in microbiological activity measured in the latter portion of the study (> 78 days). Short-chain hydrocarbons, with the exception of methane, are more difficult to degrade [68]. It is therefore conceivable that the soil bacteria had transformed the majority of longer-chained hydrocarbons to shorter intermediate chains within the first 78 days of the study. After this point, the microbial activity had decreased as the bacteria attempted, with limited success, to degrade the more recalcitrant, short-chained hydrocarbons. This limited success, or lack of success in degrading these compounds, was reflected in the decreased concentrations of O_2 utilized and CO_2 produced in the reactors headspace.

The presence of hydrocarbon compounds not detected by the TEH method could have been determined by conducting a Total Organic Carbon (TOC) analysis of the soil. Analyzing the soil for TOC, both prior to, and upon completion of the study, may have provided information on the actual concentrations of hydrocarbons degraded. A high concentration of TOC in the final soil samples would have suggested that the longer-chained hydrocarbons had indeed been transformed to shorter-chained intermediate compounds. A low, final concentration of TOC would have confirmed the accuracy of the final TEH analysis, and may have suggested an error in the method employed to

estimate the concentration of hydrocarbons based on the utilization of headspace O_2 and the production of headspace CO_2 .

5.3.2 Reactor Headspace Pressures

As described in Section 3.4.3, the concentration of O_2 and CO_2 as a percent of headspace air was determined on a Packed-Column GC. The moles of O_2 and CO_2 within the reactor headspace were derived from these values using the Ideal Gas Law, equations 3.9 and 3.10. The assumption made in utilizing this method was that the reactor headspace pressure was assumed to be ambient, 1 atm. Because of the direct relationship between pressure and moles in the Ideal Gas Law, any under or over estimation of the headspace pressure would result in an under or over estimation in the moles of gas calculated. A difference of 0.1 atm would result in an error of 10% on the number of moles calculated. As the volume of the reactor headspace was known, and the temperature also known, underestimating the headspace pressure would result in an underestimation of the actual number of gas moles present in the headspace. During the first 78 days of the study, the reactors demonstrated rapid degradation rates. It is conceivable that the gaseous by-products of microbial respiration during this period may have caused an increase in the reactor headspace pressure. Because the headspace pressure was assumed to be 1 atm, the molar concentration of O_2 or CO_2 calculated would have been underestimated. This would result in underestimating the moles of $C_{14}H_{24}$ degraded during that sample period. The accumulation of this error during every sampling session throughout the study would have resulted in a substantial under estimation of the moles of $C_{14}H_{24}$ degraded. For

future studies implementing the same protocol, the pressure within the reactor headspace should be measured and utilized in the calculation of headspace gas concentrations.

5.3.3 Estimation of Microbial Metabolism

A third item that may have contributed to the underestimation of the concentration of hydrocarbons degraded is the estimated fraction of degraded organic contaminant associated with conversion to microbial cells, as described in section 3.4.2. The fraction of organic material converted to microbial cells (f_s) chosen, 0.5, may not have accurately estimated the actual conversion of the hydrocarbon into cells. An f_s of 0.6 [57] would increase the number of moles of $C_{14}H_{24}$ degraded for every mole of O_2 utilized and CO_2 produced by 10%. Although the 10% error attributed to the value of f_s is minor, when coupled with any cumulative error associated with the under estimation of headspace gas concentrations, the actual concentration of $C_{14}H_{24}$ degraded during the duration of the study might have been substantially underestimated.

6.0 Conclusions

The following conclusions can be derived from this study:

1. The contaminated soil sample obtained from a Manitoba Hydro site in Churchill, Manitoba, was successfully bioremediated in the study's bioreactors, utilizing passive aeration. Over 98% contaminant removal rates were demonstrated in all of the study's bioreactors. Degradation rates of 13 mg $C_{14}H_{24}$ / kg_{soil}*day to 27 mg $C_{14}H_{24}$ / kg_{soil}*day, based on reactor headspace O_2 utilization and CO_2 production, were observed in this study. These rates are comparable to degradation rates found in literature. Field implementation of soil nutrient and moisture amendments, in conjunction with a passive aeration treatment such as biopiles, should effectively remediate the site from which the original contaminated soils were obtained. Remediation efforts in the field may be tracked by monitoring the by-products of microbial respiration. However, initial, intermediate, and final TEH and TOC analysis of the soil may be required to accurately quantify the results of the respiration monitoring.

2. Freezing and thawing did beneficially effect the degradation of hydrocarbon contaminated soils in this study. Freezing and thawing did increased the microbial activity in the soil. Reactors subject to freeze-thaw treatments showed estimated degradation increases of 35% to 335% over pre-freeze-thaw rates. During the same time

period, the reactors not subjected to freeze-thaw treatments demonstrated a 20% to 66% decrease in their estimated degradation rates.

3. Generally, the benefit observed from freeze-thaw treatments increased with the number of freeze-thaw cycles. The greatest increase in post freeze-thaw degradation rates were observed in the reactors subjected to 9 freeze-thaw cycles. The reactors subjected to 6 freeze-thaw cycles had the second greatest increase in degradation rates, while the reactors subjected to 1 freeze-thaw cycle demonstrated the third largest increase. The reactors subjected to 3 freeze-thaw cycles demonstrated the lowest increase in post treatment degradation rates. With the exception of the reactors subjected to 3 freeze-thaw cycles, the post freeze-thaw treatment degradation rates increased with increasing freeze-thaw cycles.

4. The final contaminant concentrations in the reactor soils estimated by the by-products of microbial respiration were an average of 50 times larger than the concentrations determined by the final TEH analysis. These result may be explained by the degradation mechanisms employed by soil bacteria. As the soil bacteria metabolize the contaminant compounds, long hydrocarbon chains are broken-up, forming shorter chained compounds. These shorter chained compounds may not have been long enough to have been detected by the TEH method, which concentrates on compounds within a C_{10} to C_{19} range. A TOC analysis of the final soil samples might have revealed high concentrations of organic carbon, which would have suggested that the TEH method had

indeed underestimated the actual concentration of contaminants present. A low TOC concentration would have suggested an error in the method used to estimate the contaminant concentration from the monitoring of soil bacterial respiration.

References

1. Schneider D.R., Billingsley R.J., 1990. Bioremediation: A Desk Manual for the Environmental Professional. Cahners Publishing Company, Des Plaines, Illinos.

2. Atlas R.M., 1991. Bioremediation of Fossil Fuel Contaminated Sites. In Situ Bioreclamation: Applications and Investigations for Hydrocarbon and Contaminated Site Remediation. Butterworth-Heinemann, United States of America. pp. 14-32 [pg. 14]

3. Cookson, J.T. Jr, Bioremediation Engineering Design and Application, McGraw-Hill Inc., 1995: pg. 3.

4. Talley J.W., Sleeper P.M., 1997. Roadblocks to the Implementation of Bioremediation Strategies. Annals of the New York Academy of Sciences, Vol 829: pp.16-29. [pg. 18]

5. Talley J.W., Sleeper P.M., 1997. Roadblocks to the Implementation of Bioremediation Strategies. Annals of the New York Academy of Sciences, Vol 829: pp.16-29. [pg. 25]

6. Blackburn J.W., Halker W.R., 1993. The Impact of Biochemistry, Bioavailability and Bioactivity on the Selection of Bioremediation Techniques. Trends in Biotechnology, Vol. 11: pp. 328-333. [pg. 330]

7. Rowell M.J., Ashworth J., Qureshi A.A., Bioremediation of Petroleum Hydrocarbons in Soil Environments, Norwest Soil Research Ltd., Edmonton AB, 1992: pg. 20.

8. Calabrese E.J., Kostecki P.T., Hydrocarbon Contaminated Soils and Groundwater, Lewis Publishers Inc, 1992. pp. 337-340

9. Margesin R., Schinner F., 1997. Bioremediation of Diesel-Oil-Contaminated Alpine Soils at Low Temperatures. Applied Microbiology and Biotechnology, Vol. 47: pp. 462-468.

10. Margesin R, Schinner F., 1997. Efficiency of Indigenous and Inoculated Cold-adapted Soil Microorganisms for Biodegradation of Diesel Oil in Alpine Soils. Applied and environmental Microbiology, Vol. 63(7): pp. 2660-2664.

11. D. D. Bell, D. M. Heinrichs, T. Sarauskas, Dr. J. A. Oleskiewicz, and G. Strong. "On-site Bioremediation in Northern Climates." Paper Presented at Techniques and Technologies for Hydrocarbon Remediation in Cold and Arctic Climates, Royal Military College of Canada, Kingston, Ontario, June 6 to 7, 1995.

12. Alexander, M., "Research Needs in Bioremediation", Environ. Sci. Technol., Vol. 25, pp. 1972-1973, 1991.

13. Blackburn, J., and Halker, W.R., "The Impact of Biochemistry, Bioavailability and Bioactivity on the Selection of Bioremediation Techniques", Bioremediation, Vol. 11, pp. 328-333, 1993.

14. Tabak H.A., Govind R., 1997. Bioavailability and Biodegradation Kinetics Protocol for Organic Pollutant Compounds to Achieve Environmentally Acceptable Endpoints during Bioremediation. Annals of the New York Academy of Sciences, Vol 829: pp. 36-61.

15. Zhang W., Bouwer E.J., Ball W.P., 1998. Bioavailability of Hydrophobic Organic Contaminants: Effects and Implications of Sorption-Related Mass Transfer on Bioremediation. Ground Water Monitoring and Remediation, Vol 18(1): pp. 126-128. [pg. 127]

16. Wu, S.C., and Gschwend, P.M., " Sorption Kinetics of Hydrophobic Organic Compounds to Natural Sediments and Soils" , Environ. Sci. Technol., Vol. 20, pp. 717-725, 1986.

17. Zhang W., Bouwer E.J., Ball W.P., 1998. Bioavailability of Hydrophobic Organic Contaminants: Effects and Implications of Sorption-Related Mass Transfer on Bioremediation. Ground Water Monitoring and Remediation, Vol 18(1): pp. 126-128. [pg. 128]

18. Zhang W., Bouwer E.J., Ball W.P., 1998. Bioavailability of Hydrophobic Organic Contaminants: Effects and Implications of Sorption-Related Mass Transfer on Bioremediation. Ground Water Monitoring and Remediation, Vol 18(1): pp. 126-128. [pg. 135]

19. Jones W.L., Dockery J.D., Vogel C.R., Sturman P.J., 1993. Diffusion and reaction within porous packing media: A phenomenological model. Biotechnology and Bioengineering, Vol 41: pp. 947-956. [pg. 953]

20. Zhang W., Bouwer E.J., Ball W.P., 1998. Bioavailability of Hydrophobic Organic Contaminants: Effects and Implications of Sorption-Related Mass Transfer on Bioremediation. Ground Water Monitoring and Remediation, Vol 18(1): pp. 126-128. [pg. 127]

21. Steinberg, S.M., Pignatello, J.J, and Sawhney, B.L., "Persistence of 1,2-Dibromoethane in Soils: Entrapment in Intra-particle Micropores", Environ. Sci. Technol., Vol. 21, pp. 1201-1208, 1987.

22. Hatzinger, P.B., and Alexander, M., "Effect of Aging of Chemicals in Soil on their Biodegradability and Extractability", Environ. Sci. Technol., Vol. 29, pp. 537-545, 1991.

23. Kelsey J.W., Kottler B.D, Alexander M., 1997. Selective Chemical Extractants to Predict Bioavailability of Soil-Aged Organic Chemicals. Environmental Science and Technology, Vol 31: pp. 214-217. [pg. 584]

24. Kelsey J., Alexander M., 1997. Declining Bioavailability and Inappropriate Estimation of Risk of Persistent Compounds. Environmental Toxicology and Chemistry, Vol 16(3): pp. 582-585. [pg. 584]

25. Fu M.H., Mayton H., Alexander M., 1994. Desorption and Biodegradation of Sorbed Styrene in Soil and Aquifer Solids.. Environmental Toxicology and Chemistry, Vol 13 (5): pp. 749-753.

26. Bosma T.N.P., Middeldorp J.M., Schraa G., Zehnder A.J.B., 1997. Mass Transfer Limitation of Biotransformation: Quantifying Bioavailability. Environmental Science and Technology, Vol 31: pp. 248-252.

27. Rasiah V., Vorony R.P., Kachanoski R.G., 1992. Bioavailability of Stabilized Oily Waste Organics in Ultrasonified Soil Aggregates. Water Air and Soil Pollution, Vol. 63 (1-2): pp. 179-186.

28. Gregorich E.G., Kachanoski R.G., Voroney R.P., 1989. Carbon Mineralization in Soil Fractions after various Amounts of Aggregate Disruption. Journal of soil Science, Vol. 40: pp. 649-659.

29. Wang F.L., Bettany J.R., 1993. Influence of Freeze-Thaw and Flooding on the Loss of Soluble Organic Carbon and Carbon Dioxide from Soil. Journal of Environmental Quality, Vol 22: pp. 709-714.

30. Foth, H.D., Fundamentals of Soil Science, John Wiley & Sons, pg. 40, 1978.

31. Edwards A.C., Cresser M.S., 1992. Freezing and Its Effect on Chemical and Biological Properties of Soil. Advanced Soil Science, Vol. 18: pp. 59-79.

32. Lehrsch G.A., Sojka R.E., Carter D.L., Jolley P.M., 1991. Freezing Effects on Aggregate Stability Affected by Texture, Mineralogy, and Organic Matter. Soil Science Society of America Journal, Vol 55: pp. 1401-1406.

33. Edwards, L.M., The Effect of Alternate Freezing and Thawing on Aggregate Stability and Aggregate Size Distribution of some Prince Edward Island Soils, J. Soil Sci., Vol. 42, pp. 193-204, 1991.

34. Sillanpaa, M., and Webber, L.R., The effect of Freezing-Thawing and Wetting-Drying Cycles on Soil Aggregation, Can. J. Soil Sci., Vol. 41, pp. 182-187, 1961.

35. Eigenbrod K.D., 1996. Effects of cyclic freezing and thawing on volume changes and permeabilities of soft fine-grained soils. Canadian Geotechnical Journal, Vol 33: pp. 529-537.

36. Vaz M.D.R., Edwards A.C., Shand C.A., Cresser M.SW., 1994. Changes in the chemistry of soil solution and acetic-acid extractable P following different types of freeze/thaw episodes. European Journal of Soil Science, Vol 45: pp. 353-359.

37. Christensen S., Christensen B.T., 1991. Organic matter available for denitrification in different soil fractions: effect of freeze/thaw cycles and straw disposal. Journal of Soil Sciences, Vol 42: pp. 637-647.

38. Mack, A.R., "Biological Activity and Mineralization of Nitrogen in three soils as Induced By Freezing and Drying", Can. J. Soil Sci., Vol. 43, pp. 316-324, 1963.

39. Hugo, W.B., Inhibition and Destruction of the Microbial Cell, Academic Press Inc., pp. 62-63, 1971.

40. Morley C.R., Trofymow J.A., Coleman D.C., Cambardella C., 1983. Effects of Freeze-thaw Stress on Bacterial populations in Soil Microcosms. Microbial Ecology, Vol 9: pp. 329-340.

41. Skogland, T., Lomeland, S., and Goksoyr, J., "Respiratory Burst After freezing and Thawing of soil: Experiments with Soil Bacteria", Soil Biol. Biochem., Vol. 20, pp. 851-856, 1988.

42. Peterson G.. Overburden Logs from a drilling program conducted at the Churchill Tank Farm, conducted 23 to 29 August, 1995. Manitoba Hydro, 1995.

43. Prescott L.M., Harley J.P., Klien D.A.. Microbiology. Wm. C. Brown Publishers, 1990. pp. 122-123.

44. Rowell M.J., Ashworth J., Qureshi A.A., Bioremediation of Petroleum Hydrocarbons in Soil Environments, Norwest Soil Research Ltd., Edmonton AB, 1992: pp. 65-66.

45. Riser-Roberts E., Bioremediation of Petroleum Contaminated Sites. C.K. Smoley, 1992: pp. 88-89.

46. Cookson, J.T. Jr, Bioremediation Engineering Design and Application, McGraw-Hill Inc., 1995: pp. 89-90.

47. Cookson, J.T. Jr, Bioremediation Engineering Design and Application, McGraw-Hill Inc., 1995: pg. 100.

48. Riser-Roberts E., Bioremediation of Petroleum Contaminated Sites. C.K. Smoley, 1992: pg. A-144.

49. Cookson, J.T. Jr, Bioremediation Engineering Design and Application, McGraw-Hill Inc., 1995: pg. 87.

50. Riser-Roberts E., Bioremediation of Petroleum Contaminated Sites. C.K. Smoley, 1992: pg. A-174.

51. Rowell M.J., Ashworth J., Qureshi A.A., Bioremediation of Petroleum Hydrocarbons in Soil Environments, Norwest Soil Research Ltd., Edmonton AB, 1992: pg. 64.

52. Calabrese E.J., Kostecki P.T., Hydrocarbon Contaminated Soils and Groundwater, Lewis Publishers Inc, 1992. pg. 273.

53. Riser-Roberts E., Bioremediation of Petroleum Contaminated Sites. C.K. Smoley, 1992: pg. A-140.

54. Hosler K.R., Booth R.M., Interim Report on the Development of a Protocol for Performing Bench-Scale Soil Bioremediation Treatability Studies. A Protocol prepared for the Development and Demonstration of Site Remediation Technology (DESRT) Program.

55. Hosler K.R., Booth R.M., Interim Report on the Development of a Protocol for Performing Bench-Scale Soil Bioremediation Treatability Studies. A Protocol prepared for the Development and Demonstration of Site Remediation Technology (DESRT) Program: pg. 30.

56. Remediation Technology Laboratory, Groundwater Technology Inc. Final Report: Laboratory Treatability Evaluation for Bioremediation of Contaminated Soils. Concord CA: Groundwater Technology Inc., 1991.

57. Cookson, J.T. Jr, Bioremediation Engineering Design and Application, McGraw-Hill Inc., 1995: pp. 471-484.

58. Saberiyan A.G., MacPherson J.R. Jr., Andrilenas R.M., Pruess A.J., A Bench-Scale Biotreatibility Methodology to Evaluate Field Remediation. Monitoring and Verification of Bioremediation, ed. Hinchee R.E., Douglas G.S., Ong S.K., Columbus, Ohio: Battelle Press, 1995: pp. 185-191.

59. Hosler K.R., Booth R.M., Interim Report on the Development of a Protocol for Performing Bench-Scale Soil Bioremediation Treatability Studies. A Protocol prepared for the Development and Demonstration of Site Remediation Technology (DESRT) Program: pg. 31.

60. Calabrese E.J., Kostecki P.T., Hydrocarbon Contaminated Soils and Groundwater, Lewis Publishers Inc, 1992. pp. 162-163.

61. ASTM, D2487-93: Standard Classification of Soils for Engineering Purposes (Unified Soil Classification System). American Society for Testing and Materials, West Consshohocken PA, 1997.

62. Foth H.D., Fundamentals of Soil Science, 6th ed. John Wiley and Sons Inc., 1978: pg. 43.

63. Cookson, J.T. Jr, Bioremediation Engineering Design and Application, McGraw-Hill Inc., 1995: pp. 226-227.

64. Hinchee R.E., Ong S.K., A Rapid In Situ Respiration Test for Measuring Aerobic Biodegradation Rates of Hydrocarbons in Soil. Journal of Air Waste Management Association, Vol 42: pp. 1305-1312.

65. Davis G.B., Johnston C.D., Patterson B.M., Barber C., Bennett M., Sheehy A., Dunbavan M., "Monitoring Bioremediation of Weathered Diesel NAPL Using Oxygen Depletion Profiles," in Monitoring and Verification of Bioremediation, ed. Hinchee R.E., Douglas G.S., and Ong S.K.. Battelle Press, 1995: pp. 193-201.

66. Davis G.B., Johnston C.D., Barber C., Bennett M., Estimation of Biodegradation Rates Using Respiration Tests During In Situ Bioremediation of Weathered Diesel NAPL. Ground Water Monitoring and Remediation, Vol 8, No. 2, Spring 1998: pp. 123-132.

67. Riser-Roberts E., Bioremediation of Petroleum Contaminated Sites. C.K. Smoley, 1992: pg. A-9.

68. Cookson, J.T. Jr, Bioremediation Engineering Design and Application, McGraw-Hill Inc., 1995: pp. 100-101.

69. Calabrese E.J., Kostecki P.T., Hydrocarbon Contaminated Soils and Groundwater, Lewis Publishers Inc, 1992. pp. 264-266.

APPENDIX A:

INITIAL SOIL CHARACTERIZATION

Initial Soil Characterization

The following values are the results of the intial physical and chemical lab characterization of the composite Manitoba Hydro soil sample from Churchill, Manitoba. Note that all of the presented values are an average of three samples.

1. $pH_{supernatant} = 8.33$
   $pH_{sediment} = 8.13$

2. VOC = 1.1854×10^{-2} g VOC / g Soil

3. Moisture Content = 9.9391×10^{-2} g H_2O / g Soil

4. Bioavailable Nitrogen = 0.00367 mg NO_3 - N / g Soil

5. Bioavailable Phosphorous = 0.00033 mg PO_4 - P / g Soil

6. Soil Texture = SW-SM *

7. Porosity = 38 %

8. Soil Density = 1425.67 kg / m^3

9. Initial Total Extractable Hydrocarbons (TEH) = 3883.81 mg TEH / kg Soil
   = 3883.81 ppm

* SW-SM = well graded sand with silt

Table A.1 presents the results of the initial composite soil sample sieve analysis.

Table A.1: Sieve Analysis Results for Composite Soil Sample

Sieve Size	Approximate Diameter (mm)	% Retained (by weight)	Soil Classification
1/2	12.7	0	Fine Gravel
No. 4	4.75	10.8	Coarse Sand
No. 8	2.36	12.9	Coarse Sand
No. 10	2.0	2.4	Medium Sand
No. 16	1.18	10.4	Medium Sand
No. 20	0.85	7.5	Medium Sand
No. 30	0.6	10.7	Medium Sand
No. 40	0.425	15.1	Fine Sand
No. 60	0.25	15.4	Fine Sand
No. 100	0.15	4.0	Fine Sand
No. 200	0.075	3.6	Fine Sand
< No. 200	< 0.75	7.2	Silts and Clays

APPENDIX B:

REACTOR HEADSPACE DATA

APPENDIX B1:

REACTOR HEADSPACE OXYGEN DATA

Reactor Headspace Oxygen Content (% O_2)

	Days											
Reactor	**0**	**2**	**4**	**7**	**7 X-Change**	**9**	**9 X-Change**	**18**	**18 X-Change**	**22**	**25**	**25 X-Change**
ABIOTIC	21	19.744275	19.570881	18.618257	18.618257	18.516921	21	18.599174	21	19.401235	18.9	21
F/T 1	21	17.767176	14.850202	10.224066	10.224066	8.3760684	21	12.119835	21	17.003086	15.518065	21
F/T 3	21	18.034351	15.190283	11.008299	11.008299	9.7045962	21	9.2561983	21	15.425926	13.616129	21
F/T 6	21	18.114504	16.610891	14.027939	14.877178	13.012821	19.474359	12.097796	21	15.70679	13.480645	21
F/T 9	21	18.408397	16.267206	12.257261	12.257261	10.978632	21	11.049587	21	16.938272	15.422581	21
F/T CNTL	21	18.087786	15.275304	10.514523	10.514523	8.974359	21	10.557851	21	15.555556	13.593548	21

Note: "# X-Change" indicates a Reactor Headspace Exchange. The headspace oxygen content was brought up to approximately atmospheric concentrations, insuring aerobic respiration.

Reactor Headspace Temperature

	Days											
	0	**2**	**4**	**7**	**7 X-Change**	**9**	**9 X-Change**	**18**	**18 X-Change**	**22**	**25**	**25 X-Change**
Deg C	26	25	26	23	23	22	22	24.5	24.5	24	24	24
Deg K	299.15	298.15	299.15	296.15	296.15	295.15	295.15	297.65	297.65	297.15	297.15	297.15

Reactor Headspace Oxygen Content (% O_2)

	Days											
Reactor	**29**	**32**	**37**	**41**	**41 X-Change**	**43**	**46**	**51**	**56**	**62**	**66**	**66 X-Change**
ABIOTIC	19.627287	19.122016	18.442013	18.126483	21	20.275862	19.461207	18.615044	18.073276	17.648448	17.094406	21
F/T 1	18.209302	16.613333	14.571098	12.68559	21	19.581897	18.375	16.446903	15.056034	13.302222	12.287943	21
F/T 3	17.139535	15.41855	13.5625	12.532751	21	19.219828	18.179598	16.177581	15.086207	14.237778	13.821277	21
F/T 6	16.953488	15.166667	12.6875	11.524017	21	20.063865	17.469828	15.331858	13.910556	12.816705	12.242553	21
F/T 9	16.930233	15.707856	14.552632	13.266376	21	19.672414	18.284483	16.911504	15.719828	14.786402	14.089362	21
F/T CNTL	17.488372	15.68	13.055921	11.860262	21	18.918103	17.258621	15.115044	13.125	12.133333	11.348936	21

Note: "# X-Change" indicates a Reactor Headspace Exchange. The headspace oxygen content was brought up to approximately atmospheric concentrations, insuring aerobic respiration.

Reactor Headspace Temperature

	Days											
	29	**32**	**37**	**41**	**41 X-Change**	**43**	**46**	**51**	**56**	**62**	**66**	**66 X-Change**
Deg C	24	25.5	24	23	23	24	25	24	24	25	24	24
Deg K	297.15	298.65	297.15	296.15	296.15	297.15	298.15	297.15	297.15	298.15	297.15	297.15

Reactor Headspace Oxygen Content (% O_2)

	Days											
Reactor	**70**	**74**	**78**	**84**	**88**	**91**	**91 X-Change**	**94**	**100**	**100 x-change**	**105**	**108**
ABIOTIC	20.624876	19.037162	18.550923	18.02085	17.846245	17.757218	17.546217	16.786805	15.189802	21	19.915013	19.7948
F/T 1	19.811927	17.878378	15.877483	14.27541	13.524272	13.187097	21	20.932258	20.932258	21	20.870128	19.429927
F/T 3	20.082367	18.327703	17.013245	16.238614	15.94822	15.765591	21	21	20.954839	21	20.263333	19.168917
F/T 6	20.293578	18.918919	17.5	17.062	16.740129	16.308602	21	21	21	21	20.85503	19.294687
F/T 9	20.518349	19.108108	17.546358	17.12306	16.831715	16.778495	21	20.932258	20.932258	21	20.83432	19.891682
F/T CNTL	19.651376	16.97973	16.271523	15.193443	15.042071	14.745161	14.745161	14.515723	14.1983	21	19.157895	18.875126

Note: "# X-Change" indicates a Reactor Headspace Exchange. The headspace oxygen content was brought up to approximately atmospheric concentrations, insuring aerobic respiration.

Reactor Headspace Temperature

	Days											
	70	**74**	**78**	**84**	**88**	**91**	**91 X-Change**	**94**	**100**	**100 x-change**	**105**	**108**
Deg C	24	23	24	24	25	24.5	24.5	24	28	26	25	25.5
Deg K	297.15	296.15	297.15	297.15	298.15	297.65	297.65	297.15	299.15	299.15	298.15	298.65

Reactor Headspace Oxygen Content (% O_2)

	Days											
Reactor	**111**	**113**	**118**	**121**	**126**	**130**	**134**	**146**	**154**	**163**	**169**	**175**
ABIOTIC	19.678718	19.36316	19.047601	18.831458	18.666544	18.414738	18.311425	18.192252	18.160269	18.128287	18.107896	18.087504
F/T 1	17.901274	17.633758	15.826408	15.111111	14.220894	13.761041	13.66401	13.017921	12.584459	12.400892	12.347391	12.29389
F/T 3	18.414013	18.033797	17.771017	17.663889	17.584519	17.455323	17.303647	17.114664	17.046667	16.964939	16.914074	16.863209
F/T 6	18.748392	18.205709	17.656457	17.395686	17.302793	17.191194	17.053767	16.919301	16.804345	17.041996	16.921414	16.800832
F/T 9	18.949045	18.652782	17.218837	17.020627	16.961034	16.808907	16.713495	16.613015	16.531532	16.426867	16.36201	16.297153
F/T CNTL	18.592357	18.480892	17.044321	16.469444	16.241698	15.985075	15.836031	15.795996	15.75596	15.715925	15.705916	15.695908

Note: "# X-Change" indicates a Reactor Headspace Exchange. The headspace oxygen content was brought up to approximately atmospheric concentrations, insuring aerobic respiration.

Reactor Headspace Temperature

	Days											
	111	**113**	**118**	**121**	**126**	**130**	**134**	**146**	**154**	**163**	**169**	**175**
Deg C	24	25	24	23	23	24	22	22	22	23.5	22	21.5
Deg K	297.15	298.15	297.15	296.15	296.15	297.15	295.15	295.15	295.15	296.65	295.15	294.65

Reactor Headspace Oxygen Content (% O_2)

	Days							
Reactor	**181**	**187**	**196**	**203**	**210**	**216**	**221**	**229**
ABIOTIC	18.067113	18.046721	18.014739	17.982756	17.960631	17.950838	17.933426	17.939308
F/T 1	12.240389	12.186887	12.075987	12.03641	11.97907	11.921089	11.840791	11.763853
F/T 3	16.812344	16.761479	16.667964	16.518244	16.456666	16.337238	16.206379	16.087807
F/T 6	16.680251	16.559669	16.470851	16.366087	16.311473	16.200149	16.05705	15.973691
F/T 9	16.232296	16.167439	16.129032	16.014489	15.956666	15.938315	15.777083	15.713017
F/T CNTL	15.685899	15.67589	15.635855	15.59582	15.555784	15.529095	15.503472	15.51575

Note: "# X-Change" indicates a Reactor Headspace Exchange. The headspace oxygen content was brought up to approximately atmospheric concentrations, insuring aerobic respiration.

Reactor Headspace Temperature

	Days							
	181	**187**	**196**	**203**	**210**	**216**	**221**	**229**
Deg C	22	21	21.5	22	21	21	22	22
Deg K	295.15	294.15	294.65	295.15	294.15	294.15	295.15	295.15

Reactor Headspace Oxygen Concentration (Moles O_2)

	Days											
Reactor	**0**	**2**	**4**	**7**	**7 X-Change**	**9**	**9 X-Change**	**18**	**18 X-Change**	**22**	**25**	**25 X-Change**
ABIOTIC	0.0147139	0.0138804	0.0137126	0.0131772	0.0131772	0.0131499	0.0149133	0.0130974	0.014788	0.0136852	0.0133316	0.0148129
F/T 1	0.0147139	0.0124905	0.010405	0.0072362	0.0072362	0.0059483	0.0149133	0.0085347	0.014788	0.0119936	0.0109461	0.0148129
F/T 3	0.0147139	0.0126783	0.0106432	0.0077912	0.0077912	0.0068918	0.0149133	0.0065181	0.014788	0.0108811	0.0096045	0.0148129
F/T 6	0.0147139	0.0127347	0.0116386	0.0099284	0.0105295	0.0092411	0.0138298	0.0085192	0.014788	0.0110792	0.0095089	0.0148129
F/T 9	0.0147139	0.0129413	0.0113978	0.0086752	0.0086752	0.0077965	0.0149133	0.007781	0.014788	0.0119479	0.0108787	0.0148129
F/T CNTL	0.0147139	0.0127159	0.0107028	0.0074417	0.0074417	0.0063732	0.0149133	0.0074348	0.014788	0.0109725	0.0095886	0.0148129

Moles of Oxygen Utilized (Moles O_2)

	Days											
Reactor	**0**	**2**	**4**	**7**	**9**	**18**	**22**	**25**	**29**	**32**	**37**	**41**
ABIOTIC	0	0.0008334	0.0010013	0.0015366	0.001564	0.0033799	0.0044827	0.0048363	0.0058046	0.0062287	0.0066406	0.00682
F/T 1	0	0.0022234	0.0043089	0.0074777	0.0087656	0.0151442	0.0179386	0.0189861	0.0209546	0.0221392	0.0235209	0.0248207
F/T 3	0	0.0020355	0.0040706	0.0069227	0.0078221	0.0162172	0.0201242	0.0214008	0.0235497	0.0248183	0.0260895	0.026786
F/T 6	0	0.0019792	0.0030753	0.0047855	0.0060738	0.0113845	0.0150933	0.0166636	0.0195179	0.020832	0.022527	0.0233203
F/T 9	0	0.0017726	0.0033161	0.0060387	0.0069173	0.0140496	0.0168898	0.0179589	0.0208296	0.0217446	0.0225038	0.0233795
F/T CNTL	0	0.001998	0.0040111	0.0072721	0.0083407	0.0158192	0.0196347	0.0210187	0.0234957	0.0248268	0.0266222	0.0274374

Reactor Headspace Oxygen Concentration (Moles O_2)

	Days											
Reactor	**29**	**32**	**37**	**41**	**41 X-Change**	**43**	**46**	**51**	**56**	**62**	**66**	**66 X-Change**
ABIOTIC	0.0138446	0.0134205	0.0130086	0.0128292	0.0148629	0.0143021	0.0136814	0.0131306	0.0127485	0.0124071	0.012058	0.0148129
F/T 1	0.0128444	0.0116598	0.0102781	0.0089783	0.0148629	0.0138126	0.0129178	0.0116013	0.0106202	0.0093516	0.0086676	0.0148129
F/T 3	0.0120898	0.0108213	0.0095667	0.0088702	0.0148629	0.0135572	0.0127805	0.0114113	0.0106415	0.0100093	0.0097492	0.0148129
F/T 6	0.0119586	0.0106445	0.0089495	0.0081562	0.0148629	0.0141526	0.0122815	0.0108147	0.0098122	0.0090103	0.0086356	0.0148129
F/T 9	0.0119422	0.0110243	0.0102651	0.0093894	0.0148629	0.0138765	0.0128542	0.011929	0.0110884	0.010395	0.0099383	0.0148129
F/T CNTL	0.0123359	0.0110048	0.0092093	0.0083942	0.0148629	0.0133444	0.012133	0.0106618	0.0092581	0.0085299	0.0080053	0.0148129

Moles of Oxygen Utilized (Moles O_2)

	Days											
Reactor	**43**	**46**	**51**	**56**	**62**	**66**	**70**	**74**	**78**	**84**	**88**	**91**
ABIOTIC	0.0073808	0.0080015	0.0085523	0.0089345	0.0092759	0.0096249	0.0098896	0.0109641	0.0113525	0.0117264	0.0118918	0.0119334
F/T 1	0.025871	0.0267658	0.0280824	0.0290634	0.030332	0.031016	0.031854	0.0331753	0.0346293	0.0357594	0.0363212	0.0365427
F/T 3	0.0280917	0.0288685	0.0302376	0.0310075	0.0316396	0.0318997	0.032547	0.033741	0.0347119	0.0352583	0.0355008	0.0355905
F/T 6	0.0240306	0.0259017	0.0273685	0.028371	0.0291729	0.0295476	0.0300459	0.0309705	0.0320164	0.0323181	0.0325847	0.0328277
F/T 9	0.024366	0.0253883	0.0263135	0.0271541	0.0278475	0.0283042	0.0286439	0.0295931	0.0307403	0.0310389	0.0312842	0.0313018
F/T CNTL	0.0289559	0.0301673	0.0316385	0.0330422	0.0337704	0.034295	0.0352463	0.0370904	0.0376304	0.0383908	0.0385332	0.0387245

Reactor Headspace Oxygen Concentration (Moles O_2)

	Days											
Reactor	**70**	**74**	**78**	**84**	**88**	**91**	**91 X-Change**	**94**	**100**	**100 x-change**	**105**	**108**
ABIOTIC	0.0145483	0.0134737	0.0130854	0.0127115	0.0125461	0.0125045	0.0123559	0.011841	0.0106429	0.0147139	0.0140005	0.0138927
F/T 1	0.0139749	0.0126536	0.0111996	0.0100695	0.0095077	0.0092862	0.014788	0.0147651	0.0146664	0.0147139	0.0146719	0.0136366
F/T 3	0.0141656	0.0129716	0.0120007	0.0114543	0.0112118	0.011102	0.014788	0.0148129	0.0146822	0.0147139	0.0142453	0.0134534
F/T 6	0.0143146	0.01339	0.0123441	0.0120351	0.0117685	0.0114844	0.014788	0.0148129	0.0147139	0.0147139	0.0146613	0.0135417
F/T 9	0.0144732	0.0135239	0.0123768	0.0120782	0.0118329	0.0118153	0.014788	0.0147651	0.0146664	0.0147139	0.0146468	0.0139607
F/T CNTL	0.0138616	0.0120176	0.0114776	0.0107171	0.0105747	0.0103834	0.0103834	0.0102391	0.0099482	0.0147139	0.0134682	0.0132472

Moles of Oxygen Utilized (Moles O_2)

	Days											
Reactor	**94**	**100**	**105**	**108**	**111**	**113**	**118**	**121**	**126**	**130**	**134**	**146**
ABIOTIC	0.0124483	0.0136464	0.0143598	0.0144676	0.0144793	0.0147477	0.0149245	0.0150321	0.0151488	0.0153709	0.0153563	0.0154409
F/T 1	0.0365656	0.0366643	0.0367062	0.0377416	0.038751	0.0389814	0.0402146	0.0406831	0.0413132	0.0416714	0.0416746	0.0421334
F/T 3	0.0355657	0.0356963	0.0361649	0.0369568	0.0374214	0.0377323	0.037875	0.0379085	0.0379646	0.0380976	0.0381219	0.0382561
F/T 6	0.0328028	0.0329018	0.0329544	0.034074	0.034391	0.0348169	0.0351612	0.0353037	0.0353695	0.0354894	0.0355048	0.0356003
F/T 9	0.0313247	0.0314234	0.0314905	0.0321766	0.0327711	0.0330242	0.0339915	0.0340908	0.034133	0.0342807	0.0342681	0.0343394
F/T CNTL	0.0388689	0.0399	0.0404054	0.0406264	0.040759	0.0408814	0.041851	0.0422172	0.0423784	0.0425981	0.0426276	0.0426473

Reactor Headspace Oxygen Concentration (Moles O_2)

	Days											
Reactor	**111**	**113**	**118**	**121**	**126**	**130**	**134**	**146**	**154**	**163**	**169**	**175**
ABIOTIC	0.0138809	0.0136125	0.0134357	0.0133281	0.0132114	0.0129893	0.013004	0.0129193	0.0128966	0.0128088	0.0128594	0.0128668
F/T 1	0.0126271	0.0123967	0.0111636	0.010695	0.010065	0.0097067	0.0097036	0.0092448	0.0089369	0.008762	0.0087686	0.0087454
F/T 3	0.0129888	0.012678	0.0125353	0.0125018	0.0124456	0.0123126	0.0122883	0.0121541	0.0121058	0.0119868	0.0120116	0.0119958
F/T 6	0.0132247	0.0127988	0.0124545	0.0123119	0.0122462	0.0121263	0.0121108	0.0120154	0.0119337	0.0120413	0.0120169	0.0119515
F/T 9	0.0133662	0.0131131	0.0121458	0.0120465	0.0120043	0.0118566	0.0118692	0.0117978	0.01174	0.0116067	0.0116196	0.0115932
F/T CNTL	0.0131146	0.0129923	0.0120227	0.0116564	0.0114952	0.0112755	0.0112461	0.0112176	0.0111892	0.0111043	0.0111537	0.0111655

Moles of Oxygen Utilized (Moles O_2)

	Days											
Reactor	**154**	**163**	**169**	**175**	**181**	**187**	**196**	**203**	**210**	**216**	**221**	**229**
ABIOTIC	0.0154636	0.0155514	0.0155008	0.0154935	0.0155298	0.0155007	0.0155453	0.0155897	0.015562	0.015569	0.0156247	0.0156205
F/T 1	0.0424412	0.0426161	0.0426096	0.0426327	0.0426856	0.0426941	0.0427878	0.0428304	0.0428422	0.0428835	0.0429693	0.043024
F/T 3	0.0382636	0.0383825	0.0383577	0.0383735	0.03843	0.0384256	0.0385124	0.0386388	0.0386428	0.0387279	0.0388603	0.0389445
F/T 6	0.035682	0.0355744	0.0355988	0.0356642	0.0357701	0.0358158	0.035899	0.0359932	0.0359926	0.0360719	0.0362127	0.0362719
F/T 9	0.0343973	0.0345306	0.0345177	0.0345441	0.0346098	0.0346168	0.0346637	0.0347645	0.034767	0.0347801	0.0349331	0.0349786
F/T CNTL	0.0426758	0.0427606	0.0427113	0.0426995	0.0427255	0.0426948	0.0427422	0.0427895	0.0427804	0.0427994	0.0428551	0.0428464

Reactor Headspace Oxygen Concentration (Moles O_2)

	Days							
Reactor	**181**	**187**	**196**	**203**	**210**	**216**	**221**	**229**
ABIOTIC	0.0128305	0.0128596	0.012815	0.0127706	0.0127982	0.0127912	0.0127355	0.0127397
F/T 1	0.0086926	0.008684	0.0085904	0.0085477	0.0085359	0.0084946	0.0084088	0.0083542
F/T 3	0.0119394	0.0119437	0.011857	0.0117305	0.0117265	0.0116414	0.0115091	0.0114249
F/T 6	0.0118456	0.0117999	0.0117167	0.0116225	0.0116231	0.0115438	0.011403	0.0113438
F/T 9	0.0115275	0.0115204	0.0114736	0.0113728	0.0113703	0.0113572	0.0112042	0.0111587
F/T CNTL	0.0111394	0.0111702	0.0111227	0.0110755	0.0110846	0.0110656	0.0110099	0.0110186

Concentration of Total Extractable Hydrocarbons in Reactor Soil (mg_{TEH} / kg_{SOIL})

	Days											
Reactor	**0**	**2**	**4**	**7**	**9**	**18**	**22**	**25**	**29**	**32**	**37**	**41**
ABIOTIC	3790.7133	3758.6519	3752.1933	3731.6002	3730.5492	3660.6935	3618.2681	3604.6671	3567.4184	3551.1017	3535.2559	3528.3548
F/T 1	3394.7	3309.1697	3228.9404	3107.0407	3057.4984	2812.1207	2704.6213	2664.3251	2588.5993	2543.0284	2489.8764	2439.8753
F/T 3	3523.48	3445.1753	3366.8868	3257.1727	3222.5724	2899.6202	2749.3245	2700.2155	2617.5482	2568.7477	2519.8464	2493.0524
F/T 6	3640.0333	3563.8962	3521.7308	3455.9409	3406.381	3202.0851	3059.4107	2999.0041	2889.2016	2838.6491	2773.4437	2742.9284
F/T 9	3053.1	2984.911	2925.5339	2820.7979	2786.9978	2512.6275	2403.3694	2362.241	2251.8076	2216.6079	2187.4016	2153.7145
F/T CNTL	3414.4067	3337.547	3260.1051	3134.6555	3093.5498	2805.8587	2659.0805	2605.8412	2510.5529	2459.3456	2390.278	2358.9204

Concentration Ratio of Total Extractable Hydrocarbons (TEH) in Reactor Soils (C_{TEH} / $C_{O_{TEH}}$)

	Days											
Reactor	**0**	**2**	**4**	**7**	**9**	**18**	**22**	**25**	**29**	**32**	**37**	**41**
ABIOTIC	1	0.9914606	0.9897379	0.9841437	0.9839108	0.9650987	0.9536595	0.9499838	0.9400014	0.9355258	0.931104	0.9290708
F/T 1	1	0.9748033	0.9509209	0.9146999	0.8998772	0.8296233	0.7989923	0.7876268	0.7659721	0.7531198	0.7380617	0.7238662
F/T 3	1	0.9774929	0.9552022	0.9248	0.9155136	0.8255374	0.783218	0.7699315	0.7475571	0.7341619	0.7208363	0.713329
F/T 6	1	0.9791283	0.9675705	0.9495388	0.9360458	0.8801797	0.841155	0.8246756	0.7946606	0.780856	0.7631176	0.7548294
F/T 9	1	0.9776425	0.958272	0.9239991	0.9129862	0.8234975	0.7877374	0.7742644	0.7370944	0.7260903	0.7170236	0.7059526
F/T CNTL	1	0.9766532	0.9531723	0.9151896	0.9026818	0.8165824	0.7726124	0.7562096	0.727446	0.7111498	0.6914697	0.6821148

Concentration of Total Extractable Hydrocarbons in Reactor Soil (mg_{TEH} / kg_{SOIL})

	Days											
Reactor	**43**	**46**	**51**	**56**	**62**	**66**	**70**	**74**	**78**	**84**	**88**	**91**
ABIOTIC	3506.7811	3482.9042	3461.7147	3447.0138	3433.8798	3420.452	3410.273	3368.9346	3353.9961	3339.6126	3333.2504	3331.6495
F/T 1	2399.4708	2365.0492	2314.4025	2276.6612	2227.8607	2201.5488	2169.3103	2118.4814	2062.5489	2019.0765	1997.4634	1988.9439
F/T 3	2442.8231	2412.9419	2360.2715	2330.657	2306.339	2296.333	2271.433	2225.4993	2188.1521	2167.1324	2157.8011	2154.3503
F/T 6	2715.6022	2643.6228	2587.1988	2548.6316	2517.7834	2503.3703	2484.2014	2448.6334	2408.3975	2396.7912	2386.5337	2377.1889
F/T 9	2115.7662	2076.4405	2040.8487	2008.5124	1981.8381	1964.2696	1951.2	1914.6838	1870.5548	1859.0685	1849.631	1848.9539
F/T CNTL	2300.5038	2253.9029	2197.3075	2143.3075	2115.2943	2095.1139	2058.5189	1987.5793	1966.8063	1937.5525	1932.076	1924.7162

Concentration Ratio of Total Extractable Hydrocarbons (TEH) in Reactor Soils (C_{TEH} / Co_{TEH})

	Days											
Reactor	**43**	**46**	**51**	**56**	**62**	**66**	**70**	**74**	**78**	**84**	**88**	**91**
ABIOTIC	0.9232335	0.9167462	0.9110349	0.9070474	0.9034097	0.899691	0.8969902	0.885601	0.8816585	0.877634	0.8759243	0.875442
F/T 1	0.7122943	0.7025238	0.6882756	0.6776491	0.663889	0.6565066	0.6471761	0.6321812	0.615576	0.6027122	0.5963193	0.5937209
F/T 3	0.6991534	0.6912009	0.6767782	0.6687258	0.662149	0.6594476	0.6526533	0.6400049	0.629699	0.624141	0.6216771	0.620717
F/T 6	0.747338	0.7276734	0.7122963	0.7017819	0.6934012	0.6894768	0.6842708	0.6745372	0.663518	0.6603772	0.6575881	0.6549873
F/T 9	0.693572	0.6806852	0.6689795	0.6583494	0.6495069	0.6436348	0.639291	0.6274032	0.612786	0.6089662	0.6058026	0.6055795
F/T CNTL	0.6644466	0.6503906	0.6334602	0.61696	0.6088494	0.6027686	0.5917727	0.5701451	0.5640398	0.5548688	0.553184	0.5511645

Concentration of Total Extractable Hydrocarbons in Reactor Soil (mg_{TEH} / kg_{SOIL})

	Days											
Reactor	**94**	**100**	**105**	**108**	**111**	**113**	**118**	**121**	**126**	**130**	**134**	**146**
ABIOTIC	3311.8426	3265.7521	3238.3081	3234.1608	3233.7087	3223.3837	3216.5833	3212.4437	3207.9536	3199.4105	3199.9741	3196.7184
F/T 1	1988.063	1984.2656	1982.6517	1942.8231	1903.9911	1895.1271	1847.6894	1829.6644	1805.4267	1791.6455	1791.525	1773.8746
F/T 3	2155.3076	2150.2806	2132.2566	2101.7912	2083.9193	2071.9609	2066.4716	2065.1831	2063.0222	2057.9052	2056.9711	2051.8083
F/T 6	2378.1461	2374.3364	2372.3143	2329.2427	2317.0486	2300.6659	2287.4188	2281.9367	2279.4075	2274.7939	2274.2005	2270.527
F/T 9	1848.073	1844.2756	1841.6933	1815.3	1792.4324	1782.6957	1745.4831	1741.6642	1740.0417	1734.3596	1734.8438	1732.0988
F/T CNTL	1919.1625	1907.9734	1860.0539	1851.552	1846.4516	1841.745	1804.4455	1790.3552	1784.1544	1775.7027	1774.5703	1773.8099

Concentration Ratio of Total Extractable Hydrocarbons (TEH) in Reactor Soils (C_{TEH} / Co_{TEH})

	Days											
Reactor	**94**	**100**	**105**	**108**	**111**	**113**	**118**	**121**	**126**	**130**	**134**	**146**
ABIOTIC	0.8702104	0.8574615	0.8499275	0.8488179	0.8486834	0.8458082	0.8438638	0.8426944	0.8414531	0.8390946	0.8391926	0.8382739
F/T 1	0.5934899	0.5923657	0.5919405	0.5801425	0.5686788	0.566115	0.5519299	0.5466232	0.5391849	0.5352609	0.5352253	0.5296479
F/T 3	0.6209911	0.619594	0.6148632	0.6065534	0.601248	0.597842	0.5962915	0.5959928	0.5954058	0.5939921	0.5938258	0.5924531
F/T 6	0.6552504	0.6542035	0.6536602	0.6419278	0.6385606	0.6340496	0.6304018	0.6289227	0.6282346	0.6269781	0.6268303	0.6258321
F/T 9	0.6053203	0.6040704	0.6031844	0.5945715	0.5871195	0.584045	0.5715606	0.5703809	0.5698726	0.5679537	0.5681294	0.5672439
F/T CNTL	0.5496644	0.5458265	0.5312305	0.5284647	0.5267232	0.52531	0.5137428	0.5094118	0.5073623	0.5046082	0.5040919	0.5038383

Concentration of Total Extractable Hydrocarbons in Reactor Soil (mg_{TEH} / kg_{SOIL})

	Days											
Reactor	**154**	**163**	**169**	**175**	**181**	**187**	**196**	**203**	**210**	**216**	**221**	**229**
ABIOTIC	3195.8447	3192.4667	3194.4139	3194.6953	3193.2997	3194.4187	3192.7041	3190.9952	3192.0588	3191.7904	3189.6475	3189.8082
F/T 1	1762.0328	1755.3049	1755.5564	1754.6647	1752.6332	1752.3034	1748.7017	1747.0607	1746.6068	1745.0174	1741.7166	1739.6147
F/T 3	2051.5227	2046.9465	2047.9004	2047.2926	2045.1213	2045.2884	2041.9497	2037.0868	2036.933	2033.6592	2028.567	2025.3277
F/T 6	2267.3865	2271.5248	2270.5847	2268.0694	2263.9964	2262.2402	2259.0394	2255.4138	2255.4367	2252.3851	2246.9712	2244.694
F/T 9	1729.8727	1724.7442	1725.2416	1724.2253	1721.6979	1721.4276	1719.6246	1715.7477	1715.65	1715.1469	1709.262	1707.5118
F/T CNTL	1772.7162	1769.4515	1771.3491	1771.8033	1770.8022	1771.9847	1770.1599	1768.3413	1768.6923	1767.9607	1765.8185	1766.1539

Concentration Ratio of Total Extractable Hydrocarbons (TEH) in Reactor Soils (C_{TEH} / Co_{TEH})

	Days											
Reactor	**154**	**163**	**169**	**175**	**181**	**187**	**196**	**203**	**210**	**216**	**221**	**229**
ABIOTIC	0.8380427	0.8371527	0.8376696	0.8377483	0.8373859	0.837685	0.8372325	0.8367816	0.8370669	0.8369925	0.8364201	0.8364717
F/T 1	0.5258638	0.5238773	0.5239379	0.5236626	0.5230524	0.5229421	0.5218151	0.5213307	0.5212052	0.5207447	0.5197851	0.5191668
F/T 3	0.5923645	0.5910919	0.5913822	0.5912215	0.5906093	0.5906726	0.589766	0.588444	0.5884128	0.5874654	0.5860158	0.5851337
F/T 6	0.6249795	0.6260867	0.6258388	0.6251576	0.6240477	0.6235755	0.6227032	0.6217152	0.6217248	0.6208908	0.6194081	0.6187898
F/T 9	0.5665209	0.5647731	0.5649184	0.5645653	0.563715	0.5636074	0.5630292	0.5617829	0.56176	0.5615921	0.5596914	0.5591148
F/T CNTL	0.5034751	0.5024641	0.5030211	0.5031474	0.5028395	0.503183	0.5026016	0.5020223	0.5020897	0.5018467	0.5011941	0.5012958

APPENDIX B2:

REACTOR HEADSPACE CARBON DIOXIDE DATA

Reactor Headspace Carbon Dioxide Content (% CO_2)

	Days											
Reactor	**0**	**2**	**4**	**7**	**7 X-Change**	**9**	**9 X-Change**	**18**	**18 X-Change**	**22**	**25**	**25 X-Change**
ABIOTIC	0	0.42854	0.91829	1.71956	0	1.75218	0	1.81497	0	1.07021	1.64537	0
F/T 1	0	1.95979	4.3832	7.61956	0	8.93331	0	6.85734	0	3.13271	4.19722	0
F/T 3	0	1.74104	4.3832	7.48178	0	8.33418	0	9.62571	0	4.63271	5.69167	0
F/T 6	0	1.7775	4.20776	7.55289	0	5.91588	1.24211	9.24435	0	4.86188	5.94167	0
F/T 9	0	1.54833	3.5718	6.27511	0	7.59344	0	7.69068	0	3.35146	4.48796	0
F/T CNTL	0	1.77229	4.25162	7.41956	0	8.63919	0	8.83475	0	4.58063	5.98796	0

Note: "# X-Change" indicates a Reactor Headspace Exchange. The headspace Carbon Dioxide content was brought down to approximately atmospheric concentrations, insuring aerobic respiration.

Reactor Headspace Temperature

	Days											
	0	**2**	**4**	**7**	**7 X-Change**	**9**	**9 X-Change**	**18**	**18 X-Change**	**22**	**25**	**25 X-Change**
Deg C	26	25	26	23	23	22	22	24.5	24.5	24	24	24
Deg K	299.15	298.15	299.15	296.15	296.15	295.15	295.15	297.65	297.65	297.15	297.15	297.15

Reactor Headspace Carbon Dioxide Content (% CO_2)

	Days											
Reactor	**29**	**32**	**37**	**41**	**41 X-Change**	**43**	**46**	**51**	**56**	**62**	**66**	**66 X-Change**
ABIOTIC	0.64688	1.23938	1.97677	2.43535	0	1.18609	1.67813	2.11601	2.2696	2.47097	2.77427	0
F/T 1	2.04271	3.29591	4.64343	5.65505	0	2.14409	3.49531	3.88185	4.87366	5.7286	5.92557	0
F/T 3	3.04271	4.25107	5.78485	6.05283	0	2.86588	3.87292	4.58685	5.23352	5.34448	5.4597	0
F/T 6	3.18854	4.48499	6.47172	6.99697	0	3.1021	4.63385	5.24444	5.90449	6.3705	6.60081	0
F/T 9	2.28229	3.42261	4.94646	5.52879	0	2.35407	3.51406	3.98594	4.38815	4.57793	4.86742	0
F/T CNTL	2.69896	4.09513	6.59293	6.96263	0	3.03648	4.89427	5.40317	6.02169	6.16779	6.37319	0

Note: "# X-Change" indicates a Reactor Headspace Exchange. The headspace Carbon Dioxide content was brought down to approximately atmospheric concentrations, insuring aerobic respiration.

Reactor Headspace Temperature

	Days											
	29	**32**	**37**	**41**	**41 X-Change**	**43**	**46**	**51**	**56**	**62**	**66**	**66 X-Change**
Deg C	24	25.5	24	23	23	24	25	24	24	25	24	24
Deg K	297.15	298.65	297.15	296.15	296.15	297.15	298.15	297.15	297.15	298.15	297.15	297.15

Reactor Headspace Carbon Dioxide Content (% CO_2)

	Days											
Reactor	**70**	**74**	**78**	**84**	**88**	**91**	**91 X-Change**	**94**	**100**	**100 x-change**	**105**	**108**
ABIOTIC	1.47112	1.90776	2.02286	2.07012	2.25601	2.36312	2.13869	2.22513	2.40123	0.0407	0.89888	1.15971
F/T 1	3.08168	4.46455	4.53528	4.60601	5.66288	5.84078	0	0	0	0	0.3464	1.74512
F/T 3	2.64538	3.40764	3.47052	3.5334	3.91275	4.22584	0	0	0	0	0.22494	1.50108
F/T 6	2.41011	2.88725	2.92771	2.96818	3.26182	3.36085	0	0	0	0	0.25193	1.62714
F/T 9	2.32251	2.80602	2.8768	2.94759	3.10392	3.3088	0	0	0	0	0.2047	1.18411
F/T CNTL	3.20384	4.04992	4.12169	4.19347	4.82103	4.79757	4.79757	4.82843	4.85929	0	1.71466	2.10977

Note: "# X-Change" indicates a Reactor Headspace Exchange. The headspace Carbon Dioxide content was brought down to approximately atmospheric concentrations, insuring aerobic respiration.

Reactor Headspace Temperature

	Days											
	70	**74**	**78**	**84**	**88**	**91**	**91 X-Change**	**94**	**100**	**100 x-change**	**105**	**108**
Deg C	24	23	24	24	25	24.5	24.5	24	26	26	25	25.5
Deg K	297.15	296.15	297.15	297.15	298.15	297.65	297.65	297.15	299.15	299.15	298.15	298.65

Reactor Headspace Carbon Dioxide Content (% CO_2)

	Days											
Reactor	**111**	**113**	**118**	**121**	**126**	**130**	**134**	**146**	**154**	**163**	**169**	**175**
ABIOTIC	1.23055	1.41276	1.51464	1.56076	1.56953	1.59804	1.63695	1.66311	1.68717	1.71161	1.71835	1.72509
F/T 1	2.53604	3.1371	3.75318	3.99649	4.29762	4.63186	5.03037	5.13327	5.33639	5.5398	5.56282	5.58583
F/T 3	1.78068	2.04361	2.3044	2.48212	2.54003	2.64023	2.68072	2.82024	2.94535	2.96807	2.98615	3.00423
F/T 6	1.93875	2.12509	2.48202	2.57378	2.62922	2.75897	2.81053	2.92447	2.9524	3.09655	3.1102	3.12384
F/T 9	1.60573	2.28198	2.57426	2.66322	2.7475	2.80652	2.92499	3.00191	3.08206	3.13898	3.16399	3.189
F/T CNTL	2.38851	2.45531	2.95335	3.06873	3.20994	3.37569	3.4112	3.44604	3.48357	3.53781	3.54994	3.56207

Note: "# X-Change" indicates a Reactor Headspace Exchange. The headspace Carbon Dioxide content was brought down to approximately atmospheric concentrations, insuring aerobic respiration.

Reactor Headspace Temperature

	Days											
	111	**113**	**118**	**121**	**126**	**130**	**134**	**146**	**154**	**163**	**169**	**175**
Deg C	24	25	24	23	23	24	22	22	22	23.5	22	21.5
Deg K	297.15	298.15	297.15	296.15	296.15	297.15	295.15	295.15	295.15	296.65	295.15	294.65

Reactor Headspace Carbon Dioxide Content (% CO_2)

	Days							
Reactor	**181**	**187**	**196**	**203**	**210**	**216**	**221**	**229**
ABIOTIC	1.73183	1.73857	1.79229	1.94513	1.98544	2.08222	2.179	2.27578
F/T 1	5.60885	5.63187	5.77969	5.87481	5.99625	6.02531	6.05437	6.08343
F/T 3	3.0223	3.04038	3.1621	3.37306	3.53182	3.67112	3.81042	3.94972
F/T 6	3.13749	3.15114	3.20124	3.37592	3.449	3.49692	3.54484	3.59276
F/T 9	3.21401	3.23902	3.34209	3.42948	3.51309	3.66896	3.82483	3.98071
F/T CNTL	3.57421	3.58634	3.62617	3.65645	3.7159	3.72711	3.73832	3.74953

Note: "# X-Change" indicates a Reactor Headspace Exchange. The headspace Carbon Dioxide content was brought down to approximately atmospheric concentrations, insuring aerobic respiration.

Reactor Headspace Temperature

	Days							
	181	**187**	**196**	**203**	**210**	**216**	**221**	**229**
Deg C	22	21	21.5	22	21	21	22	22
Deg K	295.15	294.15	294.65	295.15	294.15	294.15	295.15	295.15

Reactor Headspace Carbon Dioxide Concentration (Moles CO_2)

	Days											
Reactor	**0**	**2**	**4**	**7**	**7 X-Change**	**9**	**9 X-Change**	**18**	**18 X-Change**	**22**	**25**	**25 X-Change**
ABIOTIC	0	0.0003	0.00064	0.00122	0	0.00124	0	0.00128	0	0.00075	0.00116	0
F/T 1	0	0.00138	0.00307	0.00539	0	0.00634	0	0.00483	0	0.00221	0.00296	0
F/T 3	0	0.00122	0.00307	0.0053	0	0.00592	0	0.00678	0	0.00327	0.00401	0
F/T 6	0	0.00125	0.00295	0.00535	0	0.0042	0.00088	0.00651	0	0.00343	0.00419	0
F/T 9	0	0.00109	0.0025	0.00444	0	0.00539	0	0.00542	0	0.00236	0.00317	0
F/T CNTL	0	0.00125	0.00298	0.00525	0	0.00614	0	0.00622	0	0.00323	0.00422	0

Moles of Carbon Dioxide Utilized (Moles CO_2)

	Days											
Reactor	**0**	**2**	**4**	**7**	**9**	**18**	**22**	**25**	**29**	**32**	**37**	**41**
ABIOTIC	0	0.0003	0.00064	0.00122	0.00124	0.00252	0.00328	0.00368	0.00414	0.00455	0.00508	0.00541
F/T 1	0	0.00138	0.00307	0.00539	0.00634	0.01117	0.01338	0.01413	0.01557	0.01645	0.01741	0.01814
F/T 3	0	0.00122	0.00307	0.0053	0.00592	0.0127	0.01596	0.01671	0.01886	0.0197	0.02079	0.021
F/T 6	0	0.00125	0.00295	0.00535	0.0042	0.00983	0.01326	0.01402	0.01627	0.01717	0.01859	0.01897
F/T 9	0	0.00109	0.0025	0.00444	0.00539	0.01081	0.01317	0.01397	0.01558	0.01638	0.01746	0.01789
F/T CNTL	0	0.00125	0.00298	0.00525	0.00614	0.01236	0.01559	0.01658	0.01848	0.01945	0.02123	0.02151

Reactor Headspace Carbon Dioxide Concentration (Moles CO_2)

	Days											
Reactor	**29**	**32**	**37**	**41**	**41 X-Change**	**43**	**46**	**51**	**56**	**62**	**66**	**66 X-Change**
ABIOTIC	0.00046	0.00087	0.00139	0.00172	0	0.00084	0.00118	0.00153	0.00182	0.0017	0.00173	0
F/T 1	0.00144	0.00231	0.00328	0.004	0	0.00151	0.00246	0.00274	0.00344	0.00403	0.00418	0
F/T 3	0.00215	0.00298	0.00408	0.00428	0	0.00202	0.00272	0.00324	0.00369	0.00376	0.00385	0
F/T 6	0.00225	0.00315	0.00456	0.00495	0	0.00219	0.00326	0.0037	0.00416	0.00448	0.00466	0
F/T 9	0.00161	0.0024	0.00349	0.00391	0	0.00166	0.00247	0.00281	0.0031	0.00322	0.00343	0
F/T CNTL	0.0019	0.00287	0.00465	0.00493	0	0.00214	0.00344	0.00381	0.00425	0.00434	0.0045	0

Moles of Carbon Dioxide Utilized (Moles CO_2)

	Days											
Reactor	**43**	**46**	**51**	**56**	**62**	**66**	**70**	**74**	**78**	**84**	**88**	**91**
ABIOTIC	0.00624	0.00659	0.00693	0.00723	0.00711	0.00714	0.00818	0.00849	0.00857	0.0086	0.00873	0.00881
F/T 1	0.01965	0.02059	0.02087	0.02157	0.02216	0.02232	0.02449	0.02548	0.02551	0.02556	0.02634	0.02638
F/T 3	0.02302	0.02372	0.02423	0.02469	0.02475	0.02485	0.02671	0.02726	0.02729	0.02734	0.0276	0.02782
F/T 6	0.02116	0.02223	0.02267	0.02314	0.02345	0.02363	0.02533	0.02567	0.02569	0.02572	0.02598	0.02599
F/T 9	0.01955	0.02036	0.0207	0.02098	0.02111	0.02132	0.02296	0.02331	0.02335	0.0234	0.0235	0.02365
F/T CNTL	0.02365	0.02495	0.02532	0.02576	0.02584	0.026	0.02826	0.02887	0.02891	0.02896	0.02939	0.02938

Reactor Headspace Carbon Dioxide Concentration (Moles CO_2)

	Days											
Reactor	**70**	**74**	**78**	**84**	**88**	**91**	**91 X-Change**	**94**	**100**	**100 x-change**	**105**	**108**
ABIOTIC	0.00104	0.00135	0.00143	0.00146	0.00159	0.00166	0.00151	0.00157	0.00168	2.9E-05	0.00063	0.00081
F/T 1	0.00217	0.00316	0.0032	0.00325	0.00403	0.00407	0	0	0	0	0.00024	0.00122
F/T 3	0.00187	0.00241	0.00245	0.00249	0.00275	0.00298	0	0	0	0	0.00016	0.00105
F/T 6	0.0017	0.00204	0.00207	0.00209	0.00236	0.00237	0	0	0	0	0.00018	0.00114
F/T 9	0.00164	0.00199	0.00203	0.00208	0.00218	0.00233	0	0	0	0	0.00014	0.00083
F/T CNTL	0.00226	0.00287	0.00291	0.00296	0.00339	0.00338	0.00338	0.00341	0.0034	0	0.00121	0.00148

Moles of Carbon Dioxide Utilized (Moles CO_2)

	Days											
Reactor	**94**	**100**	**105**	**108**	**111**	**113**	**118**	**121**	**126**	**130**	**134**	**146**
ABIOTIC	0.00887	0.00898	0.00959	0.00977	0.00982	0.00995	0.01002	0.01006	0.01006	0.01008	0.01012	0.01013
F/T 1	0.02638	0.02638	0.02663	0.02761	0.02817	0.02859	0.02903	0.02921	0.02943	0.02965	0.02996	0.03003
F/T 3	0.02782	0.02782	0.02798	0.02888	0.02908	0.02926	0.02945	0.02958	0.02962	0.02968	0.02973	0.02983
F/T 6	0.02599	0.02599	0.02617	0.02714	0.02736	0.02749	0.02775	0.02782	0.02786	0.02794	0.02799	0.02807
F/T 9	0.02365	0.02365	0.02379	0.02448	0.02478	0.02525	0.02547	0.02554	0.02559	0.02563	0.02573	0.02578
F/T CNTL	0.02941	0.03	0.03061	0.03089	0.03109	0.03113	0.03149	0.03158	0.03168	0.03179	0.03183	0.03186

Reactor Headspace Carbon Dioxide Concentration (Moles CO_2)

	Days											
Reactor	**111**	**113**	**118**	**121**	**126**	**130**	**134**	**146**	**154**	**163**	**169**	**175**
ABIOTIC	0.00087	0.00099	0.00107	0.0011	0.00111	0.00113	0.00116	0.00118	0.0012	0.00121	0.00122	0.00123
F/T 1	0.00179	0.00221	0.00265	0.00283	0.00304	0.00327	0.00357	0.00365	0.00379	0.00391	0.00395	0.00397
F/T 3	0.00126	0.00144	0.00163	0.00176	0.0018	0.00186	0.0019	0.002	0.00209	0.0021	0.00212	0.00214
F/T 6	0.00137	0.00149	0.00175	0.00182	0.00186	0.00195	0.002	0.00208	0.0021	0.00219	0.00221	0.00222
F/T 9	0.00113	0.0016	0.00182	0.00188	0.00194	0.00198	0.00208	0.00213	0.00219	0.00222	0.00225	0.00227
F/T CNTL	0.00168	0.00173	0.00208	0.00217	0.00227	0.00238	0.00242	0.00245	0.00247	0.0025	0.00252	0.00253

Moles of Carbon Dioxide Utilized (Moles CO_2)

	Days											
Reactor	**154**	**163**	**169**	**175**	**181**	**187**	**196**	**203**	**210**	**216**	**221**	**229**
ABIOTIC	0.01015	0.01016	0.01017	0.01018	0.01018	0.01019	0.01023	0.01033	0.01037	0.01044	0.0105	0.01057
F/T 1	0.03017	0.0303	0.03033	0.03036	0.03037	0.0304	0.0305	0.03056	0.03066	0.03068	0.03068	0.0307
F/T 3	0.02991	0.02992	0.02994	0.02996	0.02997	0.02999	0.03007	0.03022	0.03034	0.03044	0.03053	0.03063
F/T 6	0.02809	0.02818	0.0282	0.02822	0.02822	0.02824	0.02827	0.02839	0.02845	0.02849	0.02851	0.02855
F/T 9	0.02584	0.02587	0.0259	0.02592	0.02593	0.02596	0.02603	0.02609	0.02615	0.02626	0.02637	0.02648
F/T CNTL	0.03188	0.03191	0.03193	0.03194	0.03195	0.03196	0.03199	0.03201	0.03206	0.03206	0.03206	0.03207

Reactor Headspace Carbon Dioxide Concentration (Moles CO_2)

	Days							
Reactor	**181**	**187**	**196**	**203**	**210**	**216**	**221**	**229**
ABIOTIC	0.00123	0.00124	0.00127	0.00138	0.00141	0.00148	0.00155	0.00162
F/T 1	0.00398	0.00401	0.00411	0.00417	0.00427	0.00429	0.0043	0.00432
F/T 3	0.00215	0.00217	0.00225	0.0024	0.00252	0.00262	0.00271	0.0028
F/T 6	0.00223	0.00225	0.00228	0.0024	0.00246	0.00249	0.00252	0.00255
F/T 9	0.00228	0.00231	0.00238	0.00244	0.0025	0.00261	0.00272	0.00283
F/T CNTL	0.00254	0.00256	0.00258	0.0026	0.00265	0.00266	0.00265	0.00266

Concentration of Total Extractable Hydrocarbons in Reactor Soils (mg_{TEH} / kg_{SOIL})

	Days											
Reactor	**0**	**2**	**4**	**7**	**9**	**18**	**22**	**25**	**29**	**32**	**37**	**41**
ABIOTIC	3790.71	3773.81	3754.62	3722.44	3720.91	3649.2	3606.85	3584.09	3558.5	3535.3	3505.87	3487.4
F/T 1	3394.7	3317.41	3222.41	3092.16	3038.8	2767.89	2643.93	2601.8	2520.97	2472.03	2418.05	2377.26
F/T 3	3523.48	3454.81	3351.19	3226.41	3191.44	2811.18	2627.85	2585.95	2465.54	2418.57	2357.03	2345.61
F/T 6	3640.03	3569.93	3474.64	3340.14	3404.34	3088.63	2896.23	2853.5	2727.33	2676.91	2597.4	2575.68
F/T 9	3053.1	2992.03	2912.7	2803.94	2750.58	2446.75	2314.13	2269.15	2178.84	2134.4	2073.41	2049.63
F/T CNTL	3414.41	3344.51	3247.29	3119.81	3070.22	2721.2	2539.93	2484.24	2377.44	2323	2223.35	2207.79

Concentration Ratio of Total Extractable Hydrocarbons (TEH) in Reactor Soils (C_{TEH} / Co_{TEH})

	Days											
Reactor	**0**	**2**	**4**	**7**	**9**	**18**	**22**	**25**	**29**	**32**	**37**	**41**
ABIOTIC	1	0.99548	0.99031	0.98168	0.98126	0.96189	0.95048	0.94432	0.9374	0.93111	0.92306	0.918
F/T 1	1	0.97719	0.94892	0.9101	0.89426	0.81648	0.78131	0.76947	0.74647	0.73275	0.71754	0.70607
F/T 3	1	0.98045	0.95069	0.91584	0.90629	0.8003	0.74862	0.73735	0.70364	0.69065	0.67391	0.67059
F/T 6	1	0.98078	0.95472	0.91792	0.93541	0.84915	0.79657	0.78492	0.75044	0.73669	0.71501	0.70909
F/T 9	1	0.98001	0.95402	0.91849	0.90101	0.80189	0.75858	0.74373	0.71428	0.69975	0.67972	0.67206
F/T CNTL	1	0.97877	0.94925	0.91052	0.89552	0.78987	0.73482	0.71881	0.68643	0.66998	0.63939	0.63544

Concentration of Total Extractable Hydrocarbons in Reactor Soil (mg_{TEH} / kg_{SOIL})

	Days											
Reactor	**43**	**46**	**51**	**56**	**62**	**66**	**70**	**74**	**78**	**84**	**88**	**91**
ABIOTIC	3440.46	3421.21	3401.77	3385.12	3391.83	3390.08	3331.87	3314.33	3310.03	3308.16	3301.11	3296.72
F/T 1	2292.42	2239.41	2223.65	2184.4	2151.33	2142.78	2020.83	1965.51	1963.31	1960.51	1916.9	1914.57
F/T 3	2232.21	2192.87	2164.1	2138.51	2134.83	2129.56	2024.88	1994.26	1992.23	1989.74	1975.25	1962.62
F/T 6	2452.93	2392.93	2368.15	2342.03	2324.44	2314.48	2219.1	2199.84	2198.62	2197.02	2182.36	2181.71
F/T 9	1956.48	1911.04	1891.9	1875.98	1869.08	1857.02	1765.11	1745.6	1743.18	1740.37	1734.6	1726.3
F/T CNTL	2087.63	2014.76	1993.97	1969.5	1964.53	1955.59	1828.81	1794.78	1792.48	1789.64	1765.45	1766.06

Concentration Ratio of Total Extractable Hydrocarbons (TEH) in Reactor Soils (C_{TEH} / Co_{TEH})

	Days											
Reactor	**43**	**46**	**51**	**56**	**62**	**66**	**70**	**74**	**78**	**84**	**88**	**91**
ABIOTIC	0.90529	0.90003	0.8948	0.89033	0.89188	0.89139	0.87552	0.87059	0.8695	0.869	0.86705	0.86581
F/T 1	0.68209	0.6672	0.66276	0.65173	0.64244	0.64001	0.60444	0.58813	0.58748	0.58664	0.57404	0.57318
F/T 3	0.63898	0.62838	0.62066	0.61396	0.61273	0.61119	0.58245	0.57427	0.57369	0.57298	0.56909	0.56542
F/T 6	0.67552	0.65916	0.6524	0.6453	0.6405	0.63779	0.61172	0.60649	0.60616	0.60572	0.60175	0.60157
F/T 9	0.64175	0.62672	0.62038	0.61497	0.6128	0.60902	0.5789	0.57234	0.57154	0.57062	0.56878	0.56593
F/T CNTL	0.59939	0.57772	0.57114	0.56403	0.56237	0.55932	0.52102	0.51061	0.50991	0.50905	0.50139	0.50155

Concentration of Total Extractable Hydrocarbons in Reactor Soil (mg_{TEH} / kg_{SOIL})

	Days											
Reactor	**94**	**100**	**105**	**108**	**111**	**113**	**118**	**121**	**126**	**130**	**134**	**146**
ABIOTIC	3293.16	3286.83	3252.98	3242.77	3239.73	3232.71	3228.49	3226.46	3226.11	3225.19	3223.21	3222.17
F/T 1	1914.57	1914.57	1900.91	1845.86	1814.21	1790.84	1766.05	1755.89	1743.93	1731.28	1714.16	1710.06
F/T 3	1962.62	1962.62	1953.75	1903.52	1892.15	1882.02	1871.43	1864.07	1861.77	1858.14	1855.82	1850.26
F/T 6	2181.71	2181.71	2171.77	2117.64	2104.98	2097.89	2083.49	2079.51	2077.31	2072.53	2069.73	2065.19
F/T 9	1726.3	1726.3	1718.23	1679.68	1662.76	1636.3	1624.43	1620.56	1617.21	1615.24	1609.77	1606.7
F/T CNTL	1764.52	1725	1696.96	1681.51	1670.06	1667.75	1647.71	1642.74	1637.13	1631	1628.68	1627.29

Concentration Ratio of Total Extractable Hydrocarbons (TEH) in Reactor Soils (C_{TEH} / Co_{TEH})

	Days											
Reactor	**94**	**100**	**105**	**108**	**111**	**113**	**118**	**121**	**126**	**130**	**134**	**146**
ABIOTIC	0.86481	0.86307	0.85382	0.85104	0.85017	0.84815	0.84694	0.84636	0.84628	0.84601	0.84548	0.8452
F/T 1	0.57318	0.57318	0.56913	0.55281	0.54351	0.5366	0.5294	0.52641	0.52294	0.51899	0.51391	0.5127
F/T 3	0.56542	0.56542	0.56298	0.54924	0.54599	0.54341	0.54056	0.5386	0.53794	0.53698	0.5363	0.5348
F/T 6	0.60157	0.60157	0.59886	0.58404	0.58059	0.57865	0.57475	0.57366	0.57306	0.57176	0.571	0.56975
F/T 9	0.56593	0.56593	0.56322	0.55052	0.54494	0.53641	0.53244	0.53119	0.53013	0.52951	0.52774	0.52675
F/T CNTL	0.50103	0.494	0.48037	0.47567	0.47189	0.47113	0.46456	0.46325	0.46137	0.45934	0.45862	0.45822

Concentration of Total Extractable Hydrocarbons in Reactor Soil (mg $_{TEH}$ / kg $_{SOIL}$)

	Days											
Reactor	**154**	**163**	**169**	**175**	**181**	**187**	**196**	**203**	**210**	**216**	**221**	**229**
ABIOTIC	3221.21	3220.58	3219.97	3219.58	3219.43	3218.93	3216.9	3210.93	3209.06	3205.19	3201.62	3197.76
F/T 1	1701.97	1694.98	1692.95	1691.65	1691.11	1689.43	1683.91	1680.52	1674.87	1673.7	1673.36	1672.2
F/T 3	1845.28	1844.97	1843.65	1842.73	1842.21	1841.08	1836.43	1828.24	1821.43	1815.86	1810.81	1805.26
F/T 6	2064.08	2058.96	2057.79	2057.04	2056.71	2055.74	2053.95	2047.21	2043.83	2041.91	2040.48	2038.57
F/T 9	1603.51	1601.88	1600.25	1599.03	1598.25	1596.82	1592.93	1589.67	1585.86	1579.63	1573.92	1567.71
F/T CNTL	1625.8	1624.35	1623.15	1622.43	1622.19	1621.22	1619.87	1618.91	1616.04	1615.59	1615.65	1615.2

Concentration Ratio of Total Extractable Hydrocarbons (TEH) in Reactor Soils (C_{TEH} / Co_{TEH})

	Days											
Reactor	**154**	**163**	**169**	**175**	**181**	**187**	**196**	**203**	**210**	**216**	**221**	**229**
ABIOTIC	0.84493	0.84478	0.84461	0.8445	0.84445	0.84431	0.84378	0.84222	0.84173	0.84073	0.83982	0.83883
F/T 1	0.51035	0.50821	0.50759	0.50719	0.50701	0.5065	0.50483	0.50377	0.50214	0.50181	0.50173	0.50141
F/T 3	0.53331	0.53322	0.53284	0.53256	0.53239	0.53206	0.53081	0.52863	0.52659	0.52494	0.52343	0.52178
F/T 6	0.56945	0.56805	0.56774	0.56753	0.56744	0.56717	0.56669	0.56485	0.56393	0.56341	0.56301	0.56249
F/T 9	0.52572	0.52519	0.52467	0.52428	0.52404	0.52358	0.52235	0.52132	0.5201	0.51791	0.51589	0.51371
F/T CNTL	0.45775	0.45728	0.45691	0.45668	0.4566	0.4563	0.45589	0.45558	0.45469	0.45454	0.45455	0.4544

APPENDIX B3:

TOTAL EXTRACTABLE HYDROCARBON RESULTS

Initial and Final Reactor Soil
Total Extractable Hydrocarbons (TEH) Concentrations

Reactor	Initial TEH Concentrations (mg_{TEH}/kg_{SOIL})	Final TEH Concentrations (mg_{TEH}/kg_{SOIL})
ABIOTIC	3790.71	57.59
F/T 1	3394.70	31.96
F/T 3	3523.48	25.64
F/T 6	3640.036	50.97
F/T 9	3053.10	55.02
F/T CNTL	3414.41	35.46

Note: All above values are the average of three soil samples.

APPENDIX C:

EXAMPLE CALCULATIONS

Sample Calculations

Sample calculations for the Reactor Respiration Monitoring will be demonstrated below. The sample calculations will demonstrate how one obtains a degradation rate for hydrocarbons (as $C_{14}H_{24}$) from a bio-reactor's headspace O_2 and CO_2 content.

The steps involved are as follows:

1. Convert reactor headspace O_2 and CO_2 content from a percentage to moles;

2. Determine the number of moles of O_2 utilized and CO_2 produced since the previous sample period;

3. Convert moles of O_2 utilized and CO_2 produced into moles of $C_{14}H_{24}$ degraded during the sampling period;

4. Convert moles of $C_{14}H_{24}$ degraded to a concentration ratio of C/Co;

The calculations will be demonstrated on the data obtained for the 9 freeze-thaw cycle treated reactors (F/T 9), for the time period Day 0 to Day 4. The data is provided in Table C.1 below.

Table C.1: Sample Calculation Data for Reactor F/T 9

	Reactor Headspace O_2 Content (% O_2)		
Reactor	**Day 0**	**Day 2**	**Day 4**
F/T 9	21	18.41	16.27
	Reactor Headspace CO_2 Content (% CO_2)		
Reactor	**Day 0**	**Day 2**	**Day 4**
F/T 9	0	1.55	3.57
	Reactor Temperature		
Reactor	**Day 0**	**Day 2**	**Day 4**
°C	26	25	26
°K	299.15	298.15	299.15

Step 1: Convert Reactor Headspace O_2 and CO_2 Content from a Percentage to Moles

The content of O_2 and CO_2 in the reactor headspace is obtained as a percentage. By utilizing the Ideal Gas Law, these percentage values can be converted into moles. The Ideal Gas Law formulas were presented in Section 3.4.3 previously, and have been reproduced again below.

$$Moles\ O_2 = \frac{P * V_{O_2}}{R * T} \tag{3.9}$$

P	=	*Headspace Pressure (atmospheres)*
V_{O_2}	=	*Volume of Headspace Oxygen (litres)*
	=	*Volume of Air * % O_2*
	=	*1.72 litres * % O_2*
R	=	*Ideal Gas Constant*
	=	*0.08206 (atm*L / °K * moles)*
T	=	*Temperature (Kelvin)*

$$Moles\ CO_2 = \frac{P * V_{CO_2}}{R * T} \tag{3.10}$$

P	=	*Headspace Pressure (atmospheres)*
V_{CO_2}	=	*Volume of Headspace Carbon Dioxide (litres)*
	=	*Volume of Air * % CO_2*
	=	*1.72 litres * % CO_2*
R	=	*Ideal Gas Constant*
	=	*0.08206 (atm*L / °K * moles)*
T	=	*Temperature (Kelvin)*

The following assumptions are made in utilizing Equations 3.9 and 3.10:

1. Headspace pressures were assumed to be ambient (1 atm);

2. Soil pore space does not contribute to the total headspace volume;

By substituting the values in Table C.1 for Day 0 into Equations 3.9 and 3.10, one obtains the following:

$$Moles\,O_2\underset{Day0}{} = \frac{(1\_atm)*(1.72\_L*0.21)}{(0.8206\_atm*L/^\circ K*moles)*(299.15\_^\circ K)} = 0.0147\_Moles\ O_2$$

and

$$Moles\,CO_2\underset{Day0}{} = \frac{(1\_atm)*(1.72\_L*0.0)}{(0.8206\_atm*L/^\circ K*moles)*(299.15\_^\circ K)} = 0.0\_Moles\ CO_2$$

Utilizing the same procedure for Days 2 and 4, one would obtain the results presented in Table C.2.

Table C.2: Moles of Reactor Headspace O_2 and CO_2

	Moles of O_2 in Reactor Headspace (Moles O_2)		
Reactor	**Day 0**	**Day 2**	**Day 4**
F/T 9	0.0147	0.0129	0.0114
	Moles of CO_2 in Reactor Headspace (Moles CO_2)		
Reactor	**Day 0**	**Day 2**	**Day 4**
F/T 9	0.0	0.001	0.003

Step 2: Determine Moles of O_2 Utilized and CO_2 Produced

To determine the moles of O_2 utilized and CO_2 produced, the Equations A-C.1 and A-C.2 are utilized:

$$\textit{Moles } O_2 \textit{ Utilized @ } t_n = (\textit{Moles} - \textit{Moles } O_2 \textit{ @ } t_n) + \textit{Moles } O_2 \textit{ Utilized @ } t_{n-1} \quad \text{(A-C.1)}$$

$$\textit{Moles } CO_2 \textit{ Produced @ } t_n = (\textit{Moles } CO_2 \textit{ @ } t_n - \textit{Moles } CO_2 \textit{ @ } t_{n-1}) + \textit{Moles } CO_2 \textit{ Produced @ } t_{n-1} \quad \text{(A-C.2)}$$

By assuming that there is zero O_2 utilization and CO_2 production at Day 0, and by substituting the values in Table C.2 for Day 2 into Equations A-C.1 and A-C.2, one obtains the following:

Moles O_2 Utilized @ t_n = (0.0147 moles - 0.0129 moles) + 0.0 moles = 0.0018 moles utilized

Moles CO_2 Produced @ t_n = (0.0011 moles - 0.0 moles) + 0.0 moles = 0.0011 moles produced

Utilizing the same procedure for Day 4, one would obtain the results presented in Table C.3.

Table C.3: Moles Headspace O_2 Utilized and CO_2 Produced

	Moles of O_2 Utilized in Reactor Headspace (Moles O_2)		
Reactor	**Day 0**	**Day 2**	**Day 4**
F/T 9	0.0	0.0018	0.0033
	Moles of CO_2 Produced in Reactor Headspace (Moles CO_2)		
Reactor	**Day 0**	**Day 2**	**Day 4**
F/T 9	0.0	0.0011	0.0025

Step 3: Convert Moles of O_2 Utilized and CO_2 Produced into Moles of $C_{14}H_{24}$ Degraded

To determine the moles of $C_{14}H_{24}$ degraded based on the number of moles of O_2 utilized and CO_2 produced, the relation in Equation 3.7 is utilized.

$$C_{14}H_{24} + 10O_2 + \frac{10}{7}NO_3^+ + \frac{10}{7}H^+ = \frac{10}{7}C_5H_7O_2N + \frac{48}{7}CO_2 + \frac{54}{7}H_2O \qquad (3.7)$$

To summarize equation 3.7, for every mole of hydrocarbon ($C_{14}H_{24}$) degraded:

- 10 moles of O_2 are utilized
- 1.43 moles of NO_3^- are utilized
- 6.86 moles of CO_2 are produced
- 1.43 moles of Microbial mass are produced
- 7.71 moles of H_2O are produced

or

$$C_{14}H_{24}:O_2:CO_2 = 1:10:6.86 \qquad (3.8)$$

The concentration of hydrocarbons within the bioreactors can be estimated utilizing the following equations:

Equation$(A-C3)$:

$$[C_{14}H_{24}]\ @\ t_n = [C_{14}H_{24}]\ @\ t_{n-1} - \left(\frac{Moles\ O_2\ utilized\ @\ t_n}{10\dfrac{moles\ O_2\ utilized}{mole\ C_{14}H_{24}\ degraded}}\right) * 192{,}344.56\frac{mg\ C_{14}H_{24}}{mole\ C_{14}H_{24}} * \frac{1}{0.5\ Kg_{soil}}$$

and

Equation$(A-C4)$:

$$[C_{14}H_{24}]\ @\ t_n = [C_{14}H_{24}]\ @\ t_{n-1} - \left(\frac{Moles\ CO_2\ utilized\ @\ t_n}{6.86\dfrac{moles\ CO_2\ produced}{mole\ C_{14}H_{24}\ degraded}}\right) * 192{,}344.56\frac{mg\ C_{14}H_{24}}{mole\ C_{14}H_{24}} * \frac{1}{0.5\ Kg_{soil}}$$

Given an initial concentration of 3053.1 mg $C_{14}H_{24}$ / Kg Soil within the example reactor (see Appendix B3), and an initial mass of 500 g of soil, one obtains the following, by substituting the values in Table C.3 for Day 2 into Equations A-C.3 and A-C.4:

$$[C_{14}H_{24}]@Day2 = 3053.1\frac{mg_{C14H24}}{kg_{soil}} - \left(\frac{0.0018 moles\ O_2\ utilized}{10\frac{moles\ O_2\ utilized}{mole C_{14}H_{24}\ degraded}}\right) * 192{,}344.56\frac{mg C_{14}H_{24}}{mole} * \frac{1}{0.5 Kg_{soil}} = 2984.91$$

and

$$[C_{14}H_{24}]@Day2 = 3053.1\frac{mg_{C14H24}}{kg_{soil}} - \left(\frac{0.0011 moles\ CO_2\ utilized}{6.86\frac{moles\ CO_2\ utilized}{mole C_{14}H_{24}\ degraded}}\right) * 192{,}344.56\frac{mg C_{14}H_{24}}{mole} * \frac{1}{0.5 Kg_{soil}} = 2992.03$$

Utilizing the same procedure for Day 4, one would obtain the results presented in Table C.4.

Table C.4: Concentration of $C_{14}H_{24}$ in Reactor Estimated from Moles of Reactor Headspace O_2 Utilized and CO_2 Produced

	Concentration of $C_{14}H_{24}$ Based on Moles of O_2 Utilized in Reactor Headspace (mg $C_{14}H_{24}$ / Kg_{soil})		
Reactor	**Day 0**	**Day 2**	**Day 4**
F/T 9	3053.1	2984.9	2925.5
	Concentration of $C_{14}H_{24}$ Based on Moles of CO_2 Produced in Reactor Headspace (mg $C_{14}H_{24}$ / Kg_{soil})		
Reactor	**Day 0**	**Day 2**	**Day 4**
F/T 9	3053.1	2992.0	2912.7

Step 4: Convert Moles of $C_{14}H_{24}$ Degraded to a Concentration Ratio (C/Co)

To equally compare the results of $C_{14}H_{24}$ degraded between reactors with different initial contaminant concentrations, the concentrations of contaminant over time need to be converted into a concentration ratio. This is accomplished by utilizing Equations A-C.5 .

$$\frac{C}{Co}@t_n = \frac{Conc\ of\ C_{14}H_{24}\ @\ t_n}{Conc\ of\ C_{14}H_{24}\ @\ Day\ 0} \qquad \text{(A-C.5)}$$

$$With: \quad Conc\ of\ C_{14}H_{24}\ @\ Day\ 0 = 3053.1 \frac{mg\ C_{14}H_{24}}{Kg\ Soil}$$

Applying Equation A-C.5 to the values in Table C.4, one would obtain the results presented in Table C.5.

Table C.5: Concentration Ratio (C/Co) of $C_{14}H_{24}$ in Reactor Estimated from Moles of Reactor Headspace O_2 Utilized and CO_2 Produced

	Concentration Ratio of $C_{14}H_{24}$ Based on Moles of O_2 Utilized in Reactor Headspace		
Reactor	**Day 0**	**Day 2**	**Day 4**
F/T 9	1.0	0.978	0.958
	Concentration Ratio of $C_{14}H_{24}$ Based on Moles of CO_2 Produced in Reactor Headspace		
Reactor	**Day 0**	**Day 2**	**Day 4**
F/T 9	1.0	0.980	0.954