Assessment and Optimization of Ex-Situ Bioremediation of Petroleum Contaminated Soil under Cold Temperature Conditions

By

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ABSTRACT

Current prices and demand for petroleum hydrocabons have generated an increase of oil spills around the country and the world. Health and environmental impacts associated to these organic pollutants represent a huge concern for the general public, leading the public and private sector to develop new technologies and methods to minimize or eliminate those risks.

Ex-Situ bioremediation through biopiles, as a main remediation technique to treat a wide range of hydrocarbons, has been a topic of considerable research interest over the last years. It provides an economical and environmental solution to restore the environment to background levels. Nevertheless, successful bioremediation under cold climate conditions is of considerable concern in countries like Canada, as low temperatures can delay the rate of bioremediation of oil hydrocarbons, thus limiting the operation of soil treatment facilities to certain times of the year. Recent research has found out that bioremediation could be conducted even at low or cold temperatures with larger periods of times. And even more, the addition of petroleum degrading microorganisms (bioaugmentation) and nutrients or biosurfactants (biostimulation) could enhance the process in some cases.

In the present study, a comprehensive assessment of bioaugmentation and biostimulation strategies for ex-situ bioremediation of petroleum contaminated soil under cold climate conditions is proposed. Field scale biopiles were constructed and subjected to different concentrations of commercial microbial consortia and mature compost, as bioaugmentation and biostimulation strategies, in a soil treatment facility at Moose Creek, Ontario over a period of 94 days (November 2012 to February 2013). Assessment and comparison of the biodegradation rates of total petroleum hydrocarbons (TPH) and their fractions were investigated. Furthermore, a response surface methodology (RSM) based on a factorial design to investigate and optimize the effects of the microbial consortia application rate and amount of compost on the TPH removal was also assessed.

Results showed that biopiles inoculated with microbial consortia and amended with 10:1 soil to compost ratio under aerobic conditions performed the best, degrading 82% of total petroleum hydrocarbons (TPHs) with a first-order kinetic degradation rate of 0.016 d^{-1}, under cold temperature conditions. The average removal efficiencies for TPHs after 94 days for control biopiles, with no amendments or with microbial consortia or compost only treatments were 48%, 55%, and 52%, respectively. Statistical analyses indicated a significant difference ($p < 0.05$) within and between the final measurements for TPHs and a significant difference between the treatment with combined effect, and the control biopiles. On the other hand, the modeling and optimization statistical analysis of the results showed that the microbial consortia application rate, compost amendment and their interactions have a significant effect on TPHs removal with a coefficient of determination (R^2) of 0.88, indicating a high correlation between the observed and the predicted values for the model obtained. The optimum concentrations predicted via RSM were 4.1 ml m^{-3} for microbial consortia application rate, and 7% for compost amendment to obtain a maximum TPH removal of 90.7%. This research contributes to provide valuable knowledge to practitioners about cost-effective and existing strategies for ex-situ bioremediation under cold weather conditions.

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CHAPTER 1

INTRODUCTION

Over the last years, the petroleum industry has played an important role in the world economy and society, but it has also caused several negative environmental impacts around the world. Many of the compounds and products generated by this industry are recognized by US EPA and Environment Canada as priority pollutants due to their toxicity and potential carcinogenic effects. Inappropriate use, improper handling and disposal, and accidental spills have resulted in environmental contamination in several cases, causing direct and indirect health risk to humans and living organisms. According to Zhu et al. (2001), human activities and human failures constitute more than 90% of oil pollution causes, affecting directly the natural environment. Just recently, several train derailments due to human failures across Canada have impacted our groundwater and soil resources by thousands of litres of crude oil, representing a potential threat to human health and the environment (Young, 2014).

During the last few years, the increasing attention to health risks associated with the exposition of chemicals and toxic products, such as petroleum hydrocarbons, has caused that the soil contamination is considered a major concern. For this reason, remediation of contaminated soil has become an important environmental activity, as a viable solution to restore the environment to background levels. Until recently, the common approach to soil remediation was excavation and disposal at a regulated landfill, but changes in remediation techniques, increasing disposal costs and regulatory constraints have decreased the employment of the dig and dump method as a soil cleanup alternative (Steinberg, 2009; Sasek et al, 2003).

Bioremediation has been a topic of considerable research interest over the last few decades. It exploits the ability of microorganisms to degrade and/or detoxify hydrocarbon-contaminated soils, mitigating the risk to human health and the environment (Sharma and Rehman, 2009; Adenkule, 2011). Bioremediation has been established as an efficient, economic, versatile, and environmental technique to remove environmental pollutants in water and soil.

This method aims at the complete degradation of hydrocarbons into carbon dioxide and water, without affecting or damaging the natural environment (Adekunle, 2011).

In most cases, the treatment of contaminated soils involves two main strategies: bioaugmentation whereby petroleum degrading microorganisms are added to the soil matrix, and biostimulation, which introduces essential nutrients or biosurfactants to stimulate microbial petroleum degradation (Whang et al., 2008). Both biostimulation and bioaugmentation can be accomplished separately or in combination (Kriipsalu et al., 2007; Bento et al., 2005; Tsai et al., 2009).

Microbial metabolism and hence microbial degrading activities and concomitantly the performance of bioremediation technologies are greatly affected by temperature; they can be sped up or slowed down depending on temperature (Mohn and Stewart, 2000). Cold conditions are of considerable concern as they can delay both the onset and the rate of biodegradation of oil hydrocarbons. Assessment of bioremediation strategies for cold climates, such as in Canada, and methods of enhancing cold weather degradation is of great importance for decontamination of polluted soils and it is being recognized as an attractive approach for cold regions (Aislabie et al., 2006; Børresen, 2003; Braddock et al, 1997).

1.1 STATEMENT OF THE PROBLEM

Successful bioremediation of petroleum hydrocarbons in soil under cold climate conditions has still remained a challenge after decades of research. Strategies such as biostimulation by mature compost amendment and bioaugmentation by the application of commercial microbial consortia are commercially available, but there is not enough information that compares the effectiveness of these mixtures under different temperature regimes at field scale conditions. Furthermore, due to the high cost of the commercial bacteria consortia, an optimal treatment under certain soil matrix and environmental conditions is crucial for the feasibility of ex-situ bioremediation of petroleum contaminated soil.

As such, ex-situ techniques in Ontario cold climate are often limited to the times of the year where temperatures are above freezing (April to November) and therefore soil treatment throughput is time limited. Therefore, extending the time window for soil treatment during cold weather conditions using alternative strategies could represent a significant increase in available treatment time. It also improves the productivity and reduces the operational cost of soil treatment facilities. Considering the above facts, it is very important to assess the applicability of any bioremediation technology, from a practical point of view, in field-scale and site specific conditions.

1.2 RESEARCH OBJECTIVES

The main objectives of the present study were the assessment and comparison of different microbial bioaugmentation and biostimulation strategies, and to determine the best bioremediation approach at field scale for petroleum contaminated soils under cold climate conditions.

The goals of the proposed project are to:

- Assess the feasibility of employing bioaugmentation (addition of microbial consortia) and biostimulation (addition of compost) strategies for cold climate conditions.
- Compare the performance of different strategies in terms of hydrocarbon degradation rates.
- Identify the key parameters for further assessment and analysis of ex-situ biopiles
- Statistically analyze the performance of different strategies and optimize it for cold climate conditions, using a factorial design and response surface methodology.

In different studies, the degree of degradation found in lab scale and in field studies shows considerable variation. It is expected that this research will aim to provide optimum conditions to enhance current protocols for cold weather conditions, especially for the Canadian market. These optimized procedures will assist in reducing operational costs and increasing productivity, as a result of increase in available treatment time.

1.3 RESEARCH METHODOLOGY

The research study was carried out by construction, operation and maintenance of 18 ex-situ field scale biopiles (16 m^3 each) over a period of 3-4 months during cold climate conditions. A biopile is one of the many bioremediation techniques to treat hydrocarbon-contaminated soil where the soil is piled over an air distribution system and aerated. Ex-situ biopiles of contaminated soil were set up and subjected to different amounts of bacterial augmentation (bacterial load) and compost as biostimulation agent. Considering these 2 factors, i.e. microbial consortia application rate and amount of compost amendment, a factorial design was followed to run the different biopiles. In order to be able to statistically compare the results, each biopile was triplicated. In addition, 4 control biopiles were considered: 2 with no addition of microorganisms and compost, one with addition of only compost, and one with addition of only microorganisms and no compost, for a total of 18 biopiles.

Petroleum contaminated soil with a sandy texture was used as the source. The bacteria used in this project was a commercial concentrated blend of bacteria in liquid form native to Canada. It is a commercial product classified as BioSafety Level 1, non-pathogenic and non-opportunistic blend of bacteria.

The biopiles were monitored with portable probes and gas analyzer instrument (Eagle, RKI) to determine pile temperatures, moisture content, CO_2 levels, O_2 levels and volatilization of hydrocarbons in air throughout the test periods. In addition, composite soil samples were collected and analyzed for F1-F4, pH, nutrients (N,P), heterotrophic aerobic bacteria count (log CFU g^{-1}), and total hydrocarbon degrader bacteria, using as a method the Most Probable Number (MPN), along the treatment period. The performance and efficiency of the process was determined by the extent of the reduction of contaminant concentrations. The results were then analyzed using statistical procedures.

1.4 THESIS LAYOUT

The thesis is organized in the form of technical papers, and divided into five main chapters, followed by appendices. The first chapter contains the introduction, the statement of the problem, the objectives of this research, the scope of the research and the thesis organization or layout. The second chapter discusses the theoretical background of bioremediation technologies and strategies for petroleum contaminated soil and relevant literature. The third chapter is the first technical paper entitled: “Field Scale Ex-Situ Bioremediation of Petroleum Contaminated Soil under Cold Climate Conditions”. The fourth chapter is the second technical paper entitled: “Optimization of Field Scale Biopiles for Bioremediation of Petroleum Hydrocarbon Contaminated Soil at Low Temperature Conditions by Response Surface Methodology (RSM)”. Finally, the last chapter contains a summary of important findings and conclusions that have been developed throughout this thesis. It also discusses future research approach and considerations for this subject. It is important to mention that because the thesis format is based on technical papers, some information could be repeated in different chapters.

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CHAPTER 2

LITERATURE REVIEW

2.1 INTRODUCTION

Many of the products used by humans are composed of petroleum based materials, such as gasoline, kerosene, and fuel oil that contain organic and inorganic pollutants, which have demonstrated resistance to natural degradation, and represent a toxicological threat to human health and to the environment (Juwarkar et al., 2010). Among the organic compounds, the most common ones are alkanes, cycloalkanes, benzene, toluene, xylenes, phenols and polycyclic aromatic hydrocarbons (Cerqueira et al., 2011), which are considered as primary environmental pollutants by the US environmental protection agency and the agency for toxic substances and disease registry (USA EPA, 2013; ATSDR, 2011a).

Soil contamination is a concern whose importance has been perceived only in the past couple of decades. Economic effects such as fertility loss, productivity reduction, human health effects such as skin and neurological diseases, cancer, and environmental effects such as the ecological chemical imbalance, erosion, and food chain alteration, have increased the attention of the modern society over the last decade (Juwarkar et al., 2010; Steinberg, 2009).

In the past, dig and dump practices were common to dispose the contaminated soil, hoping that contaminants will naturally degrade (Otten et al., 1997). However, current research, new legislation and recent waste management strategies have placed significant emphases on waste management, promoting recycling and remediation rather than disposal. Thus, reducing the amount of waste that is sent to the landfill, and driving the need for the development and application of remediation techniques for soil decontamination (Semple et al., 2001).

As a result of the ability of the bacteria to transform and degrade hazardous organic materials to innocuous compounds such as carbon dioxide, methane, water, and biomass, bioremediation technologies have replaced physical and chemical remediation processes as the main technique for the clean-up of contaminated soils (Juwarkar et al., 2010).

Bioremediation of petroleum hydrocarbons in contaminated soils was initially developed for the oil industry more than 30 years ago, in the search to clean up oil sludge from their operations, immobilizing the pollutants or transforming them into non-hazardous products for the environment (Hazen et al., 2003). Since then, the application of this technology has increased and has been applied in different approaches and heterogeneous environments (Boopathy, 2000; Aislabie et al., 2006).

However, several constraints such as the substrate and environmental conditions limit an effective bioremediation approach. Cold temperatures are of considerable concern as they can delay the rate of bioremediation of oil hydrocarbons. For this reason, the assessment of bioremediation strategies for cold climates, such as in Canada, and methods of enhancing cold weather degradation are of great importance for the decontamination of polluted soils. Recent research has found out that bioremediation could be conducted even at low or cold temperatures with larger periods of times. Also, the addition of petroleum degrading microorganisms (bioaugmentation) and nutrients or biosurfactants (biostimulation) could enhance the process in some cases (Braddock et al., 1997; Walworth et al., 2007; Chang et al., 2010). The success of bioremediation process depends on the operating conditions and characteristics of the soil and the source of contamination.

Other factors like bioavailability of the pollutants, nutrient source, pH, water availability, and electron donors play an important role in the performance of the bioremediation process (Suthersan and Payne, 2005; Steinberg, 2009). The assessment and proper selection of strategies could be the difference between the success or failure of the biological treatment for the remediation of the contaminated soils.

Most of the available data, until now, has come from microcosm experiments; full scale experiments could help in assessing the feasibility of bioremediation, and its effective contribution to clean-up contaminated soils (Paudyn et al., 2008; Steinberg, 2009). In the field, it is very difficult, if not impossible, to isolate the effect of temperature variability from the multitude of other rate influencing environmental factors on petroleum hydrocarbon biodegradation rates and extents. In addition, other factors, such as presence of competing or indigenous microorganisms or spatial heterogeneity may inhibit the effectiveness of the field-scale bioremediation operation. For this reason, it is very important to assess the applicability of any bioremediation technology, from a practical point of view, in field-scale and site specific conditions.

The following literature review addresses the fundamental concepts associated with soil contamination, the bioremediation technologies, and ex-situ bioremediation technologies through biopiles for the degradation of petroleum contaminated soil, using biostimulation and bioaugmentation strategies.

2.2 SOIL CONTAMINATION BY PETROLEUM HYDROCARBONS

Most of the time, soil contamination originates from anthropogenic activities or industrial sources through the release of materials on the earth surface or by introduction of contaminants into the subsurface (Juwarkar et al., 2010). But before understanding the components and concepts associated with soil contamination by hydrocarbons, it is inherent to understand the soil as a matrix system with its characteristics and properties.

The soil system, under unsaturated conditions, consists of three phases: solid, liquid and gas phases. Each of them interacts with contaminants and makes its connection with the soil matrix possible. The solid phase is divided into two distinct fractions: one mineral fraction and one organic fraction. The mineral fraction is broad in composition and properties and their particles vary depending on the grain size (Otten et al., 1997). Usually, the binding of contaminants is correlated with the surface of these particles. However, for soil particles with smaller grain size, such as clay or hydroxides or oxides, the binding mechanism is correlated with the electric charge, which influences the adsorption of substances such as organic pollutants.

On the other hand, the organic fraction consists of organic matter compounds, which have a negative charge and attract heavy metals such as lead due to its positive charge. These properties and characteristics of the different fractions influence the conditions and interaction mechanism with the liquid and gas phases (Otten et al., 1997).

The liquid and gas phases are carried out through the pores of the soil in the unsaturated (subsurface) or vadose zone and the saturated zone (Figure 2.1). These pores are partly filled with water or air, upon the phase. They serve as a means to transport nutrients or microorganisms or to content dissolved contaminants by advection and/or diffusion under the influence of concentration gradient. Their composition usually depends on physical-chemical processes and biological processes (Suthersan and Payne, 2005; Steinberg, 2009).

Thus, pollutants like petroleum hydrocarbons bind themselves and interact to the solid phase, specifically mineral and organic fractions, through a combination of physical and chemical mechanisms, such as sorption, complexation and precipitation (Megharaj et al., 2011). Polycyclic aromatic hydrocarbons (PAHs), specifically, mostly interact with organic matter and their different fractions through mechanisms such as physical and chemical adsorption, solubilization, partitioning, hydrolysis, and photosensitization, affecting their mobility and bioavailability through the soil (Sašek et al., 2003; Steinberg, 2009).

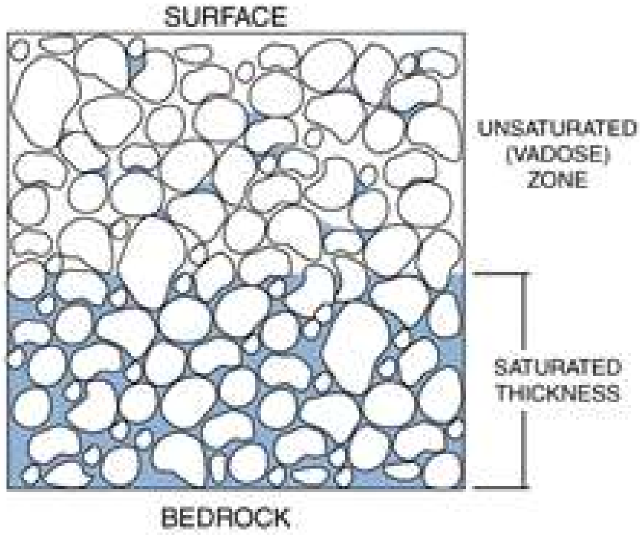

Figure 2.1. Unsaturated (Vadose) and saturated zones in soil (Buddemeier, 2000)

Petroleum hydrocarbons (PHCs) can be defined as a complex mixture of constituents, in varying proportions, composed predominantly of carbon and hydrogen. Chemicals such as hexane, benzene, toluene, naphthalene, are common examples of compounds that occur in petroleum hydrocarbons and are constituents of several petroleum products (ATSDR, 2011b).

For the purpose of standardization and for ease assessment of human and environmental health risks associated with PHC contamination in soils, petroleum hydrocarbon are subdivided into specified ranges of equivalent carbon number (ECN) or fractions. These fractions can be subdivided in sub-fractions and can be described according to their physical and chemical properties and toxicological characteristics (CCME, 2008).

Fraction 1 or F1 encompasses the range of ECN from C6 to C10, representing the volatile fraction of most hydrocarbon mixtures. Fraction 2 or F2 can de defined as the range of ECN from C>10 to C16, representing the semi-volatile fraction. Fraction 3 or F3 encompasses the range of ECN from C>16 to C34, which is considered as non-volatile fraction. Fraction 4 or F4 encompasses compounds with ECN of C>34 to C50+, and is considered as the fraction with the lowest volatility and solubility (CCME, 2008; ATSDR, 2011b).

Polycyclic aromatic hydrocarbons (PAHs) are other types of compounds, commonly found within petroleum products. PAHs are aromatic hydrocarbons with two or more fused benzene rings, which do not degrade easily under natural conditions. They are persistent towards biodegradation, and have gathered significant concern due to its potential to bio-accumulate and carcinogenic activity (Haritash and Kaushik, 2009). PAHs are assessed and managed separately due to their different physical and chemical properties in comparison to the hydrocarbon fractions (ATSDR, 2011c).

Remedial standards for petroleum hydrocarbons impacted surface and subsurface have been developed by different jurisdictions with the purpose to establish guidelines for environmental consultants. These guidelines aim to regulate the presence of the contaminants according to the exposure scenarios, site characteristics, land use, sensitivity factors, physical conditions of the soil, hydrogeological conditions, risk-based nature and toxicity of the contaminants (CCME, 2008). Tables 1 and 2 show current examples of federal (Canada) and provincial (Ontario, Canada) standards and guidelines to characterize PHCs fractions under different risk management scenarios, land use zoning, physical conditions of the soil and hydrogeological conditions.

Table 2.1. Canada-Wide Standard Tier 1 guidelines for PHC fractions for surface soil (CCME, 2008)

Land Use	Soil Texture	Fraction 1 (mg kg^{-1})	Fraction 2 (mg kg^{-1})	Fraction 3 (mg kg^{-1})	Fraction 4 (mg kg^{-1})
Agricultural	Coarse-grained	30^b	150	300	2800
	Fine-grained	210 (170^a)	150	1300	5600
Residential/ Parkland	Coarse-grained	30^b	150	300	2800
	Fine-grained	210 (170^a)	150	1300	5600
Commercial	Coarse-grained	320 (240^a)	260	1700	3300
	Fine-grained	320 (170^a)	260 (230^a)	2500	6600
Industrial	Coarse-grained	320 (240^a)	260	1700	3300
	Fine-grained	320 (170^a)	260 (230^a)	2500	6600

a=Assumes protection of potable groundwater
b=Assumes contamination near residence

Table 2.2. Provincial Soil Standards for Table 2 (Full Depth Generic Site Condition Standards in a Potable Ground Water Condition) for PHC fractions (MOE, 2011)

Land Use	Soil Texture	Fraction 1 (mg kg^{-1})	Fraction 2 (mg kg^{-1})	Fraction 3 (mg kg^{-1})	Fraction 4 (mg kg^{-1})
Agricultural	Coarse-grained	55	98	300	2800
	Fine-grained	65	150	1300	5600
Residential/Parkland	Coarse-grained	55	98	300	2800
	Fine-grained	65	150	1300	5600
Industrial/Commercial	Coarse-grained	55	230	1700	3300
	Fine-grained	65	250	2500	6600

Petroleum hydrocarbons compounds degradation depends on the environmental conditions, quantity and type of the microorganisms, and nature and chemical structure of the chemical compound being degraded. When they are biodegraded, they are transformed into less complex metabolites such as H_2O, CO_2 (aerobic processes) or CH_4 (anaerobic processes). Their rate of biodegradation depends on several factors such as pH, temperature, oxygen concentration, microbial population, degree of acclimation, accessibility of nutrients, chemical structure of the compound, cellular transport properties, and chemical partitioning in the growth medium (Kauppi et al., 2011; USA EPA, 2012b).

2.3- BIOREMEDIATION TECHNOLOGIES

There are three main steps that facilitate the management of contaminated sites (Figure 2.2). According to Juwarkar et al. (2010), this simple scheme has entailed the development of new and improved technologies, instead of conventional techniques, such as landfilling, which at the end just transfers the liability of the generator.

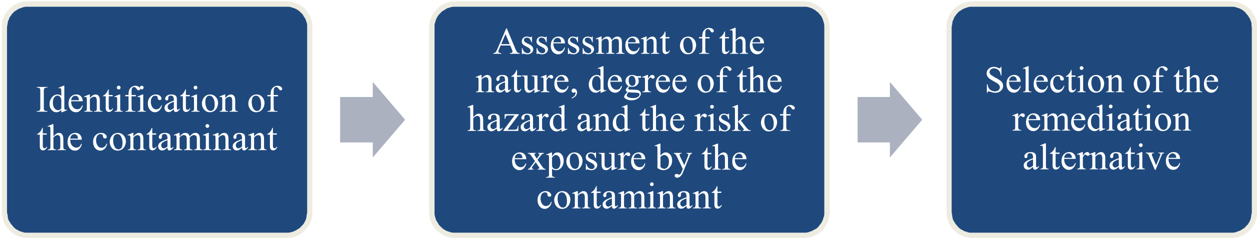

Figure 2.2. Scheme for management of contaminated sites

Several physiochemical techniques have been evaluated for the treatment of petroleum contaminated soil, which include vapor extraction, stabilization, solidification, soil flushing, soil washing, thermal desorption, incineration, etc. (Balba et al., 1998; Steinberg, 2009; Suthersan and Payne, 2005). However, most of these techniques require expensive continuous monitoring, and costly operation. For this reason, bioremediation, as a cleanup method, represents an efficient and inexpensive choice with minimal environmental damages (Al-Mutairi et al., 2008).

Bioremediation can be defined as the use of microorganisms to degrade or mineralize organic pollutants in water and soil into innocuous compounds such as carbon dioxide and water (Cerqueira et al., 2011; Khan et al., 2004). Bioremediation techniques have been used for the decontamination of surface and subsurface soils, freshwater, groundwater and marine ecosystems (Juwarkar et al., 2010). For its feasibility, it can be adapted to the site-specific conditions to minimize the effects of environmental and kinetic constraints. It has been demonstrated that it entails beneficial effects upon the environment, soil structure and fertility. But, bioremediation also has several limitations on its effectiveness, which can be overcome by understanding the microbial community, environmental conditions, and structure and physicochemical characteristics of the organic compound to be degraded (Figure 2.3) (Suthersan and Payne, 2005; Haritash and Kaushik, 2009).

Bioremediation technologies can be classified into two main categories: In-Situ and Ex-Situ techniques. In-Situ techniques are defined as the treatment processes of the contaminated material in place. Meanwhile, ex-situ techniques are defined as the involvement of the physical removal of the contaminated material for further treatment process (Hazen et al., 2003). Ex-Situ techniques will be explained in further details in the following paragraphs.

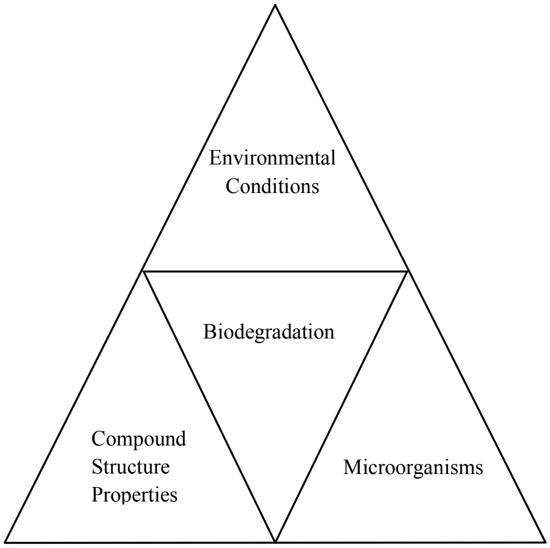

Figure 2.3 Biodegradation triangle (Suthersand and Payne, 2005)

2.3.1- EX-SITU TECHNIQUES

Ex-situ bioremediation includes all those biological treatment techniques in which the contaminated soil is physically removed from the contaminated site for further treatment (Talley and Sleeper, 2006). Some typical examples of these techniques are landfill disposal (dig and dump method), composting, landfarming, biopiles or biocells, electrobioremediation, etc. According to Talley and Sleeper (2006), although some contaminated sites can be controlled more easily and maintained with ex-situ settings, others are more effective with in-situ techniques. For this reason, a decision on the selected alternative has to be made based on a variety of technical and economic factors involved in each project.

Most organic pollutants strongly absorbed in the soil matrix, contamination situated in greater depths, difficult access by in situ techniques, time limitation, and direct exposure of the pollutants to human, can trigger the use of ex-situ techniques for remediation of contaminants (Otten et al, 1997; Trotsky and Pal, 1998; US EPA, 2012b).

As the same as in-situ techniques, there are several limiting factors including bioavailability, accessibility of pollutants, nutrients sources, and cost efficiency, which are important for the success of the remediation process. To overcome these limitations new and/or improved methods have been proposed to control the different physicochemical conditions of the soil (Juwarkar, 2010; Megharaj et al., 2011).

2.3.1.1 Landfill Disposal

A common practice for ex-situ bioremediation is the dig and dump method. It is a passive remediation technique that consists of excavation, trucking and disposal of the impacted soil in an approved landfill, in which the soil is used as a cover for natural attenuation (Kaupi et al., 2011). The main advantage is that this method will be effective at all sites in meeting remediation objectives within a reasonable time frame. However, it is not the most economical method for some cases and cost could be a prohibitive factor.

2.3.1.2- Landfarming

Landfarming is another remediation method, particular useful for remote sites due to minimal equipment requirement, which consists of the use of passive aeration by tilling the material periodically to mix it (Figure 2.4) (Paudyn et al., 2008). In this case, the contaminants are degraded, transformed and immobilized by biotic and abiotic reactions (Megharaj et al., 2011). Additional amendments or bulking agents sometimes are added to speed up the remediation process by increasing aeration, by addition of co-substrates and/or nutrients to stimulate microbial metabolism, or lime to adjust pH, or bacterial inoculations for seeding (McCarthy et al., 2004).

Although the landfarming process is a simple technique which requires very low cost, slight maintenance, almost no cleanup liabilities and minimal monitoring efforts, the process is heavily influenced by the surrounding conditions affecting the biological degradation of contaminants such as low ambient temperature or rainfall which is largely uncontrollable and may consequently affect the timing for the remediation process (Gan et al., 2009).

McCarthy et al. (2004) reported successful landfarming bioremediation of 3600 m^3 of contaminated soil in a site in Alaska during 2003, with temperatures between of 1.3 and 4.9 °C. The bioremediation study included biostimulation by additions of nitrogen and phosphorus to the contaminated soil, and an aggressive schedule of soil tilling. Despite these cold temperatures, it reached acceptable concentration of pollutants between 31 days and 55 days (< 500 mg kg^{-1} for diesel-range organics) in soil with an initial concentration of 1400-1500 mg kg^{-1}.

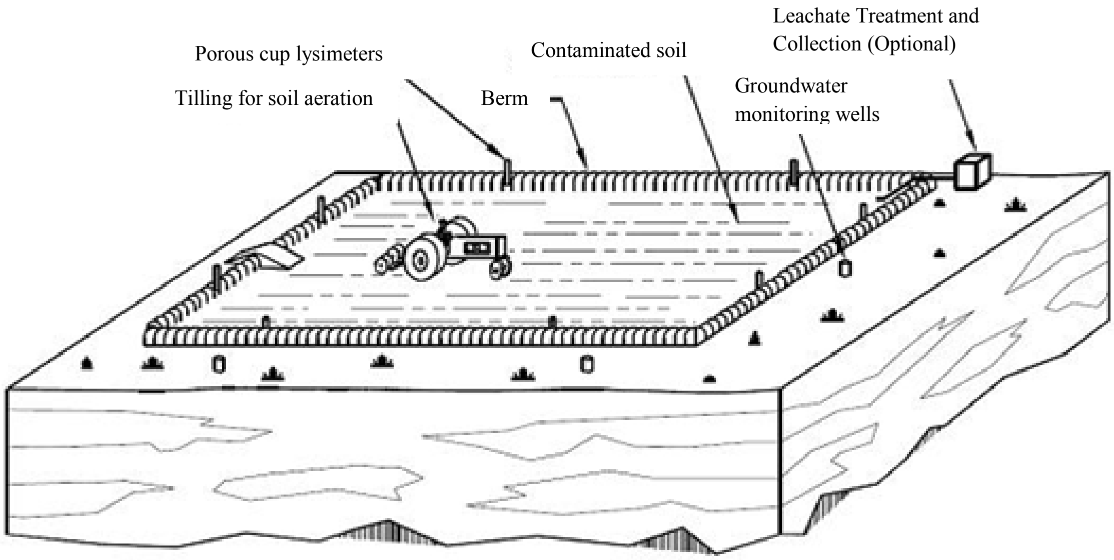

Figure 2.4 Landfarming technique (USA EPA, 2012b)

Paudyn et al. (2008) developed a landfarming bioremediation study of diesel fuel contaminated soil at a remote Canadian Arctic location, under various treatment regimes, and reported 80 % reduction of TPHs over a 3 year period with aeration of the contaminants by rototilling. However, the addition of nutrients resulted in active bioremediation and a more rapid removal of contaminants.

2.3.1.3- Electrobioremediation

Electrobioremediation constitutes one of the most recent techniques. This is a technique based on the use of bioremediation and electrokinetics together for the treatment of hydrophobic organic compounds. The bioremediation process is used for pollutant degradation with degrading microorganisms, while the electrokinetics phenomena are used for the acceleration and orientation of transport of pollutants with the use of an electric field (Megharaj et al., 2011). This technique is based on electromigration and electroosmotic principles through the application of a low level direct current electric potential through electrodes, placed into the contaminated soil See Figure 2.5 (Gan et al., 2009). The technology is particularly effective in fine-grained soils of low hydraulic conductivity and large specific surface area (Yeung et al., 2011).

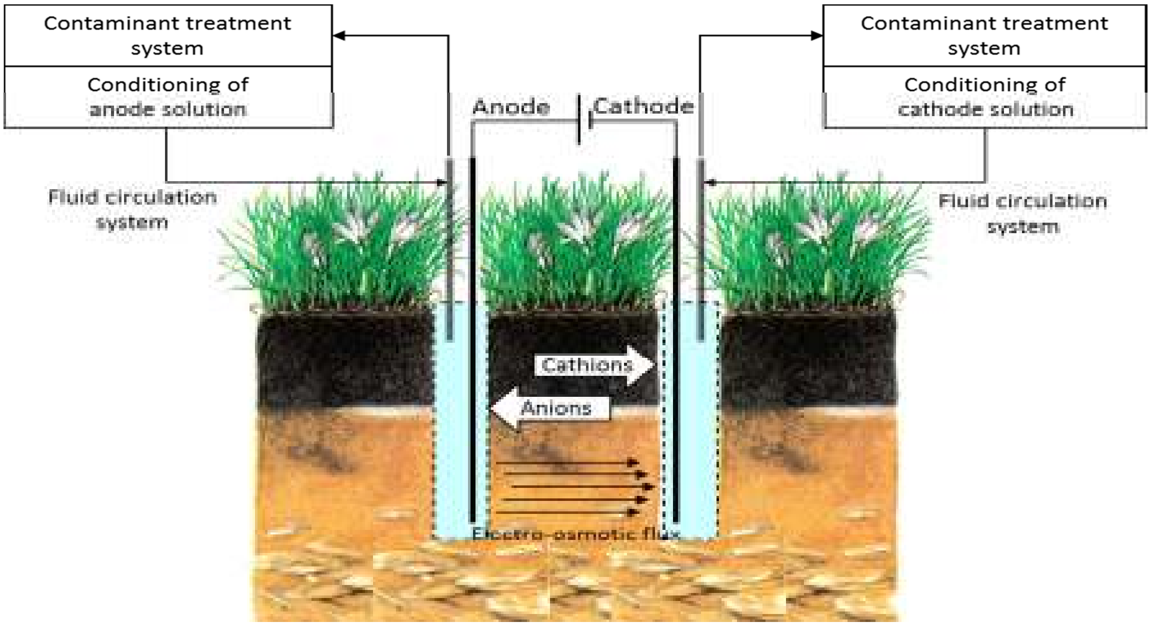

Figure 2.5 Electrobioremediation (Cameselle et al., 2013)

Reddy et al. (2006) developed a series of bench scale experiments on electrokinetic remediation of aged soil using different flushing agents including two surfactants (3% Tween 80 and 5% Igepal CA-720), a co-solvent (20% n-butylamine) and a cyclodextrin (10% hydroxypropyl-β-cyclodextrin or HPCD). During this research, PAHs were found to be solubilized in the surfactants and HPCD enhanced systems more efficiently with significant migration towards the cathode, obtaining a higher removal with Igepal CA-720 surfactant. The experiments were conducted at 2.0 VDC cm^{-1} voltage gradient and 1.4 VDC cm^{-1} hydraulic gradient.

In the same context, Fan et al. (2007) observed an increase in the bioavailability of 2,4-dichlorophenol with the use of electrokinetics. A 2-dimensional (2-D) non-uniform electric field enhanced the in-situ bioremediation process by promoting the mass transfer of organics to degrading bacteria. About 73.4% of 2,4-dichlorophenol were removed at the bidirectional mode and about 34.8% were removed at the rotational mode in 15 days.

Velazco-Alvarez et al. (2011) described how the application of an electric current and the application of Aspergillum Niger, a degrading microorganism, facilitated the degradation of hexadecane. Although, this technique has demonstrated its efficacy for the remediation process, similar to the previous techniques, it also contains several limitations such as availability of the contaminants, bioavailability of the correct type of microorganism, requirements of pore fluid as mobile phase for contaminants, heterogeneity of the soil matrix, and presence of microbial consortium potential affected by the electric current (Megharaj et al., 2011).

2.3.1.4- Composting

Compost technology or composting refers to the use of a biological system of microorganisms in a mature, cured compost to sequester or breakdown contaminants in water or soil (Figure 2.6). The use of composted organic matter provides good humus to build up soil quality and structure, supplemental nutrients and carbon, as well as mesophilic and thermophilic microorganisms to accelerate the abundance and species diversity of soil microbial community (Adekunle, 2011). These microorganisms can also lock up pollutants within the organic matrix, thereby reducing pollutant bioavailability (Semple et al., 2001).

Figure 2.6 Composting by windrows (Elcock and Veil, 2005)

During composting, the contaminant can disappear via different mechanisms such as mineralization by microbial activity, transformation to products, volatilization, and formation of non-extractable bound residues with organic matter (Semple et al., 2001; Sayara et al., 2011).

A key factor in the compost process is the tolerance of exogenous microorganism to the thermophilic temperatures (>45 °C), and also the competition with the indigenous microorganisms in the new environment that might overcome the degradation difficulty resulting from the accumulated metabolites intermediate products during the bioremediation process (Grace Liu et al, 2011, Sayara et al, 2011). The composting efficiency essentially will depend on temperature and soil-waste amendment ratio as the two important operating parameters for bioremediation. Composting has proved at both laboratory and field-scales to be a successful method to enhance aeration and microbial activity, and hence the biodegradation rate of petroleum hydrocarbon contaminants.

Antizar-Ladislao et al. (2006) investigated the degradation of PAHs in an aged coal tar contaminated soil under in-vessel composting conditions. Their results showed optimal degradation rates at the temperature of 38 °C, a soil-to-green waste ratio of 0.8:1, and a moisture content of 60%. Under those conditions, 77% of the total PAHs were removed after 98 days.

Wong et al. (2002) added pig manure at three different ratios (12.5%, 25%, 50%) to a soil spiked with 100 mg $kg^{-}1$ of the PAHs phenanthrene, anthracene, and pyrene, and investigated its effect on the degradation of these PAHs in a bench-scale composting system. They found that the manure could increase the populations of total thermophilic and mesophilic bacteria as well as PAH-degrading bacteria and enhance the amounts of soluble organic carbon, ammonia nitrogen, and soluble phosphorous in the composting mass in the early stages of the composting process. In their experiment, a manure ratio of 25% was the most effective, and 90% of the initial PAHs were removed at the end of the composting process.

2.3.1.4- Biopiles or Biocells

Another technique is the controlled solid phase biotreatment, well known as biopiles or biocells (Figures 2.7 and 2.8). This biotreatment includes the preparation of bioreactors in the form of piles or cells, allowing for the control of several factors like moisture, heat, nutrients, microbial consortia, oxygen and pH to enhance the biodegradation process. The soil is amended and piled over a piping system, which enables the supply of forced or passive air into the soil matrix, and leachate collection system for its recycle into the pile (Hwang et al., 2001; US EPA, 2012a). Over the last years, biopiles have proven being effective in reducing concentration of nearly all constituents of petroleum products, including target contaminants such non-halogenated volatile organic compounds (VOCs), semi-volatile organic compounds (SVOCs), and PAHs. It is an efficient technique that it is relatively simple to design and operate, it requires short treatment times and it is cost competitive. Biopiles requires less area than landfarming. It can be engineered to be potentially effective for any combination of site conditions and/or techniques, and most of the influential parameters can be controlled except for climatic conditions (Hwang et al., 2001; Sanscartier et al., 2009; US EPA, 2012b).

However, biopile application also presents several disadvantages: concentrations reductions higher than 95% are very difficult to achieve, it may not be effective for high constituents concentrations (>50,000 ppm TPHs), the presence of significant heavy metals concentrations may inhibit microbial growth, vapor generation during treatment may require treatment prior to discharge, and may require bottom liner installation to prevent leachate contamination (Trotsky and Pal, 1998; Mohn et al., 2001; US EPA, 2012b).

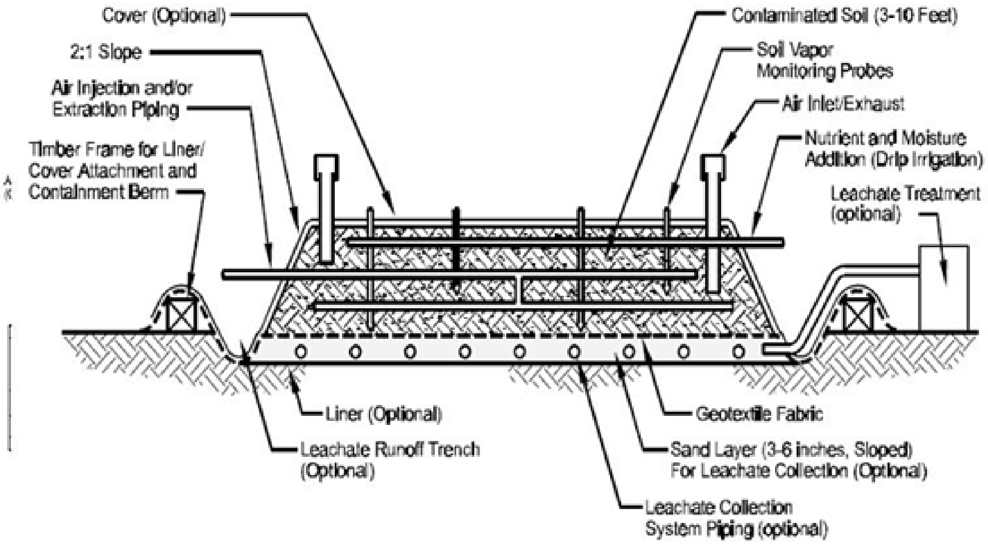

Figure 2.7 Cross-Section profile of a Biopile (US EPA, 2012b)

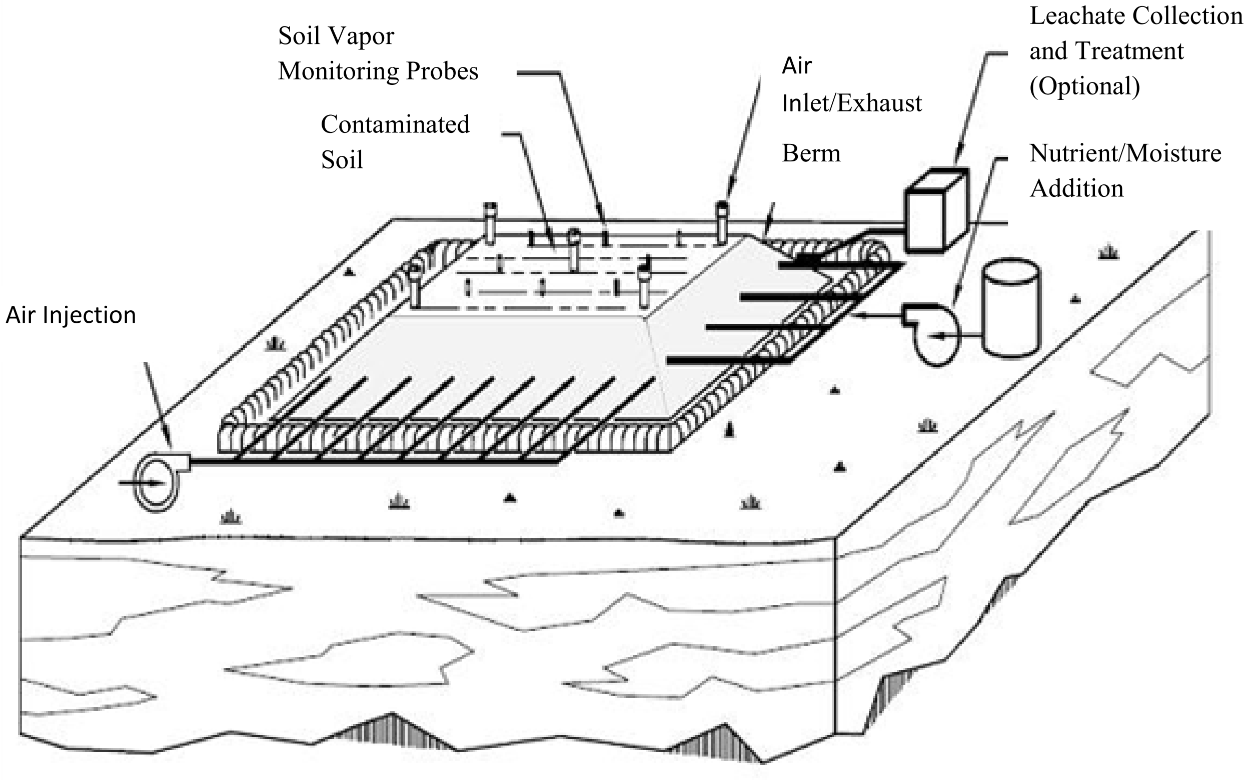

Figure 2.8 Biopile System (US EPA, 2012b)

Ex-situ remediation acts in a very complex way, due to the fact that the degradation rates are subject to petroleum compounds characteristics, physical and chemical factors of the soil, and climatic conditions. Several strategies such as heating, biostimulation and bioaugmentation aim to preserve and/or increase the biomass of degrading microorganisms present in the biopiles and thus shorten the bioremediation process, even in extreme environmental conditions (Sanscartier et al., 2009; Łebkowska et al., 2011; Beškoski et al., 2011).

Sanscartier et al. (2009) examined the effect of humidifying the air for the treatment of petroleum hydrocarbons (PHC) contaminated soil in an aerated/heated biopile system. Three biopiles were constructed with soil freshly contaminated with diesel fuel of about 11,000 ppm and operated for 10 months in Kingston, ON, Canada. One biopile was heated with an aerating/heating system. A second biopile was also aerated and heated but received water by humidifying the air prior to entering the soil pile. A third biopile was passively aerated by pipes protruding from the soil pile. Significant TPH reduction was observed in all systems, obtaining results below standards up to 500 ppm.

Thomassin-Lacroix et al. (2002) studied the effects of bioaugmentation (enrichment culture) and biostimulation (nitrogen and phosphorous amendment), in petroleum contaminated Arctic tundra soil, using laboratory microcosm and small scale (0.5 m^3) experiments. For the laboratory microcosm experiment, they obtained 79.2% removal of TPH over a period of 65 days at 7˚C; meanwhile, for the small scale biopiles, 82.7% of TPH removal was reached over a period of 65 days, with temperatures between -15˚C and 10˚C. Both the field experiments and laboratory microcosm denoted the feasibility of bioremediation of Arctic soil contaminated with weathered diesel fuel at low ambient temperatures. Biostimulation with nitrogen and phosphorus amendment demonstrated the ability to stimulate the mineralization activity of hydrocarbon degraders at low temperatures. However, bioaugmentation by enrichment culture only had no effect on hydrocarbon removal in microcosms or in biopiles.

2.4 INFLUENTIAL FACTORS IN BIOREMEDIATION

The degradation of organic contaminants and the success of the bioremediation process are directly or indirectly influenced by the pollutants properties, climatic conditions, microbial communities and by several other physical and chemical soil characteristics such as density, water retention capacity, soil pH, moisture, temperature, availability of oxygen, carbon source, and nutrients (Otten et al., 1997; Beškoski et al., 2011; Sayara et al., 2011).

2.4.1 Microorganisms

Bacteria and fungi are microorganisms capable of transforming natural and synthetic organic pollutants into the sources of energy and non-toxic raw compounds, as a part of their metabolic processes for their own growth (Haritash and Kaushik, 2009). Bacteria are the microorganisms currently involved in most of the bioremediation techniques for the degradation of organic pollutants in contaminated sites. Several strains of bacteria such as pseudomonas and rhodococcus have played a fundamental role in the degradation of soils contaminated by hydrocarbons (Zhao et al., 2011).

The energy generated by bacteria through the metabolic process is produced by the transmission of electrons from an electron donor to an electron acceptor, in order to oxidize the carbon source to carbon dioxide. For bioremediation of petroleum hydrocabons, only bacteria that are aerobic and heterotrophic are important in the process, using organic compounds (hydrocarbons) as an electron donor, which at the same time work as carbon source for cell growth and to sustain metabolic functions required for growth (Otten et al., 1997; Chikere et al., 2011). The molecular oxygen is the compound that yields the most energy and is a main factor for the aerobic degradation as the terminal electron acceptor. Furthermore, the degradation of contaminants is often catalyzed by enzymes like oxygenase that are active when oxygen is present (Otten et al., 1997). Bacteria require also nitrogen and phosphorus for cell growth (US EPA, 2009b).

The cycle of the microbial growth comprehends four different phases: the lag phase, exponential phase, stationary phase, and death phase. In the lag phase, the microbial population acclimatizes to the substrates and develops the required enzymes to consume the carbon source. In the exponential phase, the microbial population grows exponentially by consuming the food source and breaking down the contaminants. In the stationary phase, the exponential growth reaches a plateau, a point stationary of net increase or decrease. Finally, in the death phase, the microbial population decreases (Otten et al, 1997).

The potential to degrade certain organic pollutants varies among microbial groups (Megharaj et al., 2011). There are diverse microbial communities in soil or aquatic environments, acting synergistically in metabolic cooperation for the biodegradation process, with different metabolic activities, substrates and intermediate compounds. Cerqueira et al. (2011) denoted that the use of consortia may result in metabolic complementarity and be more efficient than pure cultures, due to their synergistic capacity among members of the consortia, leading to the degradation of the hydrocarbons. In many cases consortia were more effective than single strains by the fact that intermediates of a catabolic pathway of one strain may be further degraded by other strains possessing suitable catabolic pathway. They investigated and compared the biodegradation capacity of aliphatic and aromatic hydrocarbons of petrochemical oily sludge in liquid medium by a heterogeneous bacterial consortium and five pure petroleum degrading bacterial cultures. The heterogeneous bacterial consortium demonstrated the best results with excellent degradation capacity, reducing 90.7% of the aliphatic fraction and 51.8% of the aromatic fraction.

Besides bacteria, several strains of fungi have been identified as hydrocarbon degraders. Their ability to decompose several compounds is attributed to their non-specific enzymatic system (Sayara et al., 2011). They have been investigated recently as a result of their rapid adhesion to the soil matrix, and their ability to grow in environments with low nutrient concentrations, low humidity, and acidic conditions. Further, the scientists have found a synergistic degradation between fungi and bacteria, where fungi can initially clave the aromatic rings and the bacteria degrade the resulting intermediate products (Sayara et al., 2011). According to Grace Liu et al. (2011), fungal classes have been found to be the most effective degraders when using composting approaches to treat PAHs contaminated waste.

2.4.2 Oxygen

Oxygen is used as an electron acceptor to increase bioremediation activity and to enhance the aerobic biodegradation process (Boopathy, 2000). The aerobic biodegradation process breaks down the pollutants in the presence of oxygen. Aerobic bacteria use oxygen as an electron acceptor to break down both the organic and inorganic matters into smaller compounds, often producing carbon dioxide and water as the final product. Further, oxygen is also used as a substrate in oxygenase catalyzed reactions (Rike et al., 2003; Gan et al., 2009). Dawson et al.

(2007) highlighted the importance of respiration as an indicator of status for the bioremediation process, including it as a laboratory of field scale strategy for analysis.

Forced aeration by blower or fans equipment is a common approach for supplying oxygen require for the aerobic processes. The aeration equipment is usually connected to an aeration piping network, under positive or negative pressure to blow air or to suck moisture or volatiles from the contaminated soil. To ensure appropriate aeration, systems can be designed based on stoichiometric calculations and expected biodegradation rates (Sanscartier et al., 2011). Aeration rates can then be adjusted according to O_2 levels in the soil gas. However, there is no consensus on the appropriate soil-gas O_2 levels for optimal bioremediation. Reports and previous experience suggest that levels between 10- 15% should be maintained (Trostky and Pal, 1998; Hwang et al., 2006).

Nevertheless, excessive aeration can promote the volatilization of contaminants rather than their biodegradation. Forced aeration as an alternative approach enhances the oxygen concentration gradients between the gaseous and aqueous soil phases, improving the diffusion and mass transfer of O_2 in the aqueous phase and providing relatively higher oxygen concentrations in the soil void space and in deep micro-pores (Loehr et al., 2001; Sanscartier et al., 2011).

According to Sanscartier et al. (2011), injecting air in soil at high rates in cold climate conditions appears to be an interesting option to enhance biodegradation of middle-distillate fuels without promoting excessive volatilization. They suggested that this approach could be may be more cost and energy-efficient than increasing soil temperature to stimulate bioremediation.

2.4.3 Moisture Content

Soil water or moisture content within the soil is an important factor to control, as it can limit the internal transport of nutrients, organic constituents and microbes in the soil matrix, influencing the bioremediation rates (Boopathy, 2000). Excessive soil moisture may restrict the movement of air through the subsurface thereby reducing the availability of oxygen, essential for aerobic bacterial metabolic processes (Raghavarao, 2003; US EPA, 2009b). According to the US

EPA (2009b), the optimal range for soil moisture should be between 40 to 85 % of the field capacity of the soil or about 12 to 30 % by weight.

2.4.4 Soil Texture

Soil texture can affect the permeability, moisture content, and bulk density of the soil, and as a consequence, the oxygen availability for the bioremediation processes. Bali et al. (2002) affirmed that degradation rates are enhanced when the soil is granular or porous with a relatively high permeability and uniform mineralogy. Instead, rocky conditions with low permeability and complex mineralogy could negatively affect the bioremediation process. Examples of this are soils that tend to clump together such as clays, which are difficult to aerate. Further, such soils are problematic regarding the distribution of nutrients and they also attend to retain water decreasing the air void (Figure 2.8). For this reason, soil amendments and bulking materials sometimes are added to increase porosity, and thus ensure oxygen availability within the soil matrix. Wood chips, sawdust and straw are common examples of these components. These bulking agents help to maintain oxygen levels concentration constant along the different piles, enhancing microbial activity and degradation (Kauppi et al., 2011).

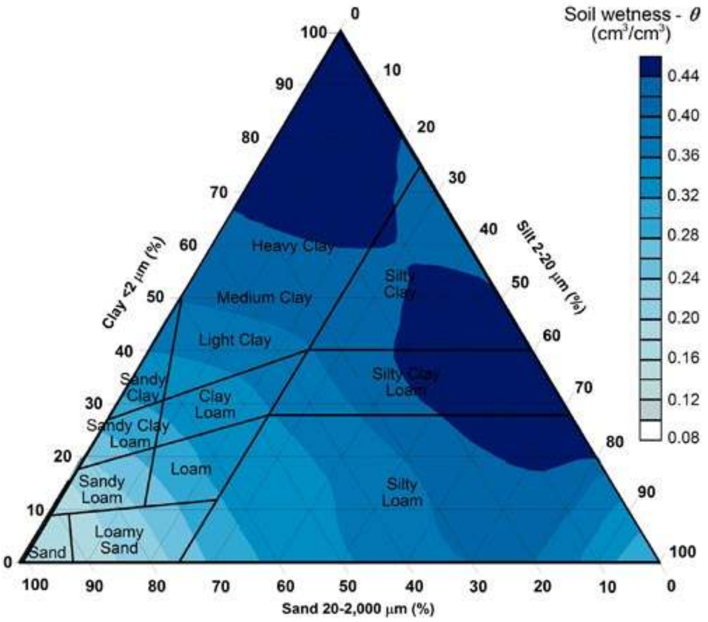

Figure 2.9 Soil Wetness based on texture classification (Triantafilis and Buchanan, 2007)

Experiments conducted by Kauppi et al. (2011) on clay loam, a type of soil with low permeability and high water retention, have shown that an enhancement of the remediation process is only possible when wood chips as a bulking agent and nutrients have been added to the process, which optimizes the C:N ratio and the oxygen availability.

Soil fractions influence the strength of the interactions with the pollutants and the activity of the microbial consortia, which is responsible for the degradation of the pollutants. Pollutants interact with different types of soil with a tendency to adsorb to particulate matter in soils and with the occurrence of others mass transfer phenomena, limiting their availability to organisms and possible biodegradation (Chang et al., 2010). Compounds characterized by low solubility and high hydrophobicity tend to be strongly sorbed to soil organic matter and are poorly available to microorganisms for degradation (Taccari et al., 2012).

Chang et al. (2010) denoted that particle composition influence the biodegradation rates, finding different TPH concentrations for coarse sized particles and macroaggregates to medium sized particles and fines. It was found that semi and non-volatile petroleum hydrocarbons can partition from a non-aqueous phase liquid and bind into soil matrix predominant in mineral phases (clay) and organic matter phases.

Desorption of the pollutants is what determines the susceptibility to microbial degradation, thus impacting on the effectiveness of the bioremediation process. For this reason, the bioavailability becomes a limiting factor in smaller particles, which make the transport of the pollutant molecule to the microbe more difficult (Megharaj et al., 2011).

Biosurfactants have been used with the aim to enhance the bioavailability of these pollutants in the contaminated areas. These surface-active agents may stimulate dissolution or desorption rates, solubilization or even emulsification of hydrocarbons (Calvo et al., 2009). According to Megharaj et al. (2011), these agents may help to lower the interfacial tension, solubilize hydrophobic organic compounds, and aid in the transfer of organic compounds from the soil-sorbed phase to the pseudo-aqueous phase. Rhamnolipids, as a surfactant, has proven to stimulate different processes involved in the degradation of organic substrates (Calvo et al., 2009).

2.4.5 Nutrients Availabity

Another factor to be considered for bioremediation is the nutrients availability, which plays a fundamental role in the performance of the microbes and their degradation pathway. Microorganisms require inorganic nutrients such as nitrogen (N) and phosphorous (P) to support cell growth and sustain metabolism processes.

Zhao et al. (2011) has demonstrated the importance of the nutrients as a limiting factor. A total viable bacteriological population was found to increase with the addition of commercially available inorganic nutrients (mainly nitrogen, phosphorous and potassium). Nutrient requirements depend on the nature of contaminants and the extent to which the polluted site has been subjected to correct the nutritional imbalance present in the soil (Beškoski et al., 2011).

Nitrogen is most often the limiting nutrient to biological hydrocarbon degradation in cold region soils. Many contaminated cold region soils are deficient in nitrogen, and the addition of the proper amount of N can increase the biodegradation rate. However, it has been demonstrated that excess nitrogen can depress the rate of microbial activity and petroleum compounds degradation in contaminated soils due to osmotic soil water potential depression (Walworth et al., 2007). Walworth et al. (2007) denoted that most of N fertilizers are composed of highly water soluble nitrate and/or ammonium salts such as ammonium nitrate (NH_4NO_3) or a non-nitrogenous salt such as sodium chloride (NaCl), which increase the salt concentration and as a consequence lowers the soil osmotic potential, and therefore can inhibit the microbial activity of the bioremediation process.

On the other hand, a rapid nitrification with urea, a common and cheap source of nitrogen used in most bioremediation applications, could entail a significant increase in pH, effects that will directly affect the viability of the consortium of microbes (Kauppi et al., 2011). The recommended C:N:P ratio of 100:10:1 has been studied for biopiles operation and a ratio of C:N:P 100:15:1 has been used in ex situ bioremediation (Zhao et al., 2011; Beškoski et al., 2011).

2.4.6 Soil pH

pH has a significant impact on bioremediation, controlling enzyme activities, transport processes and nutrient solubility (Cerqueira et al., 2011). Most natural environments have values of pH between 5.0 and 9.0, and as a result this range is optimal for microbial enhanced biodegradation of waste contamination. According to Khan et al. (2004), the rate of hydrocarbon biodegradation is greater under neutral to slightly alkaline conditions. However, pH reduction can be evidenced as a result of the release of organic acids caused by hydrocarbon degradation or by the production of extracellular polymers (Cerqueira et al., 2011).

2.4.7 Contaminants Characteristics

Volatility and chemical structure of the contaminants will determine the type of emissions control that are needed and the rate at which biodegradation will occur. Control of VOC emissions may be required, upon the site specific regulations (US EPA, 2009b). Although most of the petroleum hydrocabons products are biodegradable, the more complex the molecular structure, the more difficult and less rapid is the biodegradation. Mohamed et al. (2006) denoted that primary consumption of alkanes was observed, and it was followed by the depletion of polyaromatic fraction and asphaltenes, which are more resistant to biodegradation. Meanwhile, Cerqueira et al. (2011) concluded that the susceptibility of the aromatic fraction decreases with the number of aromatic or cyclic rings in the molecule.

2.4.8 Toxicity

Another important factor for the success of the biodegradation process is the presence of high concentrations of some contaminants. They can inhibit various cellular processes and their effects will usually involve specific chemical reactivity. For example, total petroleum hydrocabons (TPHs) in the range of 10,000 to 50,000 ppm are considered inhibitory and toxic to most microorganisms. Metals such as copper, silver, and mercury are typically very toxic, while metals such as lead, barium and iron are usually benign to the microbes at levels exceeding 2,500 ppm (US EPA, 2009b; Megharaj et al., 2011). The degree and mechanisms of toxicity vary with specific toxicants, their concentration, and the exposed microorganisms. Toxic compounds can prevent or slow metabolic reactions and often prevent the growth of new biomass to stimulate rapid contaminant removal (Talley and Sleeper, 2006).

2.4.9 Temperature

Temperature plays an important role in the bioremediation process and has to be taken into consideration during the design of the remediation system. Temperature affects the bacterial metabolism, microbial growth rates, gas solubilities, the soil matrix and physico-chemical state of the contaminants (Megharaj et al., 2011; Margesin et al., 2007).

Elevated temperature can increase the solubility of hydrophobic pollutants, decrease their viscosity, enhance their diffusion, and transfer long chain n-alkanes from the solid phase to the water phase (Aislabie et al., 2006). Haritash et al. (2009) denoted that the diffusion coefficient of water increases by 4 to 5 times with a raise of temperature from 20 to 120°C, resulting in about 150 times higher the effective diffusion coefficient within the soil.

The majority of hydrocarbon degrading bacteria are most active in the range of 20 to 30°C (Xu and Lu, 2010). Usually, when oil-contaminated soils are subjected to very low temperatures such as those on polar areas, emulsifiers or biosurfactants are of great importance because they can counter the increased viscosity and decreased water solubility of the hydrocarbons at lower temperatures (Aislabie et al., 2006; Kauppi et al., 2011).

As it was mentioned above, ambient temperatures influence the physical nature and chemical composition of hydrocarbons, rate of degradation, composition of microbial communities, mass transfer of substrate and electron acceptors availability (Yang et al., 2009; USA EPA, 2012b). Although, low temperatures affect the biodegradation rates, several authors have studied how engineering strategies could enhance and accelerate the bioremediation process and the ability of cold adapted hydrocarbon-degrading microorganisms to degrade significant amounts of petroleum hydrocarbons at temperatures around 0ºC. Techniques such as heating, composting, nutrients amendment, use of mulches, and plastic covers appear to positively impact the hydrocarbon degradation in cold climates (Coulon et al., 2005; Sanscartier et al., 2009; Chang et al., 2010).

Recent research suggests that microbial biodegradation activity does not cease at subzero temperatures. Rike et al. (2005) used soil oxygen depletion to infer the activity of cold-adapted microbes at a bulk soil temperature of -6ºC, and hydrocarbon-degrading activity at -1 to –3ºC at a contaminated permafrost site, indicating that biodegradation can occur in nominally frozen soils. Aislabie et al. (2006) observed the biodegradation of many of the components of petroleum hydrocarbons by indigenous cold-adapted microbial populations at low temperatures in hydrocarbon-contaminated soils at a slow rate. Therefore, the activity of the indigenous hydrocarbon-degrading microbes is limited, likely by a combination of unfavorable conditions including low temperature and moisture, nutrient limitation, alkalinity and potentially inhibitory hydrocarbons.

Paudyn et al. (2008) investigated the remediation of diesel-contaminated soils at a former military base at Resolution Island, Nunavut, through field and laboratory experiments. Four plots, one control, one aerated daily, one aerated every 4 days, and one aerated every 4 days with addition of nutrients, were constructed. Aeration of the contaminants by rototilling alone effectively reduced the levels by over 80% during a 3-year period with rototilling every 4 days in the summer months. Addition of nutrients resulted in active bioremediation and a more rapid removal of contaminants. The field trial has clearly demonstrated that bioremediation is enhanced when fertilizer is added and also has shown significant hydrocarbon losses due to aeration by rototilling.

McCarthy et al. (2004) reported on successful bioremediation of 3600 m^3 of diesel contaminated soil in a site in Alaska with an initial concentration of 1400-1500 mg/kg and average monthly temperatures of 1.3 - 4.9°C. The bioremediation study included biostimulation by additions of nitrogen and phosphorus to the contaminated soil, and an aggressive schedule of soil tilling using heavy equipment. Despite these cold temperatures, target soil concentrations below 500 mg/kg for diesel-range were reached after 31 days.

Ex-situ biopiles have been successfully used for the bioremediation of diesel-contaminated Arctic soils, applying combinations of biostimulation (heating, nutrients and aeration) and bioaugmentation (Filler et al., 2001; Coulon et al., 2005; Sanscartier et al., 2011). Covers such as plastic polyethylene helps to prevent influence of weather conditions and volatilization of the pile. According to Aislabie et al. (2006), soil coverage induced a small but permanent increase of the temperature in the surface soil of 2°C and favoured the degradation of alkanes over aromatics.

2.5 TREATMENT STRATEGIES

2.5.1 Bioaugmentation

The main task when treating contaminated sites is to identify and locate microorganisms native to the contaminated environment, which present excellent potential for degradation, greater adaptability, and resistance to changes in environmental conditions and lower susceptibility to genetic variation caused by environmental stress (Cerqueira et al., 2011). However, due to the absence of proper microorganisms that degrade certain hydrocarbons, bioaugmentation (addition of single strains or a consortium of microorganism) has been required to degrade a complex system of hydrocarbons in contaminated soil (Zhao et al., 2011).

2.5.2 Biostimulation

Biostimulation refers to the addition of stimulatory substrates, bulking agents, nutrients amendments or organic amendments to enhance bacterial growth and metabolic activity, and hence the biodegradation rate of contaminants. Agricultural residues, fertilizers, manure and municipal compost are common examples of substrates with excellent chemical and physical properties that add an enormous value to biological processes (Braddock et al., 1997; Molina-Barahona et al., 2004; Gandolfi et al., 2009).

2.5.2 Combined Strategies

In most cases the treatment of contaminated soil involves different technologies and combination of treatments strategies such as biostimulation and biostimulation. The site specific nature of the environmental factors will dictate the scientific and engineering approaches to the remediation of contamination. Several studies have demonstrated the use of these strategies and their successful approach.

Bioaugmentation in conjunction with aeration and biostimulation enables the success of the soil remediation at field scale, as it was noted by Beškoski et al. (2011). Braddock et al. (1997) examined the efficiency of biostimulation and denoted the biostimulation with a better nutrient approach and oxygen availability as a feasible method to enhance the growth of native microbes present in northern sites.

Sayara et. al. (2011) studied the separate and simultaneous application of both biostimulation and bioaugmentation for degradation of polycyclic aromatic hydrocarbons (PAHs) in soil under laboratory conditions with an initial concentration of 1 g of Total PAHs/kg dry soil. Bioaugmentation of the soil was carried out using a white-rot fungi *Trametes versicolor* and biostimulation was performed using two organic co-substrates, a compost derived from source-selected organic fraction of municipal solid waste (OFMSW) and rabbit food. The results indicated that bioaugmentation with *T. versicolor* did not significantly enhance the degradation of PAHs. However, biostimulation was more effective and able to improve the PAHs degradation. By the end of the composting period (30 days), 89% of the total PAHs were degraded compared to only 29.5% in the un-amended control with indigenous microorganisms and without any co-substrate.

Grace Liu et al. (2011) conducted research on biodegradation of soil contaminated with petroleum hydrocarbon oil (14,000 mg/kg) using laboratory batch experiments with different bioaugmentation and biostimulation remediation strategies at 30 °C. Bioaugmentation (selected microbial consortium and kitchen waste (KW)), biostimulation (addition of high-, or low-amounts of rhamnolipid), and combination of both strategies were used. After 140 days of treatment, the combine use of biostimulation and bioaugmentation combined (microbial consortia and KW and low-level nutrient (NEL)) achieved the highest total petroleum hydrocarbon degradation efficiency (>80%).

After 5 weeks of remediation, the results revealed that bioattenuation, bioaugmentation, biostimulation, and combined biostimulation and bioaugmentation exhibited 44.1%, 67.8%, 83.1%, and 87.3% kerosene degradation, respectively. Also, the total hydrocarbon-degrading bacteria count in all the treatments increased with time up till the second week after which it decreased. The highest bacterial growth was observed for combined biostimulation and bioaugmentation treatment strategy (Grace Liu et al., 2011).

Sayara et al. (2011) mentioned that stable compost as a co substrate from municipal solid waste showed a greater potential and benefits as a sustainable resource to enhance biodegradation of PAH’s. It will induce significant modifications to the structural and chemical properties of the humic material fraction, including loss of aliphatic materials, an increased polarity and aromatic polycondensation, resulting in a decrease in PAH-binding or desorption process (Megharaj et al., 2011).

Another favorable factor inherent to compost as a co substrate are the large amount of organic matter that are added to the system, which is considered as a major factor to lock up pollutants in the soil matrix. (Semple et al., 2001). Haritash and Kaushik (2009) observed that the supplementation of contaminated soils with compost materials can enhance biodegradation without long-term accumulation of extractable polar and more available intermediates.

Namkoong et al. (2002) thereafter found that the degradation of diesel oil was significantly enhanced by the addition of these organic amendments relative to straight soil. The degradation rates of TPH and n-alkanes were found to be greatest at the ratio of 2:1 of contaminated soil to organic amendments on wet weight basis.

Taccari et al. (2012) investigated the evolution of the bacterial community during the bioremediation of diesel-contaminated soil using laboratory scale bioreactors for 120 days, evaluating the effect of adding individually or together, mature compost, bacterial consortium and the biosurfactant (b-cyclodextrin). Results showed that the addition of compost plus a bacterial consortium caused a progressive increase in both heterotrophic cultivable aerobic bacteria and presumptive Pseudomonas. They concluded that the combined use of mature compost and of a selected microbial consortium constitutes a useful strategy for improving TPH removal, achieving a high TPH degradation (96%) at the end of the bioremediation process.

2.6 SUMMARY AND CONCLUSIONS

Bioremediation constitutes an innovative technique, in which microorganisms mitigate or reduce hazardous organic pollutants, without negatively impacting the environment. It is an alternative applicable in-situ directly in contaminated sites or ex-situ after contaminated soils have been removed. Bioremediation has demonstrated to be a feasible and cost effective alternative for the remediation of pollutants in contaminated soils, and is becoming a useful tool for the conservation of our land and soil for future generations. However, biodegradation rates are influenced by the characteristics of the pollutant, the nature of the microbial consortium, and by physical, chemical and environmental factors such as pH, water content, bioavailability of the pollutants, moisture, nutrients amendment, temperature, and oxygen. Therefore, it is important to take into account all these factors before selecting any alternative in the remediation process or in the best case optimize the current remediation practices in the contaminated sites.

Temperature plays an important role in controlling the nature and the extent of microbial metabolism and hydrocarbon bioavailability. Therefore, temperature represents an important factor that needs to be carefully considered. Cold weather conditions have remained a challenge over the last years, affecting the performance of bioremediation of petroleum contaminated soils. Previous research studies show that in most cases the combination of strategies, i.e using bioaugmentation and biostimulation together, perform the best in ex-situ bioremediation, successfully degrading the petroleum pollutants in the soil.

It is also important to point out that the application of diverse bioremediation technologies must be based on reliable scientific data obtained through research in environmental laboratories and/or in the field. In the field it is very difficult, if not impossible, to isolate the effect of temperature variability from the multitude of other influencing environmental factors, such as presence of competing or indigenous microorganisms or spatial heterogeneity that may inhibit the effectiveness of the field-scale bioremediation operation. Many research studies have been conducted in small containers under laboratory conditions with isolated microorganisms. Considering these facts, it is very important to assess the applicability of any bioremediation technology, from a practical point of view, in field-scale and site specific conditions.

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CHAPTER 3

TECHNICAL PAPER I

FIELD SCALE EX-SITU BIOREMEDIATION OF PETROLEUM CONTAMINATED SOIL UNDER COLD CLIMATE CONDITIONS[1]

Francisco Gomez, Majid Sartaj

Abstract –Assessment and development of strategies for ex-situ bioremediation in cold climates, such as in Canada, is of great importance for decontamination of contaminated soils. The purpose of this paper was to evaluate and compare the use of microbial consortia inoculant and mature organic compost as bioaugmentation and biostimulation strategies to enhance ex-situ bioremediation of a soil contaminated with heating oil under cold weather conditions. The soil was impacted as a result of leakage from an above-ground storage tank and had an initial TPH concentration was 940 ± 127 μg g^{-1} (dry weight). Aerobic biopiles of 16 m^3 each were constructed and subjected at a field scale to microbial consortia inoculant and 10:1 ratio of mature organic compost. Two biopiles (S) contained only soil as control, two biopiles (S+C) contained soil and compost to assess the individual effect of compost addition, two biopiles (S+M) contained soil and microbial consortium to assess the individual effect of microbial consortium addition, and three biopiles (S+C+M) contained soil plus compost and microbial consortium to assess the combined effect of compost and microbial consortium addition. Over a 94 days period, composite soil samples for each biopile were collected and analyzed for total petroleum hydrocarbons (TPHs), volatiles (F1), semi volatiles (F2) and non-volatiles (F3) fractions, microbial counting, and pH. Additionally, field measurements including soil temperature, moisture content, carbon dioxide (CO_2) and oxygen (O_2) were carried out. Although the ambient temperature varied from -3.5°C to -24.1°C, the internal soil temperatures for the different experimental setups maintained above freezing conditions. Results showed that biocell inoculated with microbial consortia and amended with 10:1 soil to compost ratio under aerobic conditions performed the best, degrading 82 % of total petroleum hydrocarbons (TPHs) with a

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[1] *This paper has been published in the International Biodeterioration & Biodegradation Journal (See Appendix A).*

first order kinetic degradation rate of $0.016\ d^{-1}$, in cold weather conditions. The average removal efficiencies for TPHs after 94 days for S, S+M, S+C treatments were 48%, 55%, and 52%, respectively. Statistical analysis indicated significant difference ($p < 0.05$) within and between the final measurements for TPHs and significant difference between the treatment with combined effect (S+C+M) and the control (S) as well as individual treatments of S+C and S+M. The study concluded that the combination of microbial consortia inoculation and mature compost as a biostimulant in a 10:1 soil to compost ratio would be an appropriate bioremediation approach for this type of soil matrix, reaching values below provincial standard regulations in just 40 days after construction. This research aimed to provide valuable knowledge to practitioners about cost-effective and existing strategies for ex-situ bioremediation during cold weather conditions.

Keywords: Biopiles, Total Petroleum Hydrocarbons, Compost, Cold Climate, Microbial Consortia

3.1 INTRODUCTION

Fossil fuels represent by far the largest source of energy for human activities, accounting for 81% of all energy consumption worldwide in 2010 (IEA, 2012). During storage, transportation and combustion of fossil fuels massive amounts of contaminating hydrocarbons are released into the surrounding environment with fuel spill being the major contamination route. In Canada, approximately 60% of contaminated sites involve petroleum hydrocarbon (PHC) contamination (Sanscartier et al., 2009b). Health risks and environmental impacts associated with the exposition of petroleum hydrocarbons have resulted in soil contamination to be considered as a major concern over the last few years. Soil contamination is not only a social and sanitary issue, but is also an economical concern, since it implies major costs related to decreasing productivity and monetary depreciation of the impacted sites for future use (Juwarkar et al., 2010). Several sites, among brownfields, require extensive cleanup to prevent further migration of contaminants into water, soil, air and consequent threat to human health. In Canada alone, it has been estimated that there are around 22,000 federal sites contaminated with hydrocarbons, accounting for multibillions of dollars in environmental liabilities and remediation costs (McKie, 2012).

Consequently, bioremediation of petroleum contaminated soil has become an important environmental activity, as an economical and environmentally viable solution to restore the environment to background levels in warm and temperate climates. It is also increasingly viewed as an appropriate remediation option in cold climates (Sanscartier et al., 2009b). Bioremediation exploits the ability of microorganisms to degrade or detoxify effectively and economically hydrocarbon-contaminated soils, mitigating the risk to human health and the environment (Adenkule, 2011).

Ex-situ bioremediation, as a main remediation technology to treat a wide range of hydrocarbons has been a topic of considerable research interest over the last couple of decades. In most cases the treatment of contaminated soils through ex-situ bioremediation involves two main strategies: bioaugmentation whereby petroleum degrading microorganisms are added to the soil matrix, and biostimulation, which introduces essential nutrients or biosurfactants to stimulate microbial petroleum degradation (Sayara et al., 2010). Both biostimulation and bioaugmentation can be accomplished separately or in combination, by introducing hydrocarbon degrading bacteria and/or amending contaminated soil or sludge with a heterogeneous additional material such as compost (Namkoong et al., 2002; Yuan et al., 2009; Kriipsalu et al., 2010; Adenkule, 2011). Calvo et al. (2009) reviewed the application of bioemulsifiers as a biostimulation strategy and reported that they could emulsify hydrocarbons and enhance their water solubility and increase the displacement of oily substances from soil particles. Lebkowska et al. (2011) applied multiple indigenous microorganism inoculation in soil polluted with diesel oil and aircraft fuel, obtaining 80-98% removal efficiencies of total petroleum hydrocabons (TPHs). Lin et al. (2010) used a combination of bioaugmentation and biostimulation with the reseeding strategy for ex-situ bioremediation of petroleum contaminated soil. The diesel contamination was efficiently removed by about 70% (as total petroleum hydrocarbon) over a period of 4 weeks.

Nevertheless, successful bioremediation of petroleum hydrocarbons in soil has remained a challenge, especially under cold climate conditions. Due to the cold Ontario climate, ex-situ bioremediation techniques are limited to times of the year when temperatures are above freezing, i.e. April to November, as temperature plays an important role in controlling the nature and the extent of microbial metabolism and hydrocarbon bioavailability (Zhang et al., 2008). Over the previous years, various methods have been employed to effectively degrade petroleum contaminated soil under different approaches in cold weather conditions (Aislabie et al., 2006;

Chang et al, 2011; Kauppi et al, 2011; Sanscartier et al, 2011). Chang et al. (2011) reported 55% decrease in TPHs concentrations of field aged petroleum contaminated soil by nitrogen amendment at a sub artic site with temperatures between 4.7 and 10 °C. Mohn et al. (2001) reported on degradation of 2,109 mg kg^{-1} down to 195 mg kg^{-1} of diesel in small-scale biopiles experiments after one year in the Canadian Arctic Tundra, using nutrients amendment, bulking agents and cultures of cold tolerant hydrocarbon degraders. Kauppi et al. (2011), successfully degraded 2700 mg kg^{-1} of diesel-fuel contaminated clay loam soil in a boreal climate using field scale biopiles with forced aeration and adding nutrients amendment or woodchips as a bulking agent to increase porosity over a period of 11 month. Sanscartier et al. (2009b) were able to decrease 10,000 mg kg^{-1} to approximately 300 mg kg^{-1} of TPH concentration of diesel contaminated soil by humidifying the air in an aerated/heated biopile system with average outdoor temperatures of 5.8 and 11.7 °C for 10 months. Such findings confirm the feasibility of different strategies such as biostimulation by nutrients amendment, application of bulking agents, and forced aeration, and bioaugmentation by innoculation of microorganisms to degrade hydrocarbons at significant rates under cold weather conditions (Margesin and Schinner, 2001).

Assessment of bioremediation strategies for cold climates, such as in Canada, and methods of enhancing cold weather degradation is of a great importance for an efficient and cost effective decontamination of polluted soils. Considering above, the main objective of the present study was to assess and compare biodegradation of TPHs, as well as different fractions including readily volatile (F1:C6-C10), semi-volatile (F2:C10-C16), and non-volatile (F3:C16-C34) fractions in petroleum contaminated soil using a biopile technology system under cold weather conditions. Field-scale biopiles were constructed and subjected to microbial consortia inoculation and mature compost as bioaugmentation and biostimulation strategies and monitored over a period of 94 days (November 2012 to February 2013).

3.2 MATERIAL AND METHODS

3.2.1 Contaminated Soil

Soil impacted with petroleum hydrocarbons was obtained from a contaminated site in Val-des-Bois, in the Outaouais region of Quebec, Canada, located on the eastern shores of the Du Lièvre River, north of Buckingham. Throughout the site history, heating oil (C14-C20) was stored in an above-ground storage tank (ASTs) as a fuel source. Due to leakage, the AST was removed in 1994 and subjected to environmental assessment. Current results showed a contaminated area between 11.5 and 13.5 metres below the surface, covering an approximately area of 1600 m^2. Initial samples indicated that petroleum hydrocarbon in soil were higher than the criteria C, based on guidelines issued by Quebec provincial regulations, with an estimated concentration between 6,000 and 15,000 $\mu g\ g^{-1}$ dry weight (WESA, 2012).

The contaminated soil was excavated and transported to a treatment facility in Moose Creek, Ontario, Canada in October 2012, which had a pH of 7.79 ± 0.02 (Table 3.1). The impacted soil was classified as sand, according to the USCS classification system and based on grain size analysis of five composite random samples (Table 3.1), with 12.7% of gravel, 81.3% sand, and 3.3% silt and clay. Initial chemical analysis (based on 5 composite random samples) indicated that the contaminated soil contained 924 ± 127 $\mu g\ g^{-1}$ of TPHs, with volatile fractions (F1) of 27 ± 4 $\mu g\ g^{-1}$, semi volatile (F2) fractions of 455 ± 67 $\mu g\ g^{-1}$, and non-volatile (F3) fractions of 442 ± 62 $\mu g\ g^{-1}$, based on Canadian Wide Standards Tier 1 method (CCME, 2001).

Table 3.1 Characteristics of the contaminated soil

Parameter	Value	Method
Physical Characteristics		
>19 mm (% by wt.)	8.1 ± 0.1	ASTM D2487
<19 to >4.75 mm (% by wt.)	12.7 ± 5.0	
<4.75 to >2.00 mm (% by wt.)	13.4 ± 1.7	
<2.00 to >0.425 mm (% by wt.)	49.9 ± 5.0	
<0.425 To >0.075 mm (% by wt.)	17.9 ± 2.3	
<0.075 mm (% by wt.)	3.3 ± 0.6	
General Inorganics		
pH	7.79 ± 0.02	EPA 150.1 pH probe @ 25°C
Hydrocarbons		
F1 (C6-C10) ($\mu g\ g^{-1}$)	27 ± 4	CCME PHC, CWS Tier 1-GC-FID
F2 (C10-C16) ($\mu g\ g^{-1}$)	455 ± 67	
F3 (C16-C34) ($\mu g\ g^{-1}$)	442 ± 62	
F4 (C34-C50) ($\mu g\ g^{-1}$)	ND	
TPHs (C6-C50) ($\mu g\ g^{-1}$)	924 ± 127	

The values are expressed as mean ± standard deviation.
ND: Non detectable.
TPHs are the sum of all the fractions.

3.2.2 Biopile Design

Ex-situ biopiles of 4 m x 4 m x 1 m (16 m^3) each were constructed, maintained and subjected to different amendments based on a pre-defined configuration (Table 3.2). As seen in Table 3.2, mixtures investigated included only soil (duplicated) as control, soil and compost (duplicated) to assess the individual effect of compost addition, soil and microbial consortium (duplicated) to assess the individual effect of microbial consortium addition, and soil plus compost and microbial consortium (triplicated) to assess the combined effect of compost and microbial consortium addition. All biopiles were located on 24 x 24 m pad, over an asphalted surface that included a synthetic geotextile membrane to reduce potential leachate migration to the subsurface environment. If applicable, the contaminated soil was mixed by a front end loader 10 times, after addition of mature compost (compost characteristics presented in Table 3.3) with a ratio of 10:1 (soil:compost) to obtain a uniform substrate, following design criteria (Table 3.2). Then the soil was transported and laid in layers of 30 cm height by an excavator, over a perforated pipe at the base of each biopile. After placing each layer of the soil, a microbial consortium solution with an initial concentration of 10^7 CFU ml^{-1} was sprayed on top. The process was repeated until the desired height was reached (See Appendix B).

Table 3.2 Experimental setup configuration for biopiles under cold weather conditions

Setup	Treatment
S^1	Soil
$S+M^1$	Soil + Microbial Consortia
$S+C^1$	Soil + Compost (10:1 ratio)
$S+C+M^2$	Soil + Compost (10:1 ratio) + Microbial Consortia

1: Duplicate biopiles were constructed.
2: Triplicate biopiles were constructed.

Table 3.3 Characteristics of the mature compost prior to the application

Parameter	Value
Moisture Content (%)	44.7
pH @ 25 °C	7.00
Phosphorous Total ($\mu g\ g^{-1}$)	2740
Total Kjeldahl Nitrogen ($\mu g\ g^{-1}$)	17800
C/N ratio	46.7
Org. Matter (LOI@550) (% by wt.)	73

The microbial consortium used was a commercial liquid product that contained a concentrated blend of bacteria strains natives to Canada, defined as hydrocabons degraders, which was mixed with water at 0.05% v/v dilution rate. It was considered a non-pathogenic, non-opportunistic and identified within the list of organisms on the domestic substances list of Environment Canada.

After all the biopiles were built up, monitoring and sampling pipes were installed to measure oxygen (O_2) and carbon dioxide (CO_2) inside the biopiles (Figure 3.1). The perforated piping network installed at the base of the biopiles during construction was connected to a manifold regenerative blower system (Regenair R7100r-50, Gast) to generate a low airflow injection into one side and equivalent vacuum at the other extreme thereby extracting and collecting the leachate within a knockout box and neutralizing possible fugitive emissions into the environment, as the soil could be acting as its own filter by the recirculation of readily volatiles. An average airflow rate of 30 m^3 hr^{-1} per biopile was provided on an intermittent basis in order to provide sufficient oxygen to maintain the soil above the oxygen-limiting conditions, targeting 15-20% oxygen concentration.

After their construction, the biopiles were covered by a 5mm thick black insulation membrane (Premium Silage 50158) cover to protect the biopiles from wind, precipitation, and to maintain the proper moisture and temperature necessary for bacterial growth (Figures 3.1 and 3.2).

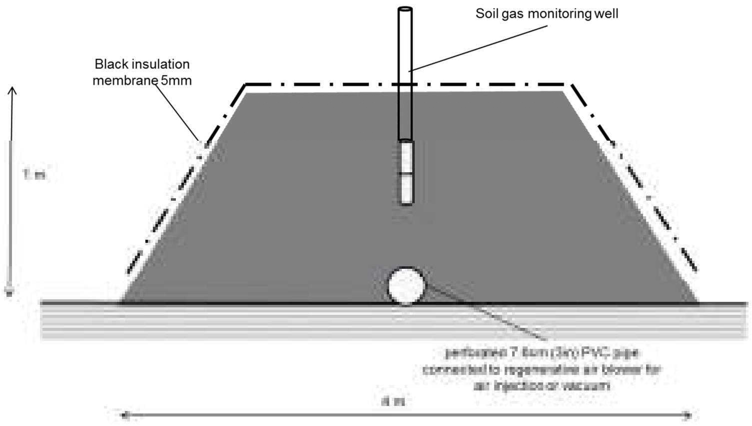

Figure 3.1 Cross section profile of each biopile

(a) (b)

Figure 3.2 (a) Biopiles during construction and (b) finished biopiles under cold weather conditions

3.2.3 Soil Sampling, Analysis and Monitoring

A monitoring program, which involved soil and air sampling, was implemented during the operating period. At the start, 5 random composite samples from contaminated soil were collected and immediately placed in airtight 250 mL glass jar containers to prevent volatilization and photodegradation and the containers were immediately placed into a cooler to preserve the samples at 4 °C and then transported to a laboratory certified by Canadian Association for Laboratory Accreditation (CALA) within a period of 24 hours. On days 6, 25, 39, 67, and 94, composite soil samples were collected from each biopile by pooling and homogenizing six sub-samples at various depths, randomly distributed throughout each biopile. A slide hammer type sampler device was used to extract the soil core. The core sampling device was pressure washed and cleaned with distilled water before next sampling to avoid cross contamination. Soil samples were subjected to different analysis including grain size (ASTM D2487), pH (EPA 150.1), heterotrophic plate counts (SM 9215C), and F1-F4 (CCME PHC), based on standard methods for the accredited lab.

Biopiles were also monitored with portable probes (Reotemp) and gas analyser instrument (Eagle, RKI) to determine pile temperatures (°C), moisture content (%), carbon dioxide levels (%Vol CO_2), and oxygen levels (%Vol O_2) in monitoring wells throughout the operation period of biopiles. All field instruments were calibrated prior to use to ensure accuracy and reliability.

QA/QC procedures were implemented both in the field and laboratory for sampling and analysis. Field blanks and duplicate samples from the same source were collected through the monitoring program to check sampling and analytical reproducibility and reliability. In addition, the lab followed its own QA/QC analytical procedures. The result of tests for each sampling event was expressed as the average of three runs with a standard deviation.

3.2.4 Statistical Methods

One-way ANOVA test was performed to determine if significant differences ($p < 0.05$) existed between the measured values for final TPHs concentrations in the four different experimental setups. However, in the case ANOVA shows a significant difference among collected data, it will not provide further information on differences between the groups of data. In this case, each pair of treatments was statistically compared for TPHs removal efficiency using t-test ($p < 0.05$).

3.3 RESULTS AND DISCUSSION

3.3.1 Temperature Profile

The ambient temperature during the treatment period varied from -3.5°C to -24.1°C, with an average temperature of -11.3°C, based on historical data obtained from Environment Canada for Moose Creek, Ontario (Environment Canada, 2013). Meanwhile, the internal soil temperature for the different experimental setups maintained above freezing conditions along the treatment duration (See Figure 3.3). The exothermic reactions by the mature compost amendment and hydrocarbon degradation helped keep and preserve the internal temperature conditions of the biopiles to promote the bacterial growth. The black insulation membrane also minimized convective heat loss, and accumulated heat solar radiation to warm up the soil, as it was reported by Mohn et al. (2001). They estimated an increase of internal biopile soil temperature by 30-49%, measured as degree-day accumulation, using a clear plastic cover. It is likely that the combination of these effects stimulated the hydrocarbon degradation over the 94 days of treatment.

Oxygen concentration and moisture content inside the different setup biopiles varied between 16 -21 % for O_2 and between 30-50% for moisture content throughout the treatment period. This represents optimum conditions to promote microbial activity and efficient petroleum hydrocarbon biodegradation (Trostky and Pal, 1998, Sanscartier et al., 2011). The plastic cover might also helped preserve the moisture conditions in the soil, as it would prevent drying effects in the soil that limits hydrocarbon degradation (Mohn et al., 2001).

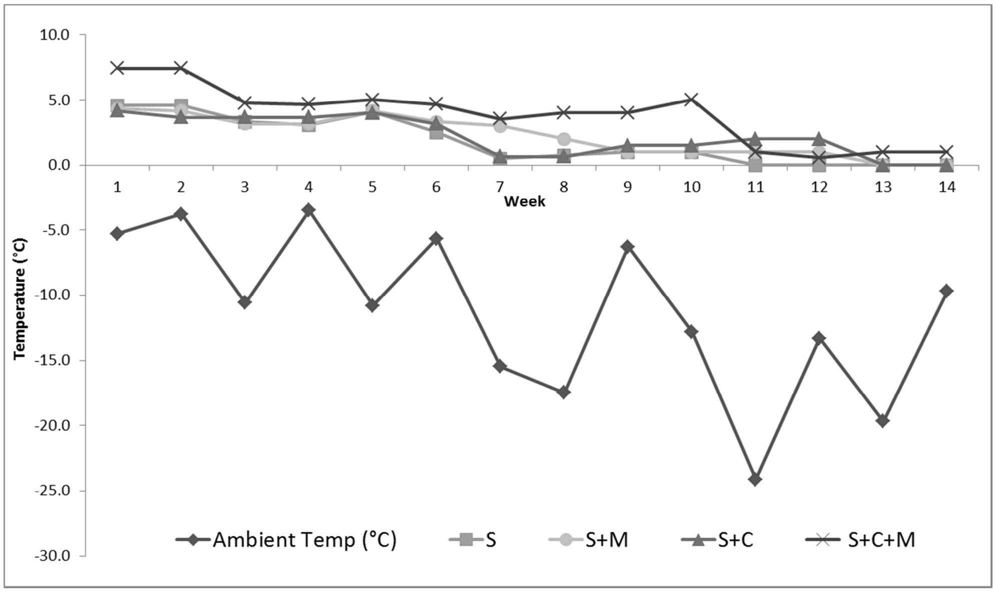

Figure 3.3 Temperature profiles for ambient and internal soil for different setup of biopiles.

3.3.2 Petroleum Hydrocarbon Degradation

The biodegradation of hydrocabons were enhanced significantly under biostimulation and bioaugmentation strategies with the combination of mature compost and innoculation of microbial consortia, resulting in higher percentages of degradation. As shown in Figure 3.4, the average removal efficiencies for TPHs after 94 days of treatment were 48%, 55%, 52%, and 82% for experimental setups S, S+M, S+C, and S+C+M, respectively. The results showed that bioaugmentation (S+M) with addition of only microbial consortia or biostimulation (S+C) with addition of only mature compost did not significantly enhance the TPHs biodegradation and resulted in only 4-5% increase in removal efficiency compared to the control biopile (S). It is likely that the degradation observed in the experimental setups S, S+C and S+M may have been influenced by the indigenous microflora from the impacted soil, compost or inoculated microbial consortia capable to degrade hydrocarbons, considering that biodegradation was the primary mechanism for hydrocarbon removal. Volatilization due to aeration could be a secondary mechanism although it is expected it was not significant due to the application of cover and the existing cold weather conditions.

Contrary to the individual application of biostimulation or bioaugmentation, the combination of biostimulation and bioaugmentation (S+C+M) significantly enhanced and promoted the hydrocarbon mineralization achieving a total 82% of TPHs removal under cold weather environment compared to only 48% TPHs removal for the control biopile (Setup S). Leaching of hydrocarbons did not appear to be the major mechanism, as the biopiles were covered, and no significant leachate was observed along the treatment.

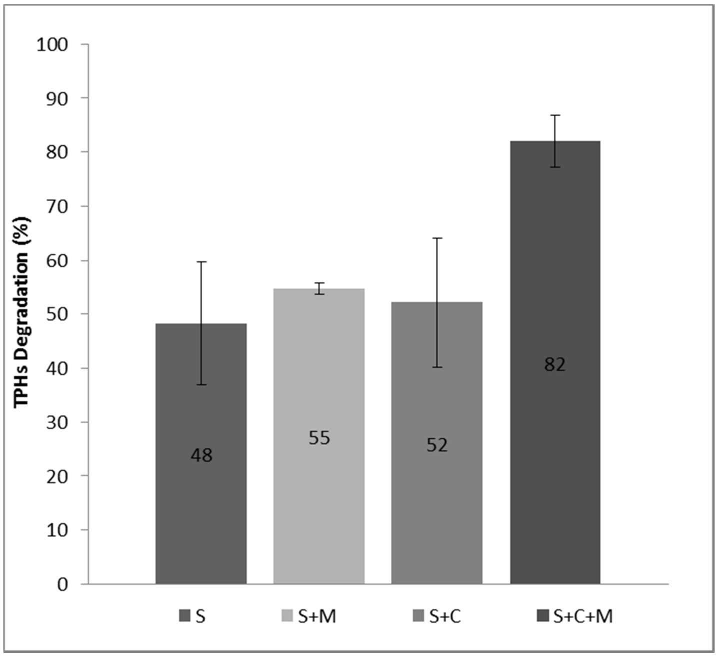

Figure 3.4 TPHs degradation percentage for different biopiles setup

Statistical analysis performed using one-way ANOVA, see Table 3.4, indicated significant differences ($p < 0.05$) between the measured values for final TPHs concentrations in the four different experimental setups. ANOVA shows whether there is significant difference among collected data or not, but does not provide further information on differences between the groups of data. Hence, each pair of treatments was statistically compared for TPHs removal efficiency using t-test. The results are presented in Table 3.5. As it can be seen, the results showed that individual effect of bioaugmentation (S+M) and biostimulation (S+C) did not significantly enhance the TPHs biodegradation as there was no significant difference observed.

There was no significant difference observed between S+M and S+C either and 4-5% increase in removal efficiency compared to the control biopile (S) observed was not statistically significant (at $p < 0.05$). However, the combined effect of bioaugmentation and biostimulation (S+C+M) was significantly different from the control (S) as well as individual treatments of bioaugmentation (S+M) and biostimulation (S+C) at $p < 0.05$.

Table 3.4 Single factor ANOVA for final TPHs measurements

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	160571	3	53523.685	9.797	0.016	5.409
Within Groups	27315	5	5463.033			
Total	187886	8				

Table 3.5 Results of t-test analysis for paired comparison of treatments for TPHs removal efficiencies

	S+M	*S*	*S+C*	*S*	*S+M*	*S+C*
Mean	54.78	48.34	*52.18*	*48.34*	54.78	52.18
Variance	1.15	129.92	*142.42*	*129.92*	1.15	142.42
Observations	2	2	*2*	*2*	2	2
Pooled Variance	65.53		*136.17*		71.78	
Hypoth. Mean Difference	0.00		*0.00*		0.00	
df	2		*2*		2	
t Stat	0.80		*0.33*		0.31	
P(T<=t) two-tail	0.51		*0.77*		0.79	
t Critical two-tail	4.30		*4.30*		4.30	
	S+C+M	S	*S+C+M*	*S+C*	*S+C+M*	S+M
Mean	82.07	48.34	82.07	*52.18*	82.07	54.78
Variance	23.12	129.92	23.12	*142.42*	23.12	1.15
Observations	3	2	3	*2*	3	2
Pooled Variance	58.72		62.89		15.80	
Hypoth. Mean Difference	0.00		0.00		0.00	
df	3		3		3	
t Stat	4.82		4.13		7.52	
P(T<=t) two-tail	0.02		0.03		0.00	
t Critical two-tail	3.18		3.18		3.18	

The results on TPHs degradation obtained in this study ranged from 48% to 82% and were comparable to the findings by Mohn et al. (2001), Sanscartier et al (2009), and Chang et al. (2010) who worked on the comparison of bioremediation strategies of petroleum contaminated soil at a field scale under cold weather conditions. Mature compost as a biostimulation agent continuously nurture nutrients and carbon for the microorganism, overcoming soil possible limitations in cold conditions (Yuan et al., 2009). It has also been proved to be an excellent bulking agent increasing porosity, oxygen diffusion and help to form water stable aggregate, enhancing the mass transfer rate of water, oxygen, nutrients, hydrocarbons availability, and microbial activity (Sayara et al., 2010; Xu and Lu, 2010).

Two weeks after the biopiles construction, soil samples were collected and analyzed for microbial assessment. Heterotrophic plate counts over 10^7 CFU per gram of soil and the presence of hydrocarbon degraders with an average of 4.7 Log_{10}MPN per gram throughout the treatment period for setup S+C+M confirmed the presence of hydrocarbon degrader adapted to the environmental internal conditions.

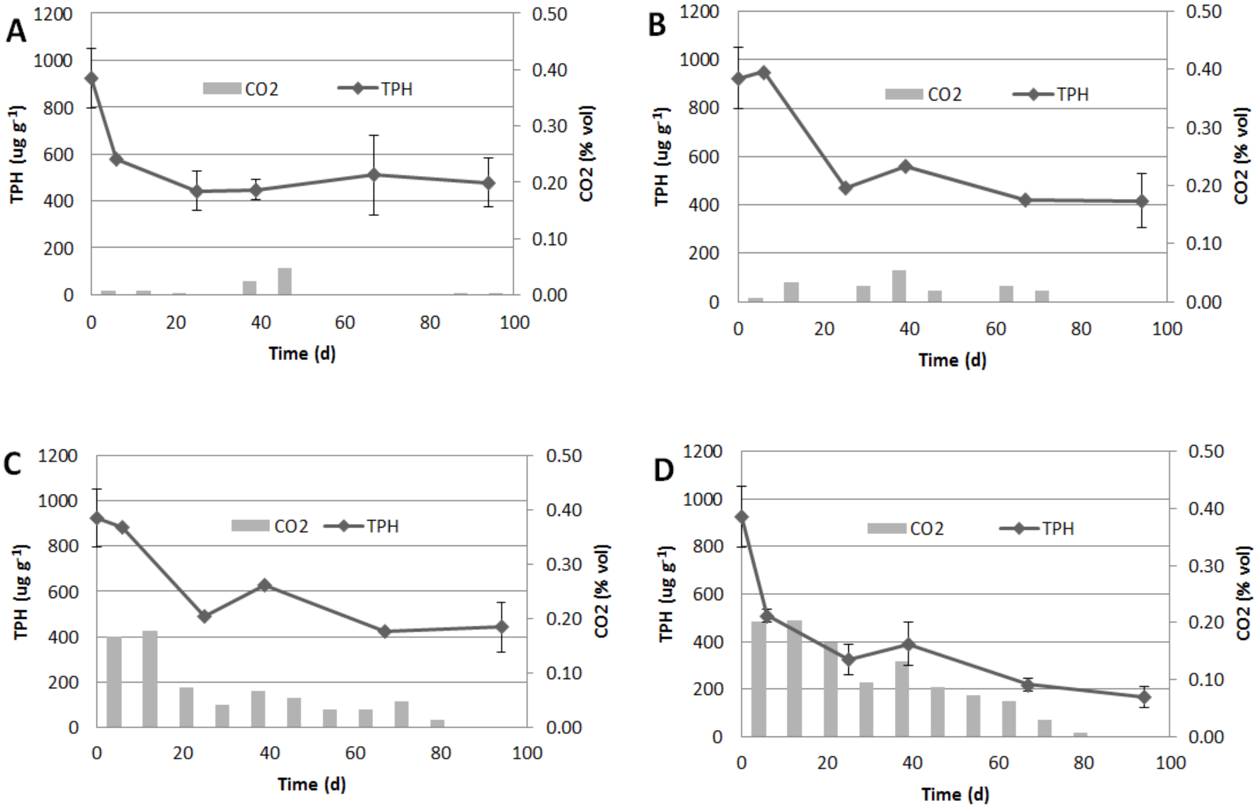

Figure 3.5 Average CO_2 production patterns versus TPHs degradation over time for (A) biopile S, (B) biopile S+M, (C) biopile S+C, and (D) biopile S+M+C

CO_2 concentrations inside the biopiles along with TPHs percentage degradation are shown in Figure 3.5. The results suggested the presence of biological activities inside the biopiles. For setups S+C, and S+C+M, higher CO_2 production patterns were observed compared to setups S, and S+M. Setup S+C+M showed the highest CO_2 production throughout the treatment with a maximum of 0.20 % vol., confirming the successful adaptation of the inoculated bacteria to the inherent conditions in the soil, which improved the efficiency of hydrocarbon degradation. These findings followed and were in agreement with the removal efficiencies, i.e., the higher the removal rate, the higher CO2 production. Chang et al. (2010) observed 49-64% degradation of TPHs in laboratory landfarming biotreatment of petroleum-contaminated soils obtained from a sub-Arctic site in Canada. They reported that the onset of TPH biodegradation was associated with and significant CO_2 production an increase in the hydrocarbon-degrading microbial populations.

3.3.3 Biodegradation rates for total petroleum hydrocarbons and hydrocarbon fractions

The data collected for this study and previous references indicated that TPHs degradation in soil was the result of microbial action, suggesting that degradation obeyed the following first-order kinetic equations:

$$(C)_t = (C)_0 \ e^{-kt}, \quad t_{½} = \ln 2 \ k^{-1}$$

where t is the time, $(C)_0$ is the initial substrate concentration, $(C)_t$ the substrate concentration at time t, and k the degradation rate constant.

Under these conditions, the first-order rate constants and half-lives for the different experimental setup were calculated and shown in Table 3.6 and Figure 3.6. After 94 days of treatment, the first-order degradation rates of TPHs for the experimental setups S, S+M, S+C, S+C+M were 0.004, 0.009, 0.008, and 0.016 day^{-1}, respectively, corresponding to half-lives of 173.3, 77, 86.6, and 43.3 days, respectively. Paudyn et al. (2008) performed an on-site pilot-scale land farming experiment at the Resolution Island site, obtaining first-order TPHs degradation rates from 0.017 to 0.026 d^{-1}. Chang et al. (2010) obtained TPHs biodegradation rates from 0.011–0.018 d^{-1} by stimulating the soil by nitrogen and phosphorous nutrients amendments in pilot scale experiment at the same site. Zytner et al. (2001) reported first-order TPHs biodegradation rate constants in the range of 0.022–0.0043 d^{-1}, from a land farming experiment at a diesel-contaminated northern site. These findings showed similar degradation rates to the experimental setup S+C+M (0.016 day^{-1}), where different strategies have been applied for the biodegradation of petroleum hydrocabons.

Table 3.6 Total petroleum hydrocarbons (TPH) and hydrocarbon fractions degradation rates (k_1) and half-lives ($t_{1/2}$) under different experimental setup conditions

Parameter	Setup Biopiles	Initial (µg g^{-1}dw)	Final (µg g^{-1}dw)	k_1 (days^{-1})	$t_{1/2}$ (days)	R^2
TPH's	S	924 ± 127	478 ± 105^{a}	0.004	173.3	0.32
	S+M		418 ± 10^{a}	0.009	77	0.72
	S+C		442 ± 110^{a}	0.008	86.6	0.72
	S+C+M		166 ± 44^{b}	0.016	43.3	0.86
F1	S	27 ± 4	ND	-	-	-
	S+M		ND	-	-	-
	S+C		ND	-	-	-
	S+C+M		ND	-	-	-
F2	S	455 ± 67	156 ± 25^{a}	0.184	3.8	0.77
	S+M		156 ± 11^{a}	0.240	2.9	0.87
	S+C		147 ± 41^{a}	0.236	2.9	0.89
	S+C+M		33 ± 11^{b}	0.491	1.4	0.97
F3	S	442 ± 62	249 ± 81^{a}	0.079	8.8	0.47
	S+M		262 ± 1^{a}	0.128	5.4	0.62
	S+C		295 ± 69^{a}	0.100	6.9	0.51
	S+C+M		128 ± 32^{b}	0.209	3.3	0.78

The values are expressed as mean ± standard deviation.
ND: Non detectable. R^2: Correlation coefficient
TPHs are the sum of all the fractions.
Values followed by the same letter are not significantly different at $P<0.05$

As shown in Table 3.6, experimental setup S showed the lowest degradation rate with 0.004 d^{-1} for TPHs, 0.184 d^{-1} for F2 fractions, and 0.079 d^{-1} for F3 fractions, respectively, and the highest half-life values of 173.3 d for TPHs, 3.8 d for F2 fractions and 8.8 d for F3 fractions, respectively, during the course of treatment. These were followed by an increasing order of degradation rates and a decreasing order of half-lives for setups S+C, S+M, and S+C+M respectively. These results suggested that the combination of bioaugmentation and biostimulation strategies observed for the setup S+C+M were more effective in promoting and enhancing biodegradation, duplicating the kinetic values obtained for the total petroleum degradation and their fractions compared to the experimental setups with bioaugmentation only (S+M) or biostimulation (S+C) only. The results also showed a considerably higher biodegradation rate of 0.491 d^{-1} for semi volatile (F2) compared to 0.209 d^{-1} for non-volatile (F3) fractions for the experimental setup S+C+M. Similar trend was observed for setups S+M and

S+C, indicating that F2 fraction was degraded at a much faster rate than F3 fraction as expected. It is likely that the degradation rates of the semi volatile (F2) fraction are preferentially promoted because of their bioavailability and lower molecular weight, in contrast to the non-volatile (F3) fraction. Similar results were observed by Børresen et al. (2003), which found that the lighter diesel components in the carbon range of C9–C12 were degraded faster than the heavier components in the carbon ranges of C12–C18 and C18–C30 in liquid cultures maintained at 5 °C. Better correlations were obtained for F2 fractions (0.77-0.97) compared to F3 fractions (0.47-0.78) for the different experimental setups conditions, suggesting a better approach to the first order kinetic equation for F2 fractions.

Figure 3.6 shows the F2 and F3 fractions as well as target provincial standards for different biopiles. As seen, only in the case of setup S+C+M, both fractions were reduced to concentration below provincial standards (Ontario Regulation 153, Table 2-Residential), which are 80 ppm for F2 fractions and 300 ppm for F3 fractions, respectively. And this happened after only 40 days of treatment. However, experimental setups S, S+M, and S+C did not meet these criteria, with concentration values still above standards for F2 fractions, and partially below standards for F3 fractions, along the treatment. Moreover, concurrent biodegradation of F2 and F3 fractions were observed (Fig. 3.6) along the 94 days of treatment for the different experimental setups, which were similar to previous findings by Chang et al. (2010), whose study of landfarming biotreatment of petroleum contaminated soils in a sub-Arctic site observed a concurrent and extent biodegradation of semi volatile and non-volatile fractions over a 60 days treatment period. Other reports suggested that there was a sequential degradation of lower molecular weight petroleum hydrocabons followed by more persistent, higher molecular weight hydrocabons. Sanscartier et al. (2009a) performed laboratory studies of artic hydrocabons contaminated soil at 22 °C and found that the degradation of F3 hydrocarbon fraction occurred after F2 hydrocarbon fraction was depleted. However, the behaviour of hydrocarbon degradation in contaminated soil could be affected by bioavailability of the fractions and characteristics and interactions of the microorganism community (Chang et al., 2010).

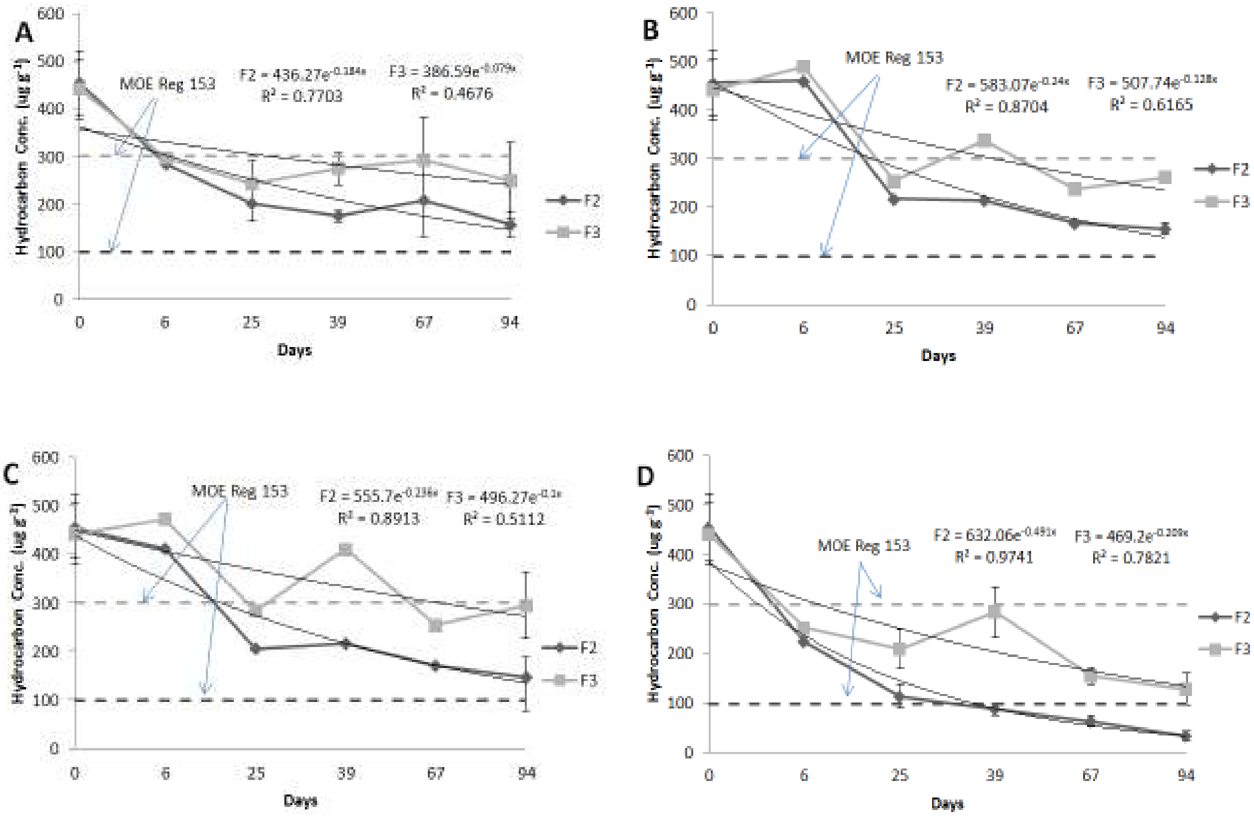

Figure 3.6 Biodegradation rates of F2 and F3 hydrocabons fractions over 94 days treatment period for (A) biopile S, (B) biopile S+M, (C) biopile S+C, and (D) biopile S+M+C

3.4. CONCLUSIONS

This study has demonstrated that the combination of microbial consortium and mature compost (10:1 compost to soil ratio) as bioaugmentation and biostimulation methods was an effective strategy to enhance the degradation of petroleum hydrocarbon contaminated sandy soil under cold weather conditions and a removal efficiency of 82% at field scale over 94 days was observed. This compared to 48% TPHs removal observed for the control biopile (Setup S), 52% for individual application of biostimulation (Setup S+C), and 55% for individual application of bioaugmentation (Setup S+M) strategies. Statistical analysis of results showed that there was no significant difference between the application of individual treatment (S+C or S+M) and the control treatment in terms of TPHs removals, however, the combined application (S+C+M) was significantly different from the control as well as individual treatments.

CO_2 production and presence of hydrocarbon degraders confirmed the adaptability of microorganism to the environmental substrate, suggesting hydrocarbon biodegradation or mineralization as the major mechanism in the treatment setups. Internal reactions in combination with black insulation plastic membrane may help preserve the temperature above freezing for the different treatment setups. Oxygen and moisture were not limiting factors along the treatment period, which helped to promote the microbial activity.

Experimental setup S+C+M experienced significantly higher degradation rates for TPHs, F2 and F3 fractions, showings 0.016 day^{-1}, 0.491 d^{-1}, and 0.209 d^{-1} degradation rates, respectively. In addition, the final concentrations obtained for F2 and F3 fractions complied with provincial regulations (Ontario Regulation Table 2: Residential) after only 40 days of treatment, allowing the soil re-use for other purposes.

Higher kinetic rate was observed for F2 compared to F3 fractions, as bioavailability and molecular weight influenced a preferential pathway for organic degradation. Concurrent biodegradation of F2 and F3 fractions were consistent with previous findings in cold weather conditions, where biodegradation played an important role in the remediation process.

The information obtained from this study indicated successful use of bioremediation strategies to biodegrade hydrocabons contaminants in ex-situ remediation projects. However, further research with different types of soil matrix at field scale still needs to be studied to assess application of these strategies under cold weather environment. Thus, in contrast to landfill and destructive treatment methods, such as incineration, the use of mature compost and consortium inoculation for bioremediation in cold weather promotes soil sustainability and re-use of petroleum hydrocabons contaminated soil.

3.5 ACKNOWLEDGEMENTS

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CHAPTER 4

TECHNICAL PAPER II

OPTIMIZATION OF FIELD SCALE BIOPILES FOR BIOREMEDIATION OF PETROLEUM HYDROCARBON CONTAMINATED SOIL AT LOW TEMPERATURE CONDITIONS BY RESPONSE SURFACE METHODOLOGY (RSM)[2]

Francisco Gomez, Majid Sartaj

Abstract –. Ex-Situ Bioremediation has been increasingly viewed as an appropriate remediation technology for hydrocarbon contaminated soils under cold climates conditions in countries like Canada. A response surface methodology (RSM) based on a factorial design was performed to investigate and optimize the effects of the microbial consortia application rate and amount of mature compost amendment on the TPH removal (964 $\mu g\ g^{-1}$ initial concentration). 18 field-scale biopiles (16 m^3 each) were constructed, maintained and subjected to different microbial consortium and mature compost application rates under cold climate conditions over a period of 94 days. TPHs removal rates in the range of 74 - 82% was observed in the treatments setups where mature compost and microbial consortia were used simultaneously, compared to an average 48% of TPHs removal in control setup.

The interaction between these two factors were studied and modeled using a statistical regression model, which showed that the microbial consortia application rate, the mature compost amendment and their interactions had a significant effect on TPHs degradation with a coefficient of determination (R^2) of 0.88. Furthermore, using a numerical optimization approach, the optimum rates predicted via RSM were estimated at 4.1 mL m^{-3} and 7% for microbial consortia and compost application rates to obtain a maximum TPH removal of 90.7%.

Keywords**:** Bioremediation, Petroleum Hydrocabons, Compost, Cold Weather, Biopiles, Consortia, Factorial design, Response surface methodology

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[2] *This paper has been accepted for publishing in the International Biodeterioration & Biodegradation Journal.*

4.1 INTRODUCTION

Contamination by industrial activities accounts for about 25% of the major Canadian urban landscape, with an estimate of 20-30 thousands brownfield sites across the country (De Sousa, 2001). Valuable real estate were often undervalued and underused in the past mainly due to considerable associated clean-up costs, representing a huge problem for the local economic development and potential reuse of the contaminated sites. However, recently there has been an increasing interest in redeveloping these sites as they are often located in the core sections of metropolitan areas and are prime candidates for urban development. Across the country, public and private sector are now using innovative policies and programs from local and provincial governments to overcome the value to remediate and redevelop contaminated sites; improving the local economic, the environment and the social benefits in a long term vision.

In Canada, approximately 60% of contaminated sites involve petroleum hydrocarbons (PHCs) contamination (Sanscartier et al., 2009). Current oil prices and growing demand have also resulted in high generation rates of oil, and subsequently an increase in oil spills around the world and in Canada. PHCs have been classified as priority environmental pollutants by the US environmental protection agency, due to their impact on human health and environment, representing a huge concern for the general public (ATSDR, 1999). As a result, government and private sectors are always in the search to develop new technologies and methods to minimize or mitigate the risks. Bioremediation is a cost-effective and environmental friendly remediation technologies that can be utilized for PHCs contaminated soils.

Bioremediation can be defined as an engineered process that uses biochemical mechanism in organisms to degrade environmental pollutants in soil, and transform them into less complex and harmless end products such as carbon dioxide and water (Coulon et al., 2010). Ex-situ bioremediation through biopiles have been considered as a feasible, cost effective and less destructive remediation technique for petroleum contaminated soils (Mohn and Stewart, 2000). Its use has been widespread over North America for the rehabilitation of different sites impacted by petroleum hydrocarbons. Biopiles can be defined as an above ground engineered system, heaping contaminated soils into piles and stimulating aerobic microbial activity by providing oxygen, nutrients and/or microbial consortia to degrade petroleum compounds adsorbed to the soil (Khan et al., 2004; Juwarkar et al., 2010; US EPA, 2012).

Although cold conditions delay bioremediation of oil hydrocarbons, bioremediation is increasingly viewed as an appropriate remediation technology for hydrocarbon contaminated soils in cold climates (Sanscartier et al., 2009; Thomassin-Lacroix et al, 2002). Margesin and Schinner (2001) denoted that even in cold regions such the Arctic and Antarctic regions, bioremediation has demonstrated to be a cost effective and non-disruptive method for contaminated soil with petroleum hydrocarbons.

Kauppi et al. (2011) investigated different biostimulation and bioaugmentation strategies for the bioremediation of diesel oil contaminated soils under cold conditions including the addition of nutrients and bulking agents, aeration, and the use of microbial inocula. They concluded that in addition to delay due to cold conditions there were other bottlenecks affecting the outcome of bioremediation. The results showed that efficient oil degradation did not depend only upon the soil microbial community and bioaugmentation alone had no additional effect; but biostimulation via optimization of nitrogen and oxygen supply could significantly improve bioremediation of oil-contaminated soil by optimizing the conditions for the continuous growth of the consortium. They reported that diesel-fuel degradation was accelerated when both nutrients and wood chips were added into contaminated boreal soil but that neither one alone made a difference and the microbial growth increase was observed when the C:N relationship was optimized. Other studies have demonstrated that fuel degradation was enhanced if nutrients and proper oxygen conditions were available (Walworth et al., 2007; Chang et al., 2010). Beškoski et al. (2011) observed that bioaugmentation in combination with aeration and biostimulation enabled the successful remediation of contaminated soil by mazut (heavy residual fuel oil) at field scale. During the 5 months of treatment, the total petroleum hydrocarbon (TPH) content of the contaminated soil was reduced to 6% of the initial value, from 5.2 to 0.3 g kg^{-1} dry matter, while for the untreated control pile the TPH was reduced only by 10%. Similarly, Thomassin-Lacroix et al. (2002) demonstrated a successful bioremediation of diesel-fuel in Artic contaminated soil by removing 83% of TPH over a period of 65 days in small-scale biopiles at low temperatures, using bioaugmentation, with an enrichment culture from the same site, and biostimulation by fertilization with phosphorous and nitrogen.

Some research studies have reported the use of compost as a source for providing nutrients to support and enhance bioremediation of organic wastes. Megharaj et al. (2011)

suggested that the addition of compost stimulated the microbial growth by supplementing nutrients and carbon source, and thus enhanced the rate of organic pollutants degradation. Namkoong et al. (2002) added sewage sludge or compost as an amendment to the contaminated soil in different ratios, obtaining the best result at a mix compost ratio of 1:0.5, with 98.5% of TPH degradation and a kinetic value of 0.113 day^{-1} for diesel oil, which was spiked at 10,000 mg kg^{-1} sample on a dry weight basis in a lab scale reactor.

Factorial experimental design and response surface methodology (RSM), as a statistical analysis approach, is an efficient and widely used methodology to analyze, compare and optimize the simultaneous application of different factors or treatment technologies in bioremediation process (Sharma et al., 2009). Zahed et al. (2010) optimized nitrogen and phosphorous concentration via RSM for removal of n-alkanes from crude oil contaminated seawater samples in batch reactors. Numerical optimization predicted 98% removal over a 20 day period in lab conditions, using nitrogen and phosphorous concentrations of 13.62 and 1.39 mg L^{-1}, respectively. Mohajeri et al. (2010) employed RSM to optimize oil concentration biomass, nitrogen and phosphorous concentrations in bioremediation of weathered crude oil sediment samples during 60 days trial, obtaining 83% removal under the optimum conditions.

There is limited existing information on the performance of biopile bioremediation of petroleum hydrocarbons and its optimization especially at field scale and under cold climate conditions. Therefore, the present study aims to evaluate the effect of different operational factors (microbial consortia application rate and mature compost amendment) on the bioremediation of petroleum contaminated soils and to optimize the process under cold conditions and at field scale using a factorial design and RSM. By determining the best possible set of parameters or factors it would be possible to achieve a higher biodegradation rate using the minimum rates of application of microbes and compost. For this purpose, field-scale biopiles were constructed and subjected to different treatments based on an experimental design to study the effect of different microbial application rate and mature compost amendment in a soil treatment facility at Moose Creek, Ontario, Canada from November 2012 to February 2013.

4.2 MATERIALS AND METHODS

4.2.1 Materials

Excavated soil from a contaminated site, in Val-des-Bois, in the Outaouais region of Quebec, Canada, containing petroleum hydrocarbons were hauled and transported to a treatment facility in Moose Creek, Ontario, Canada during October 2012. The details of the site are described elsewhere (Gomez and Sartaj, 2013). The contaminated soil was segregated, screened and stockpiled in a paved area for further analysis and treatment.

The soil was characterized and classified as sand (86.4%), based on 5 random composite samples and according to the USCS classification system (Table 4.1). Petroleum hydrocarbon analyses were conducted in accordance to accredited lab standard procedures, following Canada-Wide Standard for Petroleum Hydrocabons in soil (CCME, 2001). The TPH analysis indicated that F2 (>C10-C16) and F3 (>C16-C34) fractions accounted for almost all (96%) of extractable TPH compounds in the contaminated soil.

Table 4.1. Physical-Chemical characteristics of petroleum contaminated soil

Parameter	**Value**
pH @ 25°C	7.79 ± 0.02
Grain Size Sieve Analysis [1]	
Gravel (>2.00 mm) (%)	10.3
Sand (0.075 - <4.75 mm) (%)	86.4
Silt and Clay (<0.075 mm) (%)	3.3
Total Petroleum Hydrocarbons (μg g^{-1} dw) [2]	924 ± 127
F1 - Volatile Fractions (μg g^{-1} dw)	27 ± 4
F2 – Semi-Volatile Fractions (μg g^{-1} dw)	455 ± 67
F3 – Non-Volatile Fractions (μg g^{-1} dw)	442 ± 62

[1] Soil classified as sand, according to the USCS classification system.
[2] Based on Canadian Wide Standards Tier 1 Method. TPHs are the sum of all the fractions
Uncertainties are one standard deviation from the mean (n=5).

Mature compost used in this study was obtained from a local composting facility processing municipal solid waste. The compost characteristics are presented in Table 4.2. The microbial consortium used for the experiment was a commercial liquid product that contained a concentrated blend of bacteria strains native to Canada, non-pathogenic, and strains are identified by Environment Canada on its “List of Organisms on the Domestic Substances List”. The microbial consortium was mixed with water at 0.05% v/v dilution rate, and sprayed manually with the contaminated soil.

Table 4.2. Characteristics of mature compost

Parameter	**Value**
pH @ 25°C	7.00
Moisture content (%)	44.7
Total phosphorous ($\mu g\ g^{-1}$)	2740
Total Kjeldahl nitrogen ($\mu g\ g^{-1}$)	17800
C/N ratio	46.7
Organic matter (LOI@550) (% by wt.)	73

4.2.2 Experimental Design

Bioremediation of petroleum contaminated soils were investigated by construction, operation and maintenance of 18 field-scale biopiles under cold climate conditions over a period of 94 days. Biopiles were 4 m x 4 m x 1 m (16 m^3) and were constructed, maintained and subjected to a different microbial consortium rate and mature compost concentration based on a pre-defined three level factorial design (Table 4.3). Treatments investigated included only soil (S) as control, soil and compost (SC) to assess the individual effect of compost addition, soil and microbial consortium (SM) to assess the individual effect of microbial consortium addition, and soil plus compost and microbial consortium (SCM) to assess the combined effect of compost and microbial consortium addition. The numbers refer to the application rate of compost and/or microbial consortium.

Table 4.3. Experimental setup configurations for ex-situ biopiles

Setup	**Treatment**	
	Microbial Consortium Application Rate (mL m^{-3})	Mature Compost Amendment (%)
S[1]	-	-
SM6[1]	6	-
SC10[1]	-	10
SC5M3[2]	3	5
SC10M3[2]	3	10
SC5M6[2]	6	5
SC10M6[2]	6	10

[1] Duplicated biopiles were constructed.
[2] Triplicate biopiles were constructed.

All biopiles were located over an asphalted surface that included a synthetic geotextile membrane to reduce potential leachate migration to the subsurface environment. If applicable, the contaminated soil was mixed with compost by a front end loader 10 times to obtain a uniform substrate. Then the soil mixture was laid in layers of 30 cm height by an excavator, over a perforated pipe at the base of each biopile. After placing each layer of the soil the microbial consortium solution was sprayed on top. The process was repeated until the desired height was reached.

A low airflow injection rate of 30 m^3 hr^{-1} per biopile was supplied by regenerative blower system (Regenair R7100r-50, Gast) through a perforated piping network installed at the base of the biopiles to provide adequate O_2 levels. After their construction, the biopiles were covered by a 5 mm black membrane (Premium Silage 50158) to protect the biopiles from erosion and to maintain the proper moisture and temperature necessary for bacterial growth during both stages (See Appendix B).

Soil gas was sampled from monitoring wells of 51 mm diameter PVC piping installed 1 m below surface and backfilled with sand in each biopile. The soil gas was analysed for O_2 and CO_2 using a portable gas analyser (Eagle, RKI) instrument on site. In addition, soil temperature and moisture content were monitored using portable probes (Reotemp). For the current study, oxygen levels and moisture content were maintained between 16-21% and 30-50%, respectively, considered within the range of optimum condition to promote microbial activity and efficient hydrocarbon degradation (Trostky and Pal, 1998, Walworth et al, 2007).

At the start of the experiments, 5 random composite samples were collected and analysed to characterize the contaminated soil. Further, composite soil samples from each biopile were collected by pooling and homogenizing 6 sub-samples at various depths following a predefined schedule. A slide hammer type sampler device was used to extract the soil core. The core sampling device was pressure washed and cleaned with distilled water between biopiles to avoid cross contamination. The soil samples collected were immediately placed in an airtight 250 ml glass jar container to prevent volatilization and photodegradation. The jar containers were then immediately placed into a cooler to preserve the samples at 4 °C and transported to certified laboratories by Canadian Association for Laboratory Accreditation (CALA) the same day or within a period of 24 hours. Field blanks and duplicate samples were collected through the monitoring program to provide a check on sampling and analytical reproducibility. Additionally, the laboratory followed their own QA/QC analytical procedure, using blank, duplicate and spike methods. The collected samples were analysed for petroleum hydrocarbon (F1-F4) based on Canadian Wide Standards Tier 1 method (CCME, 2001), pH, total heterotrophic count and hydrocarbon-degrading microbial population counts analysis by a most probable number (MPN) method (Foght and Aislabie, 2005). Photos of the biopiles under operation have been presented elsewhere (Gomez and Sartaj, 2013).

4.2.3 Statistical method and data analysis

Factorial design and response surface methodology were used for experimental design and to evaluate and optimize bioaugmentation and biostimulation strategies to maximize TPH removal (Y_1) as the outcome response. A 2^3 factorial design with microbial consortia application rate (X_1) and mature compost amendment (X_2) as the independent variables were used. They were coded at three levels between -1 and +1. The ranges of the individual factors were chosen based on preliminary studies and previous experience and are presented in Table 4.4. Each setup was placed randomly and was carried out in duplicate or triplicate (see Table 4.3), with the purpose of obtaining a reliable estimate of the effects, plus to reduce the noise and bias for the outcome response.

Table 4.4. Experimental design and the levels of independent process variables

Independent Variable	**Symbol**	**Coded levels**		
		-1	**0**	**1**
Microbial consortia application rate (mL m^{-3})	X_1	0	3	6
Mature Compost mixing ratio (% v/v)	X_2	0	5	10

The statistical software MINITAB® v. 16.1.0 (Minitab Inc., Pennsylvania, USA) was used for statistical analysis of experimental data by response surface methodology of TPH removal (Y_1) as the dependent variable. TPH removal was measured as the percentage of removal in accordance with the following equation:

$$Y\,(\%) = \frac{C_o - C_t}{C_o} * 100 \qquad \text{(Eq. 4.1)}$$

where C_0 and C_t are the initial and final TPH concentrations. The behaviour of the system is explained by the following quadratic equation (Krishnaprasad and Srivastava, 2009; Montgomery, 2013), where A_0 represent the value of the fixed response at the center point of the design; A_i, A_{ii}, A_{iii} represents the linear, quadratic and interaction effects regression terms, respectively; x_i denotes the level of the independent variable; n is the number of independent variables; and ε is the random error.

$$Y = A_0 + \sum_{i=1}^{n} A_i\, x_i + \sum_{i=1}^{n} A_{ii}\, x_i^2 + \sum_{i \neq j=1}^{n} A_{ij}\, x_i x_j + \varepsilon \qquad \text{(Eq. 4.2)}$$

4.3 RESULTS AND DISCUSSION

4.3.1 Bioremediation of TPHs under cold weather conditions

Outdoor air temperatures were fluctuating between 10.2 and -25.6 °C, with an average temperature of -6.2 °C (Environment Canada, 2013) during the period of experiments. Internal soil temperatures within the biopiles were maintained above freezing conditions, with temperatures between 1 - 8 °C. Previous research has demonstrated that biodegradation of petroleum contaminated soil is still feasible, and temperature conditions below 10 °C would not affect the ability of microorganism to degrade organic contaminant in soil as a substrate (Coulon et al., 2005; Sanscartier et al., 2009).

Figure 4.1 shows the average TPH removals for different setups. During the early stage (within 4 weeks), a significant TPH removal was observed in all treatment setups. Following the rapid descend, TPH residual was removed gradually upon the treatment setups, with a higher rate in biopiles SC5M3, SC10M3, SC5M6 and SC10M6 (combination of bioaugmentation and biostimulation strategies) in contrast to the control piles S, SC10, and SM6. For the same treatment setups, significant CO_2 production patterns were observed compared to the control piles, suggesting a higher microbial activity and better adaptability to the substrate, following the addition of microbial consortia and mature compost amendment (Chang et al., 2010). As seen in Figure 4.1, TPHs removal reached values in the range of 74 - 82% in the treatments setups where mature compost and microbial consortia were used simultaneously. In control setup (S), the biopile only reached an average 48% of TPHs removal.

These results indicate that the addition of microbial consortia, with 3 or 6 mL m^{-3}, in combination with mature compost amendment at 5% or 10% under cold temperature conditions increased the removal percentage and rate of biodegradation over a 94 days period, in comparison with the control pile with no amendments or with compost or microbial consortia only. The results are in agreement with observations reported by Kauppi et al. (2011), which showed that the combined use of microbial consortia and nutrients plus aeration would achieve higher biodegradation of hydrocarbons. Beškoski et al. (2011) also observed that bioaugmentation in combination with aeration and biostimulation enabled the success of the remediation of contaminated soil by mazut (heavy residual fuel oil) at field scale by removing 94% of TPH compared to only 10% for the untreated control pile over a 5 month period. Adenkule (2011) also demonstrated the efficiency of composted municipal organic waste on degradation of soil polluted with diesel, obtaining up to 69 % of degradation with compost to soil ratio varied between 4:1 to 14:1 (w/w). Namkoong et al. (2002) added sewage sludge or compost as an amendment to the contaminated soil in different ratios, obtaining the best result at a mix compost ratio of 1:0.5, with 98.5% of TPH removal and a kinetic value of 0.113 day^{-1} for diesel oil, which was spiked at 10,000 mg kg^{-1} sample on a dry weight basis in a lab scale reactor.

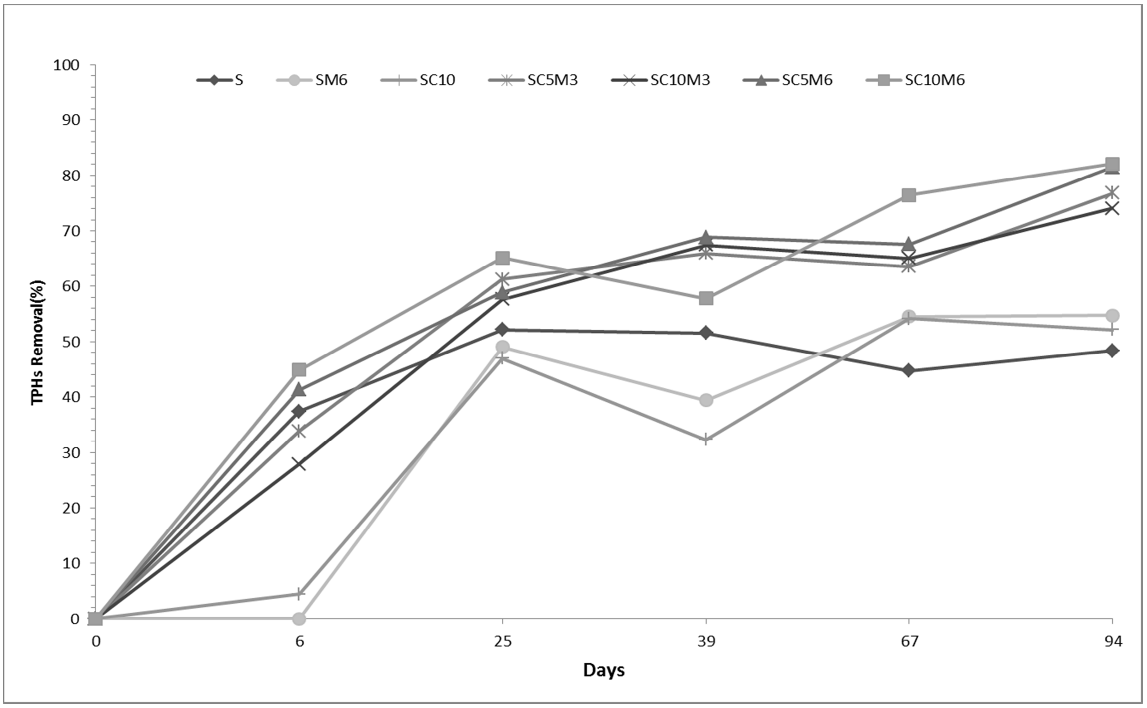

Figure 4.1. Average TPHs percentage removal for different experimental setups under cold temperature conditions over a period of 94 days

The different treatment setups were also tested for total heterotrophic count and hydrocarbon-degrading microbial population counts. Overall, the results showed culturable heterotrophs within 10^7 to 10^{10} CFU g^{-1} and hydrocarbon degraders counts between 4.5 and 6 LogMPN g^{-1} during the experimental period (Figure 4.2). Also, it can be seen that treatment setups with bioaugmentation had higher hydrocarbon degraders counts confirming the activity and adaptability to the environmental conditions of the inoculated consortia.

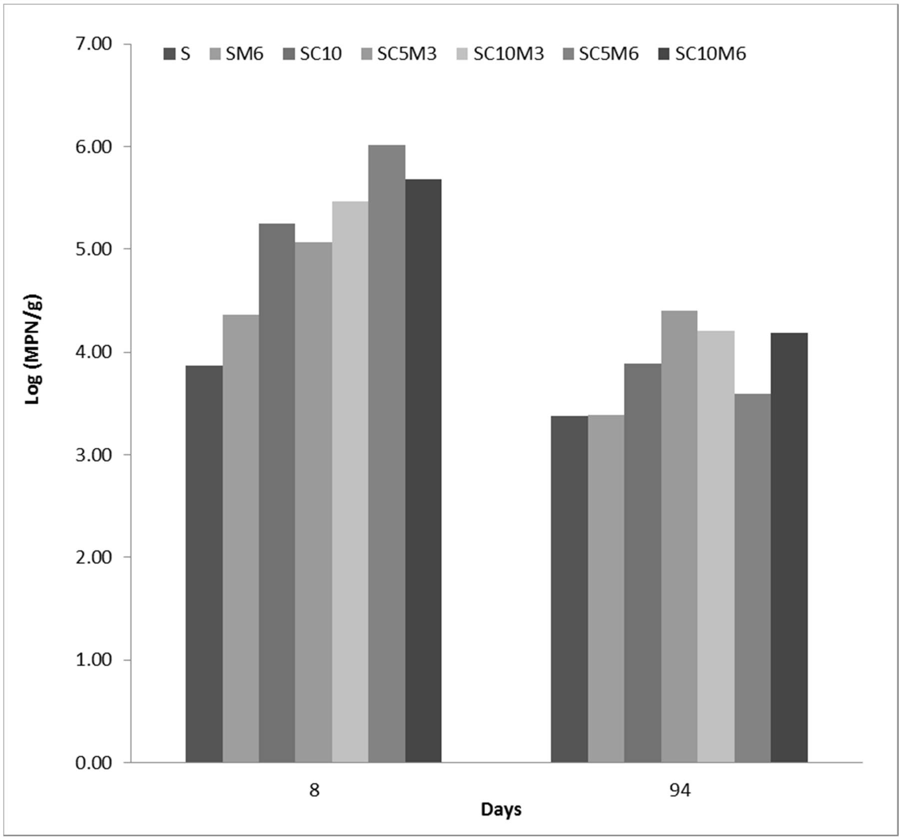

Figure 4.2. Average hydrocarbon degraders counts for different experimental setups

4.3.2 Statistical analysis and modelling

After performing 18 runs, based on a factorial design of two independent variables at three levels, the experimental results for TPH percent removal (Y1) are summarized in Table 4.5. The collected data was initially analyzed using statistical analytical techniques to detect any outlier and unreliable data. As a first step, regression analysis was used to find the best-fit model through the data. The error or residual was calculated as the difference between the predicted and the observed values and then converted to standardized residual for each data point by dividing by the standard error or deviation of the data. Approximately 95% of the standardized residuals should lie between -2 and 2. Data points with an absolute value of standardized residual greater than 2 could be considered as outlier and excluded from the matrix data. Two out of 18 observed values were identified as outliers and eliminated. The remaining results are presented in Table 4.5.

The response Y_1 was fitted with the second order polynomial equation (Eq. 4.3) in terms of coded factors and regression coefficients given in Table 4.6.

$$Y_1 = 87.61 + 9.07\,X_1 + 7.82\,X_2 - 14.75\,X_1^2 - 13.35\,X_2^2 + 5.42\,X_1X_2 \qquad \text{(Eq. 4.3)}$$

The statistical significant of the model equation was evaluated by the F-test in the analysis of variance (ANOVA), see Table 4.6. ANOVA results denoted that this quadratic model is significant at 95% confidence level ($P<0.05$). The same statistical analysis also indicated that the model parameters and their interactions were significant ($P<0.05$). The lack of fit compares the residual error to the pure error from replicated experimental design points. The p-value greater than 0.05 implies the lack of fit is insignificant relative to the pure error; there is 84.1% chance that the lack of fit occurs due to noise or random error, i.e. there was no lack of fit of the model.

Table 4.5. Experimental matrix design and results for TPH removal (%)

Run	**Factors**		**TPH removal (%), Y_1**		
	Microbial Consortia application rate (mL m^{-3}), X_1	**Mature Compost amendment (% v/v), X_2**	**Observed**	**Predicted**	**Residual**
1	0	0	56	47.8	8.2
2	0	10	61	52.7	8.3
3	3	5	82	87.6	-5.6
4	3	10	87	81.9	5.1
5	6	0	54	55.2	-1.2
6	6	5	88	81.9	6.7
7	6	10	77	81.6	-4.6
8	0	0	40	47.8	-7.8
9	0	10	44	52.7	-8.7
10	3	5	92	87.6	4.4
11	3	10	78	81.9	-3.9
12	6	0	56	55.2	0.8
13	6	5	80	81.9	-1.9
14	6	10	89	81.6	7.4
15	6	5	79	81.9	-2.9
16	6	10	78	81.6	-3.6

Table 4.6. Analysis of Variance (ANOVA) for RSM quadratic model parameters

Source	DF	Sum of Squares	F-value	Prob>F	Contribution (%)	Remarks
Model	5	3732.73	14.79	0.000		Significant
X_1	1	754.10	14.94	0.003	24.4	Significant
X_2	1	560.61	11.11	0.008	18.1	Significant
X_1^2	1	523.46	10.37	0.009	17.0	Significant
X_2^2	1	490.28	9.71	0.011	15.9	Significant
X_1*X_2	1	257.78	5.11	0.047	8.3	Significant
Residual Error	10	504.71	-	-	16.3	
Lack of Fit	1	2.38	0.04	0.841		Not Significant
Pure Error	9	502.33	-	-		
Total	15	3090.94	-	-		

DF = degrees of freedom

Contribution of the individual factors and their interactions within the model are important to understand the role and influence of each of them and to control and optimize the bioremediation process. With the purpose to determine the factors that have the greatest influence over the system response, ANOVA was used to calculate the percentage contribution of each factor and are shown in Table 4.6. Considering that all the factors are statistically significant at 95% confidence limit, the percentage of contribution for each individual factor was calculated by the ratio of adjusted sum of squares of each factor to the total sum of squares.

Among all the factors considered, the individual factors microbial application rate (X_1) and compost amendment (X_2) were the most influential within the model, accounting for 24.4 % and 18.1 %, respectively; followed by the quadratic factors (17%, 15.9%) and by the interaction factor between the independent variables (8.3%). By studying the main effects and contribution of each factor, the process could be characterized, thus the level of factor to produce the best results could be predicted (Venkata et al., 2009).

The coefficient of determination (R^2) measures the proportion of total variability explained by the model, i.e provides a measure of how well observed outcomes are replicated by the model. Figure 4.3 shows the plot of predicted versus observed values for TPH percent removal. For the TPH removal, the coefficient of determination (R^2) obtained was 0.88, indicating a high correlation between the observed and the predicted values for this model. This also implies that 88% of the sample variation is explained by the model.

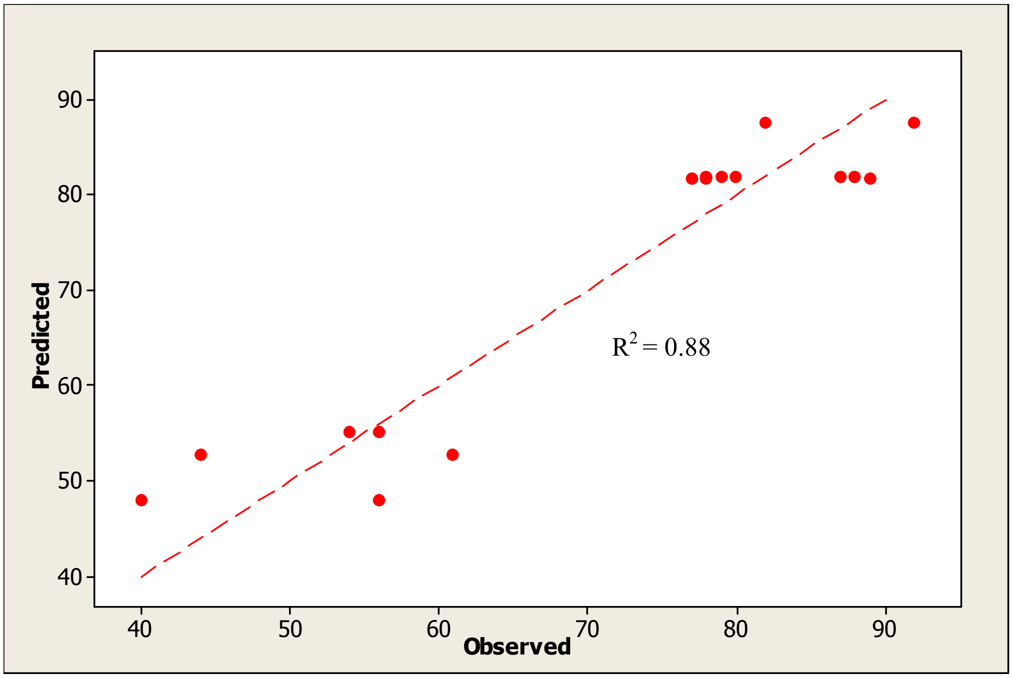

Figure 4.3. Predicted versus Observed values for TPH removal

Figures 4.4 and 4.5 show different diagnostics plots to ensure that the selected model is providing an adequate approximation to the real system. Figure 4.4 presents a reasonably good fit of the standardized residuals versus normal probability percentage, confirming that the statistical assumptions suit the analytical data. Figures 4.5a and 4.5b show standardized residual versus runs and predicted values, respectively, which revealed no obvious pattern, as the observed runs were scattered randomly within the range of residuals (-2 , 2) across the graph, confirming that the model is adequate and there is no reason to suspect any violation of the independence or constant variance assumption in all runs.

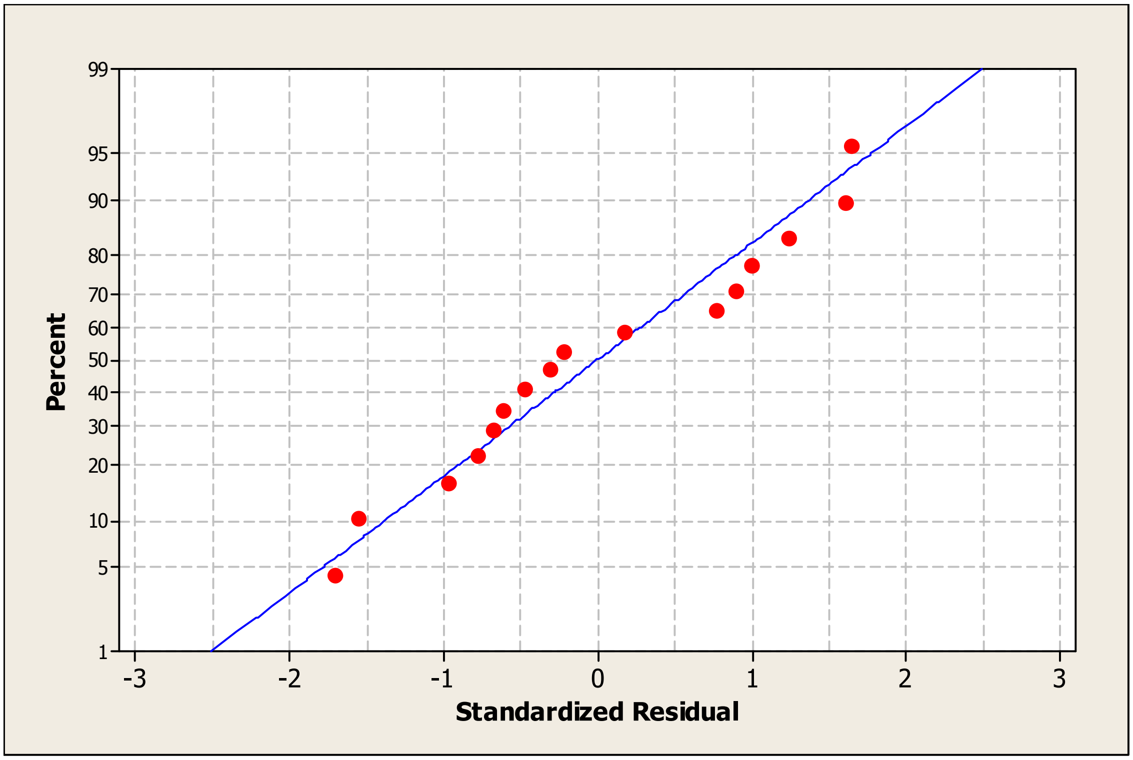

Figure 4.4 Normal probability of residuals for TPH Removal

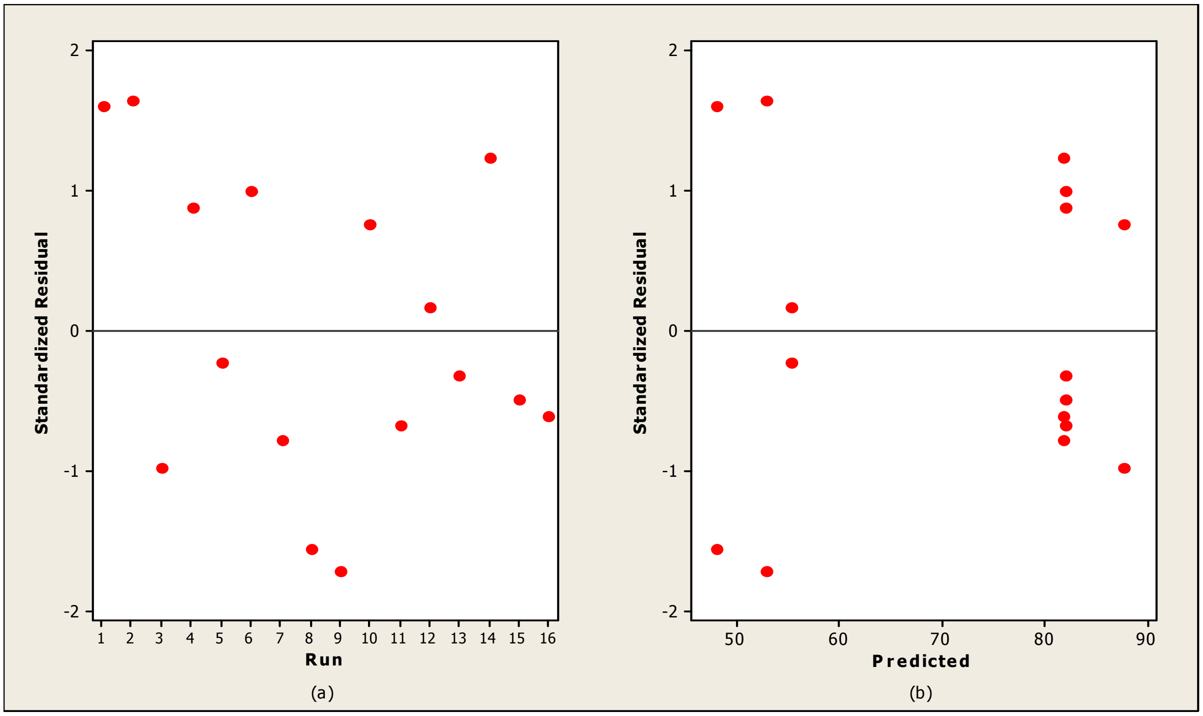

Figure 4.5. Diagnostic plots for TPH Removal (a) standardized residual versus runs, and (b) standardized residuals versus predicted

4.3.3 Response Surface Methodology

Response surface plots were used to graphically represent the quadratic model equation. A three-dimensional surface plot and two-dimensional contour plot, in Figures 4.6 and 4.7, respectively, show the effect and interaction of microbial consortia application rate and compost amendment at different levels on biodegradation of TPH. It is comprehensible from the figures that TPH degradation increased substantially, when the microbial consortia inoculation and compost application rates increased. The optimum values for microbial consortia application rate and mature compost amendment to maximize TPHs degradation appears to be approximately 0.5 (4.5 mL m^{-3}), and 0.4 (7%) in coded values, respectively. A significant synergistic interaction between these two independent variables was exhibited to success in the removal of TPHs after 94 days of treatment, in contrast to the control setups. Similar observations were also denoted by Beškoski et al. (2011), who removed 94% of TPH with combination of bioaugmentation, aeration and biostimulation strategies in mazut contaminated soil, and Kauppi et al. (2011) and Thomassin-Lacroix et al. (2002), who demonstrated that effective oil degradation did not depend only upon the bacterial community and bioaugmentation alone had no significant effect; but the synergistic relation between bioaugmentation and biostimulation via nutrients amendments and oxygen supply significantly improved the bioremediation of oil-contaminated soil, even at low temperatures.

From Figures 4.6 and 4.7, it can also be observed that excess amount of microbial consortia and compost, beyond the optimum values, did not positively enhance TPHs removal. It has been reported that the excess of carbon and nutrient source could inhibit the microbial activity for degrading the contaminants (Mohn and Stewart, 2000; Walworth et al, 2007, Namkoong et al, 2002). Furthermore, an excess of nutrients will also increase the cost of bioremediation and might cause an increase of leaching.

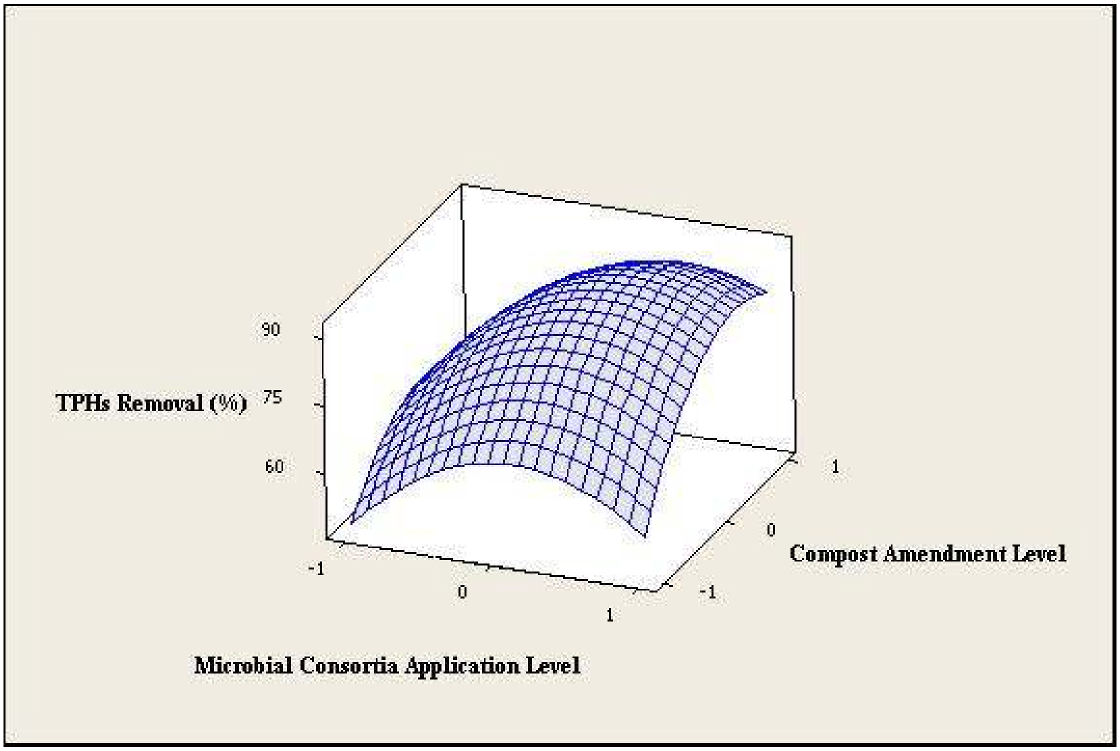

Figure 4.6. Three-dimensional surface plot by Response Surface Methodology

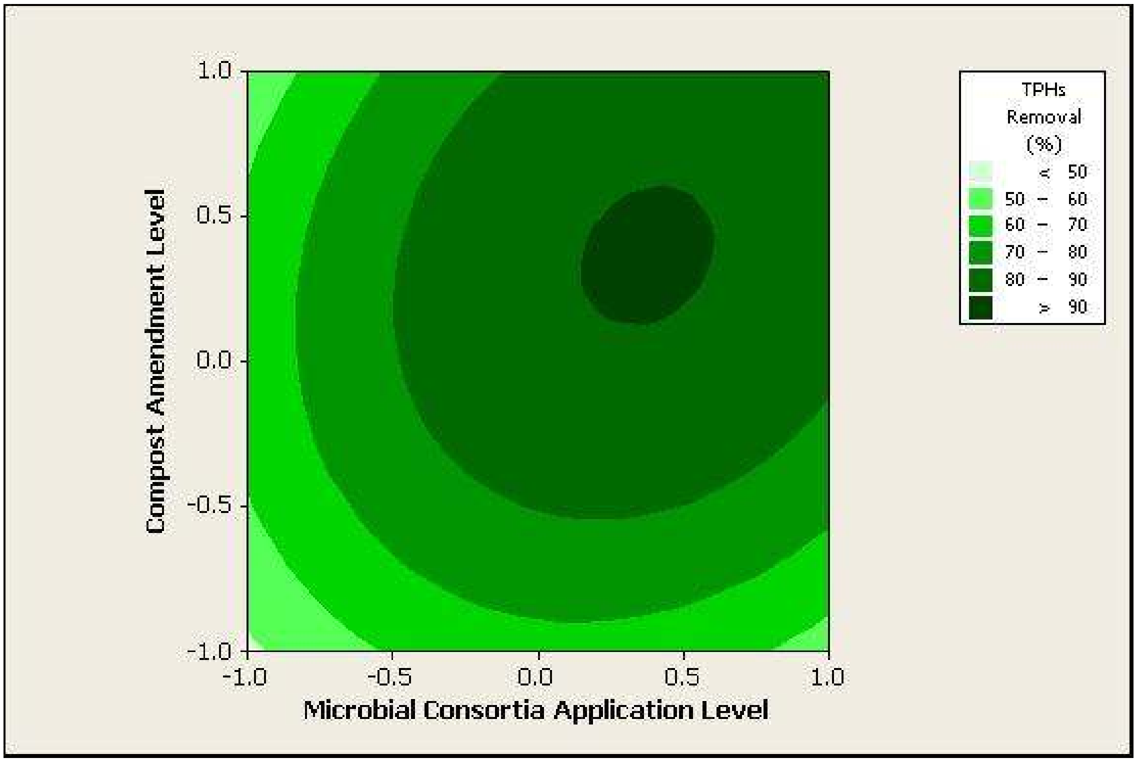

Figure 4.7. Two-dimensional Contour plot by Response Surface Methodology

A numerical optimization approach based on desirability function was performed on Minitab v. 16 to maximize TPH removal using microbial consortia application rate and compost amendment as independent variables. The independent variables were set in a coded range between -1 to +1. The optimum conditions were predicted as 4.1 mL m^{-3} for microbial consortia application rate and 7% for mature compost amendment to obtain a maximum TPHs removal of 90.7%.

4.4. CONCLUSIONS

A factorial design and response surface methodology was successfully applied for modelling and optimizing TPHs removal in ex-situ biopile remediation of hydrocarbon contaminated soils. It was observed that the simultaneous application of microbial consortia and mature compost for ex-situ biopiles under cold climate conditions enhanced the TPHs percentage removal compared to the control treatment setups. Response surface methodology had demonstrated to be a useful and practical approach to study and relate the influence of the independent variables and interactions to obtain the best performance of TPHs removal in a statistically significant manner. Results critically point out the importance and contribution of each factor during the bioremediation process for TPHs and specifically the significant influence of the microbial consortia and mature compost alone over the process performance. Surface plots and numerical optimization based on desirability function estimated the optimized conditions to enhance process performance to achieve 90.7% removal.

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CHAPTER 5

SUMMARY, CONCLUSIONS & RECOMMENDATIONS

5.1 SUMMARY

Assessment and comparison of bioaugmentation (addition of microbial consortia) and biostimulation (addition of compost) strategies at field scale for petroleum contaminated soils under cold climate conditions were investigated. A series of ex-situ field scale biopiles (16 m^3 each) were designed and constructed based on a 2^3 factorial experimental design, and operated and maintained over a period of 3-4 months during cold climate conditions (November 2012 to February 2013). These biopiles were subjected to 3 or 6 mL m^{-3} of microbial application rate (bacterial load) and 5 or 10% of amount of compost amendment. Additionally, biopiles with only soil (S) as control, soil and compost (SC), and soil and microbial consortium (SM) were also asssesed.

To investigate the performance of the different strategies, composite soil samples for each biopile were collected and analyzed for total petroleum hydrocarbons (TPHs), volatiles (F1), semi volatiles (F2) and non-volatiles (F3) fractions, heterotrophic aerobic bacteria count (log CFU g^{-1}), total hydrocarbon degrader bacteria using the Most Probable Number (MPN) method, and pH along the treatment period. Additionally, field measurements including soil temperature, moisture content, carbon dioxide (CO_2) and oxygen (O_2) were carried out. The performance and efficiency of the process was determined by the extent of the reduction of contaminant concentrations and in terms of hydrocarbon degradation rates over a period of 94 days.

A response surface methodology (RSM) based on a factorial design was used to investigate and optimize the effects of the microbial consortia application rate and amount of mature compost amendment on the TPH removal. The interactions between these two factors were studied and modeled using a statistical regression model. Finally, a numerical optimization approach via RSM was determined.

5.2 CONCLUSIONS

During this research, a field scale study of bioremediation of petroleum contaminated soil under cold climate conditions was carried out. Based on this investigation, the following conclusions can be made:

1) Combination of microbial consortium application and mature compost amendment as bioaugmentation and biostimulation strategy represents an effective method to enhance the degradation of petroleum hydrocarbon contaminated sandy soil under cold weather conditions over 94 days, in contrast to control setup or application of individual treatments.
2) One-way ANOVA indicated significant difference ($p < 0.05$) within and between the final measurements for TPHs and significant difference between the treatment with combined effect and the control as well as individual treatments.
3) Internal reactions in combination with black insulation plastic membrane may help preserve the temperature above freezing for the different treatment setups.
4) CO_2 production and presence of hydrocarbon degraders confirm the adaptability of microorganism to the environmental substrate, suggesting hydrocarbon biodegradation or mineralization in the treatment setups.
5) Paired t test statistical analysis of results ($p<0.05$) showed that there was no significant difference between the application of individual treatment and the control treatment in terms of TPHs removals. However, the combined effect of biostimulation and bioaugmentation treatments was significantly different from the control as well as individual treatments.
6) Combined strategies were more effective in promoting and enhancing biodegradation, duplicating the kinetic values obtained for the total petroleum hydrocarbon and their fractions (F2,F3) compared to the experimental setups with bioaugmentation only (S+M) or biostimulation (S+C) only.
7) Higher kinetic rate was observed for F2 compared to F3 fractions for experimental setup with combined strategies and with individual treatments, as a consequence of bioavailability and lower molecular weight.

8) Concurrent biodegradation of F2 and F3 fractions is consistent with previous findings in cold climate conditions, where biodegradation played an important role in the remediation process.
9) A factorial design and response surface methodology was successfully applied for modelling and optimizing TPHs removal in ex-situ biopile remediation of hydrocarbon contaminated soils under cold climate conditions.
10) Response surface methodology has demonstrated to be a useful and practical approach to study and relate the influence of the independent variables and interactions to obtain the best performance of TPHs removal in a statistically significant manner.
11) Results critically point out the importance and contribution of each factor during the bioremediation process for TPHs, specifically the significant influence of the microbial consortia application and mature compost alone over the process performance.
12) Surface plots and numerical optimization based on desirability function estimate the optimized conditions to enhance process performance.
13) This research also aims to provide the best combination of strategies to enhance current protocols for cold weather conditions, reducing operational costs and increasing productivity, as a result of an increase in available treatment time, especially for the Canadian market.

5.3 RECOMMENDATIONS

The information obtained from this study indicated successful use of bioremediation strategies to biodegrade hydrocarbons contaminants in ex-situ remediation project under cold climate conditions. However, the following recommendations are proposed:

1) A full factorial design with full replicates can corroborate the results obtained for the current research study, and provide more information about the main factors and their interactions (compost amendment and microbial consortia inoculation).
2) Different types of soil matrix at field scale still need to be studied to assess the application of these strategies under the same climate conditions.
3) Extensive microbiological analysis can be conducted to assess the interactions between native and foreign degraders.

4) Non-bio remedial factor such as adsorption or volatilization should be explored in more detail to evaluate their effects and contribution on TPHs removal.
5) Techniques such as electrokinetic should be considered and tested in combination with discussed strategies to enhance the results.
6) Available data should be used to evaluate numerical model created for the current research study.
7) Due to the soil variability in composition, more research should be conducted to ensure an appropriate and representative environmental sampling of contaminated soil in biopiles structure, in order to make full use of the chemical and biological test available.

Appendix A

Journal Paper I

International Biodeterioration & Biodegradation Journal

Field scale ex-situ bioremediation of petroleum contaminated soil under cold climate conditions

International Biodeterioration & Biodegradation 85 (2013) 375–382

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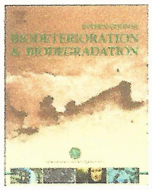

Field scale ex-situ bioremediation of petroleum contaminated soil under cold climate conditions

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ABSTRACT

Assessment and development of strategies for ex-situ bioremediation in cold climates, such as in Canada, is of great importance for decontamination of contaminated soils. The purpose of this paper was to evaluate and compare the use of microbial consortia inoculant and mature organic compost as bioaugmentation and biostimulation strategies to enhance ex-situ bioremediation of a soil contaminated with heating oil under cold weather conditions. The soil was impacted as a result of leakage from an above-ground storage tank and had an initial TPH concentration was 940+ 127 μg g^{-1} (dry weight). Aerobic biopiles of 16 m^3 each were constructed and subjected at a field scale to microbial consortia inoculant and 10:1 ratio of mature organic compost. Two biopiles (S) contained only soil as control, two biopiles (S + C) contained soil and compost to assess the individual effect of compost addition, two biopiles (S + M) contained soil and microbial consortium to assess the individual effect of microbial consortium addition, and three biopiles (S + C + M) contained soil plus compost and microbial consortium to assess the combined effect of compost and microbial consortium addition. Over a 94 days period, composite soil samples for each biopile were collected and analysed for total petroleum hydrocarbons (TPHs), volatiles (F1), semi volatiles (F2) and non-volatiles (F3) fractions, microbial counting, and pH. Additionally, field measurements including soil temperature, moisture content, carbon dioxide (CO_2) and oxygen (O_2) were carried out. Although the ambient temperature varied from −3.5 °C to −24.1 °C, the internal soil temperatures for the different experimental setups maintained above freezing conditions. Results showed that biocell inoculated with microbial consortia and amended with 10:1 soil to compost ratio under aerobic conditions performed the best, degrading 82% of total petroleum hydrocarbons (TPHs) with a first-order kinetic degradation rate of 0.016 d^{-1}, in cold weather conditions. The average removal efficiencies for TPHs after 94 days for S, S + M, S + C treatments were 48%, 55%, and 52%, respectively. Statistical analysis indicated significant difference ($p < 0.05$) within and between the final measurements for TPHs and significant difference between the treatment with combined effect (S + C + M) and the control (S) as well as individual treatments of S + C and S + M. The study concluded that the combination of microbial consortia inoculation and mature compost as a biostimulant in a 10:1 soil to compost ratio would be an appropriate bioremediation approach for this type of soil matrix, reaching values below provincial standard regulations in just 40 days after construction. This research aimed to provide valuable knowledge to practitioners about cost-effective and existing strategies for ex-situ bioremediation during cold weather conditions.

1. Introduction

Fossil fuels represent by far the largest source of energy for human activities, accounting for 81% of all energy consumption worldwide in 2010 (IEA, 2012). During storage, transportation and combustion of fossil fuels massive amounts of contaminating hydrocarbons are released into the surrounding environment with fuel spill being the major contamination route. In Canada, approximately 60% of contaminated sites involve petroleum hydrocarbon (PHC) contamination (Sanscartier et al., 2009b). Health risks and environmental impacts associated with the exposition of petroleum hydrocarbons have resulted in soil contamination to be considered as a major concern over the last few years. Soil contamination is not only a social and sanitary issue, but is also an economical concern, since it implies major costs related to decreasing productivity and monetary depreciation of the impacted sites for future use (Juwarkar et al., 2010). Several sites,

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Appendix B

Photos

Photo B.1 Mixing Contaminated Soil with Compost Amendment

Photo B.2 Biopiles construction

Photo B.3 Microbial Consortia preparation

Photo B.4 Aeration piping system for biopiles

Photo B.5 Finished biopiles with black plastic cover

Photo B.6 Inlet aeration header

Photo B.7 Soil Vapour Monitoring Wells

Photo B.8 Soil Vapour Monitoring

Photo B.9 Temperature and Moisture measurements

Photo B.10 Soil Sampling