**Large Scale Bioventing Degradation Rates of Petroleum Hydrocarbons and
Determination of Scale-up Factors**

by

Michael James Mosco

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ABSTRACT

LARGE SCALE BIOVENTING DEGRADATION RATES OF PETROLEUM HYDROCARBONS AND DETERMINATION OF SCALE-UP FACTORS

Michael James Mosco
University of Guelph,

Advisor:
Richard G. Zytner, PhD, P.Eng, FEC

Bioventing is a cutting edge, non-destructive treatment method that uses indigenous soil microorganisms in-situ to remediate petroleum hydrocarbons in the unsaturated soil zone. The application of this technology in a field environment still has some uncertainties associated with it in regards to length of remediation period.

An 80kg soil reactor system was developed, consisting of custom made reactor, climate chamber, low flow venting system and an off gas capture device. A sandy and a clayey soil were tested with known concentrations of spiked synthetic gasoline. Environmental conditions monitored included: moisture levels, pH, microbial levels, nutrient and oxygen levels. Results show a second stage degradation rate similar to the degradation rate obtained from research conducted at a 4kg scale. Research at an 80kg laboratory scale is feasible, yet not always necessary for the development of scale-up factors. In the study with aged soil contaminants, degradation rates were significantly reduced.

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LIST OF ACRONYMS

BHM = Bushnell-Hass Media

BTEX = Benzene, Toluene, Ethylbenzene, Xylene

C:N = Carbon to nitrogen ratio, weight basis

CEC = Cation exchange capacity

CFU = Colony forming unit

CRS = Column scale reactor

FCSAP = Federally Contaminated Sites Action Plan

FID = Flame ionization detector

GC = Gas chromatograph

MSR = Meso-scale reactor

OM = Organic matter content of soil

PAH = Polycyclic aromatic hydrocarbons

PDB = Petroleum degrading bacteria

PHCs = Petroleum hydrocarbons

SF = Scale up factor

SVE = Soil vapour extraction

SW = Soil water content

THB = Total heterotrophic bacteria

TPH = Total petroleum hydrocarbon

TSA = Tryptic soy agar

USEPA = United States Environmental Protection Agency

UST = Underground storage tank

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CHAPTER 1: INTRODUCTION

Soil contaminated with petroleum hydrocarbons is an environmental issue that poses a threat to human health. Humans can come in contact with contamination through exposure to contaminated surface soils, as well as through drinking contaminated groundwater. Throughout Canada there are an estimated 22,000 federally contaminated sites (OAGC, 2012). The estimated liability cost for assessment and remediation of those sites is currently set at \$4.9 billion (OAGC, 2012). FCSAP estimates that approximately 52% of the listed sites are contaminated with petroleum hydrocarbons (gasoline) and may require remediation (OAGC, 2012). Site remediation is a big business and government agencies are always looking for cost effective, yet environmentally responsible methods to clean up these sites.

Gasoline is made up of four major components: benzene, ethylbenzene, toluene and xylenes, which are also known as BTEX. Often times BTEX contamination will reach the groundwater level and migrate through the groundwater and soil and a plume. BTEX spills on the soil surface are very common during refueling operations; however, much of the contaminant will volatilize on the surface. A greater concern, is when fuel tanks containing gasoline, diesel or other petroleum hydrocarbons buried in the subsurface, unknowingly leak and contaminate large volumes of soil and the groundwater below.

BTEX in the environment, as a result of a gasoline spill, can have significant impacts on the nearby soils, waters and biological receptors. Managing these contaminated sites carries significant costs for the assessment, remediation and long term monitoring that is required. Some of these contaminated sites are in isolated areas and are difficult to access and therefore difficult to assess and remediate. Alternatively, soil sites such as a leaking underground storage tank in a

populated area may be easy to access but could pose a significant risk to people in the surrounding area through exposure to vapours and potential contamination of a local drinking water source.

The biodegradation rate of petroleum hydrocarbons are known to vary with both environmental conditions and soil type. Additionally, the degradation behaviour of aged soil contaminants is very differently than freshly contaminated soil and can significantly impact the remediation process. Understanding the differences in the degradation rates between freshly and aged contaminated soils is critical for the development of proper site remediation closer times.

1.1 Objective of the Study

This research project examines the use of bioventing soil remediation as a viable clean-up technology for the removal of petroleum hydrocarbons from unsaturated soil. Bioventing soil remediation work and studies have predominantly been conducted in the laboratory environment (Khan, 2013). However, the true value in this technology is its application to field scale remediation projects. The challenge of scaling up an experimental design and applying it to a field application is that in a laboratory setting all of the variables can be controlled and conditions are often ideal, which is not always the case in the environment. This research project will be utilizing an 80kg bioventing reactor vessel where a biodegradation rate relationship will be developed from the data. Using this relationship and comparing it to the rate constants from the experiments conducted by Eyvazi and Zytner (2009) and Khan and Zytner (2013), scale-up factors from their experiments can be created once the larger reactor experiment is conducted. Having three sets of biodegradation constants and two sets of scale-up factors will provide a better understanding of what would be expected in the field application of this technology.

There has not been significant research done with bioventing experiments at the larger (80-100kg) laboratory scale and this research will ideally develop a procedure that can be used to effectively evaluate the performance of bioventing at this scale for future work. Additionally, this experiment will determine if it is necessary to complete large scale (80kg) bioventing laboratory experiments or if a micro-scale (200g) experiment is sufficient and can be scaled-up to provide data to effectively and efficiently remediate a site.

Past research has examined laboratory scale and meso-scale bioventing soil remediation, which has led to the development of scale-up factors between the two experiments (Eyvazi and Zytner, 2009; Khan and Zytner, 2013). However, it is unclear if these scale-up factors can be directly applied to scaling up an experimental design and applying it to the data in the field. Conducting bioventing experiments using an 80kg reactor vessel to determine large scale degradation rates will allow further development of scale-up factors by comparing the new degradation rate constants to the rate constants developed by Eyvazi and Zytner (2009) and Khan and Zytner (2013). These revised scale-up factors can then be used to scale the effectiveness of bioventing in a laboratory setting compared to a field environment. As such, this research project focuses on the following objectives:

1. Develop an appropriate technique of conducting bioventing research using a large scale reactor vessel.
2. Complete larger scale laboratory studies to determine large scale degradation on soils previously studied.
3. Determine revised scale-up factors by comparing the results to previously determined degradation values.
4. Examine the effect that aged gasoline has on the degradation rate.

1.2 Layout of Thesis

This thesis contains nine chapters and three appendices. Chapter 1 briefly introduces the research topic and highlights some of the major objectives for the study. A review of past bioventing research and corresponding literature that has been reviewed in preparation for this study can be found in Chapter 2. Chapter 3 details the experimental configuration as well as the design of the large bioventing reactor. Chapter 4 details the experimental methodology of the pre-experimental trials used to refine the operational conditions prior to the biodegradation trials. Chapter 5 details the methodology used to execute the biodegradation trials. The results of the study are presented in Chapter 6. Chapter 7 concludes and discusses the major findings of the experiment. Chapter 8 identifies areas of future research for bioventing soil vapour extraction. References are provided in Chapter 9.

Appendix A contains some detailed calculations necessary for the experiment. Appendix B summarizes all of the raw experimental data collected and analyzed for this experiment. Appendix C has a series of tables outlining the equipment and supplies that were used.

CHAPTER 2: BACKGROUND AND LITERATURE REVIEW

2.1 Petroleum Hydrocarbons as Soil Contaminants

Human exposure to petroleum hydrocarbons is possible through three different exposure pathways: air, water and food. Volatilized petroleum hydrocarbons are present in the atmosphere and could be present in minimal concentrations in the drinking water supply and may be present in some of the foods that we eat. Despite receiving small doses of this contaminant from these sources, humans do not tend to absorb or accumulate it in the body (USEPA, 2013). Benzene is a known carcinogen and poses serious health risks if there is prolonged exposure. The USEPA reports that chronic exposure to benzene decreases the lymphocyte count in the body, which can lead to cancer cell development (McBean, 2014; USEPA, 2013). Exposure to high concentrations of ethylbenzene, toluene and xylenes vapours, which are non-carcinogens, can also pose serious acute health effects such as nausea, headache, dizziness and potentially death in extreme cases. Toluene and ethylbenzene primarily target the kidney and liver increasing their weight and toxicity and the calculated risk may be grouped together as both contaminants are targeting the same organs (USEPA, 2013). Xylene exposure targets the whole body, often associated with decreasing body weight.

Soil contamination as a result of a petroleum hydrocarbon spill or leak is a serious environmental concern and is very common in heavily industrialized nations such as Canada and the United States. Within the last quarter century, environmental management practices including the storage, transportation and handling of petroleum hydrocarbons have drastically improved as a result of the increase awareness of the significant environmental and health effects due to

mismanagement. However, there are many sites where legacy contamination is an issue. In addition to the legacy contamination, there are several leaking underground petroleum hydrocarbon tanks. In a recent study conducted by the USEPA, it is estimated that there are approximately 581,000 underground storage tanks (USTs) that store petroleum or hazardous substances within the United States (USEPA, 2013), of which, approximately 100,000 are actively leaking (Eyvazi *et al.*, 2009).

In Canada, under the Federally Contaminated Site Action Plan (FCSAP), there are over 5,800 active contaminated sites pending action and over 2,500 suspected contaminated sites that have yet to undergo further assessment (TBS, 2015). Approximately half of these sites are contaminated with petroleum hydrocarbons (TBS, 2015). FCSAP is a 15-year, \$3.5 billion dollar program that was established in 2005 by the Government of Canada to reduce environmental and human health risks from known federally contaminated sites (Government of Canada, 2014).

2.2 Fate of Petroleum Hydrocarbons in the Environment

2.2.1 Sorption of Chemical by Soil

Contaminants enter the soil environment through the sorption process which is a combination of the adsorption and absorption process. Research has found that adsorption is the dominant chemical process that occurs while the actual amount of absorption that occurs is minimal (Yang *et al.*, 2013). Adsorption can occur on the surface of a solid, the condensation of a gas on a soil surface or the deposition of a liquid onto the soil surface (Morrill *et al.*, 1982). The adsorption process occurs between two interfaces: liquid:liquid, gas:liquid, gas:solid, or liquid:solid (Weber and Morris, 1963).

The adsorption process occurs when there is an attraction between the adsorbate and the adsorbent material which results in a higher concentration of adsorbate at the surface –liquid interface. Depending on the particular properties of a chemical, it may be either hydrophobic or hydrophilic which will influence the adsorption process between the phases. Other factors such as ion/cation exchanges may also need to be considered.

Zytner (1994) determined the sorption and desorption characteristics for BTEX in several types of granular media. He found that the organic carbon content in the media was a key factor in the sorption and desorption process. Based on the study, the preferences for sorption were as follows: GAC, peat moss, organic top soil, clay soil and sandy loam soil. Overall, the Freundlich Isotherm for BTEX satisfactorily described the sorption and desorption behaviour (Zytner, 1994).

2.2.2 Desorption of Chemical by Soil

Desorption is the process in which a contaminant that has adsorbed to soil then releases its bond to the soil particle. Limited studies have been conducted on the synthetic organic desorption due to the length of time required to complete these studies (La Poe, 1985). Chemicals desorb from soil when there is a concentration gradient present (Krauss and Wilcke, 2005). Unlike the adsorption process, the water or air in the soil has a lower concentration of the contaminant and therefore, the concentration gradient from high to low becomes the driving force. Studies have shown that the desorption process is significantly slower than the adsorption process and losses of the contaminant may occur due to biodegradation and volatilization. Zytner (1994) found that the Freundlich Isotherm accurately describes the desorption of BTEX in soil. The ability to retain

a compound, (stay adsorbed), depends strongly on the strength of the bond between the sorbent and the sorbate.

2.2.3 Soil-Water Partition Coefficient

Another factor that is used in determining the movement of chemicals through the soil media is the soil-water partition coefficient, K_{oc} . This soil-water partition coefficient is determined by normalizing the linear partition coefficient, K_p , with the organic carbon content in the soil. This means that the adsorption of a chemical in the soil is related only to the organic carbon content present in the soil. Studies have determined that compounds that have a large K_{oc} value near 1000 are strongly bonded to the organic matter within the soil compared to compounds having a K_{oc} value less than 100 which indicate the contaminant is highly mobile (Zytner, 2014). The USEPA has found that BTEX has a K_{oc} value in the range of 26 and 656, meaning that BTEX is moderate to highly mobile in soil (Zytner, 1994). This indicates that BTEX would be highly mobile in a soil environment, particularly in soils with a high organic content.

2.2.4 NAPLS in Soil

BTEX compounds are classified as a non-aqueous phase liquid (NAPL) and as described earlier, are released into the environment from chemical spills among other releases. When a NAPL compound is released into the environment, it migrates through the unsaturated zone to the water table as a result of gravitational forces. Models have been created to explain how NAPLs move through the unsaturated zone (Pinder and Abriola, 1986). The problem with a NAPL release into the subsurface of the soil is that a percentage of it will reach the groundwater, causing potential significant groundwater contamination by infiltration of these chemicals. In order to prevent groundwater contamination by these contaminants, it is necessary to understand how the NAPL

is behaving so that it can be quickly cleaned up. However, the NAPL migration in unsaturated soil is dependent on a variety of factors: properties of the soil, properties of the NAPL, volume of NAPL spilled, the length of time in which the spill occurred and the specific area.

2.2.5 Volatilization from Soil

Volatilization is defined as the loss of a chemical from a surface to a vapour phase which eventually leads to being released into the atmosphere. The potential for a chemical to volatilize depends on the vapour pressure as well as environmental conditions and factors that exist at the mass transfer interface. Henry's Law can be used to describe the transfer between the liquid and gas phases due to volatilization (USEPA, 2012; Zytner, 2014).

There are a couple of ways in which gasoline can volatilize within the soil. The first is through passive volatilization which is a natural process whereby the contaminant is slowly desorbed from the soil and enters a vapour phase and migrates through the soil layer until it reaches the atmosphere. Passive volatilization of toluene can pose significant environmental and human health risks; however, passive volatilization is a potential in-situ soil remediation practice. Being able to accurately quantify volatilization rates, assists with the understanding of the behaviour of petroleum hydrocarbons and other contaminants as they move through the soil environment; but to date, experimental data is limited in this field (Zytner *et al.*, 1993).

Volatilization is dependent on variables such as temperature (both soil and atmospheric), concentration of contaminant in the environment and the type of soil present.

2.3 Treatment Technologies

2.3.1 Dig and Dump

A method that is frequently used is the excavation of the contaminated material from the site and then the void is replaced with clean fill; this process is commonly referred to as the “dig and dump” method. This method, though very effective, changes the natural soil structure of the area causing a disruption to the local eco-system (Khan *et al.*, 2004). Furthermore, the contaminated soil that was removed from the site must be transported to a treatment facility for proper disposal. The costs associated with the excavation, transport and processing and storage of the contaminated soil can quickly add up depending on the magnitude of the contamination. One main advantage is the quick remediation time, however, the problem has only been moved to a secondary location.

2.3.2 Soil Vapour Extraction

Soil vapour extraction is a remediation method that can effectively remove petroleum hydrocarbons from soil in the unsaturated zone. Soil vapour extraction requires placing dry wells into the contaminated soil regions and using a pump to slowly extract air out of the unsaturated soil region. This induced vapour flow allows for the faster removal of petroleum hydrocarbon contaminants from the unsaturated soil zone (Khan *et al.*, 2004). However, this process can directly vent volatilized petroleum hydrocarbons into the atmosphere which only displaces the issue. Research has shown that soil vapour extraction can be effective in removing hydrocarbons from soil initially, but soil heterogeneity and mass transfer limitations that develop over time can make it difficult to completely remediate a site. This problem is called tailing and is a significant

challenge as the soil still has contaminant levels in excess of the cleanup criteria (Gidda *et al.*, 2011).

To help better understand the desorption process in SVE, research has been conducted in the laboratory and found that the venting behaviour is strongly influenced by the presence of a non-aqueous phase liquid (NAPL) (Baehr *et al.*, 1989). In addition to this, models have been developed for coarse grained soil in order to better understand volatile organic carbon removal from the soil during the SVE treatment process (Baehr *et al.*, 1989). Furthermore, it was concluded that the smaller the grain size of the soil, the slower the mass transfer. Other studies have found that the type of soil and amount of moisture both impact the mass transfer between the soil particles and the contaminant (Gidda *et al.*, 1999). Harper *et al.* (1998) found that higher soil moisture contents slowed the remediation process as it delayed the breakthrough time.

Soil vapour extraction technologies have been used in the field with success as research has provided engineers and scientists with enough understanding to generate accurate models to predict closure/remediation times. In recent years, the use of SVE has become more costly because the vapours collected from wells need to be treated, often using a catalytic converter to oxidize the petroleum hydrocarbon vapours. A couple of decades ago the emission regulations on the direct venting of off gasses from SVE wells was permitted in some areas with certain provision on output quantities per day. Today, this practice is no longer permitted and the cost associated with the operation of the catalytic converters often puts too much of a strain on the budget to be economically viable (USEPA, 2006).

2.3.3 Bioventing

Leading the advancement in soil remediation for the clean-up of petroleum hydrocarbons is bioventing soil remediation. Bioventing builds on the existing understanding of soil vapour extraction. In addition however, nitrogen-based fertilizer is added to the soil to stimulate native microbial populations. These stimulated microbial populations in an oxygen and nutrient rich environment break down semi-volatile compounds in petroleum hydrocarbons which are often hard to remove from the soil (Zytner, 2014). Bioventing is a cutting edge, non-destructive treatment method, which if done correctly, can improve remediation efforts while lowering the cost when compared to a soil vapour extraction system. Bioventing eliminates many of the issues associated with tradition SVE remediation systems, that is, volatilized hydrocarbons that would be directly discharge to the atmosphere without the use of a catalytic converter and tailing that would occur in the soil.

Bioventing is a preferred type of in-situ soil remediation compared to the traditional SVE practices as there is no venting of volatilized organic hydrocarbons, only carbon dioxide and clean air. Additionally, there is no tailing of contaminants in the soil as the contaminants are broken down by petroleum degrading bacteria. Bioventing is biological, natural and in-situ treatment technology for the remediation of petroleum hydrocarbons. There are many other types of technologies available that are biological, in-situ and natural, but it is difficult to find one technology or practice that incorporates all three and is as effective as bioventing.

Bioventing technology has many advantages that make it a front-runner in soil remediation technologies. The technology in general is relatively simple and doesn't require sophisticated machinery. Boreholes used to collect soil and contamination samples are converted into venting

wells. The suction airflow on the venting wells is predetermined and the pumps are set to a low flow rate in order to supply adequate oxygen to the petroleum degrading bacteria, but not too high as to start volatilization of the contaminant from the soil. In order to stimulate the microorganisms, nitrogen is needed, which can be a challenge to add to soil below grade. One way to effectively deliver nitrogen to the soil profile is through anhydrous ammonia, which is injected into drill ports in the soil. The anhydrous ammonia reacts with water in the soil and creates ammonia which is required by the soil microorganisms for a nutrient source. The petroleum degrading bacteria use the nutrient and oxygen supply to degrade the petroleum hydrocarbon contamination in the soil. This remediation process is a simple, safe, environmentally sound and effective technology that can be applied to field applications.

As mentioned previously, there is a significant amount of evidence on the potential of bioventing. However, there is limited information on the decay rate of petroleum hydrocarbons based on site conditions, which leads to a difficulty in predicting the appropriate timeframe for remediation efforts (Frutos *et al.*, 2010). According to Sutton *et al.* (2013), Chien *et al.* (2009) and Kao *et al.* (2010), the results and correlations developed in a laboratory setting do not always accurately represent what occurs in the field, as there are variations caused by heterogeneity and aging of contaminants. Despite the fact that much of the work that has been done on the topic of bioventing was in small scale laboratory experiments, the results have shown promise for application to the field. There are many factors that need to be considered in order to understand the process and how small-scale experimental results can be scaled-up in order to be relevant on the macro-scale level. By understanding how petroleum degrading hydrocarbons behave in different soil types and conditions, researchers and engineers can better predict their behaviour in field scale application.

After an in-depth review of the literature on bioventing, it can be said that limited work has been conducted on the large scale application of bioventing technologies. Conducting these types of experiments on a large scale are often difficult as the systems can be quite complex, hence, more research has focussed on small scale respirometers (Chien *et al.*, 2009 and Kao *et al.*, 2010). In order to more accurately simulate bioventing soil remediation representative of the field, laboratory scale reactors have been used. An important consideration is the physical shape of the reactor vessel. Rectangular tanks have been previously used Chang *et al.* (2011). Chang's reactor held 200kg of soil that was uniformly packed. Similarly, cylindrical columns have been used by Patros (2009) containing 80kg of soil, Frutos *et al.* (2010) with 5.5kg, and Khan and Zytner (2013) with 4kg.

2.4 Factors Affecting Bioventing

As previously stated, BTEX spills and leaks in the environment poses a significant threat to air, soil, surface and groundwater. Organic chemicals present in the environment that do not mix with water exist as NAPL, which are influenced by several chemical, physical and biological factors. Processes such as advection, diffusion, adsorption, desorption, volatilization and chemical and biological degradation may occur within the unsaturated soil zone. Specific site parameters such as soil type, moisture content and temperature also play an important role in the behaviour of petroleum hydrocarbons in the environment.

2.4.1 Water Content

Soil water content is described as the amount of water present in a specific volume of soil. The water content or moisture content drastically impacts how the contaminant moves in the environment and how it can be remediated. Studies conducted by Harper *et al.* (1998) and Gidda

et al. (1999) suggest that water content limits the mass transfer for soil vapour extraction processes where the contaminant remains attached to the water and does not enter the air present in the soil pores. It was found that a non-linear relationship exists between mass transfer in the soil and the soil water content (Harper *et al.*, 1998). When the soil has a high water content, the pores in the soil are filled with water and not air, so contaminants cannot be transferred to the air and extracted easily through typical soil vapour extraction or bioventing processes (Harper *et al.*, 1998).

Microorganisms that exist in the soil require water as well as nutrients to survive. For bioventing soil remediation to be effective, the soil must contain a specific amount of soil moisture so that the microorganisms can efficiently breakdown the petroleum hydrocarbon contamination. There is a critical balance for soil moisture, too dry and the microorganisms in the soil remain dormant, too wet and there is a reduced oxygen level in the soil. It has been determined that moisture content in the soil greatly impacts the growth of the microorganisms and thus the degradation rate (Shewfelt *et al.*, 2005). Studies have suggested that the optimum water content of the soil is approximately 50% of the soil's field capacity (Harper *et al.*, 2003). However, it was found that higher water content in the soil can be a limiting factor in mass transfer which drastically impacts SVE and may have some effect of bioventing (Harper *et al.*, 2003).

2.4.2 Temperature

The temperature of the soil can also have an effect on the movement and remediation of contamination in the soil. For example, if the soil contains frost or is frozen for a portion of the year, this would drastically impact any model that is being used to predict site remediation. In a study conducted by Chang *et al.* (2010), they found that the biodegradation rates in contaminated

soils in arctic climates were very low and almost non-existent when soil temperatures were less than 4.7°C. On the contrary, it was found that microbial development and activity was higher when the temperature of the soil remained above 4.7°C. It is important to note that soil temperature does not tend to fluctuate deeper than 2 metres from the soil surface. In Ontario the average soil temperature below the frost line averages around 10°C throughout the year (Zytner, 2001, Zhang *et al.*, 2005).

2.4.3 Soil Type

The type of soil that the contaminant is travelling through plays a large role in the behaviour as it migrates through the soil. The soil environment consists of solid, liquid and gaseous phases, which work together to form a variety of physical, biological and chemical environments. Additionally, different interfaces exist, gas:liquid, liquid:solid and solid:gas, which create a more complex soil environment to study (Zytner, 2014).

Soil can be classified into four main categories by examining the grain sizes of the soil: coarse material, sand, silt and clay. Soils have the solid material (sediments) but also contain water or moisture and air. These three materials, soil, water and air, all have an effect on the contaminant movement. Soil type and composition often varies with depth from the surface and is usually not uniformly consistent in a practical field environment (Alexander, 1977). This poses a significant challenge when developing a remediation strategy as most strategies and models require the input of one soil type.

There are also three horizons within the soil layer, each having different properties associated with them that influence contaminant behaviour in the environment. Soil Horizon A makes up the surface layer which contains vegetation roots, decaying small animals, and the high

quantities of microorganisms (Coduto *et al.*, 2010). The high levels of microorganisms that are present are a direct result of the high organic matter present in this upper layer of soil. The concentration of the microorganisms and nutrients decreases in layers B and C as depth increases, with C being the naturally existing material (Coduto *et al.*, 2010).

2.4.4 Porosity

The amount of pores that are present in the soil is highly dependent on the type of soil material and the amount of compaction the soil has undergone. Soils that are heavily comprised of clay have a higher amount of pores than sand which has a lower percentage of pore space (Coduto *et al.*, 2010). However, the physical size of the pore space in sand is higher than in clays, which can impact the fate of contaminants in the environment.

2.4.5 Present Microorganism Colonies

There are many types of bacteria and microorganisms that are present in the soil. The 5 most common groups of organisms in the soil are: bacteria, actinomycetes, fungi, algae and protozoa (Zytner, 2014; Franzluebbers, 2004). However, the concentrations of bacteria that are present in the soil are highly variable with depth, which are directly related to the organic matter present at each layer. The optimum water content for microorganism growth is approximately 50-77% of the soil's field capacity (Harper *et al.*, 2003). Additionally, the bacteria present in the soil are dependent on factors such as temperature and pH of the soil. It is important to note that naturally existing bacteria in the soil are much more resilient than bacteria grown in a laboratory environment and artificially introduced into the soil.

2.4.6 Nutrients

Bioventing accelerates the biodegrading process by stimulating the microorganisms to increase their populations. This stimulation process is done through the addition of nitrogen into the soil. Shewfelt *et al.* (2005) found that the optimum carbon to nitrogen ratio was 10:1 using ammonium chloride as the nitrogen source. Shewfelt *et al.* (2005) concluded that degradation occurred at a rate five times faster than in the control group, when optimum nitrogen levels were created. In this study it was also determined that moisture content in the soil greatly impacted the growth of the microorganisms and thus the degradation rate.

2.4.7 Age of Contaminants

Often times when dealing with petroleum hydrocarbon remediation projects, the spill has often been present in the environment for an extended period of time which can have a significant impact on the remediation process. Sutton *et al.* (2013) found that aged contamination in the soil can lead to a reduction in the bioavailability due to the length of time that the contaminant has been present in the soil. In their experiment examining aged diesel fuel contamination, they found that some of the diesel was partially degraded within the soil. However, it is important to note that aged petroleum hydrocarbon contaminants reduce the bioavailability for biological degradation, which in turn would require a longer time for the remediation process compared to a non-aged contaminant in the soil (Sutton *et al.*, 2013).

2.5 Correlations and Scale-up Factors

Through bioventing experiments that have been conducted in the laboratory on the micro-scale and meso-scale, scale-up factors have been established to better predict the results that occur at

the macro-scale (field) level. Scale-up issues arise from the scale-dependent phenomena that influence the bioremediation process at the micro-scale (respirometers), meso-scale (soil columns and reactors) and macro-scale (field implementation) levels. The heterogeneity of the soil, reactor shape, age, and contaminant bio-availability were found to be major factors that influenced the scale-up factors. The study conducted by Ko (2007) suggests that field scale biodegradation is slower than the results found in the laboratory, partially due to decreased chemical diffusion in the soil, which in turn makes the contaminant less accessible to the biodegraders.

Thus far, the research and application of bioventing technologies on petroleum hydrocarbon contaminated soils has determined that it is an effective soil remediation technology. However, there is still some uncertainty with regards to the biological decay rate for a particular field site. Additionally, understanding the particular decay rates for a site will allow for the better development of total remediation time that is necessary to clean up the site. Research advancements in this area have already occurred. In 2009, Eyvazi and Zytner developed degradation rate correlation based on the type of soil grain, water content and organic matter in the soil. This correlation was developed based on laboratory research and applied to the seven classes of soil that Eyvazi and Zytner (2009) used, with each reactor holding 200g of soil. Many conditions were studied and analyzed, as soil make-up, water content, organic matter and environmental conditions greatly impact biological decay rates (Harper *et al.*, 2003).

In order to develop a biodegradation rate correlation based on soil parameters it was essential to determine the parameters that had significant impact on the biodegradation rate. Eyvazi and Zytner (2009) tested four soil variables using four different soil types. The relationship between the biodegradation rate constants and the independent soil variables as well as the soil cation-

exchange capacity (CEC) was studied separately. As all of the experiments were conducted under similar environmental conditions, C:N ratio, pH and soil moisture were excluded from the correlation (Eyvazi and Zytner, 2009). Each variable was studied by introducing one variable into the equation at a time which produced several sets of data. Several iterations of the equation were developed and the individual parameters were tested in terms of significance (SYSTAT, 1992; version 5.02). Overall, it was found that the initial population of petroleum-degrading microorganisms and the organic matter content in the soil, had a significant positive impact on the biodegradation rate. Using this process as well as linear regression, Eyvazi and Zytner (2009) were able to develop Equation 1 which corresponded to seven soils tested. A similar process was followed by Khan and Zytner (2013) to develop Equation 2.

The degradation rate developed by Eyvazi and Zytner (2009):

$$\ln(k) = 2.803 \cdot \text{PDP} + 0.210 \cdot \text{Sand} + 4.866 \cdot \text{OM} - 0.094 \cdot \text{Clay} - 0.004(\text{SW} \cdot \text{Sand}) - 0.021(\text{Sand} \cdot \text{PDP}) - 0.632(\text{OM} \cdot \text{PDP}) + 0.004(\text{SW} \cdot \text{Clay}) - 23.5 \quad (1)$$

Where:

- k = biodegradation rate constant (1/d)
- DPD = initial population of petroleum degrading microorganisms in soil (log cfu/g)
- SW = soil water content (wt%)
- Sand = soil sand content (wt%)
- OM = soil organic matter content (wt%)
- Clay = soil clay content (wt%)

Khan and Zytner (2013) used the correlation developed by Eyvazi and Zytner (2009), Equation 1, and conducted meso-scale experiments using 4kg reactors and developed a scale-up factor between the two experiments. In addition, a further refinement of the correlation developed by Eyvazi and Zytner (2009) came after the completion of Khan and Zytner's (2013) work on the 4kg reactor bioventing soil remediation project.

The degradation rate developed by Khan and Zytner (2013):

$$\ln(k_{\text{stg2-P}}) = 0.0234 \cdot \text{Silt} - 0.0355 \cdot \text{Clay} - 0.383 \cdot \text{OM} + 0.825 \cdot \text{PDB} - 0.048 \cdot \text{SW} - 6.774 \quad (2)$$

Where:

- $k_{\text{stg2-P}}$ =biodegradation rate constant (1/d)
- PDB =initial population of petroleum degrading microorganisms in soil (log cfu/g)
- SW =soil water content (wt%)
- Silt =soil silt content (wt%)
- OM =soil organic matter content (wt%)
- Clay =soil clay content (wt%)

Using the $k_{\text{stg2-P}}$ value found from Equation 2 and dividing it by the k value determined from Equation 1, a scale-up factor between the two experiments was created. The scale-up was found to be approximately 2, which demonstrated that the degradation of the petroleum hydrocarbons occurred faster in the larger reactor experiment. This result was also found by Ko *et al.* (2007) when they scaled their experiment from a batch reactor system to a larger pilot scale size. However, this was different than the findings of Sturman (1995), Davis (2003) and Wang (2008), where the bioremediation rate decreased as the reactor size increased. In the case of the experiments conducted by Eyvazi and Zytner (2009) and Khan and Zytner (2013), the scale-up factor increased as the reactor size was increased. This was expected, as the larger volume of soil/surface area ratio provided improved oxygen transfer in the soil and allowed the microorganisms better access to the carbon nitrogen sources. Overall, the results of the experiment conducted by Khan and Zytner (2013) can be used to establish a relationship and scale-up the degradation rate to the field scale level. From Khan and Zytner's (2013) experiments it was also observed that degradation follows two distinct stages. Stage 1 is 0 to 8 days where degradation occurs rapidly, while stage 2 which was observed from 8-30 days, occurs at a slower rate as detailed below in Table 2-1.

Table 2-1 The degradation rates and corresponding scale-up factors for the experiment conducted by Eyvazi and Zytner (2009) and Khan and Zytner (2013).

Soil Type	Duration (days)	Micro-scale	Meso-scale	Scale Up
Delhi	0-8	-	0.598	-
	8-30	0.045	0.123	2.73
Elora	0-8	-	0.460	-
	8-30	0.040	0.075	1.9

Completing the proposed study will help establish a procedure for working with soil on the larger scale. This bioventing study will utilize an 80kg soil reactor, which will allow for further advancement of the scale-up factor. The scale-up factor will be obtained by comparing degradation results previously obtained from the 200 g respirometers (Eyvazi and Zytner (2009) and the 4kg meso-scale reactors (Khan and Zytner (2013)). Overall, it will determine the appropriate scale to study bioventing in order to most accurately transfer the results to the field scale.

2.6 Model Development

For bioventing to be a technology that can become widely used or at least widely considered for environmental site remediation, it is essential to have some good models that accurately represent what is going on within the soil during the remediation process. Nguyen (2013) developed a 3-D model for SVE and a 1-D model has been developed by Barakat and Zytner (2008) which built on research from Gidda (2006). These models helped to improve the understanding of bioventing soil remediation; however, a full 3-D model solely describing bioventing has not yet been developed. A 3-D bioventing model using the degradation correlations and scale-up factors determined by Khan and Zytner (2013) would further help with

the understanding of applying the laboratory results to particular field sites. Through the use of models and advanced study in the area of bioventing soil remediation, research will be better able to develop closure or stop times for soil remediation projects (Carroll *et al.* 2012 and Zhao *et al.*, 2008). Additionally, if proper scale-up factors can be determined, then easier small scale experiments can be conducted instead of complex large scale ones.

CHAPTER 3: EXPERIMENTAL DESIGN

3.1 Reactor Design

The starting point of the 80kg reactor system that was used in this experiment was based on the system previously designed and used by Patros (2009). The overall column and reactor design was based on concepts established by Duggal (2005). The 80kg reactor is often referred to as the Column-Scale Reactor (CSR).

The barrel of the system is an open drum made from T-304 SS, with a wall thickness of 0.7mm (22 steel gauge), an O.D. of 41.3cm (16¼"), an I.D. of 40.6cm (16"), and a height (H) of 51.4cm (20¼"). A ¼" depressed lid for the drum with a cascaded edge and a neoprene ring makes a seal with the top of the drum. At the time of Patros's experiment there was a compression ring for the lid that was custom made to fit the barrel. However, this compression ring was replaced with three-1.5" C-clamps to clamp the lid to the drum of the barrel. The drum and lid were purchased from the General Container Corporation, Newark, NJ.

The inside of the CSR walls were hand drilled with a ½ inch drill bit and lined with an industrial grade steel mesh. At the bottom of the drum a 4" high 316 SS strip was placed along the circumference of the reactor. A 40cm diameter 316 SS plate was then placed on top of the strip creating a 4" hollow cavity in the bottom of the reactor system. This cavity was created intentionally to allow for the installation of a 316 SS vacuum well [50.8cm (20") high, 3.2cm (1¼") I.D., 3.8cm (1½") O.D., and 0.1 mm slit size] in the center of the reactor. The other end of the vacuum well had a 1 ¼" FNPT piece welded to it. In order to ensure that short circuiting did not occur in the vacuum well, a rubber stopper and Teflon tape were used to seal the portion of

the vacuum well that was below the 4" cavity. Five additional holes were drilled in the plate halfway between the center and the edge. The holes were at an angle of 72° center-to-center from each other to accommodate the five injecting-spiking wells. These five injection wells were used by Patros and left unused in this experiment. The 40cm diameter SS plate, the vacuum well and the five injection wells were all sealed in place using contractor grade silicone or a bead weld.

On the outside of the CSR, 15-1" holes were drilled through the wall. They were distributed into 3 rows x 5 columns, 72° apart from one another horizontally, center-to-center, and 18.7cm vertically, center-to-center. Into the drilled holes 15 x 3/4" FNPT 316 SS pieces were silicone into place. ten (MNPT) plugs were then screwed into 10 of the 15 FNPT ports which acted as sampling ports where soil could be extracted from the reactor during the experiment. 5 MNPT nipples were screwed into the remaining ports which were used as measurement ports where temperature, soil moisture or soil sampler ports would exist. On the outside end of the nipples a piece of rubber was cut using a utility-knife and firmly placed in the port. This allowed for sampling probes and cables to be fed into the reactor while maintaining a sufficient seal. The 10 (MNPT) plugs, 5 MNPT nipples and rubber pieces were purchased from Stan's Plumbing in Guelph, ON.

3.2 System Configuration

Figure 3-1 gives a schematic on the configuration, layout and monitoring equipment used for the bioventing experiment conducted in the laboratory. Figure 3-2 gives the layout of the experimental setup in operation in the laboratory environment.

Figure 3-1 A box diagram schematic layout of the bioventing system.

Figure 3-2 Layout of the bioventing system setup in the laboratory.

More information on the specific types of equipment used in the bioventing experiment is detailed in Chapter 4.

CHAPTER 4: METHODOLOGY PRE-EXPERIMENT OPERATIONS

4.1 Soil Collection

For this research project two different types of soil were used. Soil from the Delhi and Elora Research Station were the two selected soils as they had been previously used in the bioventing soil vapour extractions by Eyvazi and Zytner (2009) and Khan and Zytner (2013). By using the same soils that these past researchers used, it will allow for a more accurate development of scale up factors for bioventing SVE using the larger 80kg reactors. It is understood that past researchers at the University of Guelph have completed detailed soil classifications for both the Delhi and Elora soil type and a detailed classification was not required for this study.

4.1.1 Delhi Soil

The Delhi Research Station located at 711 Schafer Road, Delhi, Ontario, operated by Agriculture and Agri-Food Canada (AAFC) has been closed since December of 2012 (AAFC, 2015). In order to maintain consistency as the bioventing SVE experiments are scaled up, it was necessary to use soil from the Delhi Station as the properties of the soil were well defined. After contacting AAFC it was discovered that the Delhi Station was no longer staffed. Erin Sterling, Integrated Services Manager with AAFC assisted with access to the site on a day when a work crew lead by Joe Pratt (AAFC) would be going to the site. Ms. Sterling assisted with the site access paper work required for entry to the property. Upon arrival at the site June 25, 2015, Mr. Pratt provided directions on where to dig for the soil that was required for the experiment. The required soil was located directly across from the main administration building. The soil excavation location was about 3 meters into the winter grass crop from the small road on the east side of the field. The winter grass vegetation was pushed down with a shovel and soil was collected by digging down

approximately 30cm. After about 250kg of soil was collected and placed into poly bags, the holes were levelled out to prevent a tripping hazard to others that use the field. Table 4-1, shows the physical soil properties previously determined for the Delhi soil.

Table 4-1 Physical properties of the Delhi soil from a Laboratory Analysis.

Soil Name	Sand (%)	Silt (%)	Clay (%)	Organic Matter (%)	Water holding capacity (%)	Bulk Density (kg/m³)
Delhi	84.9	9.8	5.3	1.1	25	1550

At time of this publication, the Delhi Research Station has been sold to Titan Trailers of Delhi, Ontario. Any further access to the Delhi Research Station will require permission from Titan Trailers before proceeding (Titan Trailers, 2016).

4.1.2 Elora Soil

The Elora Research Station located at 6182 2nd Line, Pilkington Twp. R.R. #2 Ariss, Ontario is operated by the University of Guelph under the direction of Station Manager, Dave Kells. Upon arrival at the site October 5, 2015, Mr. Kells provided directions on where to dig for the soil that was required for the experiment. The soil which was recently excavated from an earth works project at the station, was stored in a large pile in a back field. About 250kg of soil was collected and placed into poly bags and transported back to the University main campus. Table 4-2, shows the physical soil properties previously determined for the Elora soil.

Table 4-2 Physical properties of the Elroa soil from a Laboratory Analysis.

Soil Name	Sand (%)	Silt (%)	Clay (%)	Organic Matter (%)	Water holding capacity (%)	Bulk Density (kg/m³)
Elora	31	50.8	18.2	3	30	1350

4.2 Soil Drying and Sorting

Once soil was collected from the field, it was dried in order to be used for the experiment. Due to the large volumes of soil and the uncooperative weather in Guelph at the time of drying, the soil was dried on a tarp in the THRN 1113, the walk-in fume hood laboratory space. The soil was turned occasionally using a small garden shovel for about 3 to 4 days or until it was deemed dry. When the soil was dry the microorganism populations went dormant and were reactivated with water was amended to the soil. In addition to drying the soil, the soil was sifted to remove any large rocks or large pieces of organic matter that were unintentionally collected from the field. A USCS Number 4 sieve with a 4.75 mm mesh size was used to remove large grain material as well as any unwanted organic matter, such as grass, from the collected soil. After the soil was sieved, it was placed back in ploy bags and stored for later use during the experiment. When sieving the soils, a dust mask was worn to prevent exposure to fine soil particulates that could have become airborne during the handling of the dry soil, posing a potential inhalation risk. This process was used for both the Delhi and Elora soils.

4.3 Mechanical Soil Mixer

Working with 80kg of soil for the purposes of a research project is often quite difficult and bioventing we do not want to volatilize the synthetic gasoline. Additionally, research work at this large of a laboratory scale had not been done previously. As part of the experiment there was a need to uniformly mix soil at two separate phases of the experiment. One phase required the mixing of the soil with water and the nutrient supplement (NH_4Cl) in order to activate the soil microorganisms and to provide them with adequate soil moisture levels for the bioventing experiment. The next phase in which mixing was required, was when the synthetic gasoline

blend was added to the soil. For both of the mixing phases it was necessary to ensure sufficient and uniform mixing of the soil. For handling of the soil in this experiment, 1-gallon paint cans were chosen to hold the soil. It was decided that the soil would need to be mixed in the 1-gallon cans before being placed into the 80kg stainless steel reactor. It was calculated that 16 1-gallon cans would be required for each experiment in order to sufficiently fill the 80kg reactor. Since the cans needed to be mixed twice for about 20 minutes each time, it was not feasible to mix the cans by hand due to physical limitations and the requirement for uniform mixing.

A mechanical mixer was designed to hold 4 1-gallon cans and tumble the soil top over bottom. A repurposed garden hose reel cart was used for the frame of the mixer and a loading can carrousel, holding 4 cans at a time, was built using plywood. The carrousel was centered in the middle of the cart around an iron pipe and originally fixed with a hand crank. After some use of the system, it was clear that the hand driven system, required significant time to mix all of the 16 cans twice. A gear driven 1/9 HP, low speed, high torque electric motor from Rolan Electric (Elmira, ON) was purchased and installed onto the cart. The motor output was 123 rpm which was still too fast for the mixing process. Using a pulley system, a 2 ½" wheel (5/8" bore) was installed on the motor head and an 8" wheel (3/4" bore) was installed on the iron bar connected to the mixer and connected with a 38" V belt. The speed of the mixing carrousel was then scaled down to a speed of about 30 rpm which was reasonable for the mixing process. The soil mixing carrousel was designed and built by M. Mosco (June 2015) and the motor and pulley system was installed by M. Mosco (August 2015). The mixing unit is seen in Figure 4-1 and 4-2.

Figure 4-1 The mechanical soil mixer which can handle 4, 1-gallon cans at a time.

Figure 4-2 A view of the drive system powering the mechanical soil mixer.

4.4 Preparation of Synthetic Gasoline

Spiking was done with synthetic gasoline consisting of 5 compounds: Isooctane (24.9 wt.%), Naphthalene (3.2 wt.%), 1,3,5- Trimethylbenzene (11.9 wt.%), m-Xylene (23.9 wt.%) and Toluene (36.0 wt. %), to represent the Canada Wide Standards for petroleum hydrocarbons. This mixture was derived from previous experimental work with Imperial Oil Ltd (Shewfelt *et al.*, 2005) and provided a good representation of the constituents in gasoline fuels. Using a synthetic gasoline mix with 5 components better allowed for the better understanding of the results in the bioventing experiment.

This was the same procedure that was used by both Eyvazi and Zytner (2009) and Khan and Zytner (2013) and was used in this experiment in order to maintain consistency when comparing and discussing the degradation results.

Table 4-3 shows the composition used for the preparation of the synthetic gasoline blend. Naphthalene was the only solid chemical compound used and the four others were in liquid form which is the reasoning behind Table 4-3 having both weights and volumes.

Table 4-3 Preparation of synthetic gasoline to mix for an initial spike of 4000mg/kg.

	Chemical	Density g/mL	Mol Weight g/mol	Used in Exp % mass	Amount gram	Amount mL
Gasoline Components	M_Xylene	0.9	106.17	0.239	76.48	84.98
	Naphthalene	0.99	128.18	0.032	10.24	10.34
	Toluene	0.87	92.14	0.361	115.52	132.78
	1,3,5 Trimethylbenzene	0.8637	120.19	0.119	38.08	44.09
	Isooctane	0.629	114.23	0.249	79.68	126.68
Vol. Total (mL)						398.87

When preparing the synthetic gasoline blend, all work was performed in a box fume hood due to the nature of the chemical compounds present. The naphthalene, being the only solid phase

chemical, was placed in the flask where trimethylbenzene and m_xylene were added. The flask was then mixed until all of the naphthalene crystals were dissolved. Toluene and isooctane were added to the mixing process (at the end) due to their high vapour pressures. Contents from the flask were poured into a 500 mL amber glass bottle and stored in the freezer for future use.

4.5 Gas Chromatography Set-up

Gas chromatography was used to analyze the concentration of petroleum hydrocarbons in the soil samples taken from the 80kg soil reactor. Gas chromatography analysis was used previously for this type of work by Eyvazi and Zytner (2009) and Khan and Zytner (2013).

4.5.1 Apparatus Operational Overview

The University of Guelph, School of Engineering has a gas chromatography machine that was utilized for this experiment. The Gas Chromatograph (GC) (Hewlett-Packard 5890 Series II) was equipped with a Flame Ionization Detector (FID) as well as an auto sampler (Hewlett-Packard 7673). It was noted early on in the experiment through the testing of the standard solutions that there was an occasional fault with the auto sampler (Hewlett-Packard 7673) as the device was “unable to retrieve vial from tray”. This equipment was used to analyze the total petroleum hydrocarbons from the soil and the ORBO 30 sorbent tubes.

The system was operated in a similar fashion as detailed by Khan and Zytner (2013) where Khan followed the procedure detailed by Eyvazi and Zytner (2010). The following details briefly overview the conditions and settings used for the operation of the GC FID. One micro litre samples from the GC vials were injected into the GC using the auto-injection system which was part of the GC FID device. The operating temperature for the injector was 225 °C and the Flame

Ionization Detector (FID) was set at 275 °C. The oven ramp program began with an initial temperature 35 °C. The system held this temperature for 3.5 minutes then gradually increased by setting the ramp at 25 °C/min to achieve an operating temperature of 200 °C. Once the system achieved the final temperature of 200 °C, it was held for 5 minutes, giving a complete run time of 15.2 minutes. Between samples runs, the syringe of GC was washed with methanol to ensure no residue of the previous sample was retained in the syringe tip (Eyvazi and Zytner, 2009; Khan and Zytner, 2013).

The capillary column used for the analysis of synthetic gasoline samples was J&W Scientific DB5 column. The column was 30 m long with inner diameter of 0.32 mm and a film thickness of 1 µm. Helium (grade: 5.0) was used as a carrier gas and its split column pressure was set at 12 psi (Eyvazi and Zytner, 2009; Khan and Zytner, 2013).

4.5.2 Creation of Standards

The soil contaminated with the synthetic gasoline was analyzed through the use of gas chromatography. In order to determine the concentrations of gasoline present in the soil samples, standard solutions with various concentrations of the synthetic gasoline blend were created to compare and develop a calibration curve. This allowed for the comparison of unknown concentrations that existed in the soil to the known concentrations based on the calibration curve.

In order to develop the standards, synthetic gasoline was prepared as detailed in Section 4.4. The solvent that was used in preparation of the samples was Methylene Chloride (CH_2Cl_2), as this solvent was used for the extraction of the synthetic gasoline out of the soil. This provides a similar area response curve when processed in the GC FID. The synthetic gasoline blend was taken from its storage in the freezer and used for the creation of the standard solutions. In order

to further reduce volatilization, about 500 mL of Methylene Chloride was placed in the freezer for one day prior to the making of the standards to cool it down.

The concentrations were prepared using details found in Table 4-4. In order to get the accurate amount of synthetic gasoline which is in a liquid form, the mass of gasoline was converted to a volume using a weighted density percentage of the synthetic gasoline blend. A 500 uL syringe was used to extract 248 uL and inject the synthetic gasoline into the first 250 mL amber glass bottle containing 100 mL of Methylene Chloride. This procedure was verified in order to ensure accuracy by injecting the volume of synthetic gasoline in the syringe into a pre-weighed glass beaker and reweighed using a Mettler Type AJ100L weigh scale.

Table 4-4 The details for the preparation of the standard solution for the GC.

Desired Concentration			Remaining Volume (mL)
2000mg/L	200mg of synth gas	Add 100ml of CH ₂ Cl ₂	35
1000mg/L	50ml of 2000mg/L	Add 50ml of CH ₂ Cl ₂	50
750mg/L	15ml of 2000mg/L	Add 40ml of CH ₂ Cl ₂	55
500mg/L	50ml of 1000mg/L	Add 50ml of CH ₂ Cl ₂	65
250mg/L	25ml of 500mg/L	Add 25ml of CH ₂ Cl ₂	50
100mg/L	10ml of 500mg/L	Add 50ml of CH ₂ Cl ₂	55
10mg/L	5ml of 100mg/L	Add 50ml of CH ₂ Cl ₂	50
1mg/L	5ml of 10mg/L	Add 50ml of CH ₂ Cl ₂	55

After the standards solutions were created in the 250 mL amber glass bottles, they were placed in the freezer overnight. A portion of the standard solutions was taken from the 250 mL bottles and placed into 2 mL GC vials and the GC vial caps were crimped closed. Each standard solution was used to make two GC vials and the remaining standard solution was returned to the freezer for storage.

4.5.3 Development of a Standards Curve

Using the standards that were prepared, a standards curve was created. This involved running the GC FID with the various GC vial standards that were prepared. The GC was initially run with pure Methylene Chloride to ensure an adequate baseline was developed. Then each of the standards were run in duplicates increasing in concentration. After each run, the GC FID baseline line was checked and the results from the previous run were quickly inspected to check for any obvious abnormalities. In order to ensure accuracy, the standard solution GC vials were tested twice on different days in order to test for any significant deviations in the results day over day.

Below Figure 4-3 is an example of a standards curve that was prepared before each batch of samples was run using the GC FID.

Figure 4-3 A Standard Samples Run showing the relationship that exists between System Response Area and Synthetic Gasoline Concentration.

As shown, the relationship between concentration and response area is linear and the R^2 value is well fitted to this relationship. Once the standard curve for each of the sample batches was developed, the concentration of the synthetic gasoline in the soil was back calculated and

reported as mg synthetic gasoline per kilogram of soil or mg of gasoline lost in the off gas venting through the air exhaust tube. Individual standard curves were used to back calculate the synthetic gasoline in the soil or activated carbon sample. Each batch of standards produced a slightly different response area due to operational factors such as gas flow and pressure despite best efforts to maintain consistent operation with the GC. The standard curves can be found in Appendix B. Initial GC FID standard curves produced for this experiment compared well with the standard curves that were created by Khan (2013) when he operated the GC FID.

4.5.4 Solvent Extraction Efficiency

When soil was sampled from the reactor it was tested using the GC FID to measure the concentration of petroleum hydrocarbons in the soil. In order to test the gasoline concentration in the soil, the gasoline had to be extracted from the soil using a solvent (methylene chloride). This solvent extraction process is not 100% efficient, meaning that not all of the gasoline can be removed from the soil. Typical solvent extraction efficiencies using the process similar to the one used by Khan (2013) achieved an extraction efficiency of approximately 75%. Keeping in mind the extraction efficiencies that can be readily achieved, the petroleum hydrocarbon concentrations were corrected to estimate the actual concentration in the soil. For the purposes of monitoring petroleum hydrocarbon degradation day over day within the soil reactor, values were detailed as change over the specified time period (the bio-degradation), making the exact extraction efficiency less critical to know.

Aged petroleum hydrocarbon soil contamination behaves significantly different than freshly contaminated soils. The chemical bonds that exist between soil particles and petroleum hydrocarbons become stronger as they age (Jain *et al.*, 1992, Sutton *et al.*, 2013). Not only does

this make the contamination harder to bio-remediate but impacts the solvent extraction efficiency. Studies have shown that extraction efficiency can drop to almost 50% for some types of petroleum hydrocarbons after a period of 28 days (Jain *et al.*, 1992).

4.6 Lab Station Overview

The main laboratory used for this bioventing SVE experiment was THRN 1113B, which was designed and built specifically for this type of work as it has a walk in fume hood. The entire 30.5m³ lab space is under negative air flow which prevents the accumulation of synthetic gasoline vapours in the lab space. The Gas Chromatography machine as well as the standard fume hoods for synthetic gasoline preparation was found in the SOE Analytical Research Lab. In addition, the SOE Analytical Research Lab freezer was used to store the synthetic gasoline and the samples. A walk-in cooler, located in the water research lab was used to refrigerate the soil prior to loading it into the reactor in order to minimize volatilization.

4.7 Air Flow in Reactor

Air flow through the 80kg soil reactor is essential for the bioventing process. The flow rate of air needed to be calculated precisely so that the microorganisms in the soil would be supplied with an adequate amount of oxygen rich air. However, too high of a flow rate would promote volatilization of the petroleum hydrocarbon compounds or rapidly dry out the soil. Studying bioventing at a large meso-scale such as this experiment with an 80kg reactor system, poses many difficulties when maintaining and monitoring the air flow through the soil. Patros (2009) and Khan and Zytner (2013) adopted a procedure used by the United States Army Corps of Engineers with regards to bioventing flow rates through soil (US Army Corps of Engineers,

2002). The guidance from the USACE was to replace 0.5 to 1.0 pore volumes (PV) of soil each 24 hour period.

When the 4kg meso-scale bioventing reactors were used, Patros (2009) calculated that 594 days of venting would require exchanging $0.412 \text{ m}^3/\text{MSR}$. In order to achieve these exchanges, Patros suggested the air flow rate 0.482 mL/min/MSR for 594 days of hydrocarbon degradation (Khan and Zytner, 2013). From this Patros determined that 594 days was a significantly long time to operate a bioventing experiment in a laboratory environment. He adjusted the flow rate on the pump drawing air from the reactor to a rate of 14 mL/min for the 4kg MSR. After running the MSR for a short period of time, Patros found that a significant amount of petroleum hydrocarbon volatilization was occurring at the high flow rate. An alternative method for the determination of the flow rate required for the degradation of petroleum hydrocarbon in the soil was to complete a stoichiometric mass balance. Qureshi (2010) used stoichiometric techniques for the replacement of one pore volume per day and suggested 0.72 mL/min air flow rate for the degradation of gasoline in meso-scale reactors (Khan and Zytner, 2013). Khan's work with the 4kg MSR reactor initially used a flow rate of 0.72 mL/min . Once again, using this higher flow rate, some volatilization of petroleum hydrocarbons was noted and the flow rate was adjusted to 0.42 mL/min by wrapping the 4kg reactors in glass wool.

For this experiment which involved a 80kg soil reactor the flow rates needed for the soil volume were calculated using the two methods detailed above, USACE and stoichiometric methodology. Using the porosity of the predetermined porosity of the Delhi soil of 0.5 and the bulk density of the soil at 1550 kg/m^3 , the USACE method was applied using the recommended 0.5 pore volume exchanges per day. It was determined that approximately 12.9 L of air would be required per day

during the operation of the 80kg reactor, which is equivalent to 8.9 mL/min as the required flow rate.

Alternately, the stoichiometric approach was applied in a simplified format having C_8H_{12} represent all of the components of the synthetic gasoline mix used in this experiment and that the gasoline would completely breakdown. It was found that in order for all of the gasoline to break down, 7.309 m^3 of air was required over 566.6 pore volume exchanges. Overall, it was determined that the volume of air required on a daily basis was approximately 12.9L or 8.95mL/min using the idea of 1 pore volume exchange per day.

These results for the calculation of air flow for the reactor system were consistent with ones found by Patros (2009) and Khan (2013). The flow rate for the 80kg reactor system was set to approximately 8.9mL/min.

In order to provide the flow rate of air for the reactor system a Gilian-Sensidyne (LFS-113DC) low flow pump was used. To monitor the flow rate of air being drawn from the soil reactor, an Omega (FMA-5606ST) Micro Flow Meter was used. To validate the accuracy of the flow readings from the Omega Micro Flow Meter, a J&W Scientific digital flow meter (Serial #1872) was connected to the system in series to the Omega Micro Flow Meter.

4.8 Soil Moisture Testing

Soil moisture in the reactor was monitored using two HOBO soil moisture probes (S-SMC-M005) connected to a HOBO Micro-Data Logger Station (H21-002). One of the two probes was located just inside the wall of the drum in the area most likely to dry out during the operation of the soil reactor. The other probe was located near the vacuum well which was another area

subject to drying out throughout the experiment. Each of the soil moisture probe tips were capable of measuring the soil moisture of a 0.3 litre volume of soil as per the specification from Onset, the probe manufacturer.

The HOBO Micro-Data Logger Station recorded the soil moisture of each probe every 10 minutes. The recorded data from the data logger was extracted and downloaded to a computer with the use of the HOBO U-Shuttle. In order to view the results, a licence for the computer software, HOBOWare Pro Version 3.7.2 was purchased. The HOBOWare Pro software neatly displayed the soil moisture readings in chart and graph format. Soil moisture was monitored throughout the experiment to ensure that the soil moisture content in the soil reactor was not less than 15 wt.% which is equivalent to 50-60% of the soils field capacity.

In order to test the effectiveness of the sensors and the HOBO Micro-Data Logger two separate tests were done. The first test was to test the simple operation of the system. After the sensors were cleaned from the previous user and the data logger batteries were replaced, the logger was launched using the U-Shuttle and the two probes were placed in a soil garden. Shortly after the data logging started, the garden was watered which, if the system was functioning correctly, would show an increase in soil moisture readings. The logger was operational for nearly 48 hours, after which the data was reviewed to ensure that the readings were representative of the watering events that occurred. The second test required the use of 4-1 gallon clean paint cans filled with soil and a known amount of water. Dry Delhi soil from the poly bag storage was weighed to approximately 3kg and placed in each of the two cans. Then two different water amounts were added to the each of two cans using information found in Table 4-5. The soil was then packed into two separate aluminum cans to the appropriate density of 1550kg/m^3 using premeasured lines on the inside of the cans.

Table 4-5 Soil Moisture Probe Testing

Can Number	Amount of Soil (g)	Amount of Water (g)	Calculated Soil Moisture (m³/m³)	Average Soil Moisture Reading from Probe (m³/m³)
1	3000	450	0.2325	0.2416 (±3%)
2	3000	600	0.31	0.3237 (±3%)

Based on the two tests that were completed, it was concluded that the soil moisture monitoring system provided enough accuracy to monitor the moisture in the soil reactor throughout the experiment. It was not critical that the soil moisture values be monitored with a high degree of precision, as the soil moisture range that was acceptable for this bioventing experiment was between 15 and 20m³/m³ or often previously referred to as approximately 50% of the soils field capacity. Of main importance was that the soil moisture levels did not significantly drop throughout the 30 day experimental period.

This system that both measured and logged the soil moisture data was selected over the previous system used by Khan and Zytner (2013) due to complications with the configuration of the gypsum plug and some key equipment missing, such as the multiplexer needed to handle the signals from the gypsum plugs. Additionally, the HOBOT system is a newer technology version and is significantly more user friendly and the HOBOT system relies on the “plug and play” concept.

4.9 Soil Moisture Experiment on Reactor

Maintaining soil moisture in the reactor throughout the experiment was critical in order to ensure that the soil microorganisms (heterotrophic bacteria) had sufficient moisture available in the soil in order to break down the synthetic gasoline contamination in the soil. The optimum water content for microorganism growth is approximately 50-77% of the soils field capacity which

equates to about 15 and 20 m³/m³ (Goring *et al.*, 1974; Harper *et al.*, 2003). Due to the design of the 80kg soil reactor and the placement of the reactor in the walk-in fume hood laboratory where there were significant air currents moving throughout, there was some initial concern about rapid drying out of the soil.

Testing of the system was completed prior to contaminating the soil with the synthetic petroleum hydrocarbons since it was previously determined by Goring *et al.* (1974) and Harper *et al.* (2003) that if soil moisture is not maintained, bioventing SVE will not be under optimal conditions.

4.9.1 Soil Moisture Test 1

Using 16 1 gallon cans, 4kg of dry Delhi soil was measured and placed in each of the 16 cans. Then 600mL of tap water was measured using a 1L glass beaker and added to each of the cans. Using the custom soil tumbler designed and built by Mosco (2015) the 1 gallon cans were tumbled, 4 at a time, for approximately 20 minutes in order to ensure that the soil was evenly mixed. The soil was then loaded into the 80kg reactor and lightly packed using a stainless steel scoop. It is important to note that for this test the lines on the soil reactor were not in place and therefore the density of the soil was likely semi-variable. As such a method was needed to ensure consistent and accurate density of the soil when being packed into the reactor was determined. Immediately after this experiment, a system was put into place to ensure more accurate packing of the soil reactor.

4.9.1.1 Uncontrolled Soil Moisture Test

After the soil was packed into the reactor, the soil moisture probes (HOBO S-SMC-M005) were placed in the soil reactor in the two areas of the reactor where the soil was mostly likely to

experience significant drying. These areas were identified as the very outside of the stainless steel reactor and the core of the reactor near the venting well. The lid of the soil reactor was secured on with the use of the 3 C-clamps and the HOBO Micro-Data Logger was launched and programed to sample each of the probes in 10 minute intervals. The soil moisture levels were monitored over a 5 day period and the results can be seen in Figure 4-4.

4.9.1.2 Details for the Soil Uncontrolled Soil Moisture Test

Total Amount of Soil: 62kg

Total Amount of Water: 9.3L (9.3kg)

Total Weight in the Reactor: 71.3kg

Volume of the Reactor: 0.051216m³

Trial 1 Density: 1392.1kg/m³

Water Content Calculated: 0.1815m³/m³

Figure 4-4 The soil moisture in the 80 kg reactor from Test 1 with Delhi soil.

Table 4-6 Tabulated results from the soil moisture experiment.

	Inside (Core) Probe (m³/m³)	Outside Probe (m³/m³)
Initial Moisture Content	0.1121	0.1739
End Moisture Content (Day 5)	0.0874	0.1397
Change Over 5 Days	0.0247	0.0342
Daily Change	0.00494	0.00684
Daily Loss of Water (mL)	253	350

Based on the results from the first experiment in monitoring the soil moisture, the soil lost between 250 and 350mL of water per day as detailed in Table 4-6. It was also determined that more moisture was lost on the outside of the soil reactor compared to the inside, which implied the outer boundary of the soil in the reactor was exchanging with air from the lab and was not fully a result of the air flow through the reactor to the venting well at the core. This posed a significant issue as the soil moisture in the reactor needed to be maintained between 15 and 20m³/m³ for the entire length of the experiment which spanned approximately 30 days.

When the 80kg reactor was designed by Patros (2009), there were five injection ports. The injection columns were at an angle of 72° center-to-center from each other to accommodate all chemical injections for the experiment conducted by Patros (2009). In order to compensate for the losses of water in the soil, 60mL of water was injected into each of the injection wells and the results were monitored through the readings on the soil moisture probes. This was repeated daily for 3 days and then the results from the HOBO Micro-Data Logger were reviewed. Unfortunately the data was relatively inconsistent. Upon initial injection of the water into each of the five ports, the soil moisture readings from the probes increased slightly for a 2 or 3 hour period before receding to the previously existing readings. Through visual inspections of the injection column area it was evident that the injected water was not being evenly distributed

throughout the soil medium in the reactor. It was clearly evident that the process of injecting water into the injection ports would not work sufficiently for the purposes of this project.

4.9.1.3 Addition of a Cotton Wrap

From the monitoring of the soil moisture with and without water injections it was determined that there was a significantly greater amount of water being lost from the outside of the stainless steel reactor as opposed to the inside or core of the reactor. In an effort to slow down soil moisture losses, in particular losses on the outer wall of the reactor, a 100% cotton wrap was purchased from Len's Mill Store in Guelph, ON. This cotton wrap was securely fit around the reactor and small holes were cut in the fabric to allow to access to the sampling ports. The experiment was continued using the cotton wrap around the outside of the reactor and the soil moisture probes continued to log the soil moisture in the reactor in 10 minute intervals. The results are depicted in Figure 4-5 and Table 4-7.

4.9.1.4 Details for the Soil Uncontrolled Soil Moisture Test

Total Amount of Soil: 62kg

Total Amount of Water: 9.3L (9.3kg)

Total Weight in the Reactor: 71.3kg

Volume of the Reactor: 0.051216m^3

Trial 1 Density: $1392.1\text{kg}/\text{m}^3$

Water Content Calculated: $0.1815\text{m}^3/\text{m}^3$

Figure 4-5 The results of the soil moisture experiment with cotton wrap.

Table 4-7 Tabulated results from the soil moisture experiment with the cotton wrap.

	Inside (Core) Probe (m³/m³)	Outside Probe (m³/m³)
Initial Moisture Content	0.0889	0.1492
End Moisture Content (Day 4)	0.0809	0.1405
Change Over 4 Days	0.008	0.0087
Daily Change	0.002	0.00218
Daily Loss of Water (mL)	102	111

It was apparent that the use of the cotton wrap was effective at slowing the moisture loss in the soil throughout the reactor. Comparing the results from the reactor with and without the cotton wrap it was observed that there was a 60-70% reduction in moisture losses when the 100% cotton wrap was used. When using the cotton wrap, the loss from the soil reactor averaged about 105mL/day compared to approximately 300mL/day without the cotton wrap. Another significant finding was that the moisture losses on the outside of the reactor were significantly lower with the use of the wrap and were much closer to the moisture loss results for the center (core) of the reactor. In the experiment involving the cotton wrap, the soil moisture loss at the core was

calculated to be approximately 102mL/day compared to the outside of the reactor at 111mL/day which was a significant improvement over the control test.

However, the soil was still losing moisture on a daily basis and would not be able to maintain the optimal soil moisture content for the entire 30-40 day experimental trial. The option to use the 5 injection ports in the reactor to add water had previously been explored and it was determined that the injection ports would not adequately distribute the water evenly throughout all of the soil in the reactor.

In order to add moisture or maintain the soil moisture levels in the reactor, a 1 litre plastic water spray bottle was purchased and used to lightly spray the outside of the cotton wrap. For each spray application, the outside of the cotton wrap was sprayed until the material was saturated (water began dripping from the cotton wrap); this was approximately 150mL. Results from the applications of water were monitored with the soil moisture probes in the reactor. The results indicated that immediately after the application of the water spray and over the next 4 hours, the moisture levels in the soil were maintained at a constant level. It was observed multiple times that the cotton wrap was dry to touch after the 4 hour period. After the four hour period, the moisture levels in the soil continued to decrease at a rate of approximately 100-110mL/day. It was concluded that the process could be plausible if the water spray was reapplied every 4 hours. However, logistically, reapplying the water spray to the outside of the reactor every 4 hours was not a reasonable process to follow.

4.9.2 Soil Moisture Test 2

After some deliberation, it was determined that an enclosure surrounding the reactor was required to maintain adequate soil moisture levels in the soil reactor for the duration of the study.

Humidified air was required to be pumped in and circulated evenly within an enclosure, allowing the soil reactor to draw air at a rate of 8-10mL/minute as required for bioventing. This implied that humidified air being supplied to the enclosure had to exceed the rate of 8-10mL/minute to ensure sufficient levels were present. Another requirement of the enclosure would be for easy disassembly to allow for complete 360 degree access to the soil reactor for soil sampling purposes. Further requirements of the enclosure required it to be in a location where the soil moisture data cables could be connected and allow for the ½” SS Swagelok pipe venting system to be connected on the top.

4.9.2.1 Design of the Enclosure

An enclosure was designed to the dimensions of 24” by 24” with a height of 28” using Plexiglas® and aluminum framing material. Using Plexiglas® allowed for the reactor to be visible at all times in order to visually inspect the system to ensure all components were operating normally. The aluminum frame of the enclosure was built using 1/8” aluminum strips which were manually riveted together. The Plexiglas® was cut to the required 24” and 28” sizes and secured to the aluminum frame with a nut, bolt and washer through predrilled pilot holes. In order for easy 360 degree access to allow for sampling, both the front and top Plexiglas® panels could be removed by pulling out the 4 secure pins. Once the top and front panels were removed, the remaining three sides could be pulled away.

Two 1 ½” holes were drilled through the Plexiglas® to allow for the entry and exit of humidified air into and out of the system. One hole was drilled on the left side of the enclosure and was connected to the humidified air source. The second hole was drilled on the top (removable panel) to allow for venting of “extra” humidified air and to allow for the cables for the soil moisture

data logger to exit the enclosure. A small cotton rag was placed in the top venting tube in order to slow air flow leaving the reactor. The main concern was creating a pressure difference within the enclosure which would impact the bioventing experiment. The design of the enclosure was not air tight and it was determined that it did not create a potential pressure difference.

Figure 4-6 A detailed schematic for the air flow system.

Figure 4-7 A picture of the Plexiglas® enclosure during experimental operation.

4.9.2.2 Humidifier System Design

As detailed previously, maintaining soil moisture within the reactor was been difficult due to variety of factors, including air currents in the laboratory and air flow/exchange within the reactor. The humidifier system was designed to work in conjunction with the Plexiglas® enclosure surrounding the reactor. The design consisted of a 120cm tall, 12cm diameter column which held approximately 10L of water as well as TopFin Air-8000 aquarium bubbler and aquarium stone capable of bubbling air into the column at a rate of 10L/min. The column, capped at the topped was connected to the left side of the Plexiglas® enclosure using 1 ½” PVC piping. The pump supplied the rock stone at the bottom with the tank air from the laboratory. The air, then saturated with water vapour entered the Plexiglas® enclosure and provided humidified air

for the bioventing system. Details of the setup can be seen below in the schematic and photo of the humidifying device used for this experiment.

Figure 4-8 The humidifier column during experimental operation.

4.9.2.3 Details for the Soil Moisture Test with Enclosure

Total Amount of Soil: 69.04kg

Total Amount of Water: 10.4L (10.4kg)

Total Weight in the Reactor: 79.44kg

Volume of the Reactor: 0.051216m^3

Trial 2 Density: $1551.06\text{kg}/\text{m}^3$

Water Content Calculated: $0.2031\text{m}^3/\text{m}^3$

Figure 4-9 Soil Moisture Testing with Enclosure.

Table 4-8 Tabulated results from the soil moisture experiment with the Plexiglas® enclosure.

	Inside (Core) Probe (m^3/m^3)	Outside Probe (m^3/m^3)
Initial Moisture Content	0.232	0.2008
End Moisture Content (Day 9.5)	0.2189	0.1884
Change Over 9.5 Days	0.0131	0.0124
Daily Change	0.00138	0.00131
Daily Loss of Water (mL)	7.1	6.7

It is apparent that use of the Plexiglas® enclosure with the use of the cotton wrap was effective at slowing the moisture loss in the soil throughout the reactor. Comparing the results of the control test to the results of the test with the enclosure system and the cotton wrap, there was about a 98% reduction in moisture loss in the reactor. When using the enclosure and the cotton wrap, the losses throughout the soil reactor averaged about 6.9mL/day compared to approximately 300mL/day as detailed in Table 4-8. Another significant finding was that the moisture loss on the outside of the reactor was slightly lower than the moisture loss in the center

(core) of the reactor. In the experiment involving the cotton wrap and the enclosure, the soil moisture loss at the core was calculated to be approximately 7.1mL/day compared to the outside of the reactor at 6.7mL/day, which was a significant improvement over the control test.

The moisture loss in the soil reactor was reasonable for the length of experiment that will be conducted. In order to account for some extra loss associated with the removal of the Plexiglas® enclosure during sampling, the cotton wrap was sprayed with approximately 50mL of water using the spray bottle before the enclosure was re-installed around the reactor. The soil moisture levels were monitored during each of the experimental runs in order to ensure the soil moisture levels were maintained in the optimum range for biodegradation.

The petroleum degrading bacteria break down the petroleum hydrocarbons contaminant in the soil into carbon dioxide and water. It is likely that through this process that some water is added back into the soil. Studying this portion of water at this experimental scale was not the mandate of the project..

4.10 Custom Soil Sampler

Initially, Patros (2009) reported some difficulty removing soil from the soil reactor and indicated that there was significant volatilization of hydrocarbon from the sample due to the time consuming soil sampling technique and the design of the traditional soil sampling equipment. The design of the push rod was based on the design developed by Khan (2013) and modified to better suit this size of soil reactor. In order to recreate the soil sampler push rod for use in this experiment, modifications were made to Khan's (2013) design and the newly designed customized soil push rod was created and built in the University of Guelph's Physics Workshop

by Mario Paroutis on July 24, 2015. The push rod takes horizontal soil cores from the reactor through access via one of the 15 sampling ports installed on the shell of the reactor barrel.

Figure 4-0-10 The soil push rod fabricated by the Physics Machine Shop.

The soil sampler was constructed using a hollow tube (10mm internal diameter and 300mm long) with a 70mm long scoopula shaped opening on one side of the tube. This opening was around 15mm above the sharp conical penetrating edge. The sampler was manually pushed down into the soil to collect around 5-10 gram of soil. The sample was then placed directly in to a preweighed 40mL amber sample vial. Soil samples were taken in two locations on the reactor.

4.11 Biohazard Permit

Through conversations with Dr. Hung Lee (School of Environmental Sciences) and Jennifer Moinoque (Environmental Health and Safety) a need for a Biohazard Permit for this project arose. Soil collected from the Delhi and the Elora Research Stations and use of the designed 80 kg soil reactor posed no biological threat. However, there was a biohazardous risk present when trying to grow the heterotrophic bacteria on agar media (TSA, BHM) for the purposes of

identifying bacterial colonies. Additionally, a soil amendment compound (Ammonium Chloride, NH_4Cl) was added to soil prior to placement of the soil in the reactor. This amendment was added in order to stimulate the natural soil microorganisms (heterogenic bacteria) so that the organisms could break down the synthetic gasoline. For this reason a biohazard permit was prepared for this project using a template permit that Dr. H. Lee submitted for studying heterotrophic bacteria in soil contaminated with toluene. The permit was prepared by Dr. R.G. Zytner and submitted to the University of Guelph Environmental Health and Safety Department in July 27, 2015. Comments were returned to Dr. Zytner on August 6, 2015 and discussed with the researcher and appropriate staff. After providing feedback to the reviewers of the permit and Jennifer Minogue on August 10, 2015, the permit was approved and issued August 11, 2015.

CHAPTER 5: METHODOLOGY FOR RUNNING THE EXPERIMENT

This section provides details regarding the methodology used during the six bioventing experimental tests. In order to ensure consistency between experimental tests, a well-planned procedure was created in advance of the first experimental run and was followed for all of the experiments. Slight modifications were made to the procedure immediately after the first experimental run to improve the efficiency and accuracy of the following tests.

5.1 Preparing the Soil

This experiment used two different soil types, a sandy loam (Delhi) and a clayey loam (Elora) for the bioventing research. Classification of both the Delhi and Elora soils was not completed as part of this research project as past research studies have extensively developed the soil characteristics. Approximately seven researchers previously classified these two soil and they all reported quite similar classification findings; hence no further classification was completed. The following section detailed the specific steps taken to prepare the soil for each of the experimental trials.

5.1.1 Soil and Water Measurements

5.1.1.1 *Delhi Soil*

The Delhi soil was used for experimental runs 1 and 2. Based on the previous analysis of the soil conducted by Khan (2013), it was found that the porosity of the soil was in the range of 0.4 to 0.43 and the bulk density was 1500 to 1600kg/m³. Optimum soil moisture conditions for bioventing SVE have been previously determined to be in the range of 50-60% of the soils field capacity, which equates to approximately 15-18wt%.

The total volume of the soil reactor, taking into account the 5 injection ports and the center venting column, was calculated to be 51216cm^3 (0.051216m^3). Based on an average bulk density of the Delhi soil (1550kg/m^3), the total weight of soil that could be placed in the soil reactor was 79.44kg . Using the optimal soil water content for bioventing SVE, the reactor required 69.04kg dry soil and 10.4kg (10.4L) of water.

Based on the quantities of soil and water that needed to be prepared and added to the reactor, 16 1-gallon epoxy coated paint cans were required. Each 1-gallon can had a volume of 3.78L and held 4.32kg of dry soil and 650mL of water including the nutrient addition (NH_4Cl) volume. Each can had a total weight of 4.97kg excluding the small amount of synthetic gasoline (25mL or 20.05g) added to each can immediately prior to the final mixing and loading into the reactor.

5.1.1.2 Elora Soil

The Elora soil that was collected was used for the experimental run 3 and 4. Based on the previous analysis of the soil conducted by Khan (2013), it was found that the porosity of the soil was in the range of 0.47 to 0.51 and the bulk density was 1300 to 1400kg/m^3 . Optimum soil moisture conditions for bioventing SVE have been previously determined to be in the range of 50-60% of the soils field capacity which equates to approximately 15-18wt%.

The total volume of the soil reactor, taking into account the 5 injection ports and the center venting column was calculated to be 51216cm^3 (0.051216m^3). Based on an average bulk density of the Elora soil (1350kg/m^3), the total weight of soil that could be placed in the soil reactor was 69.14kg . Using the optimal soil water content for bioventing, the reactor required 58.74kg dry soil and 10.4kg (10.4L) of water.

Based on the quantities of soil and water that needed to be prepared and added to the reactor, 16 1-gallon epoxy coated paint cans were required. Each 1-gallon can had a volume of 3.78L and held 3.67kg of dry soil and 650mL of water including the nutrient addition (NH_4Cl) volume. Each can had a total weight of 4.32kg excluding the small amount of synthetic gasoline (25mL or 20.05g) added to each can immediately prior to the final mixing and loading into the reactor.

5.1.2 Nutrient Addition

When soil is dry and sitting in storage, the soil microorganisms go dormant. A nitrogen based additive is required to stimulate the microorganisms in the soil in order for bioventing SVE to be effective. Previous research (Eyvazi and Zytner 2009) suggested that optimal nitrogen concentrations in the soil to stimulate the microorganisms follows the C:N ratio 10:1. Based on the initial concentration of synthetic gasoline in the soil reactor estimated at 2000mg/kg, 14.10 grams of nitrogen was required. Nitrogen was in the form of ammonium chloride (NH_4Cl). Ammonium chloride is 26.19 %wt nitrogen and a total of 53.84 grams was required for each experiment. For simplification of measuring, 54 grams of NH_4Cl was added to 800mL of water and mixed. After mixing, 50mL of the nutrient additive water was added to each of the 16 1-gallon cans. The remaining amount of water was then added to each of the cans to bring the soil to the optimum moisture level and the cans were capped and sealed with lids. The 1-gallon cans were then mixed in groups of 4 for 20 minutes using the mechanical mixer designed by Mosco (2015).

5.1.3 Microbial Activation Period

After the soil was mixed with the water and the NH_4Cl , it was stored in the laboratory at room temperature (21-23°C) for at least 5 days. This incubation period allowed for germination and

reemergence of the natural soil microorganisms required to degrade the petroleum hydrocarbons in the soil. After the minimum 5 day incubation period, all of the 16 cans were placed in the refrigerator at approximately 4°C for approximately 18-24 hours in order to allow the soil to cool. This was done in order to limit volatilization of the synthetic gasoline in the next phase.

5.1.4 Synthetic Gasoline Addition

All of the 16 1-gallon cans were removed from the refrigerator and moved to the walk-in fume hood laboratory. Using the previously prepared synthetic gasoline stored in the freezer, 25mL (20.05) was pipetted into each of the 16 1-gallon cans. For the Delhi and Elora experiments 25mL or 20.05g of synthetic gasoline equates to an approximate concentration of 4040mg/kg prior to loading the soil into the reactor. For the aged Delhi soil experiment, the soil was spiked to an initial concentration of 8080mg/kg which equates to 50mL or 40.1g of synthetic gasoline per paint can. This higher concentration was used to account for some volatilization during the long term storage of the soil. The lids of the cans were then installed and the cans were prepared for mixing. The cans were then mixed in groups of 4 for 30 minutes using the mechanical mixer designed by Mosco (2015) in order to ensure that the synthetic gasoline had been evenly mixed throughout the soil. Refrigerated soil and the synthetic gasoline from the freezer were used in order to limit volatilization during the handling of the soil.

After the soil was mixed and just before placement into the reactor, a sample of the soil from each can was taken to test the consistency of mixing. It was found that there was some variability in concentration from can to can, which can be attributed to a variety of factors. Some of these factors include variance with the consistency of the mixing process, solvent extraction and GC Analysis variability. It was determined that with the use of the mechanical mixer the variability

is reasonable. Details of the variability for each of the experimental runs completed can be found in Table 5-1 below and in Appendix B. Samples from each of the cans in the Delhi 1 experiment trial were not tested.

Table 5-1 The spiking, average concentration and standard deviation for the mixing.

Soil Experiment	Spiked Concentration (mg/kg)	Average Can Concentration (mg/kg)	Standard Deviation (mg/kg)
Delhi 1	4040	N/A	N/A
Delhi 2	4040	2698	675
Elora 1	4040	3783	312
Elora 2	2500	1508	106
Layered Soil	4040	N/A	N/A
Delhi Aged Soil	8080	6519	317

Note:

- Average can concentration values currently do not take into account extraction efficiency.
- Delhi Aged Soil concentration prior to storage for several months.

5.2 Placing Soil in Reactor

For each round of experiments, soil was loaded into the stainless steel reactor after the addition of water, the nutrient additive and the synthetic gasoline. Placing the soil into the reactor needed to be completed in a prompt manner to limit volatilization of the synthetic gasoline from the soil. Khan (2013) reported that approximately 40 to 50% of the synthetic gasoline would volatilize from the soil during the loading and handling process. Therefore, the spiking concentration added to each of the cans was approximately 4040mg/kg, meaning that when the experiment started the concentrations were expected to be approximately 2000mg/L. Based on the practices that were used for this experiment, approximately 25% of the synthetic gasoline volatilized using the mixing and soil loading procedure resulting in higher than expected initial synthetic gasoline concentration in the soil. Immediately before the gasoline mixed soil was placed into the soil reactor, an approximate 5 gram sample was taken from each of the 16 cans and placed into a 40mL amber glass vial for GC analysis. This was done for every experimental run with the

exception of Delhi 1 test and was used to examine the mixing variability. Immediately after the soil was loaded into the soil reactor, two soil samples were taken and the system was powered online. These samples were referred to as the time zero samples and are the initial synthetic gasoline concentrations in the soil.

5.2.1 Soil Experimental Runs

A total of six bioventing experiments were conducted as part of this research project. Two experiments were conducted with the Delhi soil, followed by two experiments with the Elora soils. These four experiments served as the main component of the research project. The final two tests were completed as complimentary studies to support the findings from the main study experiments and to examine bioventing behaviour using large scale laboratory testing.

The fifth experiment was conducted using a combination of the Delhi and Elora soils, referred to as the “layered” test. The layered test stacked Delhi soil on top of Elora soil to create separate soil layers. The purpose of this test was to examine the biodegradation rates that occur in the two soils simultaneously when all conditions are the same. The results of this particular bioventing test will help to confirm that biodegradation in soil is strongly dependent on the soil type.

The final bioventing experiment studied aged contaminated soil. Delhi soil contaminated at an initial concentration of 8000mg/kg in November 2015 was packed into an aluminum foil lined bin and stored until March 2016. The Delhi soil was then loaded into the reactor and the experiment was conducted using the same procedure used in previous experiments.

5.2.2 Soil Density

The overall purpose for studying soil at this large lab scale was the hope that this reactor design would represent the soil and bioventing conditions in the natural soil environment. Often times when working with soil in a laboratory environment, it is difficult to recreate the insitu conditions, in particular, the soil density (Coduto, 2010). In order to ensure, as best as possible, that the density of the soil was achieved in the reactor and to ensure uniformity throughout the 40cm tall reactor chamber, lines in 5cm intervals were drawn on the inside of the reactor column. When loading the reactor with soil, measured volumes and weights were added to the reactor and then gently packed until the soil had been packed to the corresponding line. Each 5cm interval contained a soil volume of 6.4L.

For the Delhi soil with a unit weight of 1550kg/m^3 , 9.92kg of soil were required. For simplification purposes this was measured to be the soil from exactly 2 1-gallon pails.

For the Elroa soil with a unit weight of 1350kg/m^3 , 8.64kg of soil were required. For simplification purposes this was measured to be the soil from exactly 2 1-gallon pails.

5.2.3 Setting up Monitoring Probes

The bioventing reactor is cylindrical so it was expected that the results and functions of this bioventing system would be uniform throughout the system. The placement of the two soil moisture probes were placed in the areas where soil drying was most likely to occur, which was at the outside of the reactor where the stainless steel reactor comes into contact with the lab air space and near the venting well in the core of the reactor. This process was completed during the loading of the contaminated soil into the reactor. Each of the HOBO soil moisture probes (S-

SMC-M005) are capable of monitoring about 0.3L of soil and were placed as parallel as possible to the outside and core venting areas in order to monitor any changes in the soil moisture during the experiment. As detailed in Section 4.8, the moisture probes were connected to a HOBO Micro-Data Logger Station (H21-002) where the data was reviewed on HOBOWare Pro software.

5.3 Sampling Soil for Petroleum Hydrocarbons

In order to monitor petroleum hydrocarbon degradation in the soil reactor by the microorganisms, soil samples were frequently taken throughout the bioventing SVE experiment. The time of each sample was noted in order to overlay all of the concentrations with a specific time period.

Prior to taking the soil sample, two 40mL amber glass bottles were weighed using a Mettler Type AJ100L weigh scale. Using the customized soil sampler, cores were extracted from two different locations on the soil reactor. This procedure was done in order to ensure or test that biodegradation of the petroleum hydrocarbons was occurring consistently throughout the reactor. Each of the two samples of soil were quickly mixed and about 5 grams were placed in each of the two amber 40mL glass bottles. The 40mL amber bottles were then reweighed in order to determine the exact weight of the soil in each of the vials. In each of the 40mL amber bottles, 20mL of methylene chloride was added; this solvent was used to extract the petroleum hydrocarbons from the soil. After about 20 days of reactor operation, 15mL of methylene chloride was used in order to get a more defined reading on the GC FID.

Once the methylene chloride was added to the vials, the vials were placed on a Multi-Wrist shaker on medium speed for 1 hour. After the hour, the samples were removed from the mixer

and placed in the freezer overnight to allow for the settling of sediments. The next day, the samples were poured into 2mL GC vials and prepared for analysis. Synthetic gasoline samples in methylene chloride are somewhat time sensitive and therefore cannot be left for several days before analysis. As detailed in the GC procedure, Section 4.5 of this document, a grouping of standards were run prior to each analysis to establish a baseline.

5.4 Sampling Off Gases

Sampling the off gases for the experiment was critical to characterize the amount of volatilization occurring in the soil reactor. It also provided a way to characterize the loss in the system. The only way gasoline vapour was permitted to escape was through the off venting well as air was drawn from the pump. At all times, except for the 5 minutes daily sampling period, the Plexiglas® chamber was under negative pressure due to the venting draw.

The off gases were captured online before reaching the vacuum pump using a combination of C4011-1 Target Vials (National Scientific) and OBRO-30 tubes with activated carbon. The tubes were specifically designed to capture petroleum hydrocarbon off gasses at low flows (10-300mL/min). Both the C4011-1 and the OBRO-30 tubes had two chambers, a front and back chamber separated by a porous synthetic fibre. Each chamber required separate analysis by the GC FID. The C4011-1 tubes were larger in size and were able to capture higher off gas concentrations which are present during the initial stages of the experiment. The OBRO-30 tubes were smaller and were more suitable during the latter phases of the experiment when off gas concentrations were lower.

A specialized C4011-1 and ORBO-30 device was used to break the tips of the tubes and a customized needle hook was used to pull out the whole or foam separators allowing each of the chambers to be placed in different vials.

Prior to taking the soil sample, two 40mL amber glass bottles were weighed using a Mettler Type AJ100L weigh scale. The two portions of the C4011-1 or ORBO 30 tubes were placed in separate vials. This procedure was done in order to ensure that a majority of the gasoline vapours were captured in the first chamber. The 40mL amber bottles were then reweighed in order to determine the exact weight of the GAC in each of the vials. Immediately following, 10mL of methylene chloride, which is the solvent used to extract the petroleum hydrocarbons from the GAC, was added to each of the vials. The vials were placed on a Multi-Wrist shaker for 1 hour at a medium speed. The sample vials were then removed and placed in the freezer overnight. The next day 2mL of each vial solution was transferred to GC vials and capped in preparation for GC analysis.

5.5 Monitoring Oxygen Levels

Monitoring oxygen levels throughout the experiment is essential as it is a good indicator if the soil microorganisms are consuming oxygen to break down the synthetic gasoline and producing carbon dioxide. Ideally, the experiment should have measured carbon dioxide levels throughout, but, this was not feasible. If done correctly, the oxygen level would immediately drop when the experiment started and slowly increase back to ambient lab conditions as the soil microorganisms degraded the synthetic gasoline in the soil.

It was important to monitor the oxygen levels in the air leaving the bioventing reactor through the sampling line. After the exhaust air left the pump, it was directed to a 6L glass jar. The lid of

the glass jar had three small holes: an inlet for the air, an outlet for the air and a hole for the Opogee Oxygen Meter. After all of the tubes and device cords were strung through, the holes were sealed with caulking to create an as air tight seal as possible and the lid was secured to the jar. Oxygen levels were recorded daily for each of the four experiment trials.

5.6 Sampling Soil for Petroleum Degrading Bacteria

The principal concept associated with bioventing or biological soil remediation is that the natural existing soil microorganisms will break down the petroleum hydrocarbons. When employing this remediation technology, whether in the laboratory or in the field, optimum conditions for the soil microorganisms are provided in order to increase the degradation rate. For this experiment it was important to check for the presence of these soil microorganisms to ensure colonies were available in the soil in order to have biological remediation take place.

Two particular types of soil microorganisms were of interest, total heterotrophic bacteria (THB) and petroleum-degrading bacteria (PDB). Using a spread plating technique, two different media were selected to grow the two different types of bacteria. Tryptic Soy Agar (TSA) was used for THB and Bushnell-Hass (BH) medium was used to culture the PDB.

Approximately 24 hours prior to any soil sampling, a sodium pyrophosphate (NaPP) solution was prepared using the ratio of 1 gram of NaPP to 1 litre of ultra-pure water in an auto-clave safe bottle. The solution was then mixed on a magnetic stirring plate until all of the NaPP crystals were dissolved. The solution was then autoclaved using a SANYO Autoclave (MLS-3781 L) which operated at 121 °C with a sterilization time of 35 minutes. The NaPP solution was then permitted to cool to room temperature prior to use.

Three 5 gram soil samples were taken from three locations in the soil reactor (top, middle and bottom). It was assumed that through the mixing process, microbial distribution in the soil would be rather similar. Any variation in the microbial samples would likely be a normal error of the extraction, diluting and plating process. Each of the three 5 gram samples were then placed in a 250mL Erlenmeyer flask. Immediately following, 45mL of previously prepared, room temperature NaPP solution was added to each of the three flasks. Each of the flasks were then covered with aluminum foil, then placed on a Big Bill Orbital Mixer at 300 rpm for 1 hour in order to detach the microorganisms from the soil.

In order to count the total heterotrophic microorganisms, Tryptic Soy Agar (TSA) was used as the medium to grow and cultivate the microorganisms. For each microbial plating event in the experiment fresh TSA medium was prepared to avoid any contamination or degradation of the medium. The TSA medium was prepared as detailed by the supplier in an autoclave safe bottle, which consisted of following the ratio of 40 grams of TSA powder per 1 litre of ultra-pure water. The amount of TSA medium that was required was estimated by assuming approximately 25mL of media would be used for each 100mm x 15mm plate. The appropriate amount of powder was then mixed into the ultra-pure water by gentle shaking.

In order to study the petroleum degrading microorganisms, a Bushnell-Hass medium was used. The BH medium is a nutrient broth without a carbon source; therefore a carbon source had to be added to the solution in order to test for petroleum degrading microorganisms. Synthetic gasoline was used as the carbon source which was used to isolate the soil microorganisms that degrade petroleum hydrocarbons. The BH medium then was prepared as detailed by the supplier in an autoclave safe bottle, which consisted of following the ratio of 3.27 grams of BH powder per 1 litre of ultra-pure water. For solidification of the BH medium, granulated agar was added at a

ratio of 15g/L. Similarly to the TSA solution, the amount of BH medium that was required was estimated by assuming approximately 25mL of medium would be used for each 100mm x 15mm plate. The appropriate amount of powder was then mixed into the ultra-pure water by gentle shaking.

Both of the bottles with the TSA and BH media were then autoclaved for cooking and sterilization purposes. After the two solutions were finished in the autoclave, they were placed in a hot water bath pre-heated to a temperature of 57°C in order to avoid solidification. The two solutions remained in the bath until plates were ready to be poured.

The TSA medium was plated on sterilized disposable petri dishes, where approximately 25mL of the TSA solution was free poured directly onto the plate. The plates were then covered immediately to avoid atmospheric contamination. The BH solution was removed from the 57 °C water bath and allowed to cool until the solution was approximately 40 °C. At that time 4g/L of synthetic gasoline was added to the solution and the bottle was gently mixed by hand. The BH medium was plated on sterilized glass petri dishes, where approximately 25mL of the BH solution was free poured directly onto the plate. The plates were then covered immediately to avoid atmospheric contamination. Both sets of poured plates were allowed to cool to room temperature before inoculation.

The 250mL Erlenmeyer flasks were then prepared to undergo a serial dilution in order to limit the amount of soil microorganisms on the plate so as to accurately count the colonies. Two serial dilutions were used for each 250mL Erlenmeyer flask. Using a Corning CentriStar 15mL vial, 100uL of solution from the 250mL Erlenmeyer flask was extracted and placed into 900uL of the

previously prepared NaPP water solution. The sample lid was secured and the sample was auto-vortexed for 1 minute. This process was repeated for the second serial dilution.

Once the serial dilution process was complete, 100uL of the second serial dilution was inoculated on each of the TSA and BH plates and spread evenly across the plate using a sterilized plastic disposable spreader.

All of the inoculated plates were then placed in a small Tupperware storage bin and the plates were placed in an incubator pre-set at 25 °C. The TSA plates were counted for colonies after 48 hours and then confirmed after 72 hours in the incubator. The BH plates were counted for colonies after 7 days and then confirmed after 14 days in the incubator. The results of the experiment were reported as colony forming units per gram of soil weight (CFU/g).

All waste generated in the preparation of the plates and the petri dishes themselves were autoclaved once all of the required counting was complete. Prior to autoclaving, all of the waste was placed into designated Biohazard Waste Autoclaved Bags.

CHAPTER 6: RESULTS

6.1 Bioventing Experimental Conditions

This section details the experimental conditions monitored throughout the six experimental trails. The quality in the results of these environmental conditions monitors helps to justify the degradation rates found during the experiment. The indicators measured through these experimental conditions directly correlates to microbial biodegradation and thus successful bioventing. It is important to note that these experiments were conducted under optimal environmental conditions. Oxygen, moisture and nutrients levels were supplied at the ideal conditions identified by previous researchers in the group (Shewfelt at al., 2005; Eyvazi and Zytner, 2009; Khan and Zytner, 2013) to the contaminated soil in order to produce optimal biodegradation rates.

6.1.1 Soil Moisture

Soil moisture was one of the environmental conditions that were monitored throughout the experiment. Changes in soil moisture can impact the microbial activity as well as the air flow through the reactor system. Soil moisture in the reactor was monitored using two HOBO soil moisture probes (S-SMC-M005) connected to a HOBO Micro-Data Logger Station (H21-002). One of the two probes was located just inside the wall of the drum in the area most likely to dry out during the operation of the soil reactor. The other probe was located near the vacuum well which was another area subject to drying out throughout the experiment. Each of the soil moisture probe tips were capable of measuring the soil moisture of a 0.3 litre volume of soil as per the specification from Onset, the probe manufacturer.

Figure 6-1 shows the soil moisture levels that were monitored on both the inside and the outside of the soil reactor for the Elora 1 experimental test. Readings were recorded every 10 minutes using a HOBO Data Logger by Onset. The manufacture reports 3% variability in the accuracy of the readings. This variability was not of major concern for this experiment as an overall trend was more important and the acceptable moisture ranges were also quite large.

Figure 6-1 The soil moisture plot for the Elora 1 Experimental test.

From the data presented in Figure 6-1, it is clear that there were some moisture losses throughout the 30 day experiment period; however, they were very minor and did not pose a significant impact on the biodegradation performance. The soil moisture plots for all of the experimental tests can be found in Appendix B. There were no unexpected findings in regards to soil moisture during the experimental trials. Testing, prior to the operation of the experimental tests, corrected and mitigated moisture losses for the reactor system. Table 6-1, provides a brief summary of the soil water losses for all six of the experiments conducted.

Table 6-1 Soil water losses recorded for each of the experiments.

Experiment	Location	Initial (m ³ /m ³)	Final (m ³ /m ³)	Total Loss (%)
Delhi 1	Inner	0.239	0.228	7.4
	Outer	0.254	0.238	12.7
Delhi 2	Inner	0.224	probe error	-
	Outer	0.196	0.192	2.0
Elora 1	Inner	0.152	0.139	6.9
	Outer	0.169	0.153	8.0
Elora 2	Inner	0.219	0.214	2.5
	Outer	0.176	0.164	6.0
Layered	Inner	0.207	0.175	15.1
	Outer	0.209	0.173	17.2
Aged	Inner	0.195	0.176	9.4
	Outer	0.207	0.171	17.2

Note: Initial and Final m³/m³ are direct output values taken from the HOBO Loggers. Total Loss takes into account the difference soil density and represents the total loss of moisture.

As shown in the above in Table 6-1, higher levels of moisture loss were recorded on the outside of the reactor consistently for each of the experiments. Overall, the moisture levels did not vary significantly and were still within the optimal range for the bioventing experiment, as suggested at roughly 50% of the soils field capacity (Harper *et al.*, 2003). It is noted that through the biodegradation process some water would be returned the soil as the soil microorganisms break down the petroleum hydrocarbon in to carbon dioxide and water.

A slight operational change that might help reduce the moisture losses further would be to moisten the cotton wrap every few days with a spray bottle. It is noted this might have an impact on air flow through the reactor; however, the air pumps drawn can be increased.

6.1.2 Oxygen Levels

Oxygen levels were also measured and recorded daily throughout each experiment to ensure that the bacteria were actually breaking down the gasoline. Figure 6.2 shows the recorded oxygen readings from the Delhi 1 and 2 Soil Tests which were, as expected very similar in nature.

Figure 6-2 Oxygen readings from the Delhi Soil Tests.

The initial drop in oxygen concentration in the exhaust off gases can directly correspond to the microbial bacteria in the soil beginning to degrade the synthetic gasoline. As the soil bacteria break down the petroleum hydrocarbons they consume oxygen and produce carbon dioxide and water. It would be expected that the oxygen levels leaving the soil reactor would be lower as some of the oxygen would have been used for the degradation process. Similar results were reported by Khan and Zytner, (2013) and Eyvazi and Zytner, (2009) when they had conducted their bioventing experiments. The general trend of an immediate drop in oxygen levels followed by a steady incline over the life of the experiment was noted in all of the experimental trials.

6.1.3 Off Gas Monitoring

The off gasses captured from the air drawn from the venting well were measured and documented as a part of this experiment. It was found that each of the experiments lost between 1-2% of the initial amount of gasoline over the course on the approximate 30 day operation period. This amount is considered a reasonable amount of loss for the scale of this experiment.

Figure 6-3 The synthetic gasoline off gases captured through the venting well in the Elora 1 experiment.

Figure 6-3, shows the off gasses measured daily during the Elora 1 experiment which were consistent with the trend observed in the other experimental run. Volatilization immediately spiked but then dropped off within the first 7-8 days. It is important to keep in mind that during the critical second stage of degradation, the off gasses captured were very low and were insignificant compared to the amount of synthetic gasoline in the soil reactor. Table 6-2 shows the amount of synthetic gasoline lost and captured in the GAC target vials for each of the experimental trials.

Table 6-2 The amount of synthetic gasoline lost as a result of volatilization through the venting chamber.

Soil Type	Experiment	Front Chamber (mg)	Back Chamber (mg)	Total Sampled (mg)	Extraction Efficiency (%)	Total Loss (mg)	Percent of Total (%)
Delhi	1	765.7	326.5	1092.2	75	1365.0	0.43
	2	2460.9	432.9	2893.7	75	3617.1	1.13
Elora	1	1099.4	107.1	1207.0	75	1508.8	0.47
	2	445.8	8.3	454.1	75	567.7	0.35
Mixed	-	1281.4	58.4	1253.5	75	1566.9	0.48
Aged	-	2115.3	219.1	2334.3	75	2918	0.90

The losses recorded for the first Delhi experiment, particularly for the front chamber were quite low in regards to the off gas capture in the second experiment. This was due to a limitation on the ORBO-30 GAC tubes. The target tubes became saturated and were unable to continue to capture the off gasses. For the remainder of the experimental trials, a larger C4011-1 target vial was used.

Overall, the amount of synthetic gasoline that was lost due to volatilization through the venting column was minimal in comparison to the initial amounts of gasoline at the beginning of the experiment. That being said, it is still important to capture these gasses and quantify the losses.

6.1.4 Microbial Activity

The principal concept associated with bioventing or biological soil remediation is that the natural existing soil microorganisms will break down the petroleum hydrocarbons. When employing this remediation technology, whether in the laboratory or in the field, optimum conditions for the soil microorganisms must be provided in order to increase the degradation rate. For this experiment it was important to check for the presence of these soil microorganisms to ensure colonies were available in the soil in order to have biological remediation take place.

Microbial sampling was also done for each of the experimental tests. Two particular types of soil microorganisms were of interest, total heterotrophic bacteria (THB) and petroleum-degrading bacteria (PDB). It was out of the scope of this research study to examine the specific microbial colonies present in the soil. The microbial testing carried out for this study was to simply provide an indicator that both THB and PDB microorganisms were present in the soil during bioventing. Using a spread plating technique provided by the manufacturer, two different media were

selected to grow the two different types of bacteria. Tryptic Soy Agar (TSA) was used for THB and Bushnell-Hass (BH) medium was used to culture the PDB (Difco, 2015).

Figure 6-4 Total Heterotrophic Bacteria for the Delhi soil experiments.

Figure 6-5 Total Heterotrophic Bacteria for the Delhi soil experiments.

As shown in Table 6-3, the microbial populations increased slightly as the experiment proceeded. Stimulated heterotrophic and petroleum-degrading bacteria thrive in environments where there is a constant food source, such as gasoline. If the microbial populations were to be further monitored, it would have been found that the populations would subside due to a lack of food source. The microbial populations, both heterotrophic and petroleum degrading bacteria, were higher in the Elora soil than in the Delhi soil which can be explained by the higher levels of organic matter in the Elora soil. These results are consistent both in trends, as well as approximate CFU/g counts reported by Khan and Zytner, (2009).

Table 6-3 A summary of the average microbial populations for each of the experiments conducted, reported as colony forming units per gram (CFU/g) based on a 5 gram sample.

Experiment	Type	Initial (CFU $\times 10^7$/g)	Final (CFU $\times 10^7$/g)
Delhi 1	TSA	9.5	11
	BHM	-	-
Delhi 2	TSA	8.5	9.5
	BHM	8	8.5
Elora 1	TSA	9.8	10
	BHM	12	-
Elora 2	TSA	9.5	9.5
	BHM	12	-
Layered Elora	TSA	11	13
	BHM	13.5	-
Delhi	TSA	7.5	9.5
	BHM	8.5	-
Aged	TSA	8.5	-
	BHM	9.5	-

As seen in Table 6-3, not all of the microbial tests were completed due to either lab shut downs, time availability and missing or lack of resources. Despite not having this data it is still clearly evident that the presences of both heterotrophic and petroleum-degrading bacteria existed in the soils and were necessary in the biodegradation process.

6.2 Degradation

A total of six studies were run for approximately 30 days each. Two tests were carried out with each of the Delhi and Elora soils which formed the framework for the experiment. A Layered soil experiment was conducted as a complimentary study to prove the concept that bioventing can occur radial to the venting column and is strongly dependent on soil type when environmental conditions are the same. Finally, an aged soil experiment was conducted to determine the impact that aged contaminants would have on the biodegradation rate.

One of the major objectives for this experiment was to develop degradation rates using the 80kg reactors for both the Delhi and Elora Soils. As detailed in the experimental methodology, Section 5, bio-degradation occurred under optimal conditions for this experiment. This means that overall it is expected that in the field environment, degradation would be slower as conditions would not be as optimal.

The results of the experiment showed that the concentration of the petroleum hydrocarbons degraded in an exponential format. The following equation represents the general relationship used to classify the degradation rate observed during this experiment.

$$\frac{C}{C_0} = e^{-kt}$$

Where:

C = Concentration of Synthetic Gasoline in the Soil

C₀ = Initial Concentration of Synthetic Gasoline in the Soil

k = Slope

t = Time

However, Figure 6-6 depicts an attempt to classify the degradation relationship as linear. The R^2 values for the petroleum hydrocarbon degradation are smaller than the R^2 values for the exponential degradation petroleum hydrocarbon degradation, shown in Figure 6-7, indicating that exponential degradation is a good representation of the data. This degradation pattern was previously noted in previous bioventing experiments conducted at a smaller scale (Khan and Zytner, 2013; Eyvazi and Zytner, 2009). It is important to note that the results depicted in the figures here are the sample average for each sample day, based on two soil samples collected on a daily basis throughout the entire experiment.

The concentrations reported here do not take into account the approximate 75% methylene chloride extraction efficiency. The results of the experiment are better displayed as the degradation changes over a specified time period and not a particular concentration on a specific day. It is not critical to determine the specific daily gas concentration as the overall degradation rate is reported over an entire period of time. In order to determine the extraction efficiency known concentrations were added to soil in a 1-gallon can and mixed on the mechanical mixer. Immediately after mixing, the soil and was sampled and analyzed using the GC analysis procedure.

Figure 6-6 Results of the two Delhi soil bioventing experiments showcasing if the biodegradation relationships were linear.

Figure 6-7 Results of the two Delhi soil bioventing experiments showcasing if the biodegradation relationships were exponential.

The set of Delhi experiments were the first in the series of six experiments conducted for the purposes of the research project. The results of the Delhi 1 experiment were cruder than the results of the other experiments performed, as the Delhi 1 experiment was used to refine the procedure for sampling and working with a bioventing experiment of this size in a laboratory environment. As shown in Figure 6-7, the R^2 value for the first Delhi experiment was 0.77 compared to the R^2 value for the second Delhi experiment at 0.92. However, when the results of the second Delhi soil experiment are overlaid on the same graph as showed in Figure 6-7, it can be seen that the degradation behaviour is very similar. Upon examination of the results from the Delhi experiments as shown in Figure 6-7, there is not a noticeable difference in the degradation rates between the two experiments. In order to quantify this difference, a Student's T-Test was conducted on the degradation rates comparing the results of the Delhi experiments 1 and 2. It was found that there was no statistical significant difference in the degradation results. This means that based on the two experiment tests completed, the degradation rates are from a statistics outlook, the same.

Similar to the Delhi soil experiment, the Elora soil experiments were also conducted under optimal environmental conditions. The first Elora experiment had an initial spiking concentration of 4040mg/kg of synthetic gasoline which was the same concentration used for both of the Delhi soil experiments. The second Elora soil test was spiked with an initial concentration of 2500mg/kg in order to determine if contaminate concentration levels impacted the two stage degradation rate.

Figure 6-8 The results of the two Elora soil bioventing experiments showcasing if the biodegradation relationships were exponential.

It is clear that from the results of the second Elora experiment shown in Figure 6-8, there is no noticeable visual difference in the degradation rate. The second stage degradation rate for the first experimental test was 0.071day^{-1} , compared to 0.084day^{-1} for the second experiment test. In order to quantify this difference, a Student's T-Test was conducted on the degradation rates comparing the results of the Elora experiments 1 and 2. It was found that there was not a significant difference in the degradation results. This means that based on the two experiment tests completed, the degradation rates are from a statistics outlook, the same.

The results of both the Delhi and Elora soil tests indicate that there is a clear change in the degradation rates centering on the 7-9 day period from the beginning of the experiment. For comparison purposes, Day 8 was selected as the pivotal point where degradation rates seem to change and the exponential degradation curves seemed to make the best fit based on the R^2 values. This indicates that there is a two-stage degradation rate occurring which was also found

in the bioventing experiment conducted by Khan and Zytner, 2013. In the first stage, gasoline readily available in the soil water or lightly bonded to the soil particles is easily broken down and dissolved by the microorganisms. Of most value to the research is the second stage of the degradation (the period starting at Day 8 running to the end of the experiment). Those degradation curves are the most useful to the research in order to develop closure times for remediation. The degradation half-life for the removal of petroleum hydrocarbons in the soil is in the range of 1-2 days for both the Delhi and Elora soils. The rapid half-life is consistent with the concept that the soil microorganisms easily remove the “free” or lightly bound petroleum hydrocarbons in the soil.

When conducting the two Delhi and two Elora soil bioventing experiments or tests there was an effort made to keep the environmental conditions constant throughout the approximate 30 day operation of the trial. It was important to ensure environmental conditions, such as soil moisture, air flow and bacterial levels were maintained in order to strictly quantify the differences in degradation of the petroleum hydrocarbons in the two soil types. However, there still can be some naturally variability from trial to trial. An additional experiment was conducted to confirm that degradation was dependant on soil composition when all environmental conditions were the same. A layered soil experiment was conducted by placing a layer of Delhi soil in the bottom of the reactor followed by Elora soil on top in the reactor. Based on the results of the layered experiment depicted in Figure 6-9 and detailed in Table 6-4, it can be concluded that degradation is significantly impacted by the type of soil present when environmental conditions are the same.

Figure 6-9 The layered soil bioventing experiments showing biodegradation is heavily dependent on soil type.

As shown in Figure 6-9, the second stage degradation rates for the Delhi and Elora soils tests were quite different from each other even from a visual inspection. The Elora soil, which has a large silt and clay component, has a slow second stage degradation rate of 0.072day^{-1} , when compared to the Delhi soil which is dominated by sandy loam with the second stage degradation rate of 0.112day^{-1} .

A summary of the degradation rates can be found in Table 6-4 which shows that second stage degradation rates were lower in Elora soil than the Delhi soil for each of the experimental runs. The results of the Elora and Delhi soil experiments can be confirmed by the results of the Layered soil experiment which showed second stage degradation rates in the range of the first four experiments conducted. This once again continues to build on the idea that soil type directly impacts the rate on biodegradation.

Table 6-4 The degradation rates for 80 kg large scale bioventing experiment for both of the Delhi and Elora soils as well as the Layered soil experiment.

Soil Type	Duration	80kg Exp. 1	80kg Exp. 2	Layered
Delhi	0-8	0.218	0.341	0.251
	8-30	0.141	0.101	0.112
Elora	0-8	0.389	0.384	0.362
	8-30	0.068	0.081	0.072

Note: Calculated using the average degradation rate constant for each 80kg experiment.

6.3 Aged Contaminants

As a complimentary study, an aged contaminant experiment was conducted to better understand the biodegradation rates of soil contaminated with petroleum hydrocarbons for an extend period of time. It is known that when gasoline is spilled onto soil and is in contact with the soil for an extended period of time, the bonds between the gasoline and the soil particles strengthen. This increased bond strength becomes a limiting factor for biodegradation as microorganisms are unable to easily breakdown the petroleum hydrocarbons. The reasoning behind this increased bond strength is not well documented and could be a future area of study. The soil used in this experiment was contaminated to about 8000mg/kg and stored in a refrigerated sealed container for 4 months prior to use in this experiment. The initial concentration in the soil after the 4 months in storage was measured to be approximately 2500mg/kg due the GC-FID; however, due to a 50% extraction efficiency using the methylene chloride, the concentration more accurately was in the 5000mg/L range. The results presented in Figure 6-10, show the concentrations measured by the GC FID without the extraction efficiency as day over day concentrations yield a degradation coefficient. The soil was stimulated with ammonium chloride on day 0 and immediately loaded into the reactor. Below, Figure 6-10 shows the biodegradation results measured for this aged contaminant experiment.

Figure 6-10 The results of the aged soil bioventing experiment shows that biodegradation slows when working with aged petroleum hydrocarbons in the Delhi soil.

As shown in Figure 6-10, the gasoline concentration does not drop immediately as the soil was stimulated at the start time rather than 5 days previous like the other bioventing experiments conducted. This was done since the gasoline was previously spiked and was in refrigerated storage for a period of four months prior to use in the experiment. After the 4 day period, the stimulated soil microorganisms dissolved the readily available gasoline in the soil moisture. In the second stage, degradation slowed and the process became more limited as the microorganisms began to degrade the petroleum hydrocarbons that were strongly bonded to the soil particles. In comparison to the biodegradation results from the Delhi soil experiment where the soil was freshly spiked, it is evident that the degradation rate is significantly slower, by a factor of two, in soils contaminated with gasoline for extended periods of time (aged). The second stage degradation rate for the aged contaminant experiment averaged 0.053day^{-1}

compared to the average degradation rate for the freshly contaminated Delhi soil of approximately 0.118day^{-1} .

Several studies have been completed indicating that a strong bond exists between soil particles and petroleum hydrocarbons, which can have a limiting factor on biodegradation (Chang *et al.*, 2011 and Sutton *et al.*, 2013). In freshly contaminated soil, the bonds between the soil particles and the petroleum hydrocarbons are weaker and can be dissolved back into the soil water when a concentration gradient exists. Aged contaminants require the soil microorganisms to first breakdown the petroleum hydrocarbon from the soil particles by using the microorganisms' bio-surfactants. Once the petroleum hydrocarbons are removed from the soil, it can be continuously biodegraded by the soil microorganisms.

Further study into aged contaminants and the effect they have on the biodegradation process may be appropriate as it will help researchers develop remediation closer times for sites with this specific issue.

6.4 Development of a Scale Up Factor

A major objective of this research project was to develop a scale-up factors to compare the biodegradation rates of this experiment to the biodegradation rates of past bioventing work conducted by Eyvazi and Zytner (2009) and Khan and Zytner (2013). These scale-up factors can then help to eliminate some of uncertainty with regards to the biological decay rate for a particular field site. Through bioventing experiments that have been conducted in the laboratory on the micro-scale and meso-scale, scale-up factors have been established to better predict the results that occur at the macro-scale (field) level. Additionally, understanding the particular

decay rates for a site will allow for the better development of a total remediation time that is necessary to clean up the site.

Scale-up factors were only developed for the second stage of degradation as they are required for the development of the site closure time. Table 6-5 shows the degradation rates for the experiments conducted along with the corresponding scale-up factors.

Table 6-5 The degradation rates for the micro-scale, meso-scale and large scale bioventing experiment.

Soil Type	Duration	Micro-scale	Meso-scale	Scale Up Micro to Meso	80kg Exp. 1	80kg Exp. 2	Layered Soil	Scale Up Micro to 80kg
Delhi	0-8		0.598		0.218	0.341	0.25	
	8-30	0.045	0.123	2.7	0.141	0.101	0.11	2.7*
Elora	0-8		0.460		0.389	0.384	0.36	
	8-30	0.040	0.075	1.9	0.068	0.081	0.072	1.9*

Notes:

Calculated using the average degradation rate constant for each 80kg experiment.

Micro-scale (Eyvazi and Zytner, 2009)

Meso-scale (Khan and Zytner, 2013)

Scale-up factors are calculated using the reference Micro-scale (Eyvazi and Zytner, 2009) experiment

The scale-up factors for this experiment were developed using the average of the two degradation rates for the 80kg experiment and comparing them to the degradation rate from the micro-scale experiment conducted by Eyvazi and Zytner, (2009). When comparing the scale-up factors from the micro to meso-scale (4kg) and micro to meso-scale (80kg), it is noted that they are the same. This means that the same degradation results were found for both the 4kg and 80kg bioventing experiments. Using a Student's T-Test, it was determined that there was no statistical difference in the degradation rates from the 4kg to 80kg bioventing experiments.

Soils with aged petroleum hydrocarbons take a significantly longer time to biodegrade than soils with fresh contaminants. Aged contaminants, particularly petroleum hydrocarbons, develop very strong bonds with soil particles which limit petroleum degrading bacteria from breaking down the contaminant. Results of the aged contaminant experiment with the Delhi soil produced a

second stage degradation rate of 0.053day^{-1} which was twice as slow as the degradation measured during the freshly contaminated experiments. Scale-up factors have not previously been estimated for biodegradation with aged soil contaminants as aged soil bioventing experiments with these particular soil types and methodology has not been studied. It is likely that if biodegradation rates were studied at a smaller, 4kg or even 200g reactor scale they would yield similar scale up factors as the freshly contaminated soils which made up the core of this experimental study.

Overall, this means that it is not completely necessary to conduct bioventing experiments at an 80kg scale as a 4kg scale yields the same result. Conducting bioventing experiments at a small scale makes research more feasible as working with a large scale bioventing reactor is quite complex. Conducting bioventing on a smaller scale, (4kg), can allow researchers to run multiple reactors simultaneously altering environmental conditions to test for variability in degradation rates.

CHAPTER 7: CONCLUSIONS

Soil remediation of petroleum hydrocarbons is a big business and as environmental regulations tighten, more work is required to remediate sites. Additionally, as old industrial complexes get torn down, it is necessary to clean up any contamination on the site before the site is re-purposed with a new development, which is usually done at a cost to the business that contaminated it. Unfortunately, historically, some businesses have walked away from their property rather than pay for the remediation and the land has fallen into the responsibility of the federal, provincial or municipal governments. These governments then are responsible for the liability associated with the property and have to foot the bill for remediation efforts. Developing a better understanding of soil remediation, in particular the factors that influence soil remediation, is essential for advancing the understanding of remediation technologies.

This research project examined the use of bioventing soil remediation as a viable clean-up technology for the removal of petroleum hydrocarbons from unsaturated soil. In bioventing nitrogen-based fertilizer is added to the soil to stimulate native microbial populations. These stimulated microbial populations in an oxygen and nutrient rich environment break down semi-volatile compounds in petroleum hydrocarbons which are often hard to remove from the soil. Bioventing is a non-destructive treatment method which, if done correctly, can improve remediation efforts while lowering the cost when compared to a soil vapour extraction system. Bioventing is biological, natural and in-situ treatment technology for the remediation of petroleum hydrocarbons. Traditional SVE remediation system often cause a tailing effect that occurs as the desorption process in the soil is relatively slow and it takes a while for all of the contaminants to release from their bonds. However, in aged soils with aged contaminants, the

petroleum hydrocarbon bond to the soil is so strong it requires, at least in sub-surface soils, a biological or chemical surfactant to dissolve the bond.

This experiment developed a 80 kg soil bioventing reactor system consisting of a custom-made reactor, climate chamber, low flow venting system and an off gas capture device. Environmental conditions that were monitored included; moisture levels, microbial levels, nutrient and oxygen levels, ensuring an accurate representation of natural soil environmental conditions. Gas chromatography was used to measure concentration in soil samples taken daily from the reactor.

Results from the study show that a two-stage degradation process exists and a scale-up factor similar to the results obtained from a previous study at the meso-scale (4kg) conducted by Khan and Zytner, (2013). The Delhi (sandy loam) soil had a second stage degradation rate of 0.12day^{-1} and the Elora (silt loam) soil had a degradation rate of 0.075day^{-1} . The corresponding scale up factor was 2.7 for the Delhi soil and 1.9 for the Elora soil. Through analysis it was determined that the degradation rate from the meso-scale (4kg) experiment showed no statistical difference from the degradation rate at the large (80kg) scale when the same soil was used. This similar scale-up factor indicates that there is not a significant advantage in completing bioventing experiments at a larger (80kg) scale. Completing bioventing soil remediation experiments using 4 kg soil reactors would provide researchers with a degradation correlation that could be scaled up to apply to field applications.

However, after a statistical comparison of the degradation rates for the two different soil types, it was concluded that there was a statistical difference. This is consistent with what previous research found: that soil type is a major determining factor in degradation.

An Aged soil experiment was conducted to test the impact that aged contaminants have on the degradation process. Aged contaminants, particularly petroleum hydrocarbons, develop very strong bonds with soil particles which limit petroleum degrading bacteria from breaking down the contaminant. Results of the aged contaminant experiment with the Delhi soil produced a second stage degradation rate of 0.053day^{-1} which was twice as slow as the degradation measured during the freshly contaminated experiments.

Off gasses captured from the air drawn from the venting well were measured and tacked as a part of this experiment. It was found that each of the experimental tests lost between 0.5-2% of the initial amount of gasoline over the course of the approximate 30 day operation period. These amounts are considered a reasonable amount of loss for the scale of this experiment. Microbial populations, both THB and PDB, were present for each test and counts were sustained throughout each experiment. Through daily oxygen monitoring of the off gasses from the soil reactor, it was evident that oxygen was being consumed by the microorganisms in the soil and degradation process was occurring.

Overall, it is possible to complete bioventing soil remediation experiments that mimic field conditions and provide degradation correlations and scale-up factors that can be applied to field applications. However, there are some limitations to this, as soils with aged petroleum hydrocarbons take a significantly longer time to biodegrade than soils with fresh contaminants. Aged contaminants, particularly petroleum hydrocarbons, develop very strong bonds with soil particles which limit petroleum degrading bacteria from breaking down the contaminant.

CHAPTER 8: RECOMMENDATIONS

Further research is required to explore the implications that aged contaminants have on the degradation rates for bioventing soil vapour extraction. Another area of future research could be exploring the bonding properties between petroleum hydrocarbons and soil that has been contaminated for an extended periods of time. This can allow for the development of biodegradation rates to better understand the degradation behaviour of aged contaminants.

Additionally, a 3-D bioventing model using the degradation correlations and scale-up factors determined by Khan and Zytner (2013) and confirmed by this experiment would further help with the understanding of applying the laboratory results to particular field sites. Through the use of models and advanced study in the area of bioventing soil remediation, research will be better able to predict closure or stop times for soil remediation projects.

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APPENDIX A: SAMPLE CALCULATIONS

A.1 Soil and Water Mixing

A.1-1 Delhi Soil

Soil Porosity: 0.4-0.43

Bulk Density: 1500-1600 kg/m³ (1550 kg/m³)

Water Content: 15 -18 wt% (60% of available pores filled) This equates to the recommend level of 50-70% field capacity as detailed by Khan, 2013.

Corrected Volume of Reactor: **51216 cm³ (0.051216m³)**

Total Weight of Soil in the Reactor: **79.44kg (79,440 g)**

Weight of Soil in the Reactor

69,040g (69.04 kg)

Water in the Soil Reactor

10,400g (10.4L)

Volume of Paint Can

Each Paint Can V = 3.78 L = 3780cm³

Soil (Dry) 4.32 kg/can

Water

0.15-0.18m³ water/m³ soil = 650mL water/can

Put 650mL of water into each can.

Total/Can

1 can: 4.315 kg soil

0.650 kg water (650 mL)

4.97 kg

16 Cans Required

A.1-2 Elora Soil

Soil Porosity: 0.47-0.51

Bulk Density: 1300-1400 kg/m³ (**1350 kg/m³**)

Water Content: 0.15 m³ water/m³ soil (60% of available pores filled) This equates to the recommend level of 50-70% field capacity as detailed by Khan.

Corrected Volume of Reactor: **51216 cm³ (0.051216m³)**

Total Weight of Soil in the Reactor => **69.14 kg (69,140 g)**

Weight of Soil in the Reactor

58,742 g (58.74 kg)

Water in the Soil Reactor

10,400g (10.4 L)

Volume of Paint Can

Each Paint Can V = 3.78 L = 3780cm³

Soil (Dry) 3.6714 kg/can

Water

0.15 m³ water/m³ soil = 650 mL water/can

Put 650mL of water into each can.

Total/Can

1 can: 3.6714 kg soil

0.650kg water (650mL)

4.32 kg

16 Cans Required

A.2 Nutrient Amendment Calculations

Synthetic Gasoline Blend as C_8H_{12}

$$C_8H_{12} \rightarrow 108.18 \text{ g/mol}$$

$$C = 12.01 \text{ g/mol} \times 8 = 96.08 \text{ g/mol}$$

$$H = 1.01 \text{ g/mol} \times 12 = 12.12 \text{ g/mol}$$

Carbon Portion

$$96.08 / 108.18 = 88.815\%$$

$$C_8 \rightarrow 0.8815 \text{ mole fraction of just carbon}$$

Soil Concentration

$$2000 \text{ mg/kg soil} \times 80 \text{ kg}$$

$$160 \text{ g TPH} = 114.04 \text{ g Carbon}$$

$$C:N \text{ } 10:1$$

$$141.04 \text{ g} : 14.104 \text{ g}$$

$$N \text{ as } NH_4Cl$$

Table A2-1 Chemical component breakdown of NH_4Cl .

Component	Mass (%)	Mass (g)
N	26.185	14.104
H	7.537	4.059
Cl	66.278	35.699
Total	100	53.864

Therefore 54 g of NH_4Cl [Total]

Mixing 54 g of NH_4Cl into 800 mL of water

A.3 Oxygen Requirements for Bioventing

A.3-1 Based on US Army Core of Engineers

Soil Porosity: 0.5

Bulk Density: 1550 kg/m³

The US Army Corps of Engineers recommends an optimal pore volume exchange of 1 per day.

$$\varepsilon = \frac{V_{Pores}}{V_{Total}} \Rightarrow 0.5 = \frac{V_{Pores}}{80kg \div 1550 \frac{kg}{m^3}}$$

$$V_{Pores} = 2.58 \times 10^{-2} m^3$$

Use optimal 1 pore volume exchange rate.

$$V_{Air} = 1 \times 2.58 \times 10^{-2} m^3/day$$

$$V_{Air} = 25.8 L/day$$

$$V_{Air} = 17.8 mL/min$$

This assumes a dry soil, however under optimal bioventing conditions available pore spaces should be 50% occupied by water. There remaining 50% of the pores can be used for air.

$$V_{Air} = 17.8 mL/min \times 0.50$$

$$V_{Air} = 8.9 mL/min$$

APPENDIX B: EXPERIMENTAL DATA

B.1 Delhi Soil Test 1

B.1-1 Degradation

Table B1-1 The concentration of synthetic gasoline in the soil reactor.

Experiment Date	Day	Sample Location1	Sample Location 2	Average
Sept. 9	0	-	2086.2	2086.2
Sept. 10	1	-	1269.7	1269.7
Sept. 11	2	1802.5	-	1802.5
Sept. 12	3	-	709.7	709.7
Sept. 13	4	1155.1	985.0	1070.1
Sept. 14	5	767.3	529.3	648.3
Sept. 15	6	869.1	561.7	715.4
Sept. 16	7	330.9	188.6	259.8
Sept. 17	8	479.8	364.2	422.0
Sept. 18	9	-	321.9	321.9
Sept. 19	10	440.9	150.4	295.6
Sept. 20	11	468.7	288.4	378.6
Sept. 21	12	405.6	195.5	300.5
Sept. 22	13	141.3	156.5	148.9
Sept. 23	14	127.0	30.7	78.9
Sept. 24	15	65.6	65.6	65.6
Sept. 25	16	55.6	250.3	152.9
Sept. 26	17	139.3	64.7	102.0
Sept. 27	18	104.7	36.1	70.4
Sept. 28	19	40.0	-	40.0
Sept. 29	20	46.5	47.3	46.9
Sept. 30	21	45.6	31.3	38.5
Oct.1	22	67.4	39.9	53.6
Oct.2	23	49.2	28.1	38.6
Oct.3	24	57.0	25.9	41.4
Oct.4	25	34.0	60.6	47.3
Oct.5	26	39.8	49.2	44.5

Figure B1-1 The concentration of synthetic gasoline in the soil reactor plotted with exponential degradation.

B.1-2 Off Gas Capture

Table Error! No text of specified style in document.1-2 The off gasses captured in C4011-1 and the ORBO-30 tubes with both the front and back chambers.

Experiment Date	Day	Air Front	Air Back
Sept. 9	0	-	-
Sept. 10	1	34.7	18.2
Sept. 11	2	38.9	19.0
Sept. 12	3	36.1	18.5
Sept. 13	4	33.2	16.7
Sept. 14	5	20.7	17.1
Sept. 15	6	20.9	16.8
Sept. 16	7	35.5	14.4
Sept. 17	8	34.0	17.6
Sept. 18	9	34.9	16.8
Sept. 19	10	32.0	15.5
Sept. 20	11	32.2	15.2
Sept. 21	12	29.8	13.1
Sept. 22	13	28.7	11.3
Sept. 23	14	29.9	12.2
Sept. 24	15	32.0	12.9
Sept. 25	16	30.1	11.8
Sept. 26	17	31.9	9.5
Sept. 27	18	34.8	12.8
Sept. 28	19	24.9	8.9
Sept. 29	20	28.7	8.1
Sept. 30	21	28.7	7.7
Oct.1	22	26.9	7.6
Oct.2	23	20.2	10.2
Oct.3	24	25.7	7.5
Oct.4	25	21.1	3.7
Oct.5	26	19.3	3.5

Notes: Reported concentrations do not take into account extraction efficiency

Figure B1-2 The off gasses captured in C4011-1 and the ORBO-30 tubes with both the front and back chambers plotted over the experimental run.

B.1-3 Standard Curves

Table B1-3 The GC analysis of the prepared standard samples run prior to each batch of sample results in order to back calculate synthetic gasoline concentrations in the soil.

Standards Run	Analysis Date	Standards Run	File ID	Area (Five Components)
1	Sept. 11/ 15	10	MM150911:006F0101	33613
1	Sept. 11/ 15	100	MM150911:007F0101	375306
1	Sept. 12/ 15	100	MM150911:013F0101	262503
1	Sept. 11/ 15	250	MM150911:008F0101	610591
1	Sept. 12/ 15	250	MM150911:014F0101	805157
1	Sept. 11/ 15	750	MM150911:009F0101	1769241
1	Sept. 12/ 15	1000	MM150912:012F0301	3318438
1	Sept. 11/ 15	2000	MM150911:003F0101	6249889
1	Sept. 11/ 15	2000	MM150911:003F0101	6249889
1	Sept. 12/ 15	100	MM150912:013F0301	262503
1	Sept. 12/ 15	250	MM150912:014F0301	805157
1	Sept. 12/ 15	1000	MM150912:012F0301	3318438
2	Sept. 14/15	100	MM150913:002F0101	253958
2	Sept. 14/15	250	MM150913:003F0101	805034
2	Sept. 14/15	500	MM150913:004F0101	1741438
2	Sept. 14/15	1000	MM150913:005F0201	3400592
2	Sept. 14/15	1000	MM150913:005F0202	3260164
3	Sept. 16/15	100	MM150916:002F0101	272583
3	Sept. 16/15	250	MM150916:003F0101	790086
3	Sept. 16/15	500	MM150916:004F0101	1608696
3	Sept. 16/15	1000	MM150916:005F0101	3353759
3	Sept. 16/15	1000	MM150916:005F0201	3348443
4	Sept. 18/15	100	MM150918:002F0101	392366
4	Sept. 18/15	250	MM150918:003F0101	800137
4	Sept. 18/15	500	MM150918:004F0101	1613221
4	Sept. 18/15	1000	MM150918:005F0101	3454076
4	Sept. 18/15	1000	MM150918:005F0201	3467450
5	Sept. 21/15	100	MM150921:002F0101	258208
5	Sept. 21/15	250	MM150921:003F0101	833540
5	Sept. 21/15	500	MM150921:004F0101	1659899
5	Sept. 21/15	1000	MM150921:005F0101	3597941
6	Sept. 23/15	1	MM150923:021F0801	14742
6	Sept. 23/15	10	MM150923:022F0801	25405
6	Sept. 23/15	100	MM150923:002F0101	265821
6	Sept. 23/15	250	MM150923:003F0101	811426
6	Sept. 23/15	500	MM150923:004F0101	1631922
6	Sept. 23/15	1000	MM150923:005F0101	3540057

7	Sept. 25/15	10	MM150925:006F0101	25173
7	Sept. 25/15	100	MM150925:002F0101	269060
7	Sept. 25/15	250	MM150925:003F0101	832956
7	Sept. 25/15	500	MM150925:004F0101	1638190
7	Sept. 25/15	1000	MM150925:005F0101	3617476
8	Sept. 28/15	10	MM150928:006F0101	23943
8	Sept. 28/15	100	MM150928:002F0101	260422
8	Sept. 28/15	250	MM150928:003F0101	800216
8	Sept. 28/15	500	MM150928:004F0101	1551472
8	Sept. 28/15	1000	MM150928:005F0101	3084575
9	Sept. 30/15	10	MM150930:006F0101	23905
9	Sept. 30/15	100	MM150930:002F0101	255502
9	Sept. 30/15	250	MM150930:003F0101	800933
9	Sept. 30/15	500	MM150930:004F0101	1539200
9	Sept. 30/15	1000	MM150930:005F0101	3404104
10	Oct. 2/15	10	MM151002:002F0101	26391
10	Oct. 2/15	100	MM151002:003F0101	280739
10	Oct. 2/15	250	MM151002:004F0101	854918
10	Oct. 2/15	500	MM151002:005F0101	1659047
10	Oct. 2/15	1000	MM151002:006F0101	3642999
11	Oct. 5/15	10	MM151005:002F0101	27662
11	Oct. 5/15	100	MM151005:003F0101	288426
11	Oct. 5/15	250	MM151005:004F0101	890022
11	Oct. 5/15	500	MM151005:005F0101	1692558
11	Oct. 5/15	1000	MM151005:006F0101	3674160

Figure B1-3 The analysis of the prepared standard solutions prior to samples run in the GC-FID.

Figure B1-4 The analysis of the prepared standard solutions prior to samples run in the GC-FID.

Figure B1-5 The analysis of the prepared standard solutions prior to samples run in the GC-FID.

Figure B1-6 The analysis of the prepared standard solutions prior to samples run in the GC-FID.

Figure B1-7 The analysis of the prepared standard solutions prior to samples run in the GC-FID.

Figure B1-8 The analysis of the prepared standard solutions prior to samples run in the GC-FID.

Figure B1-9 The analysis of the prepared standard solutions prior to samples run in the GC-FID.

Figure B1-10 The analysis of the prepared standard solutions prior to samples run in the GC-FID.

Figure B1-11 The analysis of the prepared standard solutions prior to samples run in the GC-FID.

Figure B1-12 The analysis of the prepared standard solutions prior to samples run in the GC-FID.

Figure B1-13 The analysis of the prepared standard solutions prior to samples run in the GC-FID.

B.1-4 Oxygen Levels

Table B1-4 The oxygen levels samples taken by the Apogee Oxygen Meter in the exhaust from the venting well.

Date	Day	Time	Volume %
Sept. 9	0	12:00	20.8
Sept. 10	1	12:00	18.7
Sept. 11	2	14:20	18.6
Sept. 12	3	12:00	18.4
Sept. 13	4	14:30	18.5
Sept. 14	5	13:00	18.6
Sept. 15	6	13:00	18.6
Sept. 16	7	11:50	18.7
Sept. 17	8	11:00	18.7
Sept. 18	9	10:00	18.5
Sept. 19	10	11:45	18.5
Sept. 20	11	11:30	18.5
Sept. 21	12	13:00	18.5
Sept. 22	13	11:45	18.7
Sept. 23	14	11:00	18.8
Sept. 24	15	12:00	18.8
Sept. 25	16	13:30	18.9
Sept. 26	17	13:00	18.8
Sept. 27	18	12:00	18.9
Sept. 28	19	11:00	19
Sept. 29	20	12:00	19
Sept. 30	21	11:30	19.1
Oct. 1	22	13:30	19.1
Oct. 2	23	13:00	19.1
Oct. 3	24	14:00	19.2
Oct. 4	25	14:00	19.2
Oct. 5	26	12:00	19.4
Oct. 6	27	13:30	19.4
Oct. 7	28	12:00	19.5

Figure B1-14 The oxygen level in the exhaust from the venting well plotted over the course of the experiment.

B.1-5 Soil Moisture

Figure B1-15 Soil moisture levels recorded in the inner and outer sections of the soil reactor.

B.1-6 Microbial Data

Table B1-5 Microbial plating using Tryptic Soy Agar (TSA) media to count Total Heterotopic Bacteria (THB) in 5 grams of soil at the beginning of the experiment.

Plate Date	Plate Time	Plate ID	Sample Location	Count Date	Count Time	Colonies	CFU x 10 ⁷ /g
Sept. 10 /15	14:30	1	Top	Sept. 12 /15	13:45	24	12
Sept. 10 /15	14:30	2	Top	Sept. 12 /15	13:45	33	16.5
Sept. 10 /15	14:30	3	Top	Sept. 12 /15	13:45	31	15.5
Sept. 10 /15	14:30	4	Bottom	Sept. 12 /15	13:45	10	5
Sept. 10 /15	14:30	5	Bottom	Sept. 12 /15	13:45	16	8
Sept. 10 /15	14:30	6	Bottom	Sept. 12 /15	13:45	12	6
Sept. 10 /15	14:30	7	Middle	Sept. 12 /15	13:45	8	4
Sept. 10 /15	14:30	8	Middle	Sept. 12 /15	13:45	22	11
Sept. 10 /15	14:30	9	Middle	Sept. 12 /15	13:45	22	11
Sept. 10 /15	14:30	10	Control	Sept. 12 /15	13:45	0	-
Sept. 10 /15	14:30	11	Control	Sept. 12 /15	13:45	3	-
Sept. 10 /15	14:30	12	Control	Sept. 12 /15	13:45	1	-

Table B1-6 Microbial plating using Tryptic Soy Agar (TSA) media to count Total Heterotopic Bacteria (THB) in 5 grams of soil at the end of the experiment.

Plate Date	Plate Time	Plate ID	Sample Location	Count Date	Count Time	Colonies	CFU x 10 ⁷ /g
Oct. 7 /15	13:00	1	Top	Oct. 9 /15	14:00	21	10.5
Oct. 7 /15	13:00	2	Top	Oct. 9 /15	14:00	18	9
Oct. 7 /15	13:00	3	Top	Oct. 9 /15	14:00	25	12.5
Oct. 7 /15	13:00	4	Bottom	Oct. 9 /15	14:00	14	7
Oct. 7 /15	13:00	5	Bottom	Oct. 9 /15	14:00	15	7.5
Oct. 7 /15	13:00	6	Bottom	Oct. 9 /15	14:00	12	6
Oct. 7 /15	13:00	7	Middle	Oct. 9 /15	14:00	26	13
Oct. 7 /15	13:00	8	Middle	Oct. 9 /15	14:00	30	15
Oct. 7 /15	13:00	9	Middle	Oct. 9 /15	14:00	28	14
Oct. 7 /15	13:00	10	Control	Oct. 9 /15	14:00	1	-
Oct. 7 /15	13:00	11	Control	Oct. 9 /15	14:00	2	-
Oct. 7 /15	13:00	12	Control	Oct. 9 /15	14:00	0	-

B.2 Delhi Soil Test 2

B.2-1 Degradation

Table B2-1 The concentration of synthetic gasoline in the soil reactor.

Experiment Date	Day	Sample Location 1 Concentration (mg/kg)	Sample Location 2 Concentration (mg/kg)	Average Concentration (mg/kg)
Oct. 19	0	2441.9	2566.1	2504.0
Oct. 20	1	1998.5	1792.1	1895.3
Oct. 21	2	1381.4	1342.9	1362.2
Oct. 22	3	962.5	911.6	937.1
Oct. 23	4	761.6	497.7	629.7
Oct. 24	5	704.2	731.8	718.0
Oct. 25	6	457.1	553.6	505.3
Oct. 26	7	353.7	469.3	411.5
Oct. 27	8	421.3	450.4	435.9
Oct. 28	9	389.6	276.6	333.1
Oct. 29	10	214.5	220.2	217.3
Oct. 30	11	165.4		165.4
Oct. 31	12	233.7	340.7	287.2
Nov. 1	13	143.2	141.1	142.1
Nov. 2	14	127.3	137.7	132.5
Nov. 3	15	173.5	160.4	166.9
Nov. 4	16	101.9	186.5	144.2
Nov. 5	17	131.9	130.0	130.9
Nov. 6	18	104.4	112.3	108.4
Nov. 7	19	111.1	100.0	105.6
Nov. 8	20	95.7	92.0	93.8
Nov. 9	21	67.2	71.7	69.5
Nov. 10	22	95.1	98.5	96.8
Nov. 11	23	79.1	54.3	66.7
Nov. 12	24	49.3	81.3	65.3
Nov. 13	25	29.2	49.1	39.1
Nov. 14	26	49.9	41.5	45.7
Nov. 15	27	31.9	55.5	43.7
Nov. 16	28	57.3	59.1	58.2
Nov. 17	29	27.2	43.4	35.3
Nov. 18	30	37.5	24.6	31.1

Figure B2-2 The concentration of synthetic gasoline in the soil reactor plotted with exponential degradation.

B.2-2 Off Gas Capture

Table B2-1 The off gasses captured in C4011-1 and the ORBO-30 tubes with both the front and back chambers.

Experiment Date	Day	Air Front (mg Syn. Gas)	Air Back (mg Syn. Gas)
Oct. 19	0	-	-
Oct. 20	1	218.4	48.4
Oct. 21	2	207.6	45.6
Oct. 22	3	196.8	42.7
Oct. 23	4	186.0	39.9
Oct. 24	5	175.2	37.0
Oct. 25	6	164.4	34.2
Oct. 26	7	153.6	31.4
Oct. 27	8	142.8	28.5
Oct. 28	9	124.5	43.5
Oct. 29	10	117.7	25.2
Oct. 30	11	125.4	33.1
Oct. 31	12	104.6	0.3
Nov. 1	13	105.1	3.3
Nov. 2	14	78.6	3.3
Nov. 3	15	65.2	0.1
Nov. 4	16	38.1	0.0
Nov. 5	17	29.6	1.0
Nov. 6	18	25.8	0.5
Nov. 7	19	25.2	0.4
Nov. 8	20	11.9	0.7
Nov. 9	21	23.5	0.8
Nov. 10	22	23.4	6.5
Nov. 11	23	17.9	0.2
Nov. 12	24	17.1	2.8
Nov. 13	25	18.0	2.0
Nov. 14	26		
Nov. 15	27	19.2	0.8
Nov. 16	28	16.8	0.5
Nov. 17	29	15.7	0.1
Nov. 18	30	12.8	0.1

Figure B2-3 The off gasses captured in C4011-1 and the ORBO-30 tubes with both the front and back chambers plotted over the experimental run.

B.2-3 Standard Curves

Table B2-3 The GC analysis of the prepared standard samples run prior to each batch of sample results in order to back calculate synthetic gasoline concentrations in the soil.

Standards Run	Analysis Date	Standards Run	File ID	Area (Five Components)
1	Oct. 23 /15	10	MM151023:001F0201	22291
1	Oct. 23 /15	100	MM151023:001F0301	235456
1	Oct. 23 /15	250	MM151023:001F0401	702213
1	Oct. 23 /15	500	MM151023:001F0501	1481445
1	Oct. 23 /15	1000	MM151023:001F0601	3088489
2	Oct. 27 /15	10	MM151027:001F0201	24858
2	Oct. 27 /15	100	MM151027:001F0301	229253
2	Oct. 27 /15	250	MM151027:001F0401	704651
2	Oct. 27 /15	500	MM151027:001F0501	1399656
2	Oct. 27 /15	1000	MM151027:001F0601	3085786
3	Oct. 30 /15	10	MM151030:001F0201	26994
3	Oct. 30 /15	100	MM151030:001F0301	237079
3	Oct. 30 /15	250	MM151030:001F0401	722034
3	Oct. 30 /15	500	MM151030:001F0501	1467811
3	Oct. 30 /15	1000	MM151030:001F0601	3200269
4	Nov. 2 /15	10	MM151102:001F0201	23896
4	Nov. 2 /15	100	MM151102:001F0301	249444
4	Nov. 2 /15	250	MM151102:001F0401	782415
4	Nov. 2 /15	500	MM151102:001F0501	1545381
4	Nov. 2 /15	1000	MM151102:001F0601	3242906
5	Nov. 5/15	10	MM151105:001F0201	26266
5	Nov. 5/15	100	MM151105:001F0301	251393
5	Nov. 5/15	250	MM151105:001F0401	776760
5	Nov. 5/15	500	MM151105:001F0501	1540035
5	Nov. 5/15	1000	MM151105:001F0601	3291896
6	Nov. 9/15	10	MM151109:001F0201	29878
6	Nov. 9/15	100	MM151109:001F0301	271958
6	Nov. 9/15	250	MM151109:001F0401	847665
6	Nov. 9/15	500	MM151109:001F0501	1679085
6	Nov. 9/15	1000	MM151109:001F0601	3531645
7	Nov. 13/15	10	MM151113:001F0201	35740
7	Nov. 13/15	100	MM151113:001F0301	302038
7	Nov. 13/15	250	MM151113:001F0401	952485
7	Nov. 13/15	500	MM151113:001F0501	1778201
7	Nov. 13/15	1000	MM151113:001F0601	3838275
8	Nov. 18/15	10	MM151118:001F0201	44000

8	Nov. 18/15	100	MM151118:001F0301	339195
8	Nov. 18/15	250	MM151118:001F0401	1082452
8	Nov. 18/15	500	MM151118:001F0501	1929078
8	Nov. 18/15	1000	MM151118:001F0601	4116658

Figure B2-4 The analysis of the prepared standard solutions prior to samples run in the GC-FID.

Figure B2-5 The analysis of the prepared standard solutions prior to samples run in the GC-FID.

Figure B2-6 The analysis of the prepared standard solutions prior to samples run in the GC-FID.

Figure B2-7 The analysis of the prepared standard solutions prior to samples run in the GC-FID.

Figure B2-8 The analysis of the prepared standard solutions prior to samples run in the GC-FID.

Figure B2-9 The analysis of the prepared standard solutions prior to samples run in the GC-FID.

Figure B2-10 The analysis of the prepared standard solutions prior to samples run in the GC-FID.

Figure B2-11 The analysis of the prepared standard solutions prior to samples run in the GC-FID.

B.2-4 Oxygen Levels

Table B2-4 The oxygen levels samples taken by the Apogee Oxygen Meter in the exhaust from the venting well.

Date	Day	Volume %
Oct. 19	0	20.7
Oct. 20	1	18.3
Oct. 21	2	18.4
Oct. 22	3	18.4
Oct. 23	4	18.6
Oct. 24	5	18.5
Oct. 25	6	18.6
Oct. 26	7	18.8
Oct. 27	8	18.7
Oct. 28	9	18.6
Oct. 29	10	18.7
Oct. 30	11	18.6
Oct. 31	12	18.6
Nov. 1	13	18.7
Nov. 2	14	18.8
Nov. 3	15	18.8
Nov. 4	16	18.9
Nov. 5	17	18.8
Nov. 6	18	18.9
Nov. 7	19	19
Nov. 8	20	19
Nov. 9	21	19.2
Nov. 10	22	19.3
Nov. 11	23	19.1
Nov. 12	24	19.3
Nov. 13	25	19.4
Nov. 14	26	19.4
Nov. 15	27	19.5
Nov. 16	28	19.4
Nov. 17	29	19.5
Nov. 18	30	19.6

Figure B2-12 The oxygen level in the exhaust from the venting well plotted over the course of the experiment.

B.2-5 Soil Moisture

Figure B2-13 Soil moisture levels recorded in the inner and outer sections of the soil reactor.

B.2-6 Microbial Data

Table B2-5 Microbial plating using Tryptic Soy Agar (TSA) media to count Total Heterotopic Bacteria (THB) in 5 grams of soil at the beginning of the experiment.

Plate Date	Plate Time	Plate ID	Sample Location	Count Date	Count Time	Colonies	CFU x 10 ⁷ /g
Oct. 27/15	14:30	1	Top	Oct. 29/15	13:30	14	7
Oct. 27/15	14:30	2	Top	Oct. 29/15	13:30	16	6
Oct. 27/15	14:30	3	Top	Oct. 29/16	13:30	15	7.5
Oct. 27/15	14:30	4	Bottom	Oct. 29/17	13:30	15	7.5
Oct. 27/15	14:30	5	Bottom	Oct. 29/18	13:30	27	13.5
Oct. 27/15	14:30	6	Bottom	Oct. 29/19	13:30	23	11.5
Oct. 27/15	14:30	7	Middle	Oct. 29/20	13:30	16	8
Oct. 27/15	14:30	8	Middle	Oct. 29/21	13:30	9	4.6
Oct. 27/15	14:30	9	Middle	Oct. 29/22	13:30	17	8.5
Oct. 27/15	14:30	10	Control	Oct. 29/23	13:30	0	-
Oct. 27/15	14:30	11	Control	Oct. 29/24	13:30	1	-
Oct. 27/15	14:30	12	Control	Oct. 29/25	13:30	0	-

Table B2-6 Microbial plating using Tryptic Soy Agar (TSA) media to count Total Heterotopic Bacteria (THB) in 5 grams of soil at the end of the experiment.

Plate Date	Plate Time	Plate ID	Sample Location	Count Date	Count Time	Colonies	CFU x 10 ⁷ /g
Nov. 17/15	10:00	1	Top	Nov. 19/15	14:00	18	9
Nov. 17/15	10:00	2	Top	Nov. 19/15	14:00	15	7.5
Nov. 17/15	10:00	3	Top	Nov. 19/15	14:00	21	10.5
Nov. 17/15	10:00	4	Bottom	Nov. 19/15	14:00	17	8.5
Nov. 17/15	10:00	5	Bottom	Nov. 19/15	14:00	14	7
Nov. 17/15	10:00	6	Bottom	Nov. 19/15	14:00	23	11.5
Nov. 17/15	10:00	7	Middle	Nov. 19/15	14:00	25	12.5
Nov. 17/15	10:00	8	Middle	Nov. 19/15	14:00	21	10.5
Nov. 17/15	10:00	9	Middle	Nov. 19/15	14:00	16	8
Nov. 17/15	10:00	10	Control	Nov. 19/15	14:00	1	-
Nov. 17/15	10:00	11	Control	Nov. 19/15	14:00	2	-
Nov. 17/15	10:00	12	Control	Nov. 19/15	14:00	2	-

Table B2-7 Microbial plating using Bushnell-Hass Media (BHM) to count Total Heterotopic Bacteria (THB) in 5 grams of soil at the beginning of the experiment.

Plate Date	Plate Time	Plate ID	Sample Location	Count Date	Count Time	Colonies	CFU x 10 ⁷ /g
Oct. 27/15	14:30	1	Top	Nov. 11/15	12:00	15	7.5
Oct. 27/15	14:30	2	Top	Nov. 11/15	12:00	19	9.5
Oct. 27/15	14:30	3	Top	Nov. 11/15	12:00	17	8.5
Oct. 27/15	14:30	4	Bottom	Nov. 11/15	12:00	10	5
Oct. 27/15	14:30	5	Bottom	Nov. 11/15	12:00	17	8.5
Oct. 27/15	14:30	6	Bottom	Nov. 11/15	12:00	12	6
Oct. 27/15	14:30	7	Middle	Nov. 11/15	12:00	15	7.5
Oct. 27/15	14:30	8	Middle	Nov. 11/15	12:00	17	8.5
Oct. 27/15	14:30	9	Middle	Nov. 11/15	12:00	20	10
Oct. 27/15	14:30	10	Control	Nov. 11/15	12:00	2	-
Oct. 27/15	14:30	11	Control	Nov. 11/15	12:00	1	-
Oct. 27/15	14:30	12	Control	Nov. 11/15	12:00	2	-

Table B2-8 Microbial plating using Bushnell-Hass Media (BHM) to count Total Heterotopic Bacteria (THB) in 5 grams of soil at the end of the experiment.

Plate Date	Plate Time	Plate ID	Sample Location	Count Date	Count Time	Colonies	CFU x 10 ⁷ /g
Nov. 17/15	10:00	1	Top	Dec. 1/15	11:30	16	8
Nov. 17/15	10:00	2	Top	Dec. 1/15	11:30	20	10
Nov. 17/15	10:00	3	Top	Dec. 1/15	11:30	17	8.5
Nov. 17/15	10:00	4	Bottom	Dec. 1/15	11:30	11	5.5
Nov. 17/15	10:00	5	Bottom	Dec. 1/15	11:30	18	9
Nov. 17/15	10:00	6	Bottom	Dec. 1/15	11:30	17	8.5
Nov. 17/15	10:00	7	Middle	Dec. 1/15	11:30	13	6.5
Nov. 17/15	10:00	8	Middle	Dec. 1/15	11:30	16	8
Nov. 17/15	10:00	9	Middle	Dec. 1/15	11:30	25	12.5
Nov. 17/15	10:00	10	Control	Dec. 1/15	11:30	1	-
Nov. 17/15	10:00	11	Control	Dec. 1/15	11:30	0	-
Nov. 17/15	10:00	12	Control	Dec. 1/15	11:30	2	-

B.3 Elora Soil Test 1

B.3-1 Degradation

Table B3-1 The concentration of synthetic gasoline in the soil reactor.

Experiment Date	Day	Sample Location 1 Concentration (mg/kg)	Sample Location 2 Concentration (mg/kg)	Average Concentration (mg/kg)
Nov. 19	0	2731.5	2783.1	2757.3
Nov. 20	1	1476.3	1453.8	1465.1
Nov. 21	2	712.9	764.8	738.9
Nov. 22	3	400.7	410.7	405.7
Nov. 23	4	316.6	334.7	325.7
Nov. 24	5	290.4	285.7	288.0
Nov. 25	6	206.9	182.9	194.9
Nov. 26	7	135.5	132.1	133.8
Nov. 27	8	105.3	100.6	102.9
Nov. 28	9	82.4	75.7	79.1
Nov. 29	10	85.7	81.5	83.6
Nov. 30	11	68.6	76.8	72.7
Dec. 1	12	78.5	68.7	73.6
Dec. 2	13	47.7	56.1	51.9
Dec. 3	14	35.2	37.8	36.5
Dec. 4	15	42.5	41.6	42.1
Dec. 5	16	37.9	42.8	40.3
Dec. 6	17	45.3	49.2	47.2
Dec. 7	18	39.6	37.3	38.5
Dec. 8	19	43.5	36.9	40.2
Dec. 9	20	68.6	36.7	52.7
Dec. 10	21	26.2	34.2	30.2
Dec. 11	22	29.4	40.7	35.1
Dec. 12	23	36.2	38.8	37.5
Dec. 13	24	30.9	27.8	29.4
Dec. 14	25	20.6	28.2	24.4
Dec. 15	26	20.3	23.2	21.7
Dec. 16	27	20.3	19.2	19.7
Dec. 17	28	19.4	23.7	21.5
Dec. 18	29	19.8	19.4	19.6

Figure B3-1 The concentration of synthetic gasoline in the soil reactor plotted with exponential degradation.

B.3-2 Off Gas Capture

Table B3-2 The off gasses captured in C4011-1 and the ORBO-30 tubes with both the front and back chambers.

Experiment Date	Day	Air Front (mg Syn. Gas)	Air Back (mg Syn. Gas)
Nov. 19	0		
Nov. 20	1	127.8	62.4
Nov. 21	2	132.9	18.0
Nov. 22	3	128.3	15.2
Nov. 23	4	136.5	10.6
Nov. 24	5	97.4	0.07
Nov. 25	6	91.5	0.04
Nov. 26	7	77.3	0.02
Nov. 27	8	47.2	0.02
Nov. 28	9	31.4	0.04
Nov. 29	10	29.2	0.02
Nov. 30	11	30.1	0.07
Dec. 1	12	20.9	0.05
Dec. 2	13	11.4	0.02
Dec. 3	14	13.4	0.01
Dec. 4	15	14.1	0.07
Dec. 5	16	7.9	0.05
Dec. 6	17	8.5	0.08
Dec. 7	18	12.9	0.11
Dec. 8	19	11.4	0.09
Dec. 9	20	10.7	0.06
Dec. 10	21	7.3	0.11
Dec. 11	22	6.5	0.10
Dec. 12	23	9.1	0.12
Dec. 13	24	7.7	0.10
Dec. 14	25	6.5	0.03
Dec. 15	26	6.9	0.10
Dec. 16	27	6.3	0.09
Dec. 17	28	4.2	0.06
Dec. 18	29	3.8	0.02

Figure B3-2 The off gasses captured in C4011-1 and the ORBO-30 tubes with both the front and back chambers plotted over the experimental run.

B.3-4 Standard Curves

Table B3-3 The GC analysis of the prepared standard samples run prior to each batch of sample results in order to back calculate synthetic gasoline concentrations in the soil.

Standards Run	Analysis Date	Standards Run	File ID	Area (Five Components)
1	Nov. 20/15	10	MM151120:001F0201	30200
1	Nov. 20/15	100	MM151120:001F0301	243726
1	Nov. 20/15	250	MM151120:001F0401	577714
1	Nov. 20/15	500	MM151120:001F0501	1317259
1	Nov. 20/15	1000	MM151120:001F0601	2724030
2	Nov. 23/15	10	MM151123:001F0201	22778
2	Nov. 23/15	100	MM151123:001F0301	260365
2	Nov. 23/15	250	MM151123:001F0401	678564
2	Nov. 23/15	500	MM151123:001F0501	1448102
2	Nov. 23/15	1000	MM151123:001F0601	3021942
3	Nov. 27/15	10	MM151127:001F0201	25232
3	Nov. 27/15	100	MM151127:001F0301	284940
3	Nov. 27/15	250	MM151127:001F0401	681195
3	Nov. 27/15	500	MM151127:001F0501	1063933
3	Nov. 27/15	1000	MM151127:001F0601	2055353
4	Dec. 2/15	10	MM151202:001F0201	21135
4	Dec. 2/15	100	MM151202:001F0301	250666
4	Dec. 2/15	250	MM151202:001F0401	415314
4	Dec. 2/15	500	MM151202:001F0501	1126500
4	Dec. 2/15	1000	MM151202:001F0601	2303890
5	Dec. 7/15	10	MM151207:001F0201	21582
5	Dec. 7/15	100	MM151207:001F0301	248091
5	Dec. 7/15	250	MM151207:001F0401	527335
5	Dec. 7/15	500	MM151207:001F0501	1230826
5	Dec. 7/15	1000	MM151207:001F0601	2551807
6	Dec.15/15	10	MM151215:001F0201	32166
6	Dec.15/15	100	MM151215:001F0301	416592
6	Dec.15/15	250	MM151215:001F0401	708733
6	Dec.15/15	500	MM151215:001F0501	1599843
6	Dec.15/15	1000	MM151215:001F0601	3062066
7	Dec.18/15	10	MM151218:001F0201	31261
7	Dec.18/15	100	MM151218:001F0301	221780
7	Dec.18/15	250	MM151218:001F0401	737357
7	Dec.18/15	500	MM151218:001F0501	2102288
7	Dec.18/15	1000	MM151218:001F0601	3798262

Figure B3-3 The analysis of the prepared standard solutions prior to samples run in the GC-FID.

Figure B3-4 The analysis of the prepared standard solutions prior to samples run in the GC-FID.

Figure B3-5 The analysis of the prepared standard solutions prior to samples run in the GC-FID.

Figure B3-6 The analysis of the prepared standard solutions prior to samples run in the GC-FID.

Figure B3-7 The analysis of the prepared standard solutions prior to samples run in the GC-FID.

Figure B3-8 The analysis of the prepared standard solutions prior to samples run in the GC-FID.

Figure B3-9 The analysis of the prepared standard solutions prior to samples run in the GC-FID.

B.3-5 Oxygen Levels

Table B3-4 The oxygen levels samples taken by the Apogee Oxygen Meter in the exhaust from the venting well.

Date	Day	Volume %
Nov. 20	0	20.9
Nov. 21	1	17.8
Nov. 22	2	17.9
Nov. 23	3	18.2
Nov. 24	4	18.3
Nov. 25	5	18.5
Nov. 26	6	18.3
Nov. 27	7	18.5
Nov. 28	8	18.5
Nov. 29	9	18.6
Nov. 30	10	18.7
Dec. 1	11	18.9
Dec. 2	12	19.1
Dec. 3	13	19.2
Dec. 4	14	19.2
Dec. 5	15	19.1
Dec. 6	16	19.3
Dec. 7	17	19.4
Dec. 8	18	19.4
Dec. 9	19	19.5
Dec. 10	20	19.7
Dec. 11	21	19.9
Dec. 12	22	19.8
Dec. 13	23	20
Dec. 14	24	20.1
Dec. 15	25	20.3
Dec. 16	26	20.2
Dec. 17	27	20.4
Dec. 18	28	20.4

Figure B3-10 The oxygen level in the exhaust from the venting well plotted over the course of the experiment.

B.3-5 Soil Moisture

Figure B3-11 Soil moisture levels recorded in the inner and outer sections of the soil reactor.

B.3-6 Microbial Data

Table B3-5 Microbial plating using Tryptic Soy Agar (TSA) media to count Total Heterotopic Bacteria (THB) in 5 grams of soil at the beginning of the experiment.

Plate Date	Plate Time	Plate ID	Sample Location	Count Date	Count Time	Colonies	CFU x 10 ⁷ /g
Nov. 23/15	13:30	1	Top	Nov. 25/15	13:00	18	9
Nov. 23/15	13:30	2	Top	Nov. 25/15	13:00	19	9.5
Nov. 23/15	13:30	3	Top	Nov. 25/15	13:00	14	7
Nov. 23/15	13:30	4	Bottom	Nov. 25/15	13:00	22	11
Nov. 23/15	13:30	5	Bottom	Nov. 25/15	13:00	25	12.5
Nov. 23/15	13:30	6	Bottom	Nov. 25/15	13:00	18	9
Nov. 23/15	13:30	7	Middle	Nov. 25/15	13:00	23	11.5
Nov. 23/15	13:30	8	Middle	Nov. 25/15	13:00	19	9.5
Nov. 23/15	13:30	9	Middle	Nov. 25/15	13:00	16	8
Nov. 23/15	13:30	10	Control	Nov. 25/15	13:00	1	-
Nov. 23/15	13:30	11	Control	Nov. 25/15	13:00	2	-
Nov. 23/15	13:30	12	Control	Nov. 25/15	13:00	0	-

Table B3-6 Microbial plating using Bushnell-Hass Media (BHM) to count Total Heterotopic Bacteria (THB) in 5 grams of soil at the beginning of the experiment.

Plate Date	Plate Time	Plate ID	Sample Location	Count Date	Count Time	Colonies	CFU x 10 ⁷ /g
Nov. 23/15	13:30	1	Top	Dec. 7/15	11:30	21	10.5
Nov. 23/15	13:30	2	Top	Dec. 7/15	11:30	22	11
Nov. 23/15	13:30	3	Top	Dec. 7/15	11:30	27	13.5
Nov. 23/15	13:30	4	Bottom	Dec. 7/15	11:30	26	13
Nov. 23/15	13:30	5	Bottom	Dec. 7/15	11:30	18	9
Nov. 23/15	13:30	6	Bottom	Dec. 7/15	11:30	23	11.5
Nov. 23/15	13:30	7	Middle	Dec. 7/15	11:30	23	11.5
Nov. 23/15	13:30	8	Middle	Dec. 7/15	11:30	26	13
Nov. 23/15	13:30	9	Middle	Dec. 7/15	11:30	28	14
Nov. 23/15	13:30	10	Control	Dec. 7/15	11:30	2	-
Nov. 23/15	13:30	11	Control	Dec. 7/15	11:30	1	-
Nov. 23/15	13:30	12	Control	Dec. 7/15	11:30	2	-

Table B3-7 Microbial plating using Tryptic Soy Agar (TSA) media to count Total Heterotopic Bacteria (THB) in 5 grams of soil in the middle of the experiment due to fear of error in previous plates.

Plate Date	Plate Time	Plate ID	Sample Location	Count Date	Count Time	Colonies	CFU x 10⁷/g
Nov. 30/15	14:00	1	Top	Dec. 2/15	13:30	21	10.5
Nov. 30/15	14:00	2	Top	Dec. 2/15	13:30	17	8.5
Nov. 30/15	14:00	3	Top	Dec. 2/15	13:30	16	8
Nov. 30/15	14:00	4	Bottom	Dec. 2/15	13:30	25	12.5
Nov. 30/15	14:00	5	Bottom	Dec. 2/15	13:30	21	10.5
Nov. 30/15	14:00	6	Bottom	Dec. 2/15	13:30	17	8.5
Nov. 30/15	14:00	7	Middle	Dec. 2/15	13:30	21	10.5
Nov. 30/15	14:00	8	Middle	Dec. 2/15	13:30	16	8
Nov. 30/15	14:00	9	Middle	Dec. 2/15	13:30	23	11.5
Nov. 30/15	14:00	10	Control	Dec. 2/15	13:30	0	-
Nov. 30/15	14:00	11	Control	Dec. 2/15	13:30	1	-
Nov. 30/15	14:00	12	Control	Dec. 2/15	13:30	1	-

Table B3-8 Microbial plating using Tryptic Soy Agar (TSA) media to count Total Heterotopic Bacteria (THB) in 5 grams of soil at the end of the experiment.

Plate Date	Plate Time	Plate ID	Sample Location	Count Date	Count Time	Colonies	CFU x 10⁷/g
Dec. 15/15	14:00	1	Top	Dec. 18/15	13:30	16	8
Dec. 15/15	14:00	2	Top	Dec. 18/15	13:30	21	10.5
Dec. 15/15	14:00	3	Top	Dec. 18/15	13:30	21	10.5
Dec. 15/15	14:00	4	Bottom	Dec. 18/15	13:30	18	9
Dec. 15/15	14:00	5	Bottom	Dec. 18/15	13:30	25	12.5
Dec. 15/15	14:00	6	Bottom	Dec. 18/15	13:30	17	8.5
Dec. 15/15	14:00	7	Middle	Dec. 18/15	13:30	18	9
Dec. 15/15	14:00	8	Middle	Dec. 18/15	13:30	25	12.5
Dec. 15/15	14:00	9	Middle	Dec. 18/15	13:30	19	8
Dec. 15/15	14:00	10	Control	Dec. 18/15	13:30	0	-
Dec. 15/15	14:00	11	Control	Dec. 18/15	13:30	0	-
Dec. 15/15	14:00	12	Control	Dec. 18/15	13:30	1	-

B.4 Elora Soil Test 2

B.4-1 Degradation

Table B4-1 The concentration of synthetic gasoline in the soil reactor.

Experiment Date	Day	Sample Location1	Sample Location 2	Average
Jan.18	0	984.8	939.7	962.3
Jan.19	1	534.3	561.2	547.7
Jan.20	2	415.3	429.9	422.6
Jan.21	3	293.8	281.6	287.7
Jan.22	4	99.1	105.5	102.3
Jan.23	5	68.8	75.0	71.9
Jan.24	6	60.2	66.7	63.5
Jan.25	7	56.6	59.3	58.0
Jan.26	8	49.8	68.8	59.3
Jan.27	9	55.1	50.7	52.9
Jan.28	10	39.3	41.0	40.1
Jan.29	11	42.0	45.3	43.6
Jan.30	12	37.5	36.9	37.2
Jan.31	13	37.4	34.2	35.8
Feb.1	14	31.2	35.4	33.3
Feb.2	15	31.0	28.2	29.6
Feb.3	16	26.5	33.3	29.9
Feb.4	17	25.9	24.1	25.0
Feb.5	18	22.9	22.0	22.4
Feb.6	19	20.6	22.7	21.7
Feb.7	20	18.6	21.7	20.2
Feb.8	21	17.8	19.8	18.8

Figure B4-1 The concentration of synthetic gasoline in the soil reactor plotted with exponential degradation.

B.4-2 Off Gas Capture

Table B4-2 The off gasses captured in C4011-1 and the ORBO-30 tubes with both the front and back chambers.

Experiment Date	Day	Air Front	Air Back
Jan.18	0		
Jan.19	1	71.2	0.05
Jan.20	2	43.2	0.05
Jan.21	3	39.4	0.04
Jan.22	4	22.3	0.05
Jan.23	5	27.9	6.25
Jan.24	6	21.7	0.02
Jan.25	7	26.4	1.07
Jan.26	8	22.6	0.10
Jan.27	9	25.2	0.09
Jan.28	10	17.0	0.07
Jan.29	11	15.8	0.06
Jan.30	12	16.4	0.06
Jan.31	13	14.4	0.05
Feb.1	14	12.3	0.05
Feb.2	15	14.0	0.04
Feb.3	16	10.4	0.03
Feb.4	17	9.7	0.02
Feb.5	18	9.2	0.02
Feb.6	19	9.9	0.16
Feb.7	20	7.9	0.01
Feb.8	21	8.9	0.01

Figure B4-2 The off gasses captured in C4011-1 and the ORBO-30 tubes with both the front and back chambers plotted over the experimental run.

B.4-3 Standard Curves

Table B4-3 The GC analysis of the prepared standard samples run prior to each batch of sample results in order to back calculate synthetic gasoline concentrations in the soil.

Standards Run	Analysis Date	Standards Run	File ID	Area (Five Components)
1	Jan.21/16	10	MM160121:001F0201	21838
1	Jan.21/16	100	MM160121:001F0301	230289
1	Jan.21/16	250	MM160121:001F0401	645456
1	Jan.21/16	500	MM160121:001F0501	1517750
1	Jan.21/16	1000	MM160121:001F0601	3158046
2	Jan.25/16	10	MM160125:001F0201	21200
2	Jan.25/16	100	MM160125:001F0301	215325
2	Jan.25/16	250	MM160125:001F0401	641421
2	Jan.25/16	500	MM160125:001F0501	1229579
2	Jan.25/16	1000	MM160125:001F0601	3204373
3	Jan.28/16	10	MM160128:001F0201	21874
3	Jan.28/16	100	MM160128:001F0301	227083
3	Jan.28/16	250	MM160128:001F0401	670724
3	Jan.28/16	500	MM160128:001F0501	1246582
3	Jan.28/16	1000	MM160128:001F0601	3270804
4	Feb.4/16	10	MM160204:001F0201	23943
4	Feb.4/16	100	MM160204:001F0301	260422
4	Feb.4/16	250	MM160204:001F0401	800216
4	Feb.4/16	500	MM160204:001F0501	1551472
4	Feb.4/16	1000	MM160204:001F0601	3084575
5	Feb.8/16	10	MM160208:001F0201	23905
5	Feb.8/16	100	MM160208:001F0301	255502
5	Feb.8/16	250	MM160208:001F0401	800933
5	Feb.8/16	500	MM160208:001F0501	1539200
5	Feb.8/16	1000	MM160208:001F0601	3404104

Figure B4-3 The analysis of the prepared standard solutions prior to samples run in the GC-FID.

Figure B4-4 The analysis of the prepared standard solutions prior to samples run in the GC-FID.

Figure B4-5 The analysis of the prepared standard solutions prior to samples run in the GC-FID.

Figure B4-6 The analysis of the prepared standard solutions prior to samples run in the GC-FID.

Figure B4-7 The analysis of the prepared standard solutions prior to samples run in the GC-FID.

B.4-4 Oxygen Levels

Table B4-4 The oxygen levels samples taken by the Apogee Oxygen Meter in the exhaust from the venting well.

Date	Day	Volume %
Jan.18	0	20.8
Jan.19	1	18.7
Jan.20	2	18.7
Jan.21	3	18.9
Jan.22	4	18.9
Jan.23	5	19.1
Jan.24	6	19.2
Jan.25	7	19.3
Jan.26	8	19.3
Jan.27	9	19.4
Jan.28	10	19.3
Jan.29	11	19.4
Jan.30	12	19.5
Jan.31	13	19.7
Feb.1	14	19.8
Feb.2	15	19.9
Feb.3	16	20.1
Feb.4	17	20.1
Feb.5	18	20.2
Feb.6	19	20.4
Feb.7	20	20.4
Feb.8	21	20.5

Figure B4-8 The oxygen level in the exhaust from the venting well plotted over the course of the experiment.

B.4-5 Soil Moisture

Figure B4-9 Soil moisture levels recorded in the inner and outer sections of the soil reactor.

B.4-6 Microbial Data

Table B4-5 Microbial plating using Tryptic Soy Agar (TSA) media to count Total Heterotopic Bacteria (THB) in 5 grams of soil at the beginning of the experiment.

Plate Date	Plate Time	Plate ID	Sample Location	Count Date	Count Time	Colonies	CFU x 10 ⁷ /g
Jan. 20/16	12:30	1	Top	Jan. 22/16	10:00	21	11
Jan. 20/16	12:30	2	Top	Jan. 22/16	10:00	17	8.5
Jan. 20/16	12:30	3	Top	Jan. 22/16	10:00	19	9
Jan. 20/16	12:30	4	Bottom	Jan. 22/16	10:00	14	7
Jan. 20/16	12:30	5	Bottom	Jan. 22/16	10:00	21	10.5
Jan. 20/16	12:30	6	Bottom	Jan. 22/16	10:00	17	8.5
Jan. 20/16	12:30	7	Middle	Jan. 22/16	10:00	18	9
Jan. 20/16	12:30	8	Middle	Jan. 22/16	10:00	15	7.5
Jan. 20/16	12:30	9	Middle	Jan. 22/16	10:00	23	12.5
Jan. 20/16	12:30	10	Control	Jan. 22/16	10:00	1	-
Jan. 20/16	12:30	11	Control	Jan. 22/16	10:00	0	-
Jan. 20/16	12:30	12	Control	Jan. 22/16	10:00	1	-

Table 4-6 Microbial plating using Bushnell-Hass Media (BHM) to count Total Heterotopic Bacteria (THB) in 5 grams of soil at the beginning of the experiment.

Plate Date	Plate Time	Plate ID	Sample Location	Count Date	Count Time	Colonies	CFU x 10 ⁷ /g
Jan. 20/16	12:30	1	Top	Feb. 3/16	10:00	25	12.5
Jan. 20/16	12:30	2	Top	Feb. 3/16	10:00	23	11.5
Jan. 20/16	12:30	3	Top	Feb. 3/16	10:00	21	10.5
Jan. 20/16	12:30	4	Bottom	Feb. 3/16	10:00	29	14.5
Jan. 20/16	12:30	5	Bottom	Feb. 3/16	10:00	25	12.5
Jan. 20/16	12:30	6	Bottom	Feb. 3/16	10:00	23	11.5
Jan. 20/16	12:30	7	Middle	Feb. 3/16	10:00	19	9.5
Jan. 20/16	12:30	8	Middle	Feb. 3/16	10:00	17	8.5
Jan. 20/16	12:30	9	Middle	Feb. 3/16	10:00	26	13
Jan. 20/16	12:30	10	Control	Feb. 3/16	10:00	3	-
Jan. 20/16	12:30	11	Control	Feb. 3/16	10:00	2	-
Jan. 20/16	12:30	12	Control	Feb. 3/16	10:00	2	-

Table B4-7 Microbial plating using Tryptic Soy Agar (TSA) media to count Total Heterotopic Bacteria (THB) in 5 grams of soil at the end of the experiment.

Plate Date	Plate Time	Plate ID	Sample Location	Count Date	Count Time	Colonies	CFU x 10⁷/g
Feb. 8/16	12:30	1	Top	Feb. 10/16	10:00	16	8
Feb. 8/16	12:30	2	Top	Feb. 10/16	10:00	14	7
Feb. 8/16	12:30	3	Top	Feb. 10/16	10:00	10	5
Feb. 8/16	12:30	4	Bottom	Feb. 10/16	10:00	15	7.5
Feb. 8/16	12:30	5	Bottom	Feb. 10/16	10:00	12	6
Feb. 8/16	12:30	6	Bottom	Feb. 10/16	10:00	18	9
Feb. 8/16	12:30	7	Middle	Feb. 10/16	10:00	19	9.5
Feb. 8/16	12:30	8	Middle	Feb. 10/16	10:00	23	11.5
Feb. 8/16	12:30	9	Middle	Feb. 10/16	10:00	17	8.5
Feb. 8/16	12:30	10	Control	Feb. 10/16	10:00	2	-
Feb. 8/16	12:30	11	Control	Feb. 10/16	10:00	2	-
Feb. 8/16	12:30	12	Control	Feb. 10/16	10:00	1	-

B.5 Mixed Soil

B.5-1 Degradation

Table B5-1 The concentration of synthetic gasoline in the soil reactor.

Sample Date	Day	Elora Soil (mg/kg)	Delhi Soil (mg/kg)
Feb.16	0	2524.65	2664.28
Feb.17	1	1883.44	1950.99
Feb.18	2	1048.22	1609.78
Feb.19	3	790.74	1080.98
Feb.20	4	557.69	872.81
Feb.21	5	421.68	742.55
Feb.22	6	312.67	535.60
Feb.23	7	162.26	310.93
Feb.24	8	154.27	349.16
Feb.25	9	151.10	298.32
Feb.26	10	130.14	254.38
Feb.27	11	124.36	194.01
Feb.28	12	100.85	187.95
Feb.29	13	103.31	157.85
Mar.1	14	90.94	178.40
Mar.2	15	85.43	148.56
Mar.3	16	83.09	114.57
Mar.4	17	76.99	115.26
Mar.5	18	79.29	102.14
Mar.6	19	63.37	81.87
Mar.7	20	46.16	71.20
Mar.8	21	57.97	74.24
Mar.9	22	55.63	69.17
Mar.10	23	42.69	52.46
Mar.11	24	49.93	47.37
Mar.12	25	45.57	47.88
Mar.13	26	48.94	48.00
Mar.14	27	41.91	43.28
Mar.15	28	33.57	32.43
Mar.16	29	30.76	33.88

Figure B5-1 The concentration of synthetic gasoline in the soil reactor plotted with exponential degradation.

B.5-2 Off Gas Capture

Table B5-2 The off gasses captured in C4011-1 and the ORBO-30 tubes with both the front and back chambers.

Sample Date	Day	Elora Soil (mg/kg)	Delhi Soil (mg/kg)	Air - Front Chamber (mg)	Air - Back Chamber (mg)
Feb.16	0	2524.65	2664.28		
Feb.17	1	1883.44	1950.99	232.16	12.20
Feb.18	2	1048.22	1563.10	222.63	10.32
Feb.19	3	677.73	1328.65	164.43	9.24
Feb.20	4	626.69	798.38	129.28	7.04
Feb.21	5	479.41	867.93	110.69	7.73
Feb.22	6	253.82	502.79	57.42	6.68
Feb.23	7	162.26	479.89	37.82	3.65
Feb.24	8	154.27	349.16	34.53	0.28
Feb.25	9	151.10	393.91	29.76	0.38
Feb.26	10	130.14	254.38	17.87	0.34
Feb.27	11	124.36	194.01	17.26	0.09
Feb.28	12	100.85	187.95	18.33	0.07
Feb.29	13	103.31	157.85	16.55	0.04
Mar.1	14	90.94	178.40	18.00	0.06
Mar.2	15	85.43	148.56	15.60	0.05
Mar.3	16	83.09	114.57	14.51	0.04
Mar.4	17	76.99	115.26	13.22	0.02
Mar.5	18	79.29	102.14	10.87	0.02
Mar.6	19	63.37	81.87	9.99	0.02
Mar.7	20	46.16	71.20	11.87	0.01
Mar.8	21	57.97	74.24	12.42	0.01
Mar.9	22	55.63	69.17	11.87	0.01
Mar.10	23	42.69	52.46	16.37	0.01
Mar.11	24	49.93	47.37	9.67	0.01
Mar.12	25	45.57	47.88	12.42	0.02
Mar.13	26	48.94	48.00	10.65	0.01
Mar.14	27	41.91	43.28	8.64	0.02
Mar.15	28	33.57	32.43	8.71	0.01
Mar.16	29	30.76	33.88	7.86	0.01

Figure B5-2 The off gasses captured in C4011-1 and the ORBO-30 tubes with both the front and back chambers plotted over the experimental run.

B.5-3 Standard Curves

Table B5-3 The GC analysis of the prepared standard samples run prior to each batch of sample results in order to back calculate synthetic gasoline concentrations in the soil.

Standards Run	Analysis Date	Standards Run	File ID	Area (Five Components)
1	Feb. 19/16	10	MM160219:001F0201	25132
1	Feb. 19/16	100	MM160219:001F0301	284698
1	Feb. 19/16	250	MM160219:001F0401	690998
1	Feb. 19/16	500	MM160219:001F0501	1051762
1	Feb. 19/16	1000	MM160219:001F0601	2054516
2	Feb. 27/16	10	MM160227:001F0201	20994
2	Feb. 27/16	100	MM160227:001F0301	249656
2	Feb. 27/16	250	MM160227:001F0401	416781
2	Feb. 27/16	500	MM160227:001F0501	1125323
2	Feb. 27/16	1000	MM160227:001F0601	2291608
3	Mar.1/16	10	MM160301:001F0201	25505
3	Mar.1/16	100	MM160301:001F0302	266086
3	Mar.1/16	250	MM160301:001F0403	809548
3	Mar.1/16	500	MM160301:001F0504	1639451
3	Mar.1/16	1000	MM160301:001F0605	3520748
4	Mar.4/16	10	MM160304:001F0201	25173
4	Mar.4/16	100	MM160304:001F0301	269060
4	Mar.4/16	250	MM160304:001F0401	832956
4	Mar.4/16	500	MM160304:001F0501	1638190
4	Mar.4/16	1000	MM160304:001F0601	3617476
5	Mar.8/16	10	MM160308:001F0301	23943
5	Mar.8/16	100	MM160308:001F0301	260422
5	Mar.8/16	250	MM160308:001F0301	802147
5	Mar.8/16	500	MM160308:001F0301	1542429
5	Mar.8/16	1000	MM160308:001F0301	3072507
6	Mar.12/16	10	MM160312:001F0301	23905
6	Mar.12/16	100	MM160312:001F0301	255502
6	Mar.12/16	250	MM160312:001F0301	800933
6	Mar.12/16	500	MM160312:001F0301	1539200
6	Mar.12/16	1000	MM160312:001F0301	3404104
7	Mar.17/16	10	MM160317:001F0301	23270
7	Mar.17/16	100	MM160317:001F0301	244149
7	Mar.17/16	250	MM160317:001F0301	805301
7	Mar.17/16	500	MM160317:001F0301	1556279
7	Mar.17/16	1000	MM160317:001F0301	3434792

Figure B5-3 The analysis of the prepared standard solutions prior to samples run in the GC-FID.

Figure B5-4 The analysis of the prepared standard solutions prior to samples run in the GC-FID.

Figure B5-5 The analysis of the prepared standard solutions prior to samples run in the GC-FID.

Figure B5-6 The analysis of the prepared standard solutions prior to samples run in the GC-FID.

Figure B5-7 The analysis of the prepared standard solutions prior to samples run in the GC-FID.

Figure B5-8 The analysis of the prepared standard solutions prior to samples run in the GC-FID.

B.5-4 Oxygen Levels

Table B5-4 The oxygen levels samples taken by the Apogee Oxygen Meter in the exhaust from the venting well.

Date	Day	Volume %
Feb.17	1	20.9
Feb.18	2	18.4
Feb.19	3	18.3
Feb.20	4	18.5
Feb.21	5	18.5
Feb.22	6	18.6
Feb.23	7	18.6
Feb.24	8	18.7
Feb.25	9	18.7
Feb.26	10	18.5
Feb.27	11	18.5
Feb.28	12	18.7
Feb.29	13	18.6
Mar.1	14	18.7
Mar.2	15	18.8
Mar.3	16	19.2
Mar.4	17	19.1
Mar.5	18	19.3
Mar.6	19	19.4
Mar.7	20	19.4
Mar.8	21	19.5
Mar.9	22	19.7
Mar.10	23	19.9
Mar.11	24	19.8
Mar.12	25	20
Mar.13	26	20.1
Mar.14	27	20.3
Mar.15	28	20.2
Mar.16	29	20.4

Figure B5-9 The oxygen level in the exhaust from the venting well plotted over the course of the experiment.

B.5-5 Soil Moisture

Figure B5-10 Soil moisture levels recorded in the inner and outer sections of the soil reactor.

B.5-6 Microbial Data

Table B5-6 Microbial plating using Tryptic Soy Agar (TSA) media to count Total Heterotopic Bacteria (THB) in 5 grams of soil at the beginning of the experiment.

Plate Date	Plate Time	Plate ID	Sample Location	Count Date	Count Time	Colonies	CFU x 10 ⁷ /g
Feb. 22/16	13:00	1	Elora	Feb. 24/16	10:00	24	12
Feb. 22/16	13:00	2	Elora	Feb. 24/16	10:00	23	11.5
Feb. 22/16	13:00	3	Elora	Feb. 24/16	10:00	19	9.5
Feb. 22/16	13:00	4	Delhi	Feb. 24/16	10:00	14	7
Feb. 22/16	13:00	5	Delhi	Feb. 24/16	10:00	13	6.5
Feb. 22/16	13:00	6	Delhi	Feb. 24/16	10:00	19	9.5
Feb. 22/16	13:00	7	Control	Feb. 24/16	10:00	0	-
Feb. 22/16	13:00	8	Control	Feb. 24/16	10:00	1	-
Feb. 22/16	13:00	9	Control	Feb. 24/16	10:00	1	-

Table B5-7 Microbial plating using Bushnell-Hass Media (BHM) to count Total Heterotopic Bacteria (THB) in 5 grams of soil at the beginning of the experiment.

Plate Date	Plate Time	Plate ID	Sample Location	Count Date	Count Time	Colonies	CFU x 10 ⁷ /g
Feb. 22/16	13:00	1	Elora	Mar. 7/16	13:30	27	13.5
Feb. 22/16	13:00	2	Elora	Mar. 7/16	13:30	31	15.5
Feb. 22/16	13:00	3	Elora	Mar. 7/16	13:30	23	11.5
Feb. 22/16	13:00	4	Delhi	Mar. 7/16	13:30	12	6
Feb. 22/16	13:00	5	Delhi	Mar. 7/16	13:30	18	9
Feb. 22/16	13:00	6	Delhi	Mar. 7/16	13:30	22	11
Feb. 22/16	13:00	7	Control	Mar. 7/16	13:30	2	-
Feb. 22/16	13:00	8	Control	Mar. 7/16	13:30	3	-
Feb. 22/16	13:00	9	Control	Mar. 7/16	13:30	1	-

Table B5-8 Microbial plating using Tryptic Soy Agar (TSA) media to count Total Heterotopic Bacteria (THB) in 5 grams of soil at the end of the experiment.

Plate Date	Plate Time	Plate ID	Sample Location	Count Date	Count Time	Colonies	CFU x 10⁷/g
Mar. 15/16	12:00	1	Elora	Mar. 17/16	10:00	27	13.5
Mar. 15/16	12:00	2	Elora	Mar. 17/16	10:00	26	13
Mar. 15/16	12:00	3	Elora	Mar. 17/16	10:00	25	12.5
Mar. 15/16	12:00	4	Delhi	Mar. 17/16	10:00	22	11
Mar. 15/16	12:00	5	Delhi	Mar. 17/16	10:00	17	8.5
Mar. 15/16	12:00	6	Delhi	Mar. 17/16	10:00	19	9.5
Mar. 15/16	12:00	7	Control	Mar. 17/16	10:00	1	-
Mar. 15/16	12:00	8	Control	Mar. 17/16	10:00	2	-
Mar. 15/16	12:00	9	Control	Mar. 17/16	10:00	0	-

BHM not completed for the end of the Aged experiment due to time constraint

B.6 Aged Soil

B.6-1 Degradation

Table B6-1 The concentration of synthetic gasoline in the soil reactor.

Sample Date	Day	Location 1 (mg/kg)	Location 2 (mg/kg)	Average
Mar.29	0	2585.77	2400.69	2493.23
Mar.30	1	2329.23	2322.52	2325.87
Mar.31	2	2164.08	2209.72	2186.90
Apr.1	3	1882.36	1955.10	1918.73
Apr.2	4	1403.58	1417.61	1410.59
Apr.3	5	1076.74	1160.20	1118.47
Apr.4	6	1038.35	995.69	1017.02
Apr.5	7	981.31	977.44	979.38
Apr.6	8	814.15	786.77	800.46
Apr.7	9	774.60	818.55	796.58
Apr.8	10	920.52	798.30	859.41
Apr.9	11	787.94	686.22	737.08
Apr.10	12	710.85	670.65	690.75
Apr.11	13	586.31	647.93	617.12
Apr.12	14	550.91	617.38	584.15
Apr.13	15	587.41	593.41	590.41
Apr.14	16	572.32	549.55	560.94
Apr.15	17	497.20	524.45	510.83
Apr.16	18	485.04	468.64	476.84
Apr.17	19	427.57	471.53	449.55
Apr.18	20	469.89	497.92	483.90
Apr.19	21	422.37	452.00	437.18
Apr.20	22	432.76	409.66	421.21
Apr.21	23	410.98	381.69	396.33
Apr.22	24	402.70	336.11	369.40
Apr.23	25	345.49	357.91	351.70
Apr.24	26	331.29	314.04	322.67
Apr.25	27	341.45	278.98	310.22
Apr.26	28	308.40	298.22	303.31
Apr.27	29	264.26	327.21	295.74
Apr.28	30	297.49	256.39	276.94
Apr.29	31	220.31	251.02	235.66
Apr.30	32	268.73	244.17	256.45
May 1	33	215.15	198.23	206.69
May 2	34	223.64	177.61	200.63

Figure B6-1 The concentration of synthetic gasoline in the soil reactor plotted with exponential degradation.

B.6-2 Off Gas Capture

Table B6-2 The off gasses captured in C4011-1 and the ORBO-30 tubes with both the front and back chambers.

Sample Date	Day	Air - Front Chamber (mg)	Air - Back Chamber (mg)
Mar.29	0		
Mar.30	1	259.65	20.24
Mar.31	2	227.41	16.16
Apr.1	3	160.28	15.42
Apr.2	4	120.01	10.99
Apr.3	5	107.77	11.40
Apr.4	6	92.27	7.25
Apr.5	7	89.15	8.96
Apr.6	8	84.87	8.18
Apr.7	9	66.81	5.31
Apr.8	10	58.73	5.36
Apr.9	11	54.40	5.14
Apr.10	12	45.88	5.96
Apr.11	13	43.27	5.41
Apr.12	14	41.03	5.17
Apr.13	15	32.26	5.19
Apr.14	16	41.33	6.30
Apr.15	17	38.34	4.63
Apr.16	18	33.42	4.92
Apr.17	19	43.51	3.89
Apr.18	20	41.83	4.92
Apr.19	21	36.56	4.37
Apr.20	22	32.68	5.34
Apr.21	23	31.53	4.08
Apr.22	24	36.40	6.04
Apr.23	25	40.53	4.12
Apr.24	26	36.18	4.95
Apr.25	27	30.65	3.93
Apr.26	28	31.07	5.50
Apr.27	29	31.40	4.65
Apr.28	30	26.96	3.46
Apr.29	31	25.89	3.03
Apr.30	32	32.38	3.78
May 1	33	17.37	2.69
May 2	34	23.45	2.41

Figure B6-2 The off gasses captured in C4011-1 and the ORBO-30 tubes with both the front and back chambers plotted over the experimental run.

B.6-3 Standard Curves

Figure B6-3 The analysis of the prepared standard solutions prior to samples run in the GC-FID.

Figure B6-4 The analysis of the prepared standard solutions prior to samples run in the GC-FID.

Figure B6-5 The analysis of the prepared standard solutions prior to samples run in the GC-FID.

Figure B6-6 The analysis of the prepared standard solutions prior to samples run in the GC-FID.

Figure B6-7 The analysis of the prepared standard solutions prior to samples run in the GC-FID.

Figure B6-8 The analysis of the prepared standard solutions prior to samples run in the GC-FID.

B.6-4 Oxygen Levels

Table B6-3 The oxygen levels samples taken by the Apogee Oxygen Meter in the exhaust from the venting well.

Date	Day	Volume %
Mar.29	0	20.6
Mar.30	1	20.4
Mar.31	2	20.2
Apr.1	3	20
Apr.2	4	19.9
Apr.3	5	19.3
Apr.4	6	19.2
Apr.5	7	19.3
Apr.6	8	19.2
Apr.7	9	19.3
Apr.8	10	19.5
Apr.9	11	19.3
Apr.10	12	19.2
Apr.11	13	19.3
Apr.12	14	19.3
Apr.13	15	19.4
Apr.14	16	19.4
Apr.15	17	19.5
Apr.16	18	19.5
Apr.17	19	19.4
Apr.18	20	19.5
Apr.19	21	19.6
Apr.20	22	19.4
Apr.21	23	19.5
Apr.22	24	19.6
Apr.23	25	19.5
Apr.24	26	19.5
Apr.25	27	19.7
Apr.26	28	19.5
Apr.27	29	19.5
Apr.28	30	19.6
Apr.29	31	19.6
Apr.30	32	19.5
May 1	33	19.7
May 2	34	19.6

Figure B6-9 The oxygen level in the exhaust from the venting well plotted over the course of the experiment.

B.6-5 Soil Moisture

Figure B6-10 Soil moisture levels recorded in the inner and outer sections of the soil reactor.

B.6-6 Microbial Data

Table B6-4 Microbial plating using Tryptic Soy Agar (TSA) media to count Total Heterotopic Bacteria (THB) in 5 grams of soil at the beginning of the experiment.

Plate Date	Plate Time	Plate ID	Sample Location	Count Date	Count Time	Colonies	CFU x 10 ⁷ /g
Apr. 5/16	11:00	1	Top	Apr. 8/16	14:00	14	7
Apr. 5/16	11:00	2	Top	Apr. 8/16	14:00	28	14
Apr. 5/16	11:00	3	Top	Apr. 8/16	14:00	21	10.5
Apr. 5/16	11:00	4	Bottom	Apr. 8/16	14:00	22	11
Apr. 5/16	11:00	5	Bottom	Apr. 8/16	14:00	17	8.5
Apr. 5/16	11:00	6	Bottom	Apr. 8/16	14:00	23	11.5
Apr. 5/16	11:00	7	Middle	Apr. 8/16	14:00	21	10.5
Apr. 5/16	11:00	8	Middle	Apr. 8/16	14:00	16	8
Apr. 5/16	11:00	9	Middle	Apr. 8/16	14:00	19	9
Apr. 5/16	11:00	10	Control	Apr. 8/16	14:00	1	-
Apr. 5/16	11:00	11	Control	Apr. 8/16	14:00	2	-
Apr. 5/16	11:00	12	Control	Apr. 8/16	14:00	0	-

Table B6-5 Microbial plating using Bushnell-Hass Media (BHM) to count Total Heterotopic Bacteria (THB) in 5 grams of soil at the beginning of the experiment.

Plate Date	Plate Time	Plate ID	Sample Location	Count Date	Count Time	Colonies	CFU x 10 ⁷ /g
Apr. 5/16	11:00	1	Top	Apr. 19/16	10:00	17	8.5
Apr. 5/16	11:00	2	Top	Apr. 19/16	10:00	21	10.5
Apr. 5/16	11:00	3	Top	Apr. 19/16	10:00	15	7.5
Apr. 5/16	11:00	4	Bottom	Apr. 19/16	10:00	12	6
Apr. 5/16	11:00	5	Bottom	Apr. 19/16	10:00	18	9
Apr. 5/16	11:00	6	Bottom	Apr. 19/16	10:00	22	11
Apr. 5/16	11:00	7	Middle	Apr. 19/16	10:00	17	8.5
Apr. 5/16	11:00	8	Middle	Apr. 19/16	10:00	26	13
Apr. 5/16	11:00	9	Middle	Apr. 19/16	10:00	18	9
Apr. 5/16	11:00	10	Control	Apr. 19/16	10:00	2	-
Apr. 5/16	11:00	11	Control	Apr. 19/16	10:00	1	-
Apr. 5/16	11:00	12	Control	Apr. 19/16	10:00	1	-

TSA/BHM not completed for the end of the Aged experiment due to laboratory shut down.

APPENDIX C: Equipment Used For Experiment

C.1 Equipment List

Table C1-1 Supplies for the CSR system.

Equipment/Part	Supplier	Quantity
T-304 SS Drum	General Container	1
Lid with neoprene ring for drum	General Container	1
¾" SS Plug for reactor	Stan's Plumbing	10
¾" SS Plug Brush FNPT	Stan's Plumbing	5
1.25 to ½ SS Reducer	Stan's Plumbing	1
2" Rubber pieces for Nipples	Stan's Plumbing	5
½" SS Piping various lengths/pieces	Swedge-Lok	Several
Tubing from flow meter to pump	Existing	1ft
Tubing from pump to vent out	Existing	5 ft
C-clamp to secure lid to base of reactor	Canadian Tire	3
Cotton Wrap 4ft x2ft	Len's Mill Store	6ft x2ft
String (to secure Cotton Wrap)	Len's Mill Store	50ft
10L Plastic Moisture Column	Existing	1
Aquarium Pump	Existing	1
Aquarium Bubbling Stone	Pet's Smart	1
2" PVC Piping (for venting chamber)	Canadian Tire	3ft
Plexiglas® Enclosure	Custom Made (Mosco, 2015)	1

Table C1-2 Chemicals used in the experiment.

Equipment/Part	Supplier	Quantity
Sodium Pyrophosphate ($\text{Na}_4\text{O}_7\text{P}_2$) -microbial extraction from soil	Fisher Scientific	-
Bushnell-Hass medium -isolation of hydro-degrading bacteria	Difco Laboratories	-
Tryptic Soy Agar -cultivation of heterotrophic bacteria	Difco Laboratories	-
Granulated Agar -solidifying agent for hydrocarbon degrading bacteria	Difco Laboratories	-
Ammonium chloride (NH_4Cl) , powder (Nutrient applied to soil as source of nitrogen)	Fisher Scientific	-
Calcium carbonate (CaCO_3) (Powder applied for enhancing level of pH in acidic soil)	Fisher Scientific	-
Calcium hydroxide $\text{Ca}(\text{OH})_2$ (Liquid applied for enhancing level of pH in acidic soil)	Fisher Scientific	-
Naphthalene (Component of synthetic gasoline)	Fisher Scientific	-
1,3,5-Trimethylbenzene (Component of synthetic gasoline)	Fisher Scientific	-
m-Xylene (Component of synthetic gasoline)	Fisher Scientific	-
Toluene (Component of synthetic gasoline)	Fisher Scientific	-
Isooctane (Component of synthetic gasoline) (2,2,4 Tri-methylpentene)	Fisher Scientific	-
Methylene chloride (CH_2Cl_2) (TPH extraction solvent)	Fisher Scientific	2-4L Bottles
Sodium sulfate (Na_2SO_4) (anhydrous) (Desiccant in extraction process)	Fisher Scientific	-
Silica Sand	Opta Minerals	4-100lb Bags
Compressed Hydrogen (Cylinder)	BOC Gases	1 Cylinder
Compressed Helium (Cylinder)	BOC Gases	1 Cylinder
Compressed Air (Cylinder)	BOC Gases	1 Cylinder
Bleach 1L (Disinfectant)	Canadian Tire	2-1L Bottle
Dish Soap 750mL	Canadian Tire	1-Bottle

Table C1-3 Sampling equipment used in the experiment.

Equipment/Part	Supplier	Part #	Quantity
Micro-Flowmeter	Omega	FMA 5606-ST	1
Low Flow Pump	Gilian-Sensidyne	(LFS-113DC)	6
Oxygen Meter	Apogee	(MO-200 Series)	1
HOBO Micro Station Data Logger	Onset Computer Corp.	H21-002	1
HOBO Soil Moisture Sensor	Onset Computer Corp.	S-SMC-M005	2
HOBO U-Shuttle	Onset Computer Corp.	U-DT-1	1
pH meter (pH measurements in soil)	Accument	(AP 125)	1
Gas Chromatograph with FID	Hewlett-Packard	5890 Series II	1
Raven PIN Multi-Mode Printer	Roland Digital Group	PR-2416	1
Smoke Pen with Cartridges	Bjornax AB, Sweden	Regin S220 Smoke Pen	1
Mettler Type PJ 12 Weigh Scale	Mettler Instruments	SNR 123068	1
Mettler Type AJ100L Weight Scale	Mettler Instruments	SNR L14590	1
Vortex Mixer	Fisherbrand	SN:1007210022	1
Isotemp 205	Fisher Scientific	SN: 1600060907190	1
Steam Autoclaving System	Sanyo	MLS-3781	1
Big Bill SE Rotator/Shaker	Thermolyne	SN:491910468547	1
Multi-Wrist Shaker (Mod. 3589)	Lab-Line	SN: 129-0449	1
USA Standard Test Sieve No.4	Fisherbrand	SN:125125834	1
Powered Soil Sifter (soil sifting)	BM&M Canada		1
1000mL Kimax Jar	Kimble	No.14395	5
250mL Erlenmeyer Flask	Kimble	No. 26500	6
1000mL Beaker	Kimble		4
25mL Graduated Cylinder	Fisherbrand		1
50mL Graduated Cylinder	Fisherbrand		1
Supelco (ORBO® 32 Small) Tubes (Entrapment of volatile hydrocarbon from off gases)	Sigma-Aldrich		4 boxes
Target Vials C4011-1 (Entrapment of volatile hydrocarbon from off gases)	National Scientific	C4011-1	2 boxes
CentriStar 15mL Tubes	Corning		1 pack
11MM PTFE/Red Rubber Seal	Fisherbrand	Cat. No. 03-391-3	5 packs
Clear Large Lid Crip Top Vial 12x32mm	Fisherbrand	Cat. No. 033915	5 packs
Mechanical mixer for mixing gasoline in paint cans	Custom Build (Mosco, 2015)		1

Tie Down Strap (budgie cord)	Canadian Tire		2
1 Gallon Paint Cans	Home Hardware		20
Rubber Mallet	Home Hardware		1
Paint Can Opener	Home Hardware		1
Spatula	Canadian Tire		1
Masher (Pack down soil)	Canadian Tire		1
Micro-Fibre Wash Cloths	Canadian Tire		1 pack
Reusable Nitrile Chemical Resistant Gloves	Canadian Tire		2 pair
Teflon Tape for threading of vials	Home Hardware	-	10 rolls
Aluminium seal crimper and decapper (used for granular activated carbon extraction)	Restek, Fisher Scientific		1
Pipettes (transfer of soil solution for serial dilution and onto the media for microbial cultivation)	Fisher Brand	20-200 ul 100-1000 ul	Multi
Poly-synthetic bags for storage	TSC Stores		10
Soil Sample Push Rod	Custom (Physics Lab)		1
Refrigerator for cooling soil before gasoline addition	In 1113		1
Respirator (Breathing protection in handling hydrocarbon)	Stanley	MC P100	Multi