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PILOT-SCALE DEMONSTRATION OF BIOSURFACTANT-ENHANCED IN-SITU BIOREMEDIATION OF A CONTAMINATED SITE IN NEWFOUNDLAND AND LABRADOR

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Pilot-Scale Demonstration of Biosurfactant-Enhanced In-Situ Bioremediation of a Contaminated Site in Newfoundland and Labrador

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EXECUTIVE SUMMARY

Soil and groundwater contamination caused by oil and chemical spills are among the most extensive and environmentally damaging pollution problems and are recognized as potential threats to human and ecosystem health. It is generally thought that spills are more damaging in cold regions such as Newfoundland and Labrador (NL), where the ecosystem recovery is slower than those in warmer climates. The contamination not only poses an adverse impact on human and environment health, but also leads to an economic loss in NL. In 2007-08, 482 of 2269 federal contaminated sites were determined in Atlantic Canada, with 331 in NL, resulting in a large number of remediation projects. The Goose Bay Remediation Project (GBRP) was one of the major projects with an investment over $258 million.

Industries have been taken efforts to solve individual problems and/or processes related to site remediation practices in Goose Bay during the past years and they are expecting effective and cost-efficient in-situ remediation technologies which can be directly applicable to NL. In-situ bioremediation has been proven as a promising technology through both experimental studies and field applications for cleaning up petroleum hydrocarbons (PHCs) from subsurface due to its low cost and the lack of toxic by-products which are commonly associated with other treatment types. However, there are challenges to apply bioremediation to NL sites, especially through an in-situ way. In NL, a number of contaminated sites are PHCs and heavy metal co-contaminated sites. The metals can inhibit the natural microbiota and hence impede the rate of PHC degradation. Moreover, bioremediation is currently still a site-dependent action, with many applications relying on demonstrating efficacy at sites of a certain region. Natural conditions in NL are different from other parts of the world (e.g., cold weather and relatively low incidence of sunlight, resulting in a decrease in both abiotic transformation and biotic degradation of contaminants). Therefore, existing in-situ bioremediation techniques are not directly suitable in the NL context.

Biosurfactants have received great attention for overcoming the above challenges of bioremediation. They are surface-active amphiphilic molecules released extracellularly or as part of the cell membrane by microorganisms. By promoting wetting, solubilisation, and emulsification of various types of organics, they can also increase the surface area between oil and water phases, thereby increasing the bioavailability of entrapped PHCs in the porous media. Heavy metals are not biodegradable and they can only be transferred from one chemical state to another, which changes their mobility and toxicity. In the heavy-metal polluted soils, biosurfactants can form complexes with metals at the soil interface, which is followed by desorption of the metals and removal from the soil surface, leading to the

potential to lower heavy metal bioavailability and/or increase microbial tolerance to heavy metals. Moreover, they have superior advantages over chemical surfactants including non-toxicity, higher substrate selectivity, biodegradable and capable of being modified by biotechnology. They are active at extreme temperatures, pH and salinity, showing high environmental compatibility. For these reasons, application of biosurfactants to in-situ bioremediation of PHC-heavy metal co-contaminated soils in NL could be really promising.

In the past few years, a biosurfactant enhanced in-situ bioremediation technology through biosufactant production, purification and characterization, as well as the bioremediation tests in the laboratory with small scales has been developed by Dr. Zhang's research group. To facilitate field applications of this newly-developed technology, a large-scale test is desired to incorporate heterogeneities in geological/hydrological characteristics and in microbial and hydrocarbon distributions of real world contaminated sites. This research thus focused on a pilot-scale demonstration of biosurfactant-enhanced in-situ bioremediation of a petroleum and heavy metal co-contaminated site in NL to address a wide range of challenges facing local site remediation actions. In-depth investigation of the effects of physicochemical, hydrological and biological factors on bioremediation performance was conducted, which plays an ever-increasing role in the implementation of the advanced bioremediation measures.

A comprehensive review was conducted, including petroleum contamination, regulation and remediation actions in NL, as well as the technical details and challenges of bioremediation and biosurfactants. Factors affecting bioremediation in NL were summarized, including but not limited to the freezing/frozen soils, temperature, bio-availability of hydrocarbons, and availability of oxygen and nutrients. Recent advances in environmental applications of biosurfactants were included. Effects of the spatial heterogeneity, advective-dispersive transport and harsh environmental conditions on bioremediation actions, especially in large environmental systems were also discussed.

A NL contaminated site was selected in this research, followed by a detailed site characterization. The target contaminated site was within the Lower Tank Farm (LTF) at 5 Wing Goose Bay. The LTW is one of the five most severe contaminated sites in Goose Bay. The majority of environmental contamination at the site can be attributed to past storage and handling practices of a broad range of environmental contaminants, particularly PHCs and heavy metals. The key factors achieved by site investigation though literature review and site visits include: (a) contaminant types and their physical and chemical characteristics (e.g., concentration, solubility, density and volatility); (b) subsurface conditions, such as soil type, hydrological/geological characteristics, homogeneity in vadose and saturated zones and soil permeability; (c) groundwater conditions, such as depth of perched water, depth of saturated groundwater and hydraulic conductivity; (d) potential extent of contamination, such as residual-phase and gaseous-phase hydrocarbons in the vadose zone, free-phase and dissolved-phase hydrocarbons in the saturated zone and the area of contamination; (e) adjacent surface conditions, such as conditions of

operating property above the contaminated zone (e.g., open space, tanks, pipes, paving and structures) and open space available for treatment; and (f) related standards including clear-up criteria.

To scale down conditions of the study site to the pilot-scale experimental system, the development of the subsurface site soil profile was conducted. Soil and groundwater conditions around and within boreholes were the inputs of this process. The Minitab software package was employed to interpolate and extrapolate the missing data and graphically represent the results. Given the heterogeneity that exists in nature, it is simply not feasible to completely define subsurface conditions at a given site. Attempting to do so will require an infinite number of borings, monitoring wells, samples and analyses. Therefore, it is feasible and necessary to make assumptions accompanied by sensitivity analysis when designing subsurface soil profile. The assumptions in this research include: (a) each cell or grid represents a single type of soil, either clay or silt or sand; (b) if two or more types of soil exist within a cell, then the soil with the highest proportion in weight is chosen; (c) the level of groundwater table is horizontal within the modeling domain; and (d) fluctuation of the groundwater table is minor and can be ignored. Based on the available data and assumptions, a conceptual model of the site subsurface was generated.

A pilot-scale stainless steel vessel (3.6m L×1.2m W ×1.4m D) was then designed and custom-manufactured, located in the Northern Region Persistent Organic Pollution Control (NRPOP) Laboratory at Memorial University, which is funded by the Canada Foundation for Innovation (CFI) and the Industrial Research and Innovation Funds (IRIF) of Newfoundland and Labrador Government. This is a completely sealed vessel, equipped with flow controller, drainage collectors, and sensors to help mimic various site conditions. Uncontaminated soils (sand, till, clay) pre-selected to ensure its inside conditions were in accordance with the target site. Then soils were filled into the vessel to simulate the real conditions of the target site following the previous generated conceptual model. The sampling outlets and monitoring/injection/extraction wells were settled within the pilot-scale experimental system to facilitate the bioremediation treatment and water/soil sample collection during the experiments.

Environmental samples were collected for screening novel biosurfactant producing microbes, including the produced water samples from oil and gas platforms, sediment samples from local coastal line in NL, and water samples from local harbours. Each collected sample was enriched with oily media and subjected to serial dilution and spread plate technique for isolation of bacteria. Isolates were then subjected to drop-collapsing test to determine their biosurfactant production ability. The isolates which can produce biosurfactants were purified and identified with 16S DNA sequencing. Biosurfactants were finally isolated and purified by cold acetone precipitation in lab.

A four-stage biosurfactant-enhanced bioremediation test was conducted in this research. The benzene, toluene, ethylbenzene, and xylenes (BTEX) and lead was determined as the target

contaminants, thus gasoline and $Pb(NO)_3$ was selected to injected into the pilot-scale system. The lab-developed biosurfactant solution was applied to the pilot-scale system as the washing agent through injection/extraction to improve removal of the co-contaminants, and as the additive in the mixing tank to enhance subsurface media conditions and microbial activities. Environmental factors (e.g., temperature, pH, nutrients, and oxygen supply) influencing behaviors of biosurfactants were examined. Concentrations of biosurfactants, heavy metals, and BTEX were obtained after the lab analysis through using the tensiometer, Flame Atomic Absorption Spectrometer (FAAS) and Gas Chromatograph-Mass Spectrometer (GC-MS). Microbial activities were also monitored. Through a number of experimental studies as well as systematic consideration of factors related to source and site conditions, the research outputs are expected to help generate an environmental friendly and economical/technical feasible alternative to solve the challenging site contamination problems in NL.

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CHAPTER 1
BACKGROUND

1.1 Soil and Groundwater Contamination in NL.

The Canadian environment industry has the annual sale of over $20 billion, and contributes 2.2% to Canada's GDP (Singh et al., 2010). Remediation is considered a part of the solid and hazardous waste management sector, comprising the second largest component (24%) of Canada's environment industry (ECO Canada, 2010). Based on programs such as Environment Canada's Green Plan, rising awareness of the need to clean-up public lands, and the expected positive image gained from establishing/enforcing regulations which mirror those of the United States Environmental Protection Agency (USEPA), the Canadian market is expected to reach $1 billion for soil and groundwater remediation. Current Canadian demand for soil remediation services and products is estimated at $250–500 million. (Flaherty, 2012). There are positive signs for further growth in Canada given the government's commitments for the next ten years of $3.5 billion for remediation of federally owned contaminated sites, $500 million for specific contaminated sites of concern across Canada for which it has shared responsibility, e.g., the Sydney Tar Ponds, and a budget of $150 million for redevelopment of municipal brown fields under the management of the Federation of Canadian Municipalities (Singh et al., 2009; FCM, 2010).

Canada has an estimated 30,000 contaminated sites, and approximately two-thirds of these sites can be economically cleaned up and redeveloped. Nevertheless, there is still great uncertainty with regard to the extent and number of contaminated sites in Canada. There is also no national legislation on contaminated land to coordinate approaches between provincial and territorial jurisdictions and create common approaches and standards. Awareness of the problem of contaminated sites is growing in Canada, as is effort to address them. According to Statistics Canada, Canadian revenues from the international environment market are in excess of $1.6 billion for exports of solid and hazardous waste management services. For large Canadian environmental consulting and engineering firms involved in remediation, approximately 10–30% of their business can come from export markets.

Soil and groundwater contaminated sites are acquiring growing attention of the public, governments and industries in Newfoundland and Labrador (NL). In 2007-08, the third operational year of the Federal Contaminated Sites Action Plan (FCSAP), 2269 sites in Canada was targeted for assessment, with 482 sites in Atlantic Canada (311 in NL) (FCSAP, 2010). These projects included the cleanup of sites as harbours and ports, military bases, former Distant Early Warning (DEW) line sites, light stations, and abandoned mines. A vast variety of contaminants were involved, ranging from heavy metals, pesticides, PAHs, petroleum hydrocarbons, to many other pollutants. Several sites in the NL domain have been targeted on the list of 57 priority federal contaminated sites funded since 2003 (CSMWG, 2005). A large-scale cleanup of PCB-contaminated soil in Canadian history was undertaken in the Saglek area of northern Labrador and approximately 20,000 cubic meters of PCB-contaminated soil were evacuated in the remediation project (CSMWG, 2005). Shea Heights/Southside Tank Farm in St. John's, another priority federal contaminated site, was

identified with extensive TPH contamination (FCSAAP, 2008). Happy Valley-Goose Bay located in the central Labrador and served as a military base for air force since the World War II (now operated by Canadian Force Command within the Department of National Defence, DND), has been contaminated with a significant amount of hazardous wastes including petroleum, PCBs, POPs, VOCs and heavy metals for years. The preliminary assessment process estimates the volume of free products could be among 15-20 million litres and the majority of the pollutants are in the deep underground (AMEC, 2008).

These contaminated sites not only pose adverse impact on human health and environmental compatibility, but also lead to financial loss and reinvestment for industries and governments in NL. Federal and provincial governments, as well as associated industries, were obliged to endeavour research effort and provide financial support for site identification, remediation, and long term monitoring. In 2007-08, $2,246,400 of the available FCSAP assessment funds were spent at 311 NL sites, grouped into 51 projects (FCSAP, 2010). DND takes the initiative of the Goose Bay Remediation Project (GBRP) with an investment more than $258 million, investigating and managing over 100 potential contaminated areas to generate a comprehensive remediation plan. This GBRP consists of 10 sub-projects with the official remediation work beginning from 2010 and being estimated to last for10 years.

1.2 Regional Policy and Challenges in Site Bioremediation in NL

Harsh environmental conditions present many engineering and design challenges. The fragile soil environment with permafrost and limited vegetation dictates that mechanical remediation technologies are unfavourable relative to technologies that enhance natural remediation processes (Mackay et al., 1980). In addition, the nature of the rugged cold region landscape poses several complicating factors for the implementation of remediation technologies. For example, transport to most NL sites is limited to air or sea, and many sea approaches are hindered by pack ice for much of the year, limiting access to heavy equipment and personnel. Technologies requiring large amounts of heavy equipment and specialized treatment apparatus therefore raise treatment costs due to the high cost of shipping.

Similarly, shipping contaminated soil or secondary contaminated waste streams off-site incurs high costs in NL. Limited seasonal availability of transport for equipment and personnel underlines the need for technologies that can provide: l) high degradation rates, and 2) short treatment seasons. In-situ technologies that can be left in place during the winter season with minimal maintenance and supervision are thus desirable (Allen, 1999). Those technologies selected in NL should also have to be cost effective, adaptable to harsh and remote conditions and meet local regulatory standards.

Industries have been taken efforts to solve individual problems and/or processes related to in-situ site remediation practices in NL during the past years. However, most of the previous efforts were dedicated to one or few existing remediation technologies for the

purposes of problem solving and/or consulting. Environmental companies tend to (a) use simple and narrow-application-scope technologies even for complicated problems, and/or (b) over-design the remediation systems to make their job easier. Consequently, the effectiveness of remediation at the contaminated sites is extremely limited, and the remediation is usually long-term and costly. This situation has hindered the efforts to effectively protect environments of this region.

Are the environmental companies rejecting new remediation technologies? The answer is absolutely a No. In this industry, a technology considered to be innovative will become "conventional" in a much shorter time frame than in many other industries as a result of the need and urgency to develop cost-effective solutions. The fact is that there was very little in-depth R&D on in-situ remediation technologies that are suitable to the NL context.

In-situ bioremediation has been proven as a promising technology through both experimental studies and field applications for cleaning up petroleum hydrocarbon (PHC)-contaminated soil because of its low cost and the lack of toxic by-products which are commonly associated with other treatment types (Kosaric 2001; Huang et al., 2006; Zhang et al., 2011). However, there are challenges to apply bioremediation to NL sites, especially through an in-situ way. (1) In NL, a number of contaminated sites are PHCs and heavy metal co-contaminated sites. The metals (e.g., As, Cd, Cr, Cu, Hg, Ni, Pb, Se and Zn) can inhibit the natural microbiota and hence impede the rate of PHC degradation (AL-Saleh and Obuekwe, 2005). (2) Bioremediation is currently still a site-dependent action, with many applications relying on demonstrating efficacy at sites of a certain region (Qin et al., 2009). Natural conditions in NL are different from other parts of the world (e.g., cold weather and relatively low incidence of sunlight, resulting in a decrease in both abiotic transformation and biotic degradation of contaminants). Therefore, existing in-situ bioremediation techniques are not directly suitable in the NL context.

Moreover, most of the studies on bioremediation in Canada were conducted in the laboratory with small scales. Such studies do not simulate field conditions well, as they don't factor in such limitations as mass transfer and distribution of nutrients/contaminants/dissolved oxygen (DO)/redox potentials, as well as changes in hydraulic conductivity in subsurface. It is, therefore, not surprising that a wide disparity between lab and field contaminant removal rates has been noted (Qin et al., 2009). Sturman et al. (1995) also indicated that though effects of nutrient conditions in soil and aquifer system petroleum degradation has been studied and reviewed extensively; research on the impact of spatial heterogeneities on nutrient availability has not. The impact of spatial heterogeneities on nutrient availability however, is important mainly in nutrient-poor aquifers (such as harsh environment in NL) where the addition of nutrients is conducted via injection or surface application. Added nutrients must flow to the site of active microorganisms and therefore are subject to transport limitations imposed by aquifer heterogeneities. While presence of significant populations of aerobic, cold-adapted bacteria in petroleum-contaminated soils from polar and alpine regions

have been reported (Eriksson et al., 2001; Whyte et al., 2001; Margesin et al., 2003), the understanding of spatial heterogeneities on nutrient availability is important to our research. On the other hand, the complexity of the hydrogeology of natural aquifers does not allow for controlled experimentation and, thus, precise delineation of the impact of various process parameters.

Large-scale treatment systems incorporate heterogeneities in soil characteristics and in microbial and hydrocarbon distributions, which are representative of field-scale systems (Sturman et al., 1995; Davis et al., 2003). Furthermore, large-scale laboratory setups combine the advantages of controlled experimentation conditions with the scale that can facilitate either direct application of the results, or precise extrapolation. However, very few pilot studies have been reported in the literatures on the remediation of the cocktail contaminants (both heavy metals and oils) and nearly no pilot-scale research targeting on the NL sites.

In general, state-of-the-art in-situ soil bioremediation technologies are highly desired, with further efforts expected for overcoming challenges including limited bioavailable PHCs due to the presence of co-toxicants especially heavy metals that inhibit biodegradation and slow reaction rates caused by environmental constraints in NL. In addition, pilot-scale demonstration of the newly developed bioremediation technologies will facilitate direct field application in the region.

Biosurfactants have received great attention for overcoming the above challenges. They are surface-active amphiphilic molecules released extracellularly or as part of the cell membrane by microorganisms (Zhang et al., 2011). By promoting wetting, solubilization, and emulsification of various types of organics, they can also increase the surface area between oil and water phases, thereby increasing the bioavailability of entrapped PHCs in the porous media (Chang et al., 2008). Heavy metals are not biodegradable; and they can only be transferred from one chemical state to another, which changes their mobility and toxicity (Lai et al., 2009). In the heavy-metal polluted soils, biosurfactants can form complexes with metals at the soil interface, which is followed by desorption of the metals and removal from the soil surface, leading to the potential to lower heavy metal bioavailability and/or increase microbial tolerance to heavy metals (Sandrin and Maier, 2003). Moreover, they have superior advantages over chemical surfactants including non-toxicity, higher substrate selectivity, biodegradable and capable of being modified by biotechnology (Tugrul and Cansunar, 2005). They are active at extreme temperatures, pH and salinity, showing high environmental compatibility (Desai and Banat, 1991). For these reasons, application of biosurfactants to in-situ bioremediation of PHC-heavy metal co-contaminated soils in NL could be really promising.

1.3 Objectives

This project aims at the design, implementation and assessment of a pilot-scale demonstration of biosurfactant-enhanced in-situ bioremediation at a PHC and heavy metal co-contaminated site in NL. Through a number of experimental studies as well as systematic consideration of factors related to source and site conditions, the proposed pilot-scale study is expected to generate environmental friendly and economical/technical feasible solutions for helping solve the challenging site contamination problem in this region; and to be directly applicable to the NL context. It entails the following research tasks:

(1) To determine a target NL contaminated site and conduct site characterization;

(2) To design subsurface soil profile and generate the conceptual model of the site subsurface based on boreholes drilling reports, the analysis of soil and water samples from surrounding boreholes, and the mathematical modeling;

(3) To realize the conceptual model and scale-down the real site conditions through the design and setup of a pilot-scale experimental system. Soil (sand, till, clay) will be selected, analyzed and loaded to the pilot-scale vessel;

(4) To produce biosurfactants in lab and conduct the pilot-scale biosurfactant-enhanced bioremediation experiments for cleaning up real-site contaminants under typical subsurface conditions within the NL site; and

(5) To examine the performance of biosurfactants and the associated bioremediation technologies during the pilot-scale test.

The proposed research and developed technologies will help to (a) obtain improved and applicable technologies for site remediation in NL; (b) reduce costs at the consulting, planning, design and operation stages associated with the site remediation practices; (c) develop multidisciplinary expertise in remediation engineering, environmental chemistry and biology, and experimental design for HQP training; and (d) demonstrate technical transfer and facilitate convenient current state and future fields of application to the industries.

CHAPTER 2
LITERATURE REVIEW

2.1 Bioremediation

2.1.1 *In-situ* Bioremediation

In-situ bioremediation has been proven as a promising technology through both experimental studies and field applications for cleaning up petroleum-contaminated soil and groundwater because of its low cost and the lack of toxic by-products which are commonly associated with other treatment types (Zhang et al., 2011). It is a managed or spontaneous process in which a biological, especially microbial, catalysis acts on pollutant compounds, thereby remedying or eliminating environmental contamination (Madsen, 1991). Harmful hydrocarbon contaminants may be assimilated by microorganisms and converted into biomass or transformed by cells or cell-free enzymes (Babel, 1994). Bacteria capable of biodegrading petroleum hydrocarbons may normally be found in subsurface soils; however, natural breakdown of the compounds will occur too slowly without intervention to prevent accumulation of the pollutants from reaching unacceptable levels (Lyman et al., 1990).

The indigenous (naturally occurring) microbes can be stimulated, or specially developed microorganisms can be added to the site to degrade, transform or attenuate organic compounds (e.g., petroleum contaminants) to low levels and nontoxic products (Catallo and Portier, 1992; Ram et al., 1993). To further improve the degradation process, oxygen and nutrients are usually added to the system to support biological growth.

Bioremediation technologies are thus developed to enhance the native capability of the microorganisms. The indigenous (naturally occurring) microbes can be stimulated, or specially developed microorganisms can be added to the site to degrade, transform or attenuate organic compounds (e.g., petroleum contaminants) to low levels and nontoxic products (Catallo and Portier, 1992; Ram et al., 1993). To further improve the degradation process, oxygen and nutrients are usually added to the system to support biological growth.

The alternative is to selectively isolate and grow specific microbial cultures which are adapted to the toxicant and thus “trained” to degrade and utilize it as a substrate. Addition of surface-active agents, especially when biodegradation of non-polar compounds is encountered, helps in the uptake and metabolism of these compounds by the microbial population. Compared to other conventional remediation technologies, bioremediation has several advantages as follows (Leavitt and Brown, 1994):

- Minimal environmental impact and liability: Unlike other technologies that temporarily displace the problem or transfer the contaminants to another medium, bioremediation attempts to render the contaminants into harmless substances (Fouhy and Shanley, 1992).
- Low contaminant levels: Often, lower residual contaminant levels are possible by bioremediation compared to those made possible by other methods.
- Reduced risk of exposure: When used In-situ, bioremediation reduces the risk of exposure during cleanups by avoiding the need for excavation.

- Reduced cost: Compared to offsite treatment methods, In-situ bioremediation could cost much less.

Table 1 illustrates the finical benefit of bioremediation compared with other technologies.

Table 1 Cost of soil treatment

Treatment	Cost per ton
Landfill disposal	$140-200
	+taxes
	+transportation
Mobile incineration	$140-150
Stabilization/fixation	$100-200
Bioremediation	$15-70

A typical In-situ approach is shown in Figure 1. In this approach, part of the ground-water can be collected at the underflow, pumped back onto the soil supplemented with nutrients and oxygen. For biodegradation of petroleum, about 3 kg oxygen is required for every kg of petroleum hydrocarbon degraded. Sparging with oxygen can deliver only 40 mg/L at the injection point while hydrogen peroxide can be dissolved and injected at concentrations > 500 mg/L and will gradually breakdown to oxygen during transport through the contaminated area.

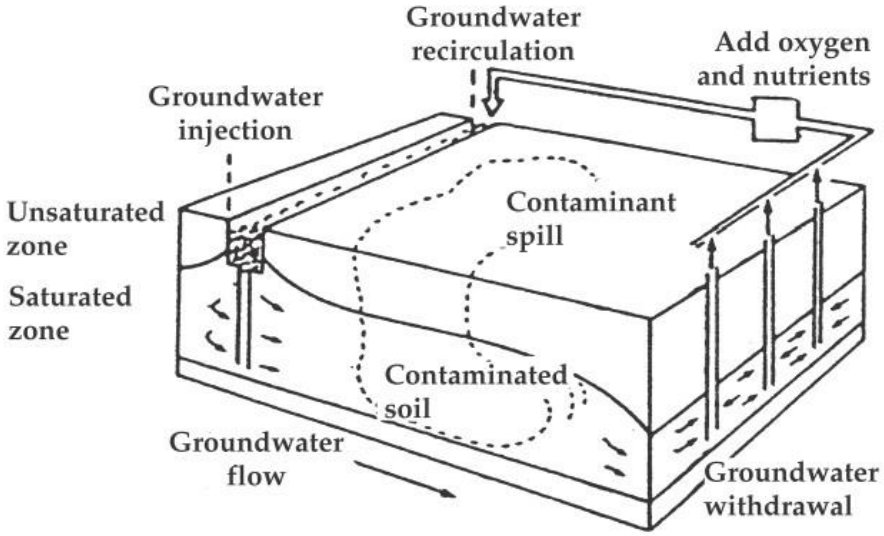


Figure 1 Schematics of the In-situ treatment of contaminated saturated soil

The success of bioremediation strategies is dependent on the presence of appropriate pollutant-degrading microorganisms as well as environmental conditions which are conducive to microbial metabolism (Khan et al., 2004). Armstrong et al. (2002) analyzed a database of groundwater chemistry results for monitoring programs at 124 contaminated sites in western Canada. The sites were mainly "upstream"oil and gas sites in Alberta, where typically the hydrocarbon contaminants in groundwater are derived from releases of crude oil or natural gas condensate. In this region groundwater temperatures typically are within the

range of 5 - 10℃. Where sufficient data were available, more than 90% of the monitored hydrocarbon plumes were either stable or shrinking, rather than expanding.

However, even when appropriate microbial strains and environmental conditions are present, the extent of biodegradation may still be severely limited by the availability of hydrophobic pollutants to microorganisms (Qin et al., 2009). Bioavailability plays a major role in limiting the degree to which soil can be decontaminated via either indigenous or augmented bioremediation (Mata-sandoval et al., 2000). Advanced approaches for enhancing pollutant bioavailability and in well conjunction with bioremediation are thus highly desired. Heavy metals in petroleum contaminated sites have been recognized in NL (AMEC, 2008).The presence of heavy metals in subsurface environments has therefore been attributed to petroleum development and mining as well as oil spills (Osuji and Onojake, 2004). These metals (e.g., As, Cd, Cr, Cu, Hg, Ni, Pb, Se and Zn) can inhibit the natural microbiota and hence impede the rate of petroleum degradation (Osuji and Onojake, 2004; Nduka et al., 2006). Studies of approaches capable of remediating sites co-contaminated with petroleum and heavy metals are thus desired. Bioremediation, the use of microorganisms or microbial process to degrade environmental contaminants, is among these new technologies. Bioremediation has been used on very large-scale application, as demonstrated by the shore-line clean-up efforts in Prince William Sound, Alaska, after the Exxon Oil spill. Although the Alaska oil-spill clean-up represents the most extensive use of bioremediation on any one site, due to its less toxicity and low cost, bioremediation has received increasingly attention and has been applied to both experimental and field studies for remediation of soil and groundwater contaminated by petroleum products and other organic materials (Zhanget al., 2011).

Bioremediation technologies have been broadly divided into two categories based on whether biodegradation is stimulated *In-situ* or carried out *ex situ* (Blackburn and Hafker, 1993; Baker and Herson, 1994). *In-situ* bioremediation involves enhancement of the biodegradation rate of organic contaminants within affected soil or groundwater environments. *Ex situ* technologies require physical removal of the contaminated material followed by treatment under contained conditions in bioreactors, biopiles, composting heaps or ponds (Blackburn and Hafker, 1993; Baker and Herson, 1994). Although *In-situ* bioremediation, by definition, assumes treatment of the contaminated material in place, "pump and treat" technologies are usually included in this category, despite the fact that they involve the removal, treatment and return of associated water from a contaminated soil zone (Blackburn and Hafker, 1993).

It is widely accepted that petroleum contamination will naturally attenuate over time even in extremely cold climate. Natural attenuation (or intrinsic bioremediation) has become a recognized and cost-effective remedial option for low risk petroleum-contaminated sites. It is not strictly a biodegradation process by indigenous microorganisms that transform contaminants into intermediate products or innocuous end products or immobilize them.

Physical and chemical phenomena such as dispersion, absorption and abiotic transformations are often important (Hinchee, 1994). However, the biodegradation rate during natural attenuation is so low in-situ that enhanced actions are needed for site cleanup.

Two approaches are applied for enhancing *In-situ* bioremediation: the microbial ecology approach and the microbiological approach (Piotrowski, 1991). The former involves altering the environment of the indigenous organisms to optimize the biodegradation of the contaminants, which is called the **Media Enhancement Approach**. The latter, on the other hand, involves supplying microorganisms that have been conditioned to degrade target compounds in the subsurface. These organisms could be prepackaged "superbugs" which are strains developed in the laboratory and shipped to a contaminated area or they could be site-specific superbugs, which have been isolated from the affected area itself and reintroduced at higher concentrations. The microbiological approach is called a **Biological Enhancement Approach**.

2.1.2 Media Enhanced Bioremediation

Various chemical and physical properties of a soil determine the nature of the environment in which microorganisms are found (Parr et al., 1983). In turn, the soil environment affects the composition of the microbiological population both qualitatively and quantitatively. The rate of decomposition of an organic waste depends primarily upon its chemical composition and upon those factors that affect the soil environment. Factors having the greatest effect on microbial growth and activity will have the greatest potential for altering the rate of residue decomposition in soil.

The most important soil factors that affect degradation are available nutrients, oxygen supply, soil temperature, water content, etc. These do not always function independently and a change in one may lead to changes in others (Parr et al., 1983). If any of the factors that affect degradation processes in soils are less than an optimum level, microbial activity will be lowered and substrate decomposition decreased (Parr et al., 1983). Effects that vary some of the main soil factors of in-situ bioremediation are reviewed in the following paragraphs.

Variation of nutrient availability: Nutrient supplementation is generally practiced for subsurface bioremediation. The requirement for the addition of inorganic nutrients depends on the nature of the contaminant and the extent to which the polluted site has previously been subjected to agricultural use. Bioremediation actions of petroleum hydrocarbons (PHC)-contaminated sites typically require nitrogen and phosphorus addition (Prince, 1992; Atlas and Bartha, 1992; Pritchard et al., 1992; Leavitt and Brown, 1994). Measurement of soil organic carbon, organic nitrogen and organic phosphorus helps determine its carbon-to-nitrogen-to-phosphorus (C: N: P) ratio and evaluate nutrient availability (Sims and Bass, 1984). If the ratio of organic C: N: P is wider than about 300:15:1 and available (extractable) inorganic forms of nitrogen and phosphorus do not narrow the ratio to within these limits, supplemental nitrogen and/or phosphorus should be added.

One of the most widely accepted values for a mixed microbial population in the soil is C: N: P = 100:10:1 (Waksman, 1924; Thompson et al., 1954). However, in reality, a complete assimilation of petroleum carbon into biomass is not achievable under natural conditions. Some of the petroleum compounds are recalcitrant or metabolized slowly over long periods. From petroleum compounds that are readily metabolized, some carbon will be mineralized to carbon dioxide. Thus, the optimal C: N: P ratios are expected to be wider than the theoretical values. Excessive nutrient supply is also not good. For example, excessive nitrogen (e.g., C: N = 1.8:1) can impair biodegradation, possibly due to ammonia toxicity (Zhou and Crawford, 1995). Therefore, nitrogen must be applied with caution to avoid excessive application (Saxena and Bartha, 1983). Furthermore, nitrate or other forms of nitrogen oxidized to nitrate in the soil may be leaked into the groundwater (nitrate is itself a pollutant limited to 45 mg/L in drinking water) (U.S. EPA, 1985). By estimation of the carbon in a spilled substance (petroleum) ending up as bacteria, it is possible to calculate the amount of nitrogen and phosphorus necessary to equate this ratio for optimum bacterial growth (Thibault and Elliott, 1980).

Proper nutrients should be water-soluble so that they can be transferred into the site with water. Ammonium phosphate $(NH_4)_3PO_4$ / $(NH_4)_2HPO_4$ / $NH_4H_2PO_4$ generally provides the nitrogen and phosphorus required for maximum growth of hydrocarbon oxidizers (Rosenberg et al., 1992). A mixture of other salts, such as ammonium sulfate $(NH_4)_2SO_4$, ammonium nitrate NH_4NO_3, ammonium chloride NH_4Cl, sodium phosphate Na_3PO_4 / Na_2HPO_4 / NaH_2PO_4, potassium phosphate K_3PO_4 / K_2HPO_4 / KH_2PO_4, and calcium phosphate, could also be used.

The mobility of nutrients themselves is also an important criterion for the selection. In general, nitrate nutrients move easily, while ammonia nitrogen is adsorbed by soil colloids and shows little movement until converted into nitrate. Phosphorus does not move in most soils. Therefore, potassium and phosphorus need to be applied or introduced to a desired point of use.

In most cases, site geology should also be considered (Raymond et al., 1976). Nutrient solution containing sodium could cause dispersion of the clays, thereby reducing permeability (U.S. EPA, 1985). The best nutrients for soil application are in the form of readily usable nitrogen and phosphorus and also in a slow-release form to provide a continuous supply of nutrients, which is beneficial in terms of nutrient savings and minimizes leaking from the oil-soil interface (Atlas, 1977).

Variation of oxygen supply: Many *In-situ* bioremediation technologies involve the provision of oxygen to enhance aerobic respiratory breakdown of organic contaminants. Oxygen is supplied either by percolation of oxygen-enriched water, air sparging, bioventing or oxygenation of returned groundwater in "pump-and-treat" systems (Pritchard et al., 1992; Blackburn and Hafker, 1993; Baker and Herson, 1994; Troy, 1994; Lu, 1994; Reisinger et al., 1995; Phelps et al., 1995). One of the most commonly used means of introducing oxygen in

subsurface or groundwater remediation applications is to add hydrogen peroxide as a potential generator of oxygen *In-situ*. Hydrogen peroxide is soluble in water. Its enzyme-catalyzed decomposition in soil yields 0.5 mol of oxygen per mol of hydrogen peroxide introduced to the contaminated site (Baker, 1994). The employment of hydrogen peroxide to supply oxygen and promote bioremediation in vadose and saturated soils as well as aquifers has been reported by Pritchard and coworkers (1992).

Variation of temperature: Temperature is a major environmental factor influencing *In-situ* bioremediation rates. As well as directly affecting bacterial metabolism and growth rates, temperature has a profound effect on the soil matrix and on the physicochemical state of the contaminants (Baker, 1994). In addition, temperature levels can fluctuate considerably during the course of a bioremediation application, varying on vertical as well as on diurnal and seasonal bases.

The vast majority of In-situ bioremediation applications have been carried out under mesophilic conditions (typically between 20 to 40 ℃). Laboratory studies of bacteria exhibiting potential remediation values have also focused on mesophilic species, mainly because of their ease of cultivation and their relatively short doubling times. Degradation of pollutants, such as petroleum hydrocarbons, is significantly decreased as the temperature is lowered below 10 ℃ (Atlas, 1975; Dibble and Bartha, 1979). On the other hand, Carss et al. (1994) demonstrated significant rates of PHC degradation in an In-situ bioremediation trial in the arctic frontier of the Northwest Territories in Canada. Despite the fact that the groundwater temperature varied from 0.2 to 8.3 ℃ and 0.3 to 2.0 ℃, respectively, the total amount of PHCs present in the groundwater decreased by 55 % in 1991 and by an additional 15 % in 1992, corresponding to a theoretical mineralization of approximately 1,200 L of petroleum products within the test site over the trial period (Carss et al., 1994). This trial highlights the fact that even modest increases in temperature may significantly increase bioremediation rates. A variety of technologies have been utilized to increase the temperature during In-situ soil bioremediation actions, such as vegetation and pumping in heated water or recirculating groundwater through a surface heating unit (Baker, 1994).

Variation of soil moisture: Biodegradation of PHCs in the soil requires water for microbial growth and for diffusion of nutrients and by-products during the breakdown process (JRB and Associates Inc., 1984). The extremes of very wet or very dry soil moisture markedly reduce waste biodegradation rates (Arora et. al., 1982). Aerobic waste hydrocarbon decomposition is diminished under saturated soil moisture conditions because of low oxygen supply; while, under very dry conditions, microbial activity is hindered due to insufficient moisture levels necessary for microbial metabolism (Arora et. al., 1982).

A typical soil is about 50 % pore space and 50 % solid matter (JRB and Associates Inc., 1984). Water entering the soil fills the pore spaces until they are full. The water then continues to move down into the subsoil, displacing air as it goes. The soil is saturated when it is at its maximum retentive capacity. Then when water drains from the pores, the soil

becomes unsaturated. Soils with large pores, such as sands, lose water rapidly whereas the smaller pores inside the aggregate retain water (Papendick and Campbell, 1981). If the soil is too impermeable, it will be difficult to circulate treatment agents or to withdraw the polluted water (Nielsen, 1983). Soils with a mixture of pore sizes, such as loamy soils, hold more water at saturation and lose water more slowly. The density and texture of the soil determine the water-holding capacity, which in turn affects the available oxygen and microbial activity (Huang et al., 2005). The actual microbial species composition of a soil is often dependent upon water availability. The migration of organisms in the soil can also be affected by pore size (Bitton and Gerba, 1985). Larger bacteria tend to be immobilized in soils by physical straining or filtering.

Control of soil moisture content can be practiced to optimize degradative and absorptive processes and may be achieved by several means (Sims and Bass, 1984). Supplemental water may be added to the site (irrigation), excess water may be removed (drainage) or the methods can be combined with other technologies for greater moisture control.

2.1.3 Biological Enhanced Bioremediation

Microorganisms are the principal agents responsible for recycling carbon in nature. In many ecosystems there is already an adequate indigenous microbial community capable of extensive oil biodegradation, provided that environmental conditions are favorable for oil-degrading metabolic activity (Atlas, 1977). It has been suggested by some researchers (Atlas, 1977; McGill, 1977) that all soils, except those that are very acidic, contain organisms capable of degrading oil products, that microbial seeding is not necessary, and that the problem is actually the supply of the necessary nutrients at the site.

Aerobic degradation in soil is dominated by various organisms, including bacteria, actinomycetes and fungi, which require oxygen during chemical degradation (Parr et al., 1983). In this process, molecular oxygen serves as the ultimate electron acceptor, while an organic component of the contaminating substance functions as the electron donor or energy source. Most aerobic bacteria use oxygen to decompose organic compounds into carbon dioxide and other inorganic compounds (Freeze and Cherry, 1979). In soil, oxygen is supplied through diffusion. If the oxygen demand is greater than the supply, the soil becomes anaerobic. Maximum degradation rates are dependent upon the availability of molecular oxygen. Aerobic biodegradation occurs via a more efficient and rapid metabolic pathway than anaerobic reactions (Zitrides, 1983). Therefore, most site decontamination is conducted under aerobic conditions.

Although hydrocarbon-degrading bacteria have been found to be naturally present, microbial inoculation is capable of substantially accelerating biodegradation when appropriate conditions are provided (Vecchioli et al., 1990). The factors that could be limiting biodegradation by the supplemented microbes (e.g., oxygen and nutrients) should be evaluated and corrected (Maxwell and Baqai, 1995). If microorganisms are to be added, they

must be hydrocarbon degraders and able to compete with the native population. The organisms may be unable to move through the soil to sites containing the chemical (Vecchioli et al., 1990). Appropriate methods must be used to ensure that the microbes can move throughout the contaminated area (Maxwell and Baqai, 1995). Substantial monitoring should then be conducted to evaluate site conditions and assess the effectiveness of the treatment.

Most laboratory studies on the degradation of organic pollutants have involved incubation temperatures of 20 to 35 ℃, resulting in the selection and enrichment of mesophilic organisms (McKenzie and Hughes, 1976). Mesophilic microorganisms are usually metabolically inactive at temperatures < 8 - 10 ℃. Cold-adapted microorganisms are then desired. Generally, their minimum, optimum and maximum temperatures for growth are 0 - 5, >15 and > 20 ℃ for psychrotrophs, and < 0, < 15 and < 20 ℃ for psychrophiles (Morita, 1975). Cold-adapted microorganisms can be very sensitive to temperature increases. Many hydrocarbon-oxidizing bacteria isolated at 10 ℃ grow well at 15 ℃ but not at all at 25 ℃; similarly a bacterium isolated below 8 ℃ failed to grow at 18 ℃ and was killed within 10 min at 25 ℃ (McKenzie and Hughes, 1976). These observations emphasize the care needed in the isolation of such organisms. Since > 80% of the biosphere show temperatures < 5 ℃, cold-adapted microorganisms are widely distributed in nature, with Gram-negative bacteria being predominant (Morita, 1975). Surprisingly, their potential for biotechnological application (Margesin and Schinner, 1999) has not yet been fully exploited.

Injection of hydrocarbon-degrading bacterial inocula has been considered as a possible bioremediation option for petroleum contaminated sites (Dott et al., 1989; Venosa et al., 1992; Mùller et al., 1995). However, various authors reported that inoculation had no positive, or only marginal, effects on oil biodegradation rates in cold regions (Dott et al., 1989; Venosa et al., 1992; Mùller et al., 1995; Allard and Neilson, 1997). Studies on experimentally (Margesin and Schinner, 1997) oil-polluted cold alpine soils demonstrated that bio-augmentation with cold-adapted bacteria was not successful. All soils investigated harboured enough hydrocarbon-degrading indigenous soil microorganisms to metabolize diesel oil at low temperatures more effectively than the cold-adapted oil-degrading microorganisms introduced into the soil. The authors assumed that the inocula might have been replaced by the indigenous microorganisms with time (Margesin and Schinner, 1997). In soils in northern Alberta, the inoculation of oil-degrading bacteria did not have any effect on the composition of recovered oil; this was attributed to the presence of indigenous oil-degrading bacteria in soils (Westlake et al., 1978). The adaptation of introducing microorganisms into the subsurface environment is essential for a successful application, which is really challenging in a cold climate region (Goldstein et al., 1985).

Some natural conditions of the contaminated sites in NL are different from other parts of the world. The cold weather and relatively low incidence of sunlight result in a decrease in both abiotic transformation and biotic degradation of contaminants. Consequently, none of the existing bioremediation technologies are directly suitable in NL (Liu et al., 2001). State-of-

the-art soil bioremediation technologies with further efforts expected for overcoming challenges including limited bioavailable PHCs and slow reaction rates caused by environmental constraints in NL are thus desired.

Table 2 Microbial genera for hydrocarbon degradation in soil

Bacteria		Actinomycetes	Fungi	Yeasts
Achromobacter	*Escherichia*	*Actinomyces*	*Aspergillus*	*Candida*
Aerobacillus	*Flavobacterium*	*Endomyces*	*Cephalosporium*	*Rhodotorula*
Alcaligenes	*Gaffkya*	*Nocardia*	*Cunninghamella*	*Torula*
Arthrobacter	*Methanobacterium*		*Torulopsis*	
Bacillus	*Micrococcus*		*Trichoderma*	
Bacterium	*Micromonospora*		*Saccharomyces*	
Beijerinckia	*Mycobacterium*			
Botrytis	*Pseudomonas*			
Citrobacter	*Sarcina*			
Clostridium	*Serratia*			
Corynebacterium	*Spirllum*			
Desulgovibrio	*Thiobacillus*			
Enterobacter				

2.2 Factors Affecting Bioremediation in NL

Oil spilled onto permafrost can influence the microbial populations (Atlas，1981), freeze-thaw processes and soil stress (Grechishchev et al., 2001), and thermal and moisture regimes (Balks et al., 2002), as well as the soi1 pH and nutrient availability. Most of all, the same levels of contamination may have a greater impact on the environments of cold regions than on the other environments, as the cold ecosystems have adapted to harsh conditions in ways that make them more sensitive (Snape et al., 2003).

In colder Antarctic and Arctic climates, trials involving bioremediation have been conducted with mixed results (Aisablie et al., 2004; McCarthy et al., 2004).Research has shown the presence of organisms adapted to cold conditions at sites where hydrocarbon contamination is present in these cold climate soils (Mohn andStewart, 2000). Hydrocarbon degrading extreme ophiles are thus ideal candidates for the biological treatment of polluted extreme habitats such as the Canadian Arctic, (Rike et al., 2001; Mohn and Stewart, 2000). A wide variety of microorganisms have been detected in the active layer in Arctic soils in northern Canada and Alaska (Deming, 2002). These cold habitats possess sufficient indigenous microorganisms for In-situ bioremediation, (Ferguson et al., 2003).They adapt rapidly to hydrocarbon contamination in the soil, as demonstrated by significantly increased numbers of oil degraders shortly after a pollution event. An increased number of the hydrocarbon degrading bacteria in response to oil spills has been reported by both Whyte et al. (1999) and Rike et al. (2001) illustrating that growth and proliferation of hydrocarbon degrading bacteria have taken place under site-specific conditions. Over the past several years, a number of studies in both Arctic and Antarctic regions have shown that microorganisms naturally occurring in

harsh environments are capable of degrading petroleum hydrocarbons (McCarthy et al., 2004; Ferguson et al., 2003).This study discussed the important factors affecting bioremediation process based on NL soil texture, for better assist the remediation process.

2.2.1 Freezing and Frozen Soils

2.2.1.1 Freezing Saline Soils

NL is located on the north-eastern corner of North America, surrounded by the Atlantic Ocean. Its long coastlines and extreme temperature makes its soil frozen in winter time. On the other hand, salt in water decreases the freezing point of a soil and increases the amount of unfrozen water. During the freezing process, salt is excluded from the ice phase and thus the solute is redistributed through the soil (Hallet 1978).

Mahar et al. (1983) reported that the rate of freeze to a certain depth increases with an increase in salinity. They attributed this phenomenon to the gradual release of latent heat over a range of temperature. Yen et al. (1991) provided an approximation for the latent heat as a function of ice salinity, which shows that the latent heat released is less than that of pure water. Visualization studies by Arenson and Sego (2004) showed that the frozen fringe becomes thicker with an increase in salt concentration, and they hypothesized that needle-like ice formations in a saturated coarse-grained soil could adversely affect soil shear strength.

Chamberlain (1983) gave evidence of reduced soil hydraulic conductivity under freezing conditions. Experiments done on saline sand columns by Baker and Osterkamp (1988) showed that significant salt rejection occurred when the columns were frozen from the top down, but that this does not occur when the columns froze from the bottom up. They attributed this contrast to gravity drainage of the brine.

Cryogenic structure of saline soils is generally characterized by the same types of cryogenic structure which are typical for soils which do not contain salts. But, as was noticed by Khimenkov and Brushkov (2003), the greater the salinity of soil the more prominent become vertical ice lenses in frozen soil. Phase equilibrium models of saline fine-grained soils have been developed (Grechishchev et al. 1998). Studies indicate that the soil-water-salt system is dynamic, and that hydraulic conductivity in saline cold soils is a function of temperature and salt exclusion.

2.2.1.2 Permeability

The permeability of a soil is its ability to accommodate liquid flow. In the past three decades it has been shown that layers of ice-rich soil (and permafrost) are not impervious to the flow of liquids, whether it is water or non-aqueous phase liquids (NAPL). Susceptibility to liquid flow is a function of the soil type, temperature, and moisture/ice content. Measuring hydraulic conductivity and permeability of frozen soils is difficult and only a few experimental methods have been developed. Burt and Williams (1976) and Anders land et al. (1996) studied lactose and decane as fluid permeants in soil. It has also been shown that water molecules can be transported through ice by regelation, which can be a significant moisture

transport mechanism in saturated soils (Wood and Williams 1985). The infiltration of NAPL into frozen soils has been studied by Wiggert et al. (1997) and McCauley et al. (2002), amongst others. Both conclude that the infiltration of fuel into a frozen soil decreases with increasing ice saturation.

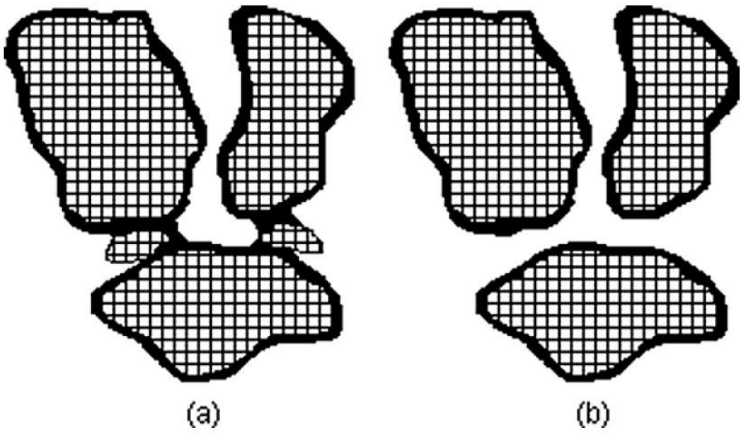


Figure 2 Comparison of pore ice formation in coarse-grained soils with (a) and without (b) the presence of smaller particles. Cross hatched areas represent soil grains and black areas represent water held by capillary forces. The scenario shown in (a) represents the creation of a dead end pore with minimal pore ice content in comparison to the scenario shown in (b) where pore channels remain open to flow. Further additions of water to the pore space shown in (a) will result in the pore becoming either filled with ice or entrapped air (Fourie et al. 2007).

In Olovin's study (1993), the results from over 3000 tests generally showed that permeability decreased by approximately two orders of magnitude with an increase in saturation of up to 0.5. Overall the results from his studies showed that the permeability of a frozen soil is an uncertain parameter that depends on initial water content of the soil prior to freezing, soil temperature, and structure. The gradation of a soil has a strong influence on soil permeability. In a coarse-grained soil, the average pore space diameter is large, and water can flow unheeded through the soil matrix. Upon freeze-up, water freezes along soil grain boundaries, thereby decreasing the average pore diameter and altering the flow of water. In a system that includes fine particles, the average pore diameter is drastically reduced and dead end pores can easily be created (Fourie et al. 2007).This process is schematically shown in Figure 2.

2.2.1.3 The Active Layer

The active layer is that part of the soil that undergoes annual freezing and thawing as a function of temperature. In a tundra environment underlain with continuous permafrost, subsoil conditions can be characterized based on time of year and precipitation (Figure 3).

In the northern hemisphere, from January to March (Figure 3(a)), winter prevails and snow accumulates with the maximum thickness occurring in depressions. Soil may not be completely frozen in the depressions as snow is a good insulator. If the soil is not completely frozen, soil water may redistribute under pressure from the advancing freeze-front. Between

April and May (Figure 3(b)), the increase in solar radiation causes some initial melting and surface runoff may occur. Precipitation as rain or snow occurs during this period. Late May and June (Figure 3(c)) marks early summer, when precipitation is generally in liquid form and evapotranspiration from the ground increases markedly. Water collects in the depressions and the resulting higher thermal conductivity increases the thaw rate. During July to September (Figure 3(d)), precipitation is predominately liquid and evapotranspiration decreases. Extreme temperature variations occur in surficial soils and this realm may dry out completely. From late September through October (Figure 3(e)), winter sets in and precipitation transitions to snow. During the early part of this time period the maximum depth of thaw exists and evapotranspiration becomes negligible. The winter period of November and December (Figure 3(f)) is marked by snowfall, deeply frozen soils, and little, if any, unfrozen soil moisture.

Freezing of the active layer causes elevation of the pressure in suprapermafrost water, which migrates with advance of the freezing front. Freezing of suprapermafrost water of the active layer is accompanied by frost heave and sometimes by the creation of frost mounds. In natural arctic settings, suprapermafrost water typically has low mineral and high organic contents. The converse is true for gravel pads and roads where a layer of fine sediment develops at the base of these manmade features, in direct proximity with suprapermafrost water. Here, suprapermafrost water may have a high mineral content.

Suprapermafrost water is a very limited source of water supply and is mainly used for technical needs. It is particularly susceptible to contaminants in general, and liquid and solid contaminants at human settlements and industrial sites. At industrial sites, this water is usually confined within or limited to the fringes of earthen pads and roads, and only later exposed after infrastructure commission.

The depth of active layer can be determined by air thawing index (ATI) and air freezing index (AFI).

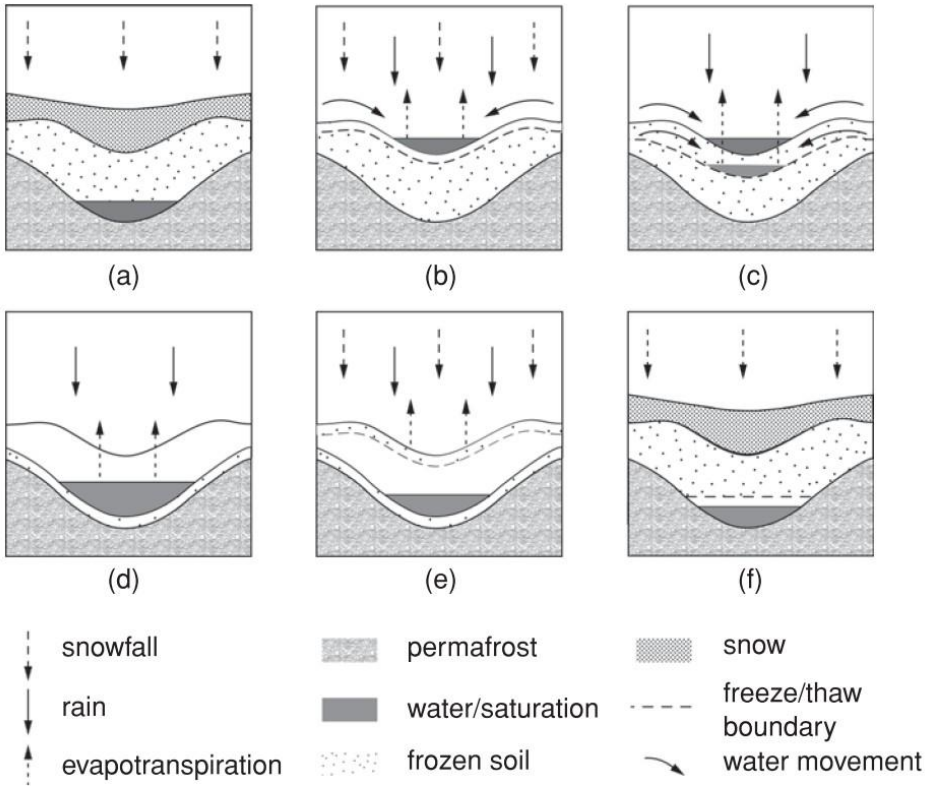


Figure 3 Subsoil conditions in a tundra environment (based on Ryden and Kostor 1977)

2.2.2 Temperature

The ambient temperature of environment influences the physical nature and chemical composition of oil, rate of hydrocarbon degradation, and composition of microbial communities, as well as the mass transfer of substrate and/or electron acceptors in frozen ground, which are crucial to the cold-adapted microbes and consequent bioremediation (Aislabie et al., 2006). Low ground temperatures retard the evaporation rate of volatile components, and thus delay the activation of oil biodegradation. The spilled oil, on the other hand, can decrease surface albedo by one half and the oil-darkened cold surfaces may warm up by 2–12 °C for six hours daily (Balks et al., 2002). In a word, the fluctuation, duration, and variable frequency of temperatures differ from site to site and the resultant biodegradation may be diverse. Ground temperatures can remarkably affect the degradation rates. For instance, the hydrocarbon degradation was over an order of magnitude faster at 25 ℃ than at 5 ℃ (Atlas, 1981). Biodegradation of heavy fuel (Bunker C) by indigenous organisms in the North Sea was four times greater in summer (18 ℃) than in winter (4 ℃) (Balks et al., 2002). In the Arctic/sub-Arctic environments, the biodegradation decreases during winter period and the temperature threshold for remarkable oil biodegradation is around 0 ℃. Although the microbial biodegradation activity does not cease at sub-zero temperatures, the optimum temperature for biodegradation is usually 15–30 ℃ for aerobic processes and 25–35 ℃ for anaerobic processes (Yang et al., 2009). In this regard, the ground temperatures are unfavorable at contaminated sites in cold regions (Aislabie et al., 2006).

Therefore, bioremediation should take advantage of the warm season in the cold regions since warmer months correlate with better degradation rates.

Besides that, biodegradation of pollutants relies on enzymes within the bacterial cell. The microorganisms can be metabolically active only when mass transfer across the cell membrane occurs. When the ambient temperature is lowered toward the freezing point, the channels in the cell membrane tend to be closed and cytoplasm is subject to cryogenic stress. If the temperature keeps dropping, the growth will diminish considerably. When the cytoplasmic matrix becomes frozen, the cell will stop functioning (Yang et al., 2009). Therefore, cryogenic stresses, resulting in closing the transport channels or freezing the cytoplasm, are very common in extreme conditions for several seasons and may restrict mass transport and limit contaminants to gain access into cells.

2.2.3 Bioavailability

Bioavailability is the tendency of individual oil components to be taken up by microorganisms. As for the microbial aspects, difficulties in bioavailability result from the obstacles for hydrocarbons transferring into cellulous enzymes and from limitations in energy for maintaining degradation.

The aqueous solubility of a pollutant is important in biodegrading contaminants because the soil adsorption of contaminants correlates directly with the octanol-water partition coefficient (K_{ow}) and inversely with the aqueous solubility (Bressler and Gray, 2003). With very low water solubility, the maximum rate of bioremediation is dictated solely by mass transfer limitations. However, mass transfer in frozen soils depends on the liquid water or water films, which is a limitation especially in permafrost environments (Ostroumov and Siegert, 1996).Therefore, when the solubility of soil is very low, especially in NL area, it indicates a strong adsorption of contaminants on soil particles and limited mass transfer of contaminants, thus decrease the bioavailability of contaminant to organisms, and impeding biodegradation.

Bioavailability plays a major role in limiting the degree to which soil can be decontaminated via either indigenous or augmented bioremediation. Advanced approaches for enhancing pollutant bioavailability and in well conjunction with bioremediation in cold regions are thus highly desired.

2.2.4 Oxygen

Oxygen is usually severed as the terminal electron acceptor in metabolism and oxygen limitation is one of the crucial reasons for bioremediation failures in cold regions. The importance of oxygen comes from the participation of oxygenases and molecular oxygen involved in the major degradation pathways for the hydrocarbons. Aerobic processes mostly yield a considerably greater potential energy yield per unit of substrate and tend to occur considerably more rapidly. Theory suggests that the mass of oxygen necessary to remediate the hydrocarbon load is about 0.3 g oxygen for each gram of oil oxidized (Atlas, 1981).

Oxygen supply, however, is a common constraint to the bioremediation in frozen ground because oxygen is scarce and the oxygen diffusion is partly or completely blocked. Within these environments, oxygen transport is considered to be the rate-limiting step in aerobic bioremediation. Oxygen may be consumed faster than it can be replaced by diffusion from the atmosphere, and the soil may become anaerobic. In this circumstance, aerobic degradation will be limited, the transformation rates will decline, and obligate anaerobic organisms gradually become the dominant populations (Atlas, 1981; Bressler and Gray, 2003). Thus, engineering techniques are often used to improve the oxygen supply of ex-situ and in-situ treatment systems.

2.2.5 Nutrients

The nutrient status of a soil directly impacts microbial activity and biodegradation. A group of nutrient elements or organic compounds is required as a source of carbon or electron donor/acceptor. Inorganic nutrients including exchangeable cations, nitrates, and phosphates are important for bioremediation. However, nitrogen, and to a less extent, phosphorus are in low concentration in cold regions such as the Arctic environments, and low concentrations of some amino acids, vitamins, or other organic molecules are also needed for bioremediation (Thomassin-Lacroix, 2000). Moreover, the spill of large quantities of petroleum contaminants tends to result in a rapid depletion of the availability of major inorganic nitrogen and phosphorus. Nitrogen and phosphorous often become limiting factors especially when the contaminant functions as a carbon source (Roling and van Verseveld, 2002). Based on Redfield stoichiometry, when nutrients are not limited, the desired ratio of C, N, P, and K is 100:15:1:1 (Filler at al., 2006).

The concentrations and distribution of these inorganic nutrients will be disturbed by the dynamic freeze-thaw processes in permafrost regions, and thus the nutrient supply will be partially influenced. Microbial activities can be constrained by the limitations of both nutrient supply and transport affected by freeze-thaw processes of soils. In some cases, slow-releasing fertilizers should be used if rapid dissolution and dilution of fertilizers in water systems fail to effectively stimulate biodegradation. Excessively high nitrogen levels, e.g., C/N ratios less than 20, may result in inhibited soil microbial activity possibly owing to nitrite toxicity (Thomassin-Lacroix, 2000). However, it is still not easy to know to what extent the microbial populations will respond to the addition of fertilizers to balance the degradation of the spilled oil with the minimal input of inorganic fertilizers in vulnerable, cold environments.

2.2.6 Toxicity

Experiments show that lichens and mosses suffer particularly heavy mortality from toxicity. A hydrophobic coat of oil, which covers the root, may disrupt the root nutrient uptake.

Spilled oil is toxic to birds, fishes, eggs, and larvae, and can transfer toxicity via the food web. Generally, the toxicity depends on the petroleum composition and concentration. Refined oil products, for example, are found to be more toxic to plant cover than crude oil (Yang et al.,

2009). Acute toxicity usually results from low-molecular-weight alkanes and aromatics, while chronic toxicity is from PAHs. Toxicity is also related to ambient temperatures and the consequent weathering of volatile compounds. With higher air temperatures, more toxic components will be lost through weathering. When oil is cooling in the ambient environment, the volatilization of toxic short-chain alkanes is reduced, and their water solubility is increased. Additionally, hydrocarbons have the potential to increase the soil hydrophobility (Balks et al., 2002). Microbes are able to degrade a contaminant when its concentration is below the toxic threshold, but their growth and viability are restricted when the contaminant is above the threshold concentration (Bressler and Gray, 2003). If a contaminated environment is nearly lethal for microbes and biodegradation cannot be implemented, special engineering methods need to be employed to extract and dilute the concentrations prior to biodegradation.

2.2.7 Other Factors

Other important factors affecting soil bioremediation include pH and salinity. The pH of soil is vary widely and acid at 5 Wing Goose Bay (AMEC, 2008). Most heterotrophic bacteria and fungi favor neutral pH, with fungi being more tolerant of acidic conditions. Studies have shown that degradation of oil increases with increasing pH, and that optimum degradation occurs under slightly alkaline conditions (Yang et al., 2009).Changes in soil pH may affect oil biodegradation through alteration of the microbial population.

Considering the location of 5 Wing Goose Bay, dramatic variation in salinity may occur in estuarine environments where marine organisms mingle with freshwater forms. Many freshwater organisms can survive for long periods in seawater although few can reproduce. In contrast, most marine species have an optimum salinity range of 2.5–3.5% and grow poorly or not at all at salinity lower than 1.5–2%.A study of hyper saline salt evaporation ponds, showed that rates of hydrocarbon metabolism decreased with increasing salinity in the range of 3.3–28.4% (Vempsaa and Zhu, 2003). More studies are required to understand the effect of salinity on oil biodegradation.

2.3 Biosurfactants

2.3.1 Surfactants

The molecules of surface active agents (surfactants) are in their most common form, constituted by a hydrocarbon portion (chain) and a polar or ionic portion (head), as illustrated schematically in Figure 4 (Rosen, 1985). The hydrocarbon chain, which can be linear or branched, is usually non-polar and hydrophobic in nature. The polar or ionic portion of the molecule, usually termed the head group, interacts strongly with the water via dipole-dipole or ion-dipole interactions, and is solvated. Consequently, the head group is called the hydrophilic moiety (Rosen, 1985). In short, surface active agents are chemical compounds of high molecular weights with hydrophobic (water-fearing) and hydrophilic (water-loving) moieties.

Surfactants are usually classified into various groups depending on the nature of the head group. It is the hydrophilic head group of the surfactant which gives it the special chemistry (Roy & Griffin, 1988). If a head group carries a negative charge, the surfactant is called anionic; if it carries a positive charge, the surfactant is called cationic; if it carries both positive and negative charges, the surfactant is called zwitterionic; and nonionic if the head has no charge (Rosen, 1985; Huang et al., 2003).

One of the characteristic features of surfactants is their tendency to adsorb at interfaces in an oriented fashion. This adsorption is characterized by the concentration of surfactant at the interface, the orientation of the surfactant at the interface and the energy change in the system. The amount of material adsorbed per unit area of interface is calculated indirectly from surface interfacial tension measurements. As a result, a plot of surface tension as a function of concentration of surfactant in one of the liquid phases is generally used to describe adsorption in a plot between the surface tension and the logarithm of concentration of the surfactant (Figure 5), the slope of the curve abruptly changes at a certain breakpoint. Until it reaches this breakpoint, surface tension reduces as the concentration increases. This indicates that saturation adsorption of the surface has been reached. Just above the breakpoint, the activity of the surface active agent remains constant, meaning even though more surfactant is added to the solution; its activity in that solution remains constant.

Thus, at a certain threshold concentration, partial derivatives of both the interfacial and bulk properties of surfactants (e.g., surface tension and conductance, respectively) with respect to the surfactant concentration display a sudden change in value. The value in the breakpoint is called the critical micelle concentration (CMC) (Rosen, 1985). At smaller surfactant concentrations, surfactant molecules exist solely as monomers. Above the CMC, surfactant molecules associate to form larger units. These associated units, which are colloidal aggregates, are called micelles. Figure 6 shows a diagrammatic representation of the monomeric and micellar forms of surfactant molecules in aqueous systems.

2.3.2 Biosurfactants

It has been well established that microorganisms can utilize hydrocarbons as a carbon and energy source. Different types of bacteria, yeasts and fungi produce metabolic products or membrane components behaving similar to surfactants when growing on substrates insoluble in water (Falatko, 1991). These substances are called biologically produced surfactants or biosurfactants (Mulligan and Gibbs, 1993). Biosurfactants could be either cell wall associated or could be excreted into the surrounding media. The excreted surfactants cause emulsification of the hydrocarbons in the solution, whereas cell wall associated surfactants facilitate hydrocarbon uptake.

Biosurfactants are made up of a large variety of chemical structures. Most of them are lipids. The lipophilic portions of lipids are always the hydrocarbon tails of one or more fatty acids which may be saturated or unsaturated and may contain cyclic structures or hydroxyl

functions. The polar, water-soluble part of a biosurfactant may be from one of the following groups (Georgiou et al., 1992): (1) Phosphate-containing portions of phospholipids; (2) A carbohydrate of glycolipids; or (3) A carboxylate group of fatty acids.

Like chemically synthesized surfactants, biosurfactants have an affinity for the interface between polar and non-polar environments where they can mediate the surface tension between two phases in a mixture such as oil and water or at the air-water interface of an aqueous solution of surface-active molecules. Besides reducing the surface tension of a liquid, biosurfactants may also have emulsion-stabilizing capabilities. This allows for the "mixing" of hydrophobic substances such as hydrocarbons in aqueous solutions.

Biosurfactants are classified mainly by their chemical composition and their microbial origin. The hydrophilic region may consist of a peptide or amino acid, an ionic region, or a mono- or disaccharide (Desai and Desai, 1993). The hydrophobic region is often a long chain fatty acid, hydroxy fatty acid, or the α-branched-β-hydroxy fatty acid such as a mycolic acid (Desai and Desai, 1993; Desai and Banat, 1997). Overall, most biosurfactants are anionic or neutral, as only those containing an amine group are cationic (Mulligan and Gibbs, 1993). Biosurfactants are divided into the following groups based on chemical composition: glycolipids; fatty acids, neutral lipids and phospholipids; lipopeptides and lipoproteins; and polymeric or particulate biosurfactants. Table 3 lists examples of the biosurfactants in these classes along with their microbial origin (Desai and Desai, 1993; Hommel, 1990).

Biosurfactants typically described as glycolipids are carbohydrates in combination with long chain aliphatic acids or hydroxy aliphatic acids. Glycolipids are the most frequently isolated and studied biosurfactants (Desai and Desai, 1993). The major types are the rhamnolipids, trehalolipids, and sophorolipids.

Rhamnolipid biosurfactants are produced by *P. aeruginosa* strains and consist of one or two rhamnose residues glycosidically linked to one or two β-hydroxydecanoic acids. L-rhamnosyl-L-rhamnosyl-β-hydroxydecanoyl-β-hydroxydecanoate is designated rhamnolipid type one; molecules possessing only one rhamnose residue are classed as rhamnolipid type two (Edward and Hayashi, 1965; Itoh and Suzuki, 1974; Mulligan, 2005). These are the principal glycolipids produced by *P. aeruginosa.* Other rhamnolipids with only one fatty acid chain and containing either one or two rhamnose residues are designated types three and four (Desai and Banat, 1997). Figure 7 shows the structure of rhamnolipid produced by *P. aeruginosa*.

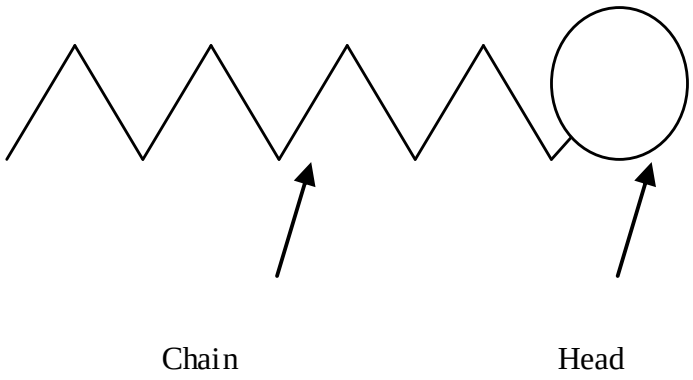


Figure 4 Schematic diagram of a surfactant molecule

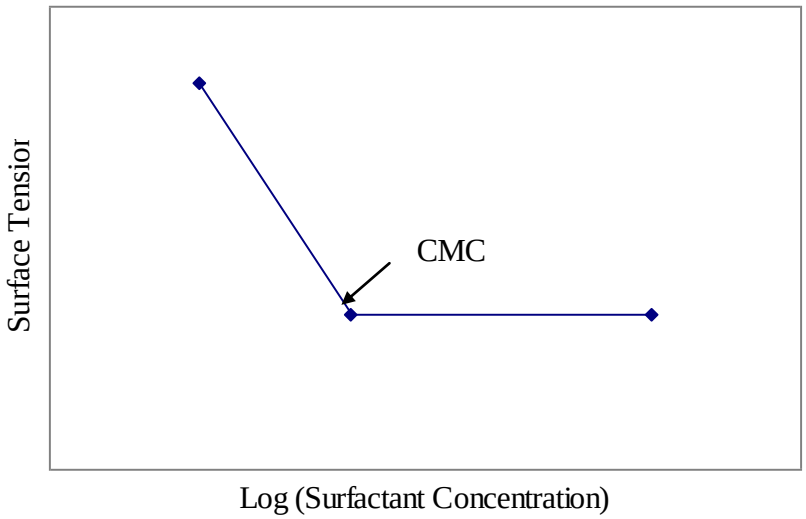
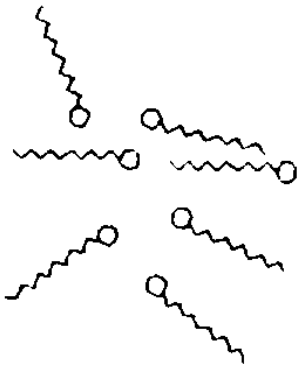
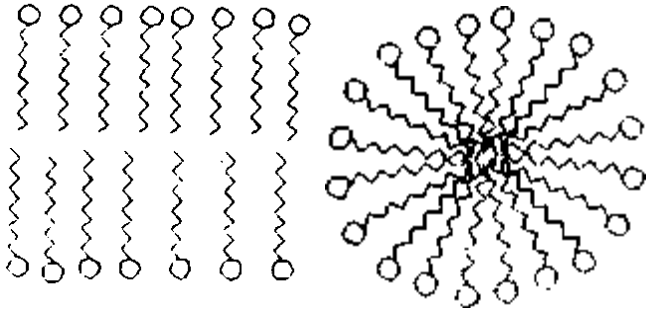


Figure 5 Plot illustrating the relation between the surface tension and surfactant concentration



Monomeric form



Micellar forms

Figure 6 Monomeric and micellar forms of surfactant molecules (Rosen, 1985)

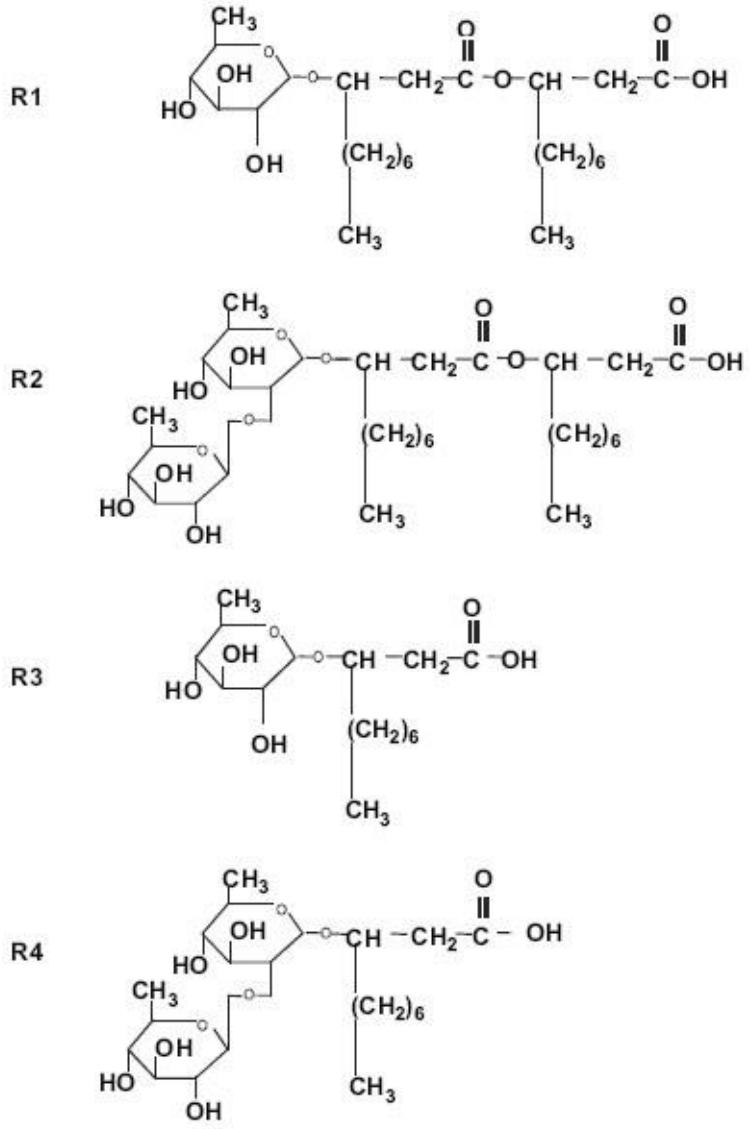


Figure 7 Chemical structures of four different rhamnolipids produced by *P. aeruginosa* (Mulligan, 2005)

Table 3 Types of biosurfactants

Biosurfactant type	Producing Species	Reference
1. Glycolipids		
Rhamnolipids	*Pseudomonas aeruginosa*	Edward and Hayashi, 1965
	Pseudomonas spp.	Lang and Wagner, 1987
Trehalolipids	*Rhodococcus erythropolis*	Rapp et al., 1979
	Nocardia erythropolis	Margaritis et al., 1979
	Nocardia spp. SFC-D	Kosaric et al., 1990
	Mycobacterium spp.	Cooper et al., 1989
Sophorolipids	*Torulopsis bombicola*	Gobbert et al., 1984
	Candida (Torulopsis) apicola	Hommel et al., 1987
	Torulopsis petrophilum	Cooper and Paddock, 1983
Glucolipids	Marine bacterial strain MM1	Cooper et al., 1989
2. Fatty acids, neutral lipids, and phospholipids		
Fatty acids	*Corynebacteria lepus*	Cooper et al., 1978
Neutral lipids	*Nocardia erythropolis*	MacDonald et al., 1981
Phospholipids	*Thiobacillus thiooxidans*	Beeba and Umbreit, 1971
3. Lipopeptides and lipoproteins		
Peptide -lipids	*Bacillus licheniformis*	Yakimov et al., 1995,
Surfactin	*Bacillus subtilis*	Arima et al., 1968
Subtilisin	*Bacillus subtilis*	Bernheimer and Avigad, 1970
Viscosin	*Pseudomonas fluorescens*	Neu et al., 1990
Gramicidins	*Bacillusbrevis*	Marahiel et al., 1977
Polymixins	*Bacilluspolymyxa*	Suzuki et al., 1965
Viscosin	*Pseudomonas fluorescens*	Nue et al., 1990
4. Polymeric biosurfactants		
Emulsan	*Acinetobacter calcoaceticus*	Rosenberg et al., 1979
Alasan	*Acinetobacter radioresistens*	Barkay et al., 1999
Biodispersan	*Acinetobacter calcoaceticus*	Rosenberg et al., 1988
Mannan-lipid-protein	*Candida, tropicalis*	Kappeli et al., 1984
Liposan	*Candida lipolytica*	Cirigliano et al., 1984
Carbohydrate-protein-lipid	*Debaryomyces polymorhpis*	Singh and Desai, 1989
	Pseudomonas. fluorescens	Desai et al., 1988
PS-33	*Rhodococcus* spp.strain No. 33	Nue et al., 1992
5. Particulate Biosurfactants		
PM factor	*Pseudomonas marginalis*	Burd and Ward, 1996
Vesicles and fimbriae	*Acinetobacter calcoaceticus*	Kappeli and Finnerty, 1979
Whole cells	Variety of Bacteria	Rosenberg, 1986

2.3.3 Advantages of Biosurfactants over Traditional Chemical Surfactants

Biosurfactants have the potential to be as effective for many applications as synthetic surfactants due to their low interfacial tensions and low CMCs (Georgiou et. al., 1992). Moreover, they have many advantages over synthetic surfactants in applications (Bruheim et al., 1997):

Resistance to environmental changes: Biosurfactants have chemical diversity which results in a wide variety of physico-chemical properties suited for applications (Mulligan and Gibbs, 1993; Thangamani and Gina, 1994). For example, the biosurfactant BL-86 was found to be an excellent dispersant for ceramic processing. Not only does it serve this specific purpose but it adapts quite well to diverse environmental conditions. BL86, when isolated from the foam produced during the growth of *Bacillus licheniformis* 86 (Horowitz et al., 1990), composed of lipopeptides, was found to be stable over a pH range of 4.0 to 13.0, a temperature range of 25 to 120 °C and salinity from 0 to 30% NaCl. Biosurfactants composed of lipopeptides from *B. licheniformis* JF-2 were stable up to temperatures of 75 ℃ for at least 140 hours (Lin et al., 1994). The biosurfactant was stable at pH values between 5.5 and 12 but slowly lost its activity under acidic conditions.

Low Toxicity and easy biodegradation: Another main advantage of biosurfactants is their lower toxicity than traditional surfactants. Chemical surfactants are mostly produced from petroleum, thus their toxicity cannot be avoided. Sometimes the process used in the production of synthetic surfactants could release toxic byproducts, as in the case of sodium dodecylbenzene sulfonate (SDS) production, where corrosive and toxic chemicals are used and quite frequently discharged as pollutants. When focusing on environmentally sound products, potentially toxic and sparing biodegradable synthetic surfactants could be replaced by biosurfactants, which are naturally produced and nontoxic. Furthermore, biosurfactants are easily biodegradable and, hence, have no environmental impact after the application (Passeri et al., 1992).

Easy application and cost-effectiveness: Because biosurfactant production is growth associated, *In-situ* production may also be possible in areas contaminated by hydrocarbons (Kosaric et al., 1987). Moreover, the production through microbial activities makes them inexpensive. Most importantly, biosurfactants can be synthesized by using renewable resources such as molasses and sugar, while the petroleum resources used to produce chemical surfactants are limited and non-renewable.

Biosurfactants have considerable potential for bioremediation application because of the above advantages. Biosurfactants are a naturally occurring, biodegradable product and, thus, may be acceptable for application at PHC-contaminated sites; biosurfactants are generally nontoxic to microorganisms, especially hydrocarbon-degrading microorganisms; industrial production is likely to be cost effective relative to synthetic surfactants and it may be possible to induce *In-situ* production of a biosurfactant at a PHC-contaminated site (Bai et al., 1997).

2.4 Biosurfactant Enhance Bioremediation

Biosurfactants are surface-active amphiphilic molecules released extracellularly or as part of the cell membrane by microorganisms (Zhang et al., 2011). By promoting wetting, solubilization, and emulsification of various types of organics, they could also increase the surface area between the oil and water phases, thereby increasing the bioavailability of entrapped oil in the porous media (Zhang et al., 2012). During application to site bioremediation, biosurfactants exist in pore throats to (i) lower the surface/interface tension between oil and water, resulting in increased system transmissivity and reduced viscosity, and (ii) stimulate and harness the power of targeted beneficial microorganisms that live in sites (Chang et al., 2008). They also have the potential to remove heavy metals (e.g., Cd, Pb and Zn) from the subsurface through desorption and complexation actions (Lai et al., 2009). Their diversity and stability enable them to perform effectively even in harsh environment such as extreme pH and salinity. For these reasons, inclusion of biosurfactants in bioremediation could be really promising (Calvo et al., 2009). Previously, a biosurfactant enhanced in-situ bioremediation approach through biosufactant production, purification, and characterization has been developed by the authors for cleaning up petroleum contaminated sites (Zhang et al., 2008; 2011; 2012).

There are many advantages of biosurfacants if compared to their chemically synthesized counterparts (Kosaric, 2001). Some of these are:

- *biodegradability*
- *generally low toxicity*
- *biocompatibility and digestibility* – which allows their application in cosmetics, pharmaceuticals and as functional food additives
- *availability of raw materials* – biosurfactants can be produced from cheap raw materials which are available in large quantities; the carbon source may come from hydrocarbons, carbohydrates and/or lipids, which may be used separately orin combination with each other
- *acceptable production economics* – depending uponapplication, biosurfactants can also be produced from industrial wastes and by-products and thisis of particular interest for bulk production (e.g. for use in petroleum-related technologies)
- *use in environmental control* – biosurfactants can be efficiently used in handling industrial emulsions, control of oil spills, biodegradation and detoxification of industrial effluents and in bioremediation of contaminated soil
- *specificity* – biosurfactants, being complex organic molecules with specific functional groups, are often specific in their action (this would be of particular interest in detoxification of specific pollutants): de-emulsification of industrial emulsions, specific cosmetic, pharmaceutical, and food applications
- *effectiveness* – at extreme temperatures, pH and salinity

Because of their potential advantages, biosurfactants are widely used in many industries such as agriculture, food production, chemistry, cosmetics and pharmaceutics. Many properties of microbial surface active compounds such as wetting, solubilization, and emulsification of various types of organics; they could also increase the surface area between the oil and water phases, thereby increasing the bioavailability of entrapped oil in the porous media (Zhang et al., 2012). Biosurfactants increase the bioavailability of hydrocarbon resulting in enhanced growth and degradation of contaminants by hydrocarbon-degrading bacteria present in polluted soil. In heavy-metal polluted soils biosurfactants form complexes with metals at the soil interface, which is followed by desorption of the metal and removal from the soil surface leading to the increase of metal ions concentration and their bioavailability in the soil solution. The new approach is the use of heavy metal-resistant bacterial strains capable of producing biosurfactants for increasing the metal-removing efficiency by phytoremediation.

2.4.1 Biosurfactant Enhanced Hydrocarbons Degradation/Remediation

Hydrocarbons, as the hydrophobic organic chemicals, exhibit limited solubility in groundwater and tend to partition to the soil matrix. This partitioning can account for as much as 90–95% or more of the total contaminant mass. As a consequence, the hydrocarbon contaminants exhibit moderate to poor recovery by physico-chemical treatments; limited bioavailability to microorganisms; and limited availability to oxidative and reductive chemicals when applied to in-situ and/or ex-situ applications (Pacwa-Płociniczak et al., 2011)

2.4.1.1 Role of Biosurfactants in Biodegradation Processes

A promising method that can improve bioremediation effectiveness of hydrocarbon contaminated environments is the use of biosurfactants. They can enhance hydrocarbon bioremediation by two mechanisms. The first includes the increase of substrate bioavailability for microorganisms, while the other involves interaction with the cell surface which increases the hydrophobicity of the surface allowing hydrophobic substrates to associate more easily with bacterial cells (Mulligan and Gibbs, 2004). By reducing surface and interfacial tensions, biosurfactants increase the surface areas of insoluble compounds leading to increased mobility and bioavailability of hydrocarbons. In consequence, biosurfactants enhance biodegradation and removal of hydrocarbons. Addition of biosurfactants can be expected to enhance hydrocarbon biodegradation by mobilization, solubilization or emulsification (Figure 3) (Neuyen et al., 2008; Raman and Rahman, 2003; Urum and Pekdemir, 2004; Nievas et al., 2008).

The mobilization mechanism occurs at concentrations below the biosurfactant CMC. At such concentrations, biosurfactants reduce the surface and interfacial tension between air/water and soil/water systems. Due to the reduction of the interfacial force, contact of biosurfactants with soil/oil system increases the contact angle and reduces the capillary force holding oil and soil together. In turn, above the biosurfactant CMC the solubilization process takes place. At these concentrations biosurfactant molecules associate to form micelles, which dramatically increase the solubility of oil. The hydrophobic ends of biosurfactant molecules connect

together inside the micelle while thehydrophilic ends are exposed to the aqueous phase on the exterior. Consequently, the interior of a micelle creates an environment compatible for hydrophobic organic molecules. The process of incorporation of these molecules into a micelle is known as solubilization (Urum et al., 2006).

Emulsification is a process that forms a liquid, known as an emulsion, containing very smalldroplets of fat or oil suspended in a fluid, usually water. The high molecular weight biosurfactants are efficient emulsifying agents. They are often applied as an additive to stimulate bioremediation and removal of oil substances from environments.

In the current literature, the latest advantages of the role of biosurfactants in interaction between hydrocarbons and microorganisms are presented. Franzetti et al. (Franzetti et al., 2010) describe proposed roles for biosurfactants with respect to their interactions between microorganisms and hydrocarbons in the content of modulation of cell surface hydrophobicity. High cell-hydrophobicity allows microorganisms to directly contact oil drops and solid hydrocarbons while low cell hydrophobicity permits their adhesion to micelles or emulsified oils (Franzetti et al., 2010). They discuss three mechanisms of interaction between microorganisms and hydrocarbons: access to water-solubilized hydrocarbons, direct contact of cells with large oil drops and contact with pseudo solubilized or emulsified oil. The authors suggest that during the different growth stages of microorganisms, biosurfactants can change hydrocarbon accession modes. In their studies, they observed that Gordonia sp. strain BS 29 grown on hydrocarbons produced cell-bound glycolipid biosurfactant and extracellular bioemulsifier, and during the phase of the growth on hexadecane the surface hydrophobicity changes were observed (Franzetti et al., 2009 and 2010).

The recent report by Cameotra and Singh (2009) throws more light on the uptake mechanism of n-alkane by Pseudomonas aeruginosa and the role of rhamnolipids in the process. The authors reported a new and exciting research for hydrocarbon uptake involving internalization of hydrocarbon inside the cell for subsequent degradation. Biosurfactant action dispersed hexadecane into micro droplets, increasing the availability of the hydrocarbon to the bacterial cells. The electron microscopic studies indicated that uptake of the biosurfactant-coated hydrocarbon droplets occurred. Interestingly, “internalization” of “biosurfactant layered hydrocarbon droplets” was taking place by a mechanism similar in appearance to active pinocytosis. This mechanism was not earlier visually reported in bacterial modes for hydrocarbon uptake. Although much work has been done by many groups to explain the role of biosurfactants in the degradation of water immiscible substrates, most processes still remain unclear.

2.4.1.2 Biodegradation Studies

The capability of biosurfactants and biosurfactant-producing bacterial strains to enhance organic contaminants’ availability and biodegradation rates was reported by many authors (Rahmanet al, 2003; Inakolluet al., 2004). Obayori et al. (2009) investigated the biodegradative properties of biosurfactant produced by Pseudomonas sp. *LP1* strain on crude

oil and diesel. The results obtained confirmed the ability of strain LP1 to metabolize the hydrocarbon components of crude and diesel oil. They reported 92.34% degradation of crude oil and 95.29% removal of diesel oil. Biodegradative properties of biosurfactant producing *Brevibacterium sp*. PDM-3 strain were tested by Reddy et al. (Reddyet al., 2010). They reported that this strain could degrade 93.92% of the phenanthrene and also had ability to degrade other polyaromatic hydrocarbons such as anthracene and fluorene.

Kang et al. (2010) used sophorolipid in studies on biodegradation of aliphatic and aromatic hydrocarbons and Iranian light, crude oil under laboratory conditions. Addition of this biosurfactant to soil increased also biodegradation of tested hydrocarbons with the rate of degradation ranging from 85% to 97% of the total amount of hydrocarbons. Their results indicated that sophorolipid may have potential for facilitating the bioremediation of sites contaminated with hydrocarbons having limited water solubility and increasing the bioavailability of microbial consortia for biodegradation.

The effective microbiological method in bioremediation of hydrocarbon polluted sites is the use of biosurfactant producing bacteria without necessarily characterizing the chemical structure of the surface active compounds. The cell free culture broth containing the biosurfactants can be applied directly or by diluting it appropriately to the contaminated site. The other benefit of this approach is that the biosurfactants are very stable and effective in the culture medium that was used for their synthesis.

The usefulness of biosurfactant producing strains in bioremediation of sites highly contaminated with crude petroleum-oil hydrocarbons were confirmed by Das and Mukherjee (Das and Mukherjee, 2007). The ability of three biosurfactant producing strains: *Bacillus subtilis* DM-04, *Pseudomonasaeruginosa* M and *Pseudomonas aeruginosa* NM to remediate petroleum crude-oil contaminated soilsamples was investigated by treating the soil samples with aqueous solutions of biosurfactants obtained from the respective bacteria strains. Additionally, the tested soil was inoculated with mineral-salts media containing a specified amount of *Bacillus subtilis* DM-04 or *Pseudomonas aeruginosa* M and NM strains. Todetermine the extent of biodegradation, the soil-phase total petroleum hydrocarbons (TPH) concentrations were analyzed after 120 days and compared to a control where the soil was treated with un-inoculated medium. Bioagumentation of studied soil with *P. aeruginosa* M and NM consortium and *B. subtilis* strain showed that TPH levels were reduced from 84 to 21 and 39 $g \cdot kg^{-1}$ of soil, respectively. In contrast, the TPH level was decreased to 83 $g \cdot kg^{-1}$ in control soil.

Joseph and Joseph (2009) separated the oil from the petroleum sludge by induced biosurfactant production by bacteria. Petroleum sludge is generated in significant amount in the refineries during crude oil processing. Crude oil is usually stored in storage tanks. Pollutants present in the oil are deposited at bottom of the tank. During cleaning of the tank the sludge is recovered and is treated as a waste. The sludge used for the investigation contained TPH in the concentration range of 850 ± 150 $g \cdot kg^{-1}$. In this study the sludge was

inoculated directly with *Bacillus sp.* strains and by addition of the cell free supernatant. Uninoculated sludge was also taken as a control. Upon inoculation of the supernatant to the sludge slurry, oil separation and reduction of TPH was observed.

The oil separation process was slow initially in the test supplied with the fresh inoculation of the bacterium compared to the samples inoculated with the supernatant, but the residual TPH of both became equal within 48 h. The efficiency of removal of the various isolates ranged from 91.67% to 97.46%. Therefore, it has been observed that the biosurfactant produced by the primary inoculum remained in the supernatant and it was enough to continue the reaction. The biosurfactant displayed the property to reduce surface and interfacial tensions in both aqueous and hydrocarbon mixtures and hence had potential for oil recovery.

Biosurfactants have often been used to enhance bioavailability and biodegradation of hydrophobic compounds but there is little knowledge available about the effect of simultaneous emulsifier production on biodegradation of complex hydrocarbon mixtures. Nievas et al. (2008) studied the biodegradation of a bilge waste which is a fuel oil-type complex residue produced in normal ship operations. Bilge waste is a hazardous waste composed of a mixture of sea-water and hydrocarbon residue, where n-alkanes, resolvent total hydrocarbons and unsolvent complex mixture are the main constituents. Unsolvent complex mixture principally is composed by branched and cyclic aliphatic hydrocarbons and aromatic hydrocarbons, which usually show the greatest resistance to biodegradation. In their studies, they investigated the biodegradation of an oily bilge wastes by anemulsifier-producing microbial consortium. As the result for both levels of oily wastes, 136 $g \cdot kg^{-1}$ of resolvent hydrocarbons and 406 $g \cdot kg^{-1}$ of unsolvent mixture, they found that all of the hydrocarbon types showed an important concentration reduction from their initial values. They observed that the extent of biodegradation followed the order n-alkanes > resolved total hydrocarbon> unsolvent complex mixture. An emulsifier-producing microbial consortium used for biodegradation of bilge wastes showed reduction of n-alkanes, resolvent hydrocarbons and unsolvent mixture around by 85%, 75% and 58%, respectively.

Barkay et al. (1999) tested the effect of a bioemulsifier alasan produced by AcinetobacterradioresistensKA53on the solubilization of polyaromatic hydrocarbons (PAHs), phenanthrene (PHE) and fluoranthene (FLA). They also studied the influence of alasan on mineralization of PHE and FLA by Sphingomonas paucimobilis EPA505. They indicated that aqueous solubility of phenanthrene and fluoranthene increased linearly in the presence of increasing concentrations of bioemulsifier (50 to 500 $\mu g \cdot mL^{-1}$) and mineralization of PAHs by S. paucimobilis EPA505 was stimulated by appearance of alasan. The presence of alasan at concentrations of up to 300 $\mu g \cdot mL^{-1}$more than doubled the degradation rate of fluoranthene and significantly increased the degradation rate of phenanthrene. Increasing the alasan concentration over 300 $\mu g \cdot mL^{-1}$ had no further stimulation on PAH smineralization, although solubilization curves showed that the apparent solubility of these compounds continued to increase linearly with alasan additions in this concentration range.

This could be explained by association of PAHs with multi-molecular structures of alasan, formed at concentrations above the CMC (about 200 $\mu g \cdot mL^{-1}$), which was not readily available for the degrading strain.

2.4.2 Biosurfactant Enhanced Metal Remediation

Contamination of soil environments with heavy metals is very hazardous for human and other living organisms in the ecosystem. Due to their extremely toxic nature, presence of even low concentrations of heavy metals in the soils has been found to have serious consequences. Nowadays, there are many techniques used to clean up soils contaminated with heavy metals. Remediation of these soils includes non-biological methods such as excavation, and disposal of contaminated soil to landfill sites or biological techniques (Aşçıet al., 2010). Biological methods are processes that use plants (phytoremedation) or microorganisms (bioremediation) to remove metals from soil. Application of microorganisms was discovered many years ago to help in reduction of metal contamination. Heavy metals are not biodegradable; they can only be transferred from one chemical state to another, which changes their mobility and toxicity. Microorganisms can influence metals in several ways. Some forms of metals can be transformed either by redox processes or by alkylation. Metals can also be accumulated by microorganisms by metabolism-independent (passive) or by intracellular, metabolism-dependent (active) uptake. Microorganisms can influence metal mobility indirectly by affecting pH or by producing or releasing substances which change mobility of the metals (Briunset al., 2010; Ledinet al., 2010).

Two following methods, “soil washing” or “soil flushing”, are involved in remediation of metal contaminated soil. The first technique used is ex situ—contaminated soil is excavated, put into the glass column and washed with biosurfactant solution. In turn, soil flushing of In-situ technologies involves use of drain pipes and trenches for introducing and collecting biosurfactant solution to and from the soil (Singh and Cameotra, 2004). Interestingly, biosurfactants can be used for metal removal from the soil.

Biosurfactants can be applied to a small part of contaminated soil in which soil is put in a huge cement mixer, biosurfactant-metal complex is flushed out, soil deposited back, and biosurfactant-metal complex treated to precipitate out biosurfactant, leaving behind the metal. The bond formed between the positively charged metal and the negatively charged surfactant is so strong that flushing water through soil removes the surfactant metal complex from the soil matrix. This method can also be carried out for deeper subsurface contamination only with more pumping activities.

Using biosurfactants have unquestionable advantages because bacterial strains able to produce surface active compounds do not need to have survival ability in heavy metal-contaminated soil. However, using biosurfactants alone requires continuous addition of new portions of these compounds. The usefulness of biosurfactants for bioremediation of heavy metal contaminated soil is mainly based on their ability to form complexes with metals. The

anionic biosurfactants create complexes with metals in a nonionic form by ionic bonds. These bonds are stronger than the metal's bonds with the soil and metal-biosurfactant complexes are desorbed from the soil matrix to the soil solution due to the lowering of the interfacial tension. The cationic biosurfactants can replace the same charged metal ions by competition for some but not all negatively charged surfaces (ion exchange). Metal ions can be removed from soil surfaces also by the biosurfactant micelles. The polar head groups of micelles can bind metals which mobilize the metals in water (Figure 8) (Mulligan and Gibbs, 2004; Singh and Cameotra, 2004; Juwarkar et al., 2007; Aşçı et al., 2008)

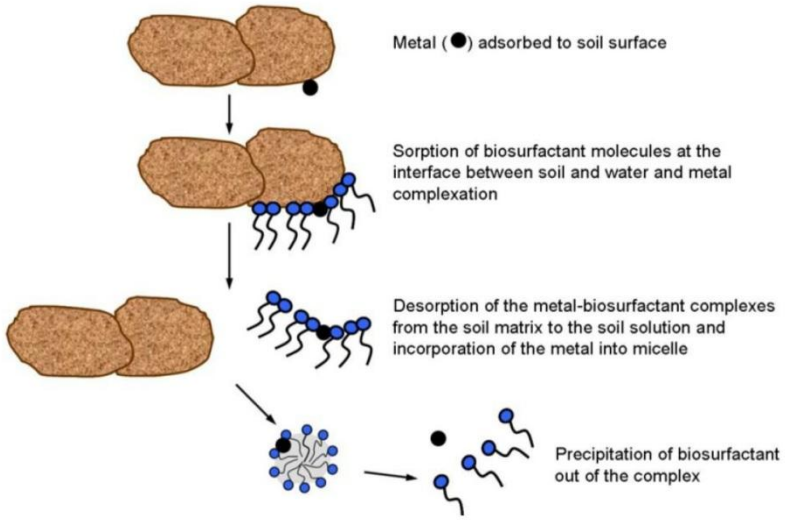


Figure 8 Mechanism of biosurfactant activity in metal-contaminated soil (Mulligan, 2005)

Biosurfactants which are used in bioremediation of metal-contaminated soils have been proposed for use in metal removal in recent years (Juwarkaret al, 2007; Aşçıet al., 2008). High potential of biosurfactants in mobilization and decontamination of heavy metal contaminated soil was confirmed by Juwarkar et al. (Juwarkar et al., 2008), who used di-rhamnolipid biosurfactant produced by Pseudomonas aeruginosa BS2 for mobilization of metals from multi-metal contaminated soil. To study the feasibility of di-rhamnolipid to remove chromium, lead, cadmium and copper from soil, a column study was conducted. Heavy metal spiked soil into a glass column was washed with 0.1% di-rhamnolipid biosurfactant solution. The results indicated that di-rhamnolipid selectively removed heavy metals from soil in the order of Cd = Cr > Pb = Cu > Ni. In turn, Das et al. (2009) investigated the possibility of using the biosurfactant produced by marine bacterium for removal of heavy metals from solutions. The positive role of marine biosurfactant in the remediation of polyaromatic hydrocarbons was reported earlier (Das et al., 2008), however there was no information about the role of this biosurfactant in heavy metal remediation. The study revealed that tested anionic biosurfactant was able to bind the metal ions and the percentage removal of Pb and Cd metals varied with the different concentrations of metals and biosurfactants. The ability of biosurfactant of marine origin to chelate toxic heavy metals and form an insoluble precipitate could be useful in treatment of heavy metal containing wastewater.

Removal of heavy metals from sediments could be enhanced by use of solution containingbiosurfactant and inorganic compounds. For example, Dahrazma and Mulligan (2007) reported the higher rate of removal of copper and nickel from sediments by adding1% NaOH to the solution ofrhamnolipid. Many metals mostly exist in the environment organic fraction, adding OH^- to the sediment solubilizes this fraction, and thus, more metals are available for removal by a rhamnolipid biosurfactant.

Another effective method for the remediation of heavy metals contaminated soil is biosurfactant foam technology. Wang and Mulligan (2004) evaluated the feasibility of using rhamnolipid foam to remove Cd and Ni from a sandy soil. They reported that the use of foam had a significant effect on the mobility of biosurfactant flowing in a porous medium and made a more uniform and efficient contact of biosurfactant with the metals. Application of rhamnolipid foam increases efficiency and allows removal of 73.2% and 68.1% of Cd and Ni, respectively, whereas the rhamnolipid solution flushed only 61.7% and 51% of Cd and Ni, respectively. The system used for the experiment is presented schematically by Wang and Mulligan (2004).

The rate of heavy metal removal from soil strongly depends on its chemical composition. The predominant constituent of the sand and silt fraction in many soils is quartz, thus quartz was chosen for the bioremediation experiment. Aşçi et al. (2010) studied recovery of the metal ions from quartz by rhamnolipid. They observed that the best recovery efficiency from quartz, approximately 91.6% of the sorbed Cd and 87.2% of the sorbed Zn, was achieved using 25 mM rhamnolipid concentration.

Biosurfactants were also used to evaluate their potential in arsenic mobilization from the mine tailings (Wang and Mulligan, 2009). The experimental results showed that introduction of rhamnolipid enhanced As mobilization from the mine tailings significantly. Themobilization increased with the concentration of biosurfactant and became relatively stable when the concentration of rhamnolipid was above100mg $\cdot L^{-1}$. It has been reported by Doong et al. (1998) that the removal of heavy metals increasedlinearly with increasing surfactant concentration below the CMC and remained relatively constant above the CMC. The CMC of the biosurfactant used by Wang and Mulligan (2009) was around 30 mg $\cdot L^{-1}$. The high concentration of rhamnolipid required in this experiment could be due to thesorption of biosurfactant to the mine tailings and the dilution and binding effects of mine tailing particles. The biosurfactant may be enhancing As mobilization by reducing the interfacial tension between As and the mine tailings, by formation of aqueous complexes or micelles and by improving the wettability of the mine tailings. The results from this research study indicated that biosurfactants have potential to be used in the remediation of As-contaminated mine tailings and they can be also effectively used to remove As from soils.

Besides the mobilization, biosurfactants can be involved in other processes connected with remediation of heavy metals. They are used, for example, in entrapping of trivalent chromium in micelles which provides bacterial tolerance and resistance towards high concentration of

Cr (III). Gnanamani et al. (2010) studied the bioremediation of chromium (VI) by biosurfactant producing, marine isolate *Bacillus sp.* MTCC 5514. The remediation carried out by this strain proceeded via two processes: reduction of Cr (VI) to Cr (III) by extracellular chromium reductase and entrapment of Cr (III) by the biosurfactants. The first process transforms the toxic state of chromium into less-toxic state and the second process prevents the bacterial cells from the exposure of chromium (III). Both reactions keep bacterial cells active all the time and provide tolerance and resistance toward high hexavalent and trivalent chromium concentrations.

Efficiency of phytoremediation of heavy metal contaminated soils can be increased by inoculation of plants by biosurfactant-producing and heavy metal-resistant bacteria. Biosurfactant-producing *Bacillus sp.* J119 strain was investigated for its capability to promote the plant growth and cadmium uptake of rape, maize, sudangrass and tomato in soil contaminated with different levels of Cd (Sheng et al., 2008). The study demonstrated that the tested strain could colonize the rhizosphere of all studied plants but its application enhanced biomass and Cd uptake only in plant tissue of tomato. This means that root colonization activity of the introduced strain is plant type influenced. However, further analyses of interactions between the plants and biosurfactant-producing bacterial strain J119 may provide a new microbe assisted-phytoremediation strategy for metal-polluted soils. Further work on the applications of biosurfactants and biosurfactants-producing bacteria in phytoremediation, especially in sites co-contaminated with organic and metal pollutants is required.

2.4.3 Biosurfactants in Co-Contaminated Site Remediation

It was estimated by the U.S. Environmental Protection Agency that 37% of the organic compound polluted sites tested were found to be polluted also with metals such as arsenic, mercury, lead, and zinc (Sandrin and Maier, 2003). The presence of toxic metals (lead, cadmium, arsenic) in some cases causes inhibition of organic compound biodegradation (Sandrin and Maier, 2003; Sandrin et al., 2000; Maslin and Maier, 2000). However, a review of the literature shows a number of possible approaches that can lower metal bioavailability and/or increase microbial tolerance to metals. These include inoculation with metal-resistant microorganisms, addition of materials like: clay minerals—kaolinite and montmorillonite, calcium carbonate, phosphate, chelating agents (EDTA), and bio- and surfactants (Sandrin and Maier, 2003). Biosurfactants produced by microorganisms show promise for enhancing organic compound biodegradation in the presence of metals. Application of biosurfactants or microorganism produced biosurfactants in In-situ co-contaminated sites bioremediation seems to be more environmentally compatible and more economical than using modified clay complexes or metal chelators.

Exploiting this property, Todd et al. (2000) studied the effectiveness of rhamnolipid biosurfactants in the remediation of cadmium and naphthalene co-contaminated site. They observed reduced cadmium toxicity by *P. aeruginosa* rhamnolipid leading to an enhanced

naphthalene biodegradation by a *Burkholderia sp*. They proposed that the mechanism by which rhamnolipid reduces metal toxicity might involve a combination of rhamnolipid complexation of cadmium and rhamnolipid interaction with the cell surface to alter cadmium uptake resulting in enhanced rates of bioremediation. Sandrin et al. (2000) showed that metal-complexing rhamnolipids reduced metal toxicity to allow enhanced organic biodegradation by Burkholderia sp. under laboratory conditions as well. This research demonstrated that rhamnolipids induced the release of lipopolisaccharide (LPS) from gram-negative bacteria, Burkholderia sp., which does not produce rhamnolipid. The authors suggested that rhamnolipid was able to reduce metal toxicity to microbial consortia in co-contaminated soils through a combination of metal complexation and in the alteration of cell surface properties through the release of lipopolisaccharide (LPS), resulting in enhanced bioremediation effect. In another co-contaminant study, it was observed that the inhibition of phenanthrene mineralization in the presence of cadmium was reduced by the pulsed addition of rhamnolipid (Maslin and Maier, 2000). Their research studied the effect of rhamnolipids produced by various *Pseudomonas aeruginosa* strains on the phenanthrene degradation by indigenous populations in two soils co-contaminated with phenanthrene and cadmium. Results showed that rhamnolipids applied had the ability to complex cationic metals, increasing the phenanthrene bioavailability. The biodegradation of phenanthrene was increased from 7.5 to 35% in one soil, and from 10 to 58% in the second soil, in response to rhamnolipids application. As biosurfactants are degraded by soil populations in 2–3 weeks, Maslin and Maier (2000) used a pulsing strategy, in which new portions of rhamnolipids were added to the system to maintain a constant level of biosurfactant during organic contaminant mineralization. Anionic biosurfactants were found to be more effective where metals are the agents to be sequestered. Surfactin, rhamnolipid, and sophorolipids, all anionic biosurfactants, were able to remove copper and zinc from a hydrocarbon-contaminated soil (Mulligan et al., 1999). One advantage in case of co-contaminated soil is that biosurfactants potentially can be produced In-situ using the organic contaminants as substrates for their production, which subsequently would lead to remediation of both the contaminants along with greatly reducing the remediation cost.

The efficiency of biosurfactants for stimulating biodegradation of contaminants is uncertain given the specificity observed between biosurfactant and organism. Addition of biosurfactant can stimulate some organisms but also can inhibit some microorganisms. So, as mentioned earlier, a strategy suitable for effective remediation would be to stimulate biosurfactants produced by indigenous population or use commercial biosurfactants produced by organisms found to be already present at the contaminated site. Further, delivery of a biosurfactant into co-contaminated sites for In-situ treatment may be more environmentally compatible and more economical than using modified clay complexes or metal chelators such as EDTA (Mulligan et al., 1999).

2.5 Pilot-Scale Experiments

In-situ bioremediation is a complex undertaking which requires an understanding of many physical, chemical and biological phenomena. Observations made at the bench or batch may not necessarily apply at the pilot-scale. Observed contaminant loss rates, for example, depend on scale. Table 4 illustrates this point with a compilation of several reports from the scientific literature in which laboratory- and pilot/field-scale rates are compared. Field measured half-lives tend to be 4-10 times longer than laboratory determined values, presumably due to scale-dependent rate limitations. Bioremediation engineering must consider all relevant phenomena to determine which will limit contaminant biodegradation rate for a particular site. Field sites are typically heterogeneous, which can cause different phenomena to limit biodegradation rates across the site. Selection of a remedial strategy should include an assessment of its effects on biodegradation rate-limiting phenomena. This assessment is useful for determining: (1) the potential for successful bioremediation; (2) whether the rate can be enhanced; (3) how to best engineer the process; and (4) how to verify bioremediation has occurred. These issues cannot be properly addressed by observations made at a single scale alone.

Table 4: Scale dependence of contaminant half-lives (Sturman et al. 1995)

Half life (days)		Ratio	Contaminants
Laboratory	Field		
42	397	9.5	aviation gas as TPH
3.6	23	6.4	gasoline, No. 2 fuel oil as TPH
28	111	7.0	benzene
6.1	42	6.8	toluene (nitrate)
5.6	55	9.8	m,p-xylenes (nitrate)
10	73	7.3	BTEX

Pilot-scale experiments under controlled conditions have been demonstrated to be very valuable in the research and development of bioremediation technologies (Gary et al., 1993; Cantafio et al., 1996; Ding et al., 2002; Seidel et al., 2004). Pilot studies of in-situ bioremediation processes can provide opportunities to evaluate the performance of bioremediation systems under site-specific conditions through systematically scaling down the concerned site (Pradhan et al., 1996). Data gathered from pilot studies are able to provide feedback for field plans, process design and action adjustments accordingly (Seidel et al., 2004). For this reason, this report gives a literature review on pilot scale soil and groundwater bioremediation experiments within recent years to better design and conduct the proposed experiment in NRPOP lab.

2.5.1 Effects of Spatial Heterogeneity on Bioremediation

Spatial heterogeneity at contaminated field sites can significantly influence contaminant movement and rate of degradation. Subsurface properties which are subject to significant spatial variation include porosity, permeability, degree of microbial colonization, and chemical properties such as nutrient and electron acceptor conditions. If significant

heterogeneities exist, different phenomena may limit the rate and extent of biodegradation across the site. For example, an often encountered soil textural heterogeneity is the presence of clay lenses in otherwise permeable sandy soils, which can reduce the local permeability by up to 5 orders of magnitude (Todd, 1980).As mentioned above, drastically reduced groundwater flow velocities generally make diffusive transport predominant within clay lenses. This may cause such lenses to act as reservoirs for contaminants, recontaminating groundwater for long periods after more permeable zones have been cleansed. Under these conditions biotransformation within the clay could be limited by molecular diffusion while the higher-permeability zones may be limited by advection.

A study evaluated the feasibility of landfarming biotreatment of petroleum-contaminated soils obtained from a sub-Arctic site at Resolution Island, Nunavut, Canada, and evaluates the changes in composition of the semi- and non-volatile petroleum hydrocarbon fractions during the biotreatment (Chang et al., 2010).This study revealed that after the 60-day treatment period, the TPH concentration was approximately 500 mg Kg^{-1}, and the residual TPH mass was largely associated with particles and aggregated particles with diameters of 0.6–2 mm, rather than the larger or finer particles and aggregates.

Another study was conducted to examine the biodegradation rate with soils characterized by high contents of clay and organic matter coupled to low hydraulic conductivity and permeability (Robles-Gonzalez, 2006). It has been suggested that the overall removal efficiency of 2,4-Dfrom soils depends on their contents of organic matter. Willems et al. (1996) have reported that2,4-D can be incorporated to the humic substances of the soils, which would render that compound less available (and less detectable) and less susceptible to further degradation.

Physical heterogeneities can also cause significant chemical heterogeneities, such as the establishment of various redox zones within the aquifer. While aerobic biotransformation may occur within advective-flow dominated areas, oxygen may be absent where diffusion is the primary transport mechanism. Under these circumstances, nitrate, sulfate, iron (III) and carbon dioxide may be sequentially utilized as electron acceptors. As the most energetically favorable alternative to oxygen, hydrocarbon degradation using nitrate as the electron acceptor has been extensively studied. Denitrification has been used to successfully biodegrade jet fuel at the field scale (Hutchins et al., 1991), though the authors found that ~ 10 times as much nitrate was utilized as could be accounted for by contaminant degradation alone. Another scale effect noted was the relative recalcitrance of sorbedJP-4 fuel oil within the aquifer. Chang et al. (2010) proved the residual TPH mass was largely associated with the size of particles. The majority of residual TPH mass remained in soil particle and aggregate sizes ranging between 0.6 and 2 mm rather than in larger or finer size fractions.

Contaminants also may exhibit significant spatial heterogeneity with regard to flow channels and contaminant phase. An example is the weathering process of a spilled gasoline or crude oil, where the lighter hydrocarbon fraction may volatilize quickly after

introduction, leaving progressively heavier fractions down the path of migration. In addition, dispersive activity and the progression of biodegradation from the edges into the center of a plume usually cause the plume to contain significantly higher (>3 orders of magnitude) contaminant concentrations in its center than at the edges, causing differences in the rate as degradation proceeds. Where biodegrading microorganisms are primarily fixed to aquifer solids, the residence time of the contaminated groundwater in the system must be sufficient to allow the reaction to proceed to completion. Aquifer physical heterogeneities which effect groundwater flow rates therefore may impact degradation rates.

2.5.2 Effects of Advective-Dispersive Transport on Biodegradation Rate

Mass transport by advection and dispersion has a significant effect on contaminant distribution and substrate/electron acceptor availability to microorganisms. Field experimentation indicates oxygen transport limitations cause contaminant degradation rates at the edge of a subsurface plume to be much higher than those in the plume center due to the maintenance of aerobic conditions at the edge (Chiang et al., 1989a; Morgan and Watkinson, 1989). Such transport limitations apply to nutrients and other electron acceptors as well, but are most often observed with oxygen due to its common stoichiometric limitation. This is usually not observed at the micro scale, where mass transport limitations are often minimized or eliminated by design.

Pilot evidence indicates contaminant persistence in areas of lower advective flow velocity or hydraulic conductivity (Sutton and Barker, 1985; Barker et al., 1987; Chiang et al., 1989a).The effects of advective and dispersive transport on biodegradation rates are becoming more evident in the modeling literature as well. To illustrate the rapid progress in this field, Lee et al. (1988) noted that although then current models did take into account the changes in solute (contaminant and electron acceptor) concentrations resulting from differences in aquifer permeability, no modeling efforts had sought to relate these variations to the inevitable effect they must have on the rate at which bioremediation takes place In-situ. More recent modeling efforts (MacQuarrie and Sudicky, 1990) have illustrated that variation in aquifer physical characteristics such as dispersivity can significantly affect advective flow and rates of biotransformation over comparatively small spatial separations.

The overall effect of increasing advective-dispersive transport within an aquifer is to enhance the process of mixing contaminants, electron acceptor and cells capable of contaminant biodegradation. Only where all three of these constituents are present concurrently can biodegradation occur. Lee et al. (1988) observe that this mixing is frequently confounded in aquifers in which contaminants adhere to solids within areas of low advective flow, while dissolved oxygen and nutrients flow within adjacent higher-permeability zones.

2.5.3 Effects of Harsh Environmental Conditions on Biodegradation in Large Scale

Field bioremediation studies conducted in Arctic and Antarcticregions have demonstrated the reduction of petroleum hydrocarbons, and at least part of the reduction is attributable to

biodegradation (Braddock et al., 1997; Thomassin-Lacroix et al., 2002;McCarthy et al., 2004; Paudyn et al., 2008). The presence of significant populations of aerobic, cold-adapted bacteria in petroleum-contaminated soils from polar and alpine regions have been reported (Eriksson et al., 2001; Whyte et al., 2001;Margesin et al., 2003), and the existence of indigenous cold-adapted hydrocarbon-degrading microorganisms at many northern sites, makes biostimulation through addition of N, P nutrients, oxygen and moisture a feasible approach for implementation of bioremediation (Braddock et al., 1997; Margesin and Schinner, 1997; Walworth et al., 2001; Whyte et al., 2001). However, given that the ground is frozen for most of the year, and that soils usually encountered in these regions often contain low levels of nitrogen (N) and phosphorous (P) nutrients to support biological growth, the implementation of bioremediation appears to be challenging, thus field-scale bioremediation experiments in cold climates are required.

Chang et al. (2010) conducted a pilot-scale bioremediation experiment of a site contaminated with petroleum located in a sub-Arctic site at Resolution Islandanks. The total petroleum hydrocarbons (TPH) biodegradation rate constants of pilot scale experiment is comparableto another field study at the same site, which indicates the pilot-scale tanks provided an environment that served as a good surrogate biodegradation system. Paudyn et al. (2008) performed an on-site pilot-scale landfarming experiment at the same Resolution Island site. Their first-order TPH degradation rates is generally in good agreement with the TPH biodegradation rates observed in Chang's research. Similar field scale landfarming experiment also conduct by Zytner et al. (2001) at a historically diesel-contaminated site in northern area, they get the similar result as the one got in pilot scale tank.

At a site near Fairbanks, AK, Reynolds et al. (1994) effectively used landfarming to remediate soils that were moderately contaminated with diesel. Within a 7-weekoperating period, during which nutrients were added and the soil periodically tilled, TPH in soil at this s total petroleum hydrocarbons site were reduced from 6200 to 280 mg/kg. Wingrove (1997) applied landfarming at a diesel-spill site near Pukatawagan, Manitoba. Prior to landfarming, soil TPH concentrations ranged up to 13,000 mg/kg. After 4 months of landfarming, during which nutrients and moisture were applied and the soil was tilled, extractable hydrocarbon concentrations in the soil were all below 250 mg/kg.

2.5.4 Case Study

Nakhlaa and Niaz (2002) evaluated the impact of groundwater velocity and dissolved oxygen (DO) on the efficiency of In-situ bioremediation to treat groundwater contaminated with benzene, toluene, and xylene (BTX). Hydrogen peroxide was added into the system to overcome oxygen solubility limitations, and contaminant concentrations. The experiment was conducted in a pilot scale tank with n 8.6m long, 30cm wide and 30 cm high. BTX served as model compounds of gasoline contamination. Groundwater velocities of 1, 2, and 4 m/d were studied. At each velocity, two concentrations of BTX were employed–10 and 50 mg/l of each of the contaminants to reflect "hot spot" conditions following a spill or major leak. Similarly

DO: BTX mass ratios of 1.5:1 and 3.2:1 were employed. The results of the study indicated that BTX removal efficiencies of 96.7 to 99.7% were achievable at a groundwater velocity of 1 m/d with final concentrations reaching as low as 30 µg/l. BTX removal efficiencies decreased to 70 to 85 percent at a velocity of 2 m/d, and to 37 to 53% at a velocity of 4 m/d. At any given groundwater velocity, BTX removal efficiencies generally increased with increasing DO and BTX concentrations. Statistical analysis of the data revealed that groundwater velocity was the most significant parameter impacting biodegradation efficiency, accounting for approximately 80% of the variability. Hydraulic conductivity of the aquifer decreased by approximately 80% over the course of the seven-month study, with 90% of the decrease occurred within the first six weeks.

Souza et al. (2009) reported an anaerobic treatment of gasoline-contaminated groundwater in a pilot-scale horizontal-flowanaerobic immobilized biomass (HAIB) reactor inoculated with a methanogenic consortium. The reactor was 3 m in length and 15 cm in diameter, and metabolic activated in laboratorial conditions. Biomass was collected from an Up-flow Anaerobic Sludge Blanket reactor (UASB reactor) and immobilized in the reactor. The HAIB reactor was then fed with pre-screened (2 mm) domestic sewage during 8 days at a hydraulic detention time of 10 h in order to foment and sustain an active methanogenic consortium. The reactor was then transported for In-situ bioremediation. The contaminated groundwater was fed at a flow rate of 1–2.5 l/h and remediation lasted for 70 days. BTEX removal rates varied from 59 to 80%, with a COD removal efficiency of 95% during the 70 days of *in-situ* trial. BTEX removal was presumably carried out by microbial syntrophic interactions, and at the observed concentrations, the interactions among the aromatic compounds may have enhanced overall biodegradation rates by allowing microbial growth instead of co-inhibiting biodegradation.

Łebkowska et al. (2011) conducted a research to estimate the efficiency of treating soils polluted with fuels by using biostimulation and bioaugmentation where the indigenous bacterial strains isolated from the polluted soils were cultured and used in high concentration as an inoculum. The bacteria used to inoculate the remediation plots were isolated from the polluted soil and proliferated in field conditions. The inoculation process was repeated every three days. The amount of biomass applied to the polluted soil was set to ensure the total number of bacteria in soil 10^7-10^8 cfu/gd.w. The multiple inoculation of soil with indigenous bacteria active in diesel oil and engine oil (plot A) degradation increased bioremediation effectiveness by 50% in comparison to the non-inoculated control soil and by 30% in comparison to the soil that was inoculated only once. The multiple inoculation of soil with indigenous microorganisms was then applied in bioremediation of the soil polluted with double high concentration of diesel oil (soil B) and in bioremediation of the soil polluted with aircraft fuel (soil C). The process efficiency was 80% and 98% removal of TPH for soil B and C, respectively.

The application of landfarming was studied extensively in recent years, and the pilot-scale researches in arctic areas were conducted in both laboratory and field. Pilot-scale landfarming experiments were conducted in a laboratory in soil tanks with 1.0m long, 0.65m wide and 0.35m deep Chang et al. (2010; 2012). Studies assessed the extent of biodegradation of semi-volatile (F2:>C10–C16) and non-volatile (F3:>C16–C34) petroleum hydrocarbon fractions in historically diesel-contaminated soils treated with adding nitrogen and phosphorus nutrient to achieve C_{TPH}: N: P molar ratio of 100:9:1, and $CaCO_3$ at 2000 $mgKg^{-1}$ for maintaining neutral pH, and periodic 10-day tilling under aerobic conditions, under representative seasonal freezing and thawing temperature regimes. The site soils were acidic and N-deficient, but contained indigenous populations of hydrocarbon-degrading microorganisms. The reduced TPH concentration was up to 64% over a 60-day period. The rate and extent ofF2 and F3 petroleum hydrocarbon fractions in the landfarms containing higher initial TPH levels (~2000 mg Kg^{-1}) and lower TPH levels (~1000 mg Kg^{-1}) were compared. Biodegradation profiles of the C14, C16 and C18 alkanes revealed that at TPH concentrations above 1000 mg Kg^{-1} these compounds are degraded concurrently, whereas below 1000 mg Kg^{-1} the higher-molecular weight alkanes are preferentially degraded. Their research also examined the changes in microbial respiration activity and population size and composition in these soils during the same temperature regimes. Research detected an increase in culturable heterotrophs and 16S rDNA copy numbers during the freezing phase, and the $^{(14)}$C-hexadecane mineralization in soil samples obtained from the nutrient-amended tank steadily increased. Hydrocarbon degrading bacterial populations identified as *Corynebacterineae*- and *Alkanindiges*-related strains emerged during the freezing and thawing phases, respectively, indicating there were temperature-based microbial community shifts.

Similarly, a simple, economical landfarming operation was implemented to treat 3600 $m^3$of soil at a site just northeast of Barrow, AK (McCarthy, 2004). Diesel-range organics (DRO) and trimethylbenzene (TMB) were the major contaminants in the soil. The landfarming operation included application of a commercial fertilizer and an aggressive schedule of soil tilling. This work demonstrates that even in extremely harsh climates, soils that are moderately contaminated with petroleum hydrocarbons can be effectively and economically remediated within reasonable timeframes via landfarming.

Yu et al. (2009 and 2011) developed an integrated mathematical modeling system for simulating biosurfactant-enhanced bioremediation (BEB) processes. A pilot-scale tank (3.6m ×1.4m ×1.2m) was constructed to simulate a western Canadian site in Saskatchewan. Rhamnolipid was injected into the system to enhance the efficiency of bioremediation. The results indicated that the developed mathematical modeling system was effective in simulating the coupled remediation processes of biodegradation and biosurfactants.

CHAPTER 3
CHARACTERIZATION OF THE STUDY SITE

Before conducting the pilot-scale bioremediation study, a comprehensive site investigation was performed to facilitate the experimental design. Obtaining this information in the complex subsurface environment is a major challenge. Transport, physical, and chemical processes as well as biological processes must be considered. Thus, when designing field experiments, and determining the experimental protocol, it is important to consider whether the biological treatment process applicable given the complexity of the subsurface environment and the many processes that are occurring (Lewis et al., 1992).

The key factors achieved by site investigation included: (1) contaminant types and their physical and chemical characteristics (e.g. concentration, solubility, density and volatility); (2) subsurface conditions, such as soil type, hydrological/geological characteristics, homogeneity in vadose and saturated zones and soil permeability; (3) groundwater conditions, such as depth of perched water, depth of saturated groundwater and hydraulic conductivity; (4) potential extent of contamination, such as residual-phase and gaseous-phase hydrocarbons in the vadose zone, free-phase and dissolved-phase hydrocarbons in the saturated zone and the area of contamination; (5) adjacent surface conditions, such as conditions of operating property above the contaminated zone (e.g., open space, tanks, pipes, paving and structures) and open space available for treatment; and (6) related standards including clear-up criteria.

3.1 Site Selection

3.1.1 Goose Bay Contaminated Sites

5 Wing Goose Bay is located in the Province of Newfoundland and Labrador, near the mouth of the Churchill River in central Labrador (NL), Canada. As Figure 9 illustrated, it was at the southwestern limit of Hamilton Inlet in central Labrador and approximately 200 kilometers away from the Labrador coast. The Base lies on the north side of the river. It was constructed on a raised beach terrace, which has an elevation of approximately 40 to 50 m above mean sea level (masl), and is situated between Terrington Basin, a salt water body, and the Churchill River, located to the north and south of 5 Wing Goose Bay, respectively. Terrington Basin is an arm of Goose Bay, itself an arm of Lake Melville, that is modestly affected by tides.

5 Wing Goose Bay was constructed as a military base in the 1940's by the United States Air Force for the purpose of staging aircraft en-route to Britain during the Cold War. It was then operated by Public Works and Government Services Canada (PWGSC) and Transport Canada (TC) on behalf of its tenants, the Canadian Forces (CF), USAF, and Allied Participants from 1976 to 1987. In May, 1987, 5 Wing Goose Bay became a Canadian Forces Base (CFB) and to this day, sustains multinational flying operations as well as supports allied low level flight training.

The current land use at 5 Wing Goose Bay is predominantly military use (i.e. commercial/industrial) with some residential (e.g. PMQs). Forest, lakes, streams and wetlands surround the Base-these areas are categorized as recreational, as access is

unrestricted and can be used for recreational purposes. No designated wetlands are located in the area. Farms are located south of the Base, between the Trans Labrador highway and the Churchill River. A golf course is located immediately southeast, adjacent to the Base. The Town of Happy Valley-Goose Bay, originally located 8 kilometers (5 miles) from the Base, has expanded to where it now is located adjacent to the Base boundary. In fact, Town property surrounds the Base on all sides.

Figure 9 Overview of general site location (Goose Bay Remediation Project, 2008)

3.1.2 Goose Bay Remediation Project

At its peak of operation during 1951's and 1960's, more than 300 million litres of fuels were brought and stored in the tank farm in CFB 5 Wing Goose Bay. Around 160 km of pipelines were buried underground for the transportation of fuel within the Air Base. The majority of environmental contamination at the Wing can be attributed to past storage and handling practices of fuel and other contaminants. In addition to the normal operation of the base over the last 60 years, the remote location as well as the inconvenience of transportation lead to the on-site disposal of waste generated during operation before 1990 (Goose Bay Remediation Project, 2008). Furthermore, oil spill incidents such as leakage and rupture of pipelines resulted in a variety of contaminants released into subsurface. Contaminants have been existed in soil, sediments, surface and groundwater as well as regional biota.

The subsurface contamination in 5 Wing Goose Bay not only posed an adverse impact on human health and environmental compatibility, but also led to financial loss and reinvestment for industries and governments in NL. Federal and provincial governments, as well as associated industries, were obliged to endeavour research efforts and provide financial

support for site identification and remediation. Department of National Defence (DND) took the initiative of the Goose Bay Remediation Project (GBRP) with an investment more than $258 million dollars, investigating and managing over 100 potential contaminated areas to generate a comprehensive remediation plan. This GBRP consists of 10 sub-projects with the official remediation work beginning from 2010 and being estimated to last for 10 years.

3.1.3 Selection of the Study Site

The history of contamination at 5 Wing Goose Bay is well known and has a high profile with the public, media, and regulatory agencies. While the major hydrocarbon plumes can be attributes to leaking of underground and aboveground tanks, leaking or ruptured pipelines, and historical general management and containment practices. Heavy metals and other chemical contamination (e.g. polychlorinated biphenyls (PCBs), volatile organic compounds (VOCs), polycyclic aromatic hydrocarbons (PAHs), pesticides etc.) are due to historical waste disposal practices and existence of numerous dumpsites (Defence Construction Canada and Department of National Defence, 2010).

After investigation of the contaminated sites, the following areas are identified as the main legacy contaminated sites:

1) The South Escarpment (SES) waste disposal sites – a series of dump sites near the southern Base boundary containing a variety of wastes. Contaminants include fuels, VOCs, PAHs, PCBs, pesticides, and heavy metals.
2) The Upper Tank Farm (UFT) – the main tank farm on the upper part of the Base. Contaminants include primarily fuels.
3) The Survival Tank Farm (SFT) – one of two tank farms located off the escarpment, to northeast of the Main Base. The SFT tanks and pipelines have been removed. Contaminants include primarily fuels and PAHs.
4) The Ex-hydrant Area – a series of four fuel hydrants and infrastructure (Heavy Bomber Hydrant, Medium Bomber Hydrant, Fighter Hydrant, and Transport Hydrant) formerly used for refuelling airplanes. Contaminants include primarily fuels and PAHs.
5) The Lower/Main Tank Farm (LTF) – the second and largest of two tank farms located off the escarpment, north of the Main Base. Some of the tanks and infrastructure remain in service. Contaminants include primarily fuels, PAH and heavy metals.

Numerous other areas such as the North Escarpment, the Former Canadian Side, and various waste disposal sites also have associated environmental issues. Figure 10 gives the generalized location of the various contaminated sites.

Figure 10 Location of various contaminated sites (Goose Bay Remediation Project, 2008)

Among the above listed contaminated sites, LTF was selected as our target contaminated site. First of all, it was one of the main areas for staging such a storage/delivery system in 5 Wing Goose Bay. Around 63,500 m^3 of PHC impacted soil and 4. 13,600 m^2 of LPH impacted soil/GW (still determining the volume) was involved in this site.

Unlike many other sites mostly constituted of sand, the LTF geology consists of glaciofluvial deposits of inter-bedded fine- to medium-grained sands and marine silts and clay. Coarse-grained sandy soils are found near the toe of the escarpment while fine-grained organic marine sediments dominate in the salt marsh environment along the shoreline of Terrington Basin. Recent environmental investigations identified the presence of a semi-confined to confined aquifer formed by a silt or silty clay layer at some areas in the LTF. This confined layer was also observed during the current investigation at select locations throughout the LTF. The silt or silty clay layer is quite variable in depth and thickness, reportedly ranging from 1.9 mbgs to 9.75 mbgs in depth and from a few centimeters to over 2 m in thickness. Artesian conditions encountered in some wells throughout the LTF confirm that the silt or silty clay layer is acting as a confining layer at some locations (AMEC 2008b, 2008c, 2008d, 2008e, 2009).Its location (offshore area) as well as its complexity of soil constitution is another reason for the selection of this site.

Thirdly, LTF just finished site investigation and characterization, and is currently developing statement of work (SOW) for procurement. The work of this report can place great practical value to the next stage of work.

LFT contains 27 tank lots (7 active) within 3 km ²area, and Tank Lot 1526, 1519 and vicinity area were chosen to conduct pilot-scale bioremediation.

3.2 Site Investigation

3.2.1 Location and Setting of the Lower Tank Farm (LTF)

3.2.1.1 Location

Lower Tank Farm (LTF) is located at this base. Due to its vast size of the LTF, the area was divided into 21 sites (referred to as Site A through Site U). The Lower Tank Farm (LTF) is located approximately 1 kilometre (km) east-northeast of the Main Base. Although LTF is located apart from the Main Base property, it is within the DND property boundary. It occupies an area of approximately 3 km^2 to the south of Terrington Basin and to the northeast and below the North Escarpment Area and the plateau on which the main components of 5 Wing Goose Bay are situated. Founded in 1941, the 5 Wing Goose Bay areas have been actively used as a military base for over 60 years.

As required by military operations, significant volumes of fuel storage, as well as a comprehensive fuel delivery system, was constructed for 5 Wing Goose Bay. One of the main areas for staging such a storage/delivery system was the LTF. Since initial development (i.e. early 1940s), there have been 27 bulk fuel aboveground storage tanks erected at the LTF. Over the years, many of the original tanks have been decommissioned and dismantled; currently, only seven tanks remain intact at the LTF. The current fuel storage capacity of the seven bulk fuel storage tanks is approximately 80,637,000 L. At its peak, the LTF fuel storage capacity totaled almost 232,000,000 L (AMEC, 2007a).

The bulk storage and transfer of petroleum products at the LTF leads to a high potential for environmental impacts through accidental releases. In the past the LTF has been the subject of various environmental investigations. The majority of these have identified significant soil and groundwater impacts including presence of liquid petroleum hydrocarbons (LPH) at the water table surface at different locations within LTF. Although subject to intense investigation, much of the historical and intrusive data is disconnected and inconsistent, resulting in numerous data gaps and some locations within the LTF have remained undelineated and/or received limited investigation. In an attempt to identify and/or highlight some of these data gaps, AMEC conducted a Historical Review (HR) and Preliminary Investigation (AMEC 2007a) of the LTF and a follow-up LTF Site Reconnaissance (AMEC, 2008a).

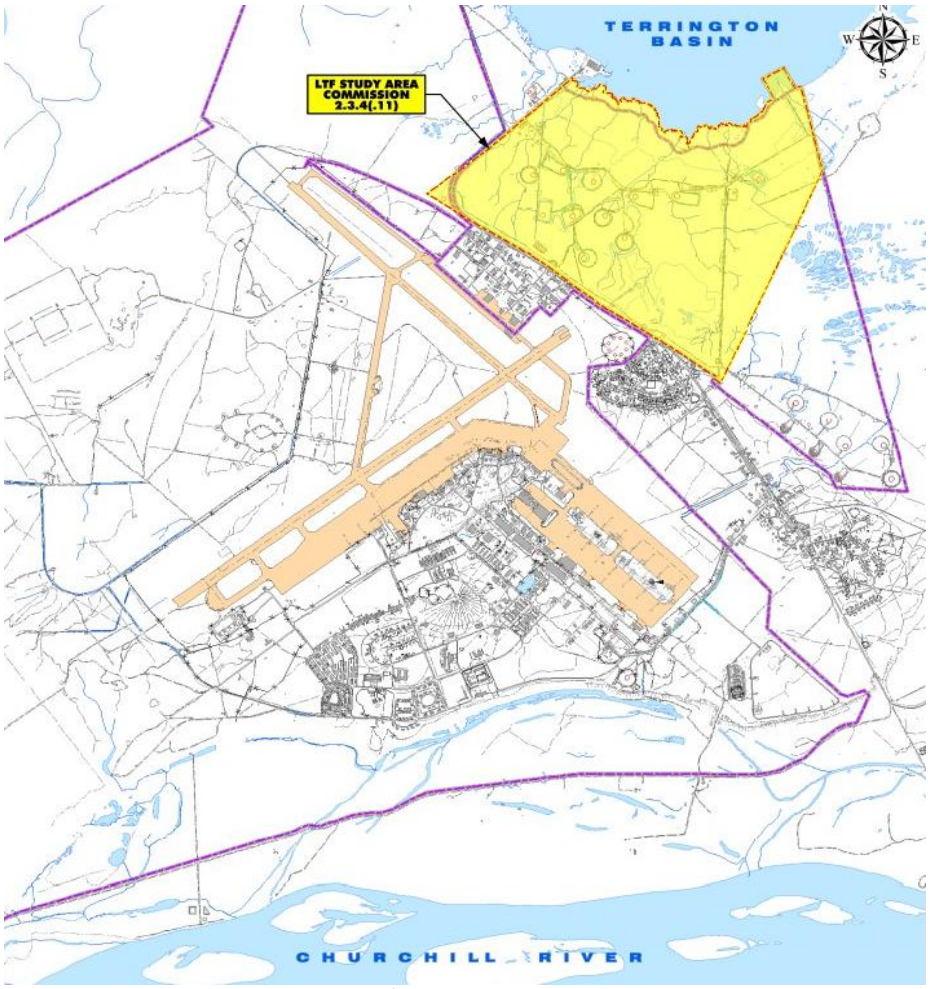


Figure 11 Location of Lower Tank Farm (AMEC, 2010)

Site E is located in the central portion of the LTF and includes existing Tank Lot 1526 (T1526) and surrounding areas (Figures E1 and E2; Attachments). Tank Lot 1526 is surrounded by other tank lot properties as follows: active Tank Lot 1518 (Site J) to the west, former Tank Lot 1520 (Site K) to the southwest, former Tank Lot 1519 (Site L) to the southeast and former Tank Lot 1531 (Site D) to the east. Undeveloped wooded lands and wetland areas are situated to the north, beyond which is Terrington Basin (as indicated above, approximately 550 m north). Immediately to the north, the Tank Lot is bordered by a pipeline corridor that is actively used to transport various fuels. LTF access roads are situated north and west of the Tank Lot.

Site L is located immediately north of the Shell Spill Site (Site S). It occupies the former Tank Lot 1519 (T1519). T1519 was installed in 1952 having a capacity of 15,911 m^3 and was used up until 1991 and was subsequently dismantled in 2006.

3.2.1.2 Climate

The climate in the Goose Bay area is not typical of a northern coastal climate because Goose Bay is located at the western end of the Terrington Basin, 200 km inland from the Labrador

Sea.According to Environment Canada records on Goose Bay, the mean annual temperature is -0.5℃, with mean minimum and mean maximum temperatures of -12.9℃ and 20.9℃ in January and July, respectively. There are, on average, 102 frost-free days a year at Goose Bay.

The average annual precipitation for the Goose Bay area is 949 mm, 59% as rain (based on a 40-year average). Snow cover normally persists from the end of October to the first week in May with a mean maximum depth of 75 to 80 cm. All of the records for extreme snow cover are more than 25 years old.

3.2.1.3 Topography and Drainage

The ground surface rises to the west across the Base. Bedrock outcrops are encountered to the west of the Base (e.g., Dome Mountain). The Goose River flows from west to east on the north side of the Base, while the Churchill River flows from west to east on the south side of the Base. Terrington Basin and the peninsula, on which the Town of Happy Valley-Goose Bay is located, lie to the east of the Base.

The Base lies on a raised terrace (upper plateau) that slopes gradually down towards escarpments to the south and north, which drop by as much as 35 m. The low-lying lands to the south are occupied by a number of lineal surface water bodies (called Stillwaters) and those to the north by the LTF and the STF.

Surface water flow is controlled by man-made ditches/drainage systems on the upper plateau portion of the Base. Flow is directed toward Terrington Basin (north and east portions of the Base) and toward the Churchill River (south side of the Base). Further to the north, creeks also drain northward to the Goose River.

Over most of the Base, surficial sands promote rapid infiltration of rainwater, recharging local groundwater. The highly permeable sands of the upper plateau promote rapid infiltration of rainwater which may discharge as groundwater to the low-lying areas to the east and south of the escarpments. Runoff from paved areas is channelled to storm drains and roadside ditches. Land cover at the present time consists of both open and forested areas, wetlands and several large diked areas around active tanks. In the areas below the terrace (LTF, STF and Stillwater areas), more surface water bodies exist. These include the Stillwaters running parallel to the Churchill River, streams, wetlands (e.g., marshes) and the Churchill and Goose Rivers.

It is anticipated that several hydrologic divides exist across the Base. These separate the three main watersheds (Churchill River, Goose River and Terrington Basin/Lake Melville). Surface and groundwater flow from the north-northwest portion of the Base would be toward the Goose River, from the northeast toward Terrington Basin and from the south to the Churchill River.

The LTF topography slopes northeast from 24 metres above sea level (masl) at the toe of the North Escarpment to near sea level at Terrington Basin. Vegetation varies from short brush

and moss near Terrington Basin to scrub and fully developed coniferous and deciduous trees towards the North Escarpment. There is a series of small natural and manipulated (i.e. manmade) watercourses and drainage channels throughout the LTF, which in some cases terminate at high water tables and wet areas, prior to draining into Terrington Basin. Of particular note is a large man-made drainage channel that flows from south to north along the eastern side of the LTF. This drainage channel traverses through sub-sites U and C (along the main LTF access road) and drains towards Terrington Basin to the north.

3.2.1.4 Geology and Hydrogeology

Ice flows covering Labrador and the Goose Bay area during the most recent period of glaciation spread eastward from northern Quebec, pushing along debris and gouging the land surface with embedded rock particles dragged along with the moving ice. Before the most recent glaciation, the region was a low plateau but the pre-glacial landscape was effectively destroyed.

The easily eroded sandstones infilling the Lake Melville and Churchill River rifts were scraped away and glaciers carved the U-shaped valleys. The bedrock of the Mealy Mountains to the south were resistant to glacial action, hence they remain as mountains. When the glaciers melted about 8,000 years ago, melt water laden with sand filled the Churchill River and a massive delta formed at the mouth. Sea levels rose and filled Lake Melville. Following removal of the great weight of ice, the land rebounded.

The present wetlands along the shorelines, which surround the Base, were once submerged coastline. Happy Valley-Goose Bay is built on the amalgamated sandy deltas of the Goose and Churchill rivers. Gravel and sand deposits are examples of glacial-fluvial sediments. The boulders strewn about the area are erratics, rocks carried along by the moving ice. The raised terrace at 5 Wing Goose Bay is made up of glacial fluvial sand deposits, which collectively exceed 50 m in thickness. The deposits are composed of fine-to-medium sand with trace silt and occasional discontinuous silt lenses and coarse-grained sand interbeds. Silty sand generally predominates below a depth of 25 m below ground surface (mbgs). Generally, bedrock is not encountered on Base property, with the exception of mountains to the west and north (e.g., Dome Mountain) where bedrock is at/near surface. Groundwater beneath the Base is unconfined and flows south to southeast towards the Churchill River and northeast to east towards the Goose River and Terrington Basin. The water table ranges from approximately 10 to 30 mbgs across the terrace and is typically less than 5 mbgs along the low lying lands surrounding the terrace.

AMEC (2008c) describes the groundwater underlying the Base. This report states that "Groundwater beneath 5 Wing Goose Bay Base flows south to southeast towards the Churchill River and northeast to east towards the Goose River form a groundwater divide. The divide is positioned approximately diagonally across the Base. The water table ranges from approximately 10 to 30 mbgs across the terrace and is typically less than 5 mbgs along the low lying lands surrounding the terrace. Annual water table fluctuations beneath the

terrace are on the order of 2m to 3m. The hydraulic conductivity (K) of the water table aquifer ranges from 2×10^{-5} cm/s to 3×10^{-2} cm/s with a geometric mean of 5×10^{-5} cm/sec. The effective porosity reportedly ranges from 0.15 to 0.25. The average groundwater seepage velocity across is estimated at 75 m/year but is higher near embankments ranging from 100m/year to 200m/year.

The LTF geology consists of glaciofluvial deposits of inter-bedded fine- to medium-grained sands and marine silts and clay. Coarse-grained sandy soils are found near the toe of the escarpment while fine-grained organic marine sediments dominate in the salt marsh environment along the shoreline of Terrington Basin (Serco, 2003). The sand thickness varies from over 10 m at the toe of the North Escarpment to less than 1 m at Terrington Basin. Thesand unit is underlain by silt and clay units of varying thickness and lateral continuity. Previously, the porosity at the LTF has been estimated as 0.34 to 0.46 (Serco, 2003), however based on the soil conditions observed during the current investigations (i.e. sand and silts) a porosity range of 0.25 to 0.50 has been used for most locations when calculating groundwater velocity. However, at a few select locations a lower porosity value (i.e. 0.15) has also been used based on local soil conditions.

The shallow aquifer in the LTF is generally characterized as an unconfined, unconsolidated, fine- to medium-grained sand aquifer; however some recent environmental investigations identified the presence of a semi-confined to confined aquifer formed by a silt or silty clay layer at some areas in the LTF. This confined layer was also observed during the current investigation at select locations throughout the LTF. The silt or silty clay layer is quite variable in depth and thickness, reportedly ranging from 1.9 mbgs to 9.75 mbgs in depth and from a few centimeters to over 2 m in thickness. Artesian conditions encountered in some wells throughout the LTF confirm that the silt or silty clay layer is acting as a confining layer at some locations (AMEC 2008b, 2008c, 2008d, 2008e, 2009). At other locations, where the layer is very thin, confining or semi-confining conditions may not exist.

Groundwater elevation data collected during the most recently conducted fluid level monitoring event (i.e. December 2010) indicates that the groundwater elevation at the LTF area varies from 0.61 masl (Site A) in the northeast of the LTF to 24.26 masl (Site S) along the southern border of the LTF (immediately north of the North Escarpment). The hydraulic conductivity (K) values determined within the LTF vary from 2.30×10^{-5} cm/sec (Site E) to 8.01×10^{-3} (Site S) cm/s.

Using the 2010 groundwater elevation data, the horizontal hydraulic gradient has been estimated to vary from 0.0086 m/m (at Site P) to 0.172 m/m (at Site I). Using a porosity range of 0.15 to 0.50 the groundwater velocity was calculated across the LTF area to vary from 0.36 m/yr (Site L) to 651 m/yr (Site I). The general groundwater flow direction across the LTF was observed to be influenced by features (e.g. streams, ditches, and wetlands) encountered across the LTF, but generally groundwater flow is towards the north or northeast towards Terrington Basin. A more detailed analysis of the prevailing local hydrogeological

conditions encountered at each of the LTF sites is presented in each of the individual site summaries.

Hydraulic conductivity at site E**:** The K test results obtained during previous work at the Site E were used in combination with the current groundwater elevation data to calculate an average groundwater velocity across the Site. The table below provides a summary of the K test results obtained during previous investigations:

Table 5 Hydraulic conductivity at site E

Station ID	Bottom of Screen Elevation (masl)	Calculated K (cm/s)
09-MW291-N1E1	4.25	5.42×10^{-5}
09-MW297-N1E1	4.33	9.83×10^{-6}

The geometric mean of the K values stated above was calculated to be 2.30×10^{-5} cm/s. The average horizontal hydraulic gradient (i) at the Site is estimated to be on the order of 0.026 m/m. Using an effective porosity (n) range of 0.25 to 0.50, which is an acceptable range for the soil conditions identified at the Site (i.e. sand to silt/clay), the average linear groundwater velocity in this area of the Site was calculated using V = Ki/n and is on the order of 0.75 m/yr to 0.38 m/yr.

Hydraulic conductivity at site L: Previous reports (i.e. AMEC 2009 and Franz 2001) indicate that monitoring wells LTF2030-2007-MW94S and LTF2030-2007-MW89S2 are situated in the deeper aquifer and the monitoring well LTF2030-2007-MW89S3 is situated in the shallow aquifer. The geometric mean of the hydraulic conductivity results for wells within the deeper aquifer was determined to be 5.26×10^{-5} cm/s. The monitoring wells LTF2030-2007-MW89S and 98-LTF-04 were screened through intersecting both the shallow and deep aquifers and therefore were not considered during the groundwater velocity calculations.

Table 6 Hydraulic conductivity at site L

Station ID	Calculated K (cm/s)
LTF2030-2007-MW94S	1.33E-06
LTF2030-2007-MW89S	1.07E-03
LTF2030-2007-MW89S2	2.08E-03
LTF2030-2007-MW89S3	6.32E-05
98-LTF-04	1.18E-05

The average horizontal hydraulic gradient at the Site is estimated using the current groundwater elevation for newly installed wells in the shallow aquifer and is on the order of 0.009 m/m. Using an effective porosity range of 0.25 to 0.50, which is an acceptable range for the soil conditions identified at the Site (i.e. sand to silt/clay), the average linear groundwater velocity in this area for the shallow aquifer of the Site was calculated using V = Ki/n and is in the order of 0.36 m/yr to 0.72 m/yr.

The groundwater elevations at site L obtained from the fluid level monitoring were used to generate groundwater contours and an inferred groundwater flow at the Site. The inferred

direction of horizontal groundwater flow is generally directed north towards the tank lot 1526 (i.e. Site E) and subsequently to the Terrington Basin, which is consistent with findings presented in previous reports pertaining to the Site

3.2.2 Site Use

The LTF was constructed on undeveloped, forested lands circa 1942 for the USAF with the erection of the first five fuel storage tanks (Tanks 1, 2, 3, 4 and 83), followed by an additional 22 tanks over the ensuing 10 to 15 years. Out of the 27 fuel storage tanks, only seven tanks (Tanks 1509, 1516, 1518, 1526, 1536, 1540 and 1541) remain intact today. As described in AMEC 2007a, a few of the former tanks (i.e. Tanks 1, 2 and 4) were located outside of current DND property boundary. In 1959-1965, four of the first five tanks were dismantled (Tanks 1, 2, 3 and 4). Five fuel storage tanks (Tanks 101, 102, 103, 104 and 105) were dismantled post-1987 and 10 tanks (Tanks 1511, 1515, 1517, 1519, 1520, 1531, 1534, 1535, 1537 and 1539) were dismantled between the years 1991-1996. Tank 83, a one-million gallon underground storage tank (UST), was also previously abandoned, however the year in which this occurred is unknown. Throughout its history, LTF has always been used as a commercial/industrial property mainly for the bulk storage of petroleum hydrocarbons such as gasoline, avgas, jet and diesel fuels. The former POL dock (Site A) and constructed jetty was utilized for transporting petroleum products from tankers to the storage tanks through pipelines, which were installed pre-1954. Reportedly, these pipelines were dismantled post-1973 (exact date unknown).

Building 77, which was utilized as a central heating and power plant was constructed prior to 1953 and dismantled in 2002 (AMEC, 2006). The known fuels used and stored in B77 are Bunker A and Bunker C.

For convenience of the current investigation, the LTF was divided into 21 sites (referred as Site A to Site U). The following table (information taken from AMEC 2007a and other historical documentation) summarizes site IDs, description of the main study areas including tank lot/building numbers, year of installation, capacity, historical/current contents and year of dismantling (if applicable). The history use of the sites E and L in LTF were listed in the table 7.

3.2.3 Review of History Investigation

Historical usage of LTF： Dillon (2009a) conducted a holistic review of the historical data/reports (1989-2008) pertaining to the LTF in an attempt to identify any data gaps and/or outstanding issues that could be addressed via a holistic short term plan. This review included a prioritized summary of recommended additional work required at the LTF to bring all sites to step 6 of the Federal Approach to Contaminated Sites process (Dillon, 2009b). Historically, LPH was detected at a number of Sites at the LTF. Recent investigation activities, conducted since 2005, suggest that historical LPH detections in a few monitoring wells may have been falsely identified (i.e. LPH <1 mm that was not bailer confirmed). Sporadic appearance of

LPH in a number of monitoring wells might be associated with one or more of the following factors: the fluctuation of groundwater table, inappropriate screen interval, inappropriate or absence of sand pack aroundthe well casing, migration of the LPH and disappearance of small volumes of LPH due to source removal and biodegradation.

As reported in Dillon 2009a and subsequent investigation reports, historical concentrations of BTEX/PHC, PAH and metals in soil and groundwater were in excess of the applicable guidelines, standards and/or criteria throughout the main LTF study area (e.g., tanklots, buildings, pipeline junctions) at each of the sites in the LTF. Prior to 2010, the extents of the soil and/or groundwater impacted areas were not fully delineated.

Historically, limited surface water and sediment sampling was carried out during site investigations/risk assessments at a few locations within the LTF; some of which identified various impacts in surface water (e.g., PHC F1 at Site H; SNC-Lavalin, 2009) and sediment (e.g., PCB at Site Q; AMEC, 2007b). However, surface water and sediment quality in most of the surface water bodies within the LTF had never been investigated and represented a significant data gap.

Historical usage of site E： A thorough review completed by AMEC, 2007a found that Tank Lot 1526 was installed in 1952 and has a storage capacity of 18,800 m^3. The tank lot was upgraded in 1996-97 to meet guidelines current at that time governing the bulk storage and transfer of petroleum products. It is currently used for storage of Jet A1 fuel. The known historical uses include Jet B and Avgas (AMEC, 2007a).

Drainage within the active tank lot is controlled by grading, which directs surface water towards two control weirs on the north side of the tank lot berm. Surface water drainage is diverted to a constructed drainage ditch that surrounds the perimeter of the tank lot. Ultimately, drainage from the drainage ditch is to tributaries and streams that drain to the Terrington Basin, which is located approximately 550 m north of the Site.

Due to the fact that Tank Lot 1526 is currently an active lot, intrusive investigation within the limits of the tank lot boundaries is prohibited (the tank lot contains an impermeable liner with bermed area). As such, all work carried out under the 2010 work program was completed beyond the boundaries of the Tank Lot.

Historical usage of site L: The known historical use of T1519 was storage of Avgas in 1953 and Jet A1 at a later date. The former tank is surrounded by a vegetated and swampy ground surface with abundant standing water. The remainder of the Site contains a mixture of spruce/birch forest and wetland with occasional ponding and small streams and creeks. Tank Lot 1519 is bounded within the LTF by former Tank Lot 1520 to the northwest, Tank Lot 1526 to the north, a vegetated portion of the LTF to the east, the location of the former Shell spill to the south, and Tank Lot 1509 to the southwest.

Table 7 History use of this LTF

Site ID	Description of the Main Study Area	Year Installed	Capacity (m^3)	Known Current/ Historical Contents	Year Dismantled
E	Active Tank Lot 1526 and nearby existing pipelines	1952	18,800	Jet A1, Jet B, Avgas	Exists and active
L	Former Tank Lot 1519 (T1519) and nearby existing pipelines	1952	15,911	Avgas, Jet A1	2006

3.2.4 Site Classifications/ Guidelines

The applicable regulatory criteria to compare against analytical data are determined, in part, by features of the Site such as land use, presence of potable water supplies and distance to other possible receptors. The land use at the LTF is considered to be commercial. The surface and sub-surface soil at the Site is coarse-grained. There are no potable water wells known to be located within 1 km of the LTF. There are drainage ditches, creeks, streams, standing waters and wetlands present throughout the LTF. Groundwater from the LTF is generally shallow and the potential exists for discharge to local drainage channels/streams and wetland areas. Therefore, criteria protective of Fresh Water Aquatic Life (FWAL) have been selected as a conservative measure where it was deemed to be applicable.

3.2.4.1 Soil Criteria

Given the above, the following soil criteria were adopted for comparison purposes:

- BTEX, Metals, PCBs, Pesticides and VOCs: CCME SQG. Canadian soil quality guidelines for the protection of environmental and human health: Summary tables, updated September 2007. Guidelines for commercial land use and coarse-textured surface soil. Canadian Council of Ministers of the Environment (CCME, 2007a). Note, Interim Soil Quality Criteria were used when soil quality guidelines have not been developed for a given parameter as suggested in CCME, 2007a.
- PHC (Tier1): CCME Tier 1 PHC CWS. Canada-wide standards for petroleum hydrocarbons (PHC) in soil: Table 1. Revised January 2008. Standards for Tier 1 levels for coarse-grained surface soils and commercial land use in a non-potable groundwater condition. Canadian Council of Ministers of the Environment (CCME, 2008a).
- PAHs: CCME SQG. The Canadian Soil Quality Guidelines for the Protection of Environmental and Human Health - Polycyclic Aromatic Hydrocarbons (PAHs). Revised July 2010. Table 2: Soil Quality Guidelines for Carcinogenic and Other PAHs. Guideline for Benzo[a]pyrene TPE (10^{-5}) = human health guidelines (SQG DH) based on carcinogenic effects of PAHs for direct contact based on an incremental lifetime cancer risk (ILCR) of 1 in 100,000 (10^{-5}) for commercial land use; guidelines for other PAHs =

Environmental health guidelines (SQG E) based on non-carcinogenic effects of PAHs for commercial land use. Canadian Council of Ministers of the Environment (CCME, 2010).

3.2.4.2 Surface Water and Groundwater Criteria

The following criteria have been adopted for comparison purposes:

- BTEX, PAHs, Metals, inorganic and indicator parameters: CCME WQG-FWAL. Canadian water quality guidelines for the protection of aquatic life: Summary tables. Updated December 2007, Guidelines for freshwater aquatic life. Canadian Council of Ministers of the Environment (CCME, 2007b). Long-Term Exposure Guideline for Boron has been adopted (CCME, 2009).
- PHC F1 and PHC F2: GBIAC – SW & GW. Interim aquatic life and wildlife water use criteria for PHC F1 and F2 in surface water and groundwater at Goose Bay recommended by AMEC 2007. Commission 83, Petroleum Hydrocarbon Assessment Criteria Review – Surface Water and Groundwater, AMEC Earth and Environmental (AMEC, 2007c).

It is noteworthy that all of the groundwater samples were analyzed for ARBCA Tier 1 PHC fractions (C6-C10 less BTEX, >C10-C21 and >C21-C32), and the laboratory reported results have been converted to the CCME CWS PHC fractions (F1 less BTEX: C6-C10, F2: >C10-C16 and F3: >C16-C34) using the Health Canada spreadsheet calculator (Health Canada, 2009) for comparing PHC F1 and F2 results against the applicable GBIAC (AMEC, 2007c).

It is also important to note that the GBIAC considered development of risk based PHC F1 and PHC F2 criteria, in analogy to the Alberta Tier 1 Soil and Groundwater Remediation Guidelines (AENV, 2007), for the following ecological receptors: (i) aquatic life, via lateral groundwater transport and discharge into a surface water body; and (ii) wildlife, drinking water from a surface water body potentially connected to contaminated groundwater. The criteria development took into account natural attenuation in groundwater only, and assumed that there is a 10 m distance between the groundwater contaminant plume and the surface water in order to allow natural attenuation to take place. Therefore, groundwater samples collected from monitoring wells located within 10 m of a surface water body (e.g., drainage ditch, stream, wetland) were assessed against GBIAC-SW. Groundwater samples collected from monitoring wells located beyond 10 m of a surface water body were assessed against GBIAC-GW. Furthermore, if there was any uncertainty regarding the distance of a monitoring well from the surface water body or if the surface water body was non-perennial; the more conservative GBIAC-SW was applied.

3.2.4.3 Sediment Criteria

The following sediment criteria have been adopted for comparison purposes:

- PAHs, Pesticides, PCBs, and metals: Canadian Sediment Quality Guidelines for the Protection of Aquatic Life: Summary tables – Interim Sediment Quality Guidelines

(ISQG) and Probable Effects Levels (PEL), updated 2002. Canadian Council of Ministers of the Environment (CCME, 2002).

It is noteworthy that there are no comparable sediment criteria for BTEX/PHC or TPH.

3.2.5 Impact across target site in LTF

The following table (Table 8) provides an overview of impacts/compounds of concern identified in each of the Sites within the LTF during the current investigation.

Table 8 Site impact

Site ID	LPH (√/×)	Media / Compounds of Concern			
		Soil	Groundwater	Sediment	Surface Water
E	×	EX/PHC (F1, F2); PAH (Naph)	BE/PHC (F1,F2); PAH (Naph); metals (Cr, Hg, Pb)	Elevated TPH; PAHs; metals (Cd, Cu, Hg, Pb, Zn); pesticides	PHC (F1); PAHs; metals (Cu, Pb)
L	×	PHC (F1, F2); PAH (Naph)	BE/PHC (F1,F2); PAH (Naph); metals (Cd, Cr, Hg)	Elevated TPH; PAHs	PHC (F1)

3.2.5.1 Impact of Site E

1) Soil:

BTEX/PHCs： As indicated above, two of the eight soil samples collected during the investigation were found to exceed applicable criteria for one or more parameters of BTEX/PHC. Based on the current analytical results, the inferred extent of BTEX/PHC impacted soil is shown in Figure E5 (Attachments). The majority of the BTEX/PHC impacts in soils have been delineated, with the exception being to the south inside the tank lot and to the north as defined by the impacts at 10-MW216-N1E1. The impacts noted in sample 10-MW216-N1E1 extend the inferred soil impacts north of those previously defined. A soil sample could not be obtained during the installation of the drive point piezometer installed northwest of the tank lot, as drive-point piezometer installations do not require removal of soils. However based on the field observations, no PHC impacts were noted at this location. No impacts were noted in the soils obtained from 10-MW210-N1E1 which is located south of the drive point piezometer. To the south the extent of soil impacts has not been fully investigated due to the fact that the tank lot is active and as such, intrusive investigation within the tank lot impoundment is not permitted at this time. Based on the analytical data the primary compounds of concern in soils are a mixture of the BTEX constituents and PHCs in the F1 and F2 fractions. The hydrocarbon signature present in the Site soils has been identified as being predominantly a product resembling gasoline.

Petroleum Degrading Bacteria (PDB)： Three soil samples have been analyzed from Site E for the presence of PDB; one sample from the current investigation (10-MW212-N1E1) and two samples (09-MW294-N1E1 and MW299) during the previous investigation (i.e. AMEC,

2010). Based on the analytical results, PDB counts in Site soil ranged from <100 cfu/g (09-MW299-N1E1) to 31,000 cfu/g (09-MW294-N1E1). Samples obtained during AMEC, 2010 were collected from areas outside of identified PHC impacts, while the sample from 10-MW212-N1E1, which had a PDB count of 9900 cfu/g, was collected from an area where known PHC impacts were identified. There is quite a variation in the PDB counts in the Site soils and from within impacted and non-impacted areas of the Site. Due to the variation of PBD counts across the Site, it cannot be determined if PBD counts are sufficient to sustain active bioremediation/degradation of hydrocarbons by microbes in the impacted areas of the Site.

2) Groundwater

BTEX/PHC: Hydrocarbon odour and/or sheen noted in groundwater during well development and/or groundwater sampling programs during the current investigation indicated that hydrocarbon impacts in groundwater likely existed beyond the previously defined area of impact. Groundwater samples from six of the nine newly installed monitoring wells at the Site were found to exceed applicable criteria for one or more parameters of BTEX/PHC. Groundwater samples from two of the six existing monitoring wells sampled in 2010 were found to exceed applicable criteria for more than one parameters of BTEX/PHC.

The inferred areal extent of the BTEX/PHC plume based on the current and previous investigations is shown in Figure E8 (Attachments). As indicated, the dissolved BTEX/PHC impacts at Site E encompass a large area of the Site. The majority of the impacts are noted immediately north of the Tank Lot where the plume is spread (west and east) along the pipeline corridor and north towards the LTF access road, both of which are situated north of the Tank Lot. This main area of the plume has been delineated to the west and to the east; however the majority of the plume to the north and south has not been delineated during the current investigation.

At the conclusion of the previous investigation at Site E (i.e. AMEC 2010b), the inferred PHC plume was shown to extend slightly north along the northeast section of the plume. After the current investigation the plume has now been extended even further. Most of the newly installed monitoring wells in the northern portion of the Site are within 10 metres of a creek/stream that resides in this area of the Site (water from these locations is assessed against the GBIAC-SW). The northern sections of the plume have been extended northwest, north and northeast; however, due to a lack of additional monitoring wells, the plume remains undelineated beyond these margins.

The PHC plume has also been extended to the southwest and west of the Tank Lot. Previously, this portion of the plume was not delineated. Two monitoring wells were installed in this area of the Site to help achieve delineation. Apparent impacts in both 10-MW211-N1E1 and –MW210 has extended the plume southwest from the main area of the plume. Due to the presence of a stream as well as wetland areas west of the Tank Lot the groundwater

from both of these wells are compared against the GBIAC-SW. The plume extension southwest has not been delineated in any direction. No investigation to the east, within the tank lot, could be carried out to confirm if impacts extend beyond the tank berm and into the tank lot in this direction. Further delineation work west of the tank lot would be required to fully delineate this portion of the plume. Based on the current analytical results, the inferred PHC impacts across the Site resemble gasoline.

PAHs: Fourteen groundwater samples were collected from across Site E; nine from the newly installed wells and five from existing wells. Of the 14 samples collected, six samples (all from the newly installed wells) were found to exceed the applicable criteria for naphthalene.

The inferred aerial extent of the dissolved PAH (naphthalene) plume is shown in Figure E9 (Attachments). As indicated on Figure E9 (Attachments) the dissolved PAH plume at the Site is quite extensive, covering a large area north of the Tank Lot. Moving north and northeast the plume becomes stretched or elongated by sampling points 10-MW214-N1E1 and –MW215. The plume is nearly delineated, with the exception of a small region north of 10-MW217-N1E1 and to the northeast beyond monitoring wells 10-MW214-N1E1 and –MW215. To the south, no wells have been installed or sampled due the fact that the tank lot is currently active. The extent of PAH impacts in groundwater have not been delineated to the south. Further delineation to the north, northwest and south would be required to fully delineate the existing PAH plume.

Metals: Metals in groundwater appear to be widespread across the Site. Groundwater samples from each of the newly installed monitoring wells were found to exceed applicable CCME guidelines for one or more of the metal parameters analyzed. Table F-12 in Appendix F contains the analytical results that exceeded that applicable CCME guidelines and Figure E10 in Attachments illustrates the locations where the different metals exceeded during the current investigation. Iron was found exceeding at every location, indicating that iron is prevalent at Site E (and across the LTF). Aluminum was found at three of the nine monitoring locations, chromium at two locations, mercury at one location and lead at three locations. Iron and aluminum exceedences are widespread across the LTF and surrounding area suggesting that they are likely related to an elevated natural background.

3) Sediment

A total of 10 sediment samples (plus 1 duplicate) were gathered from various areas of the Site. Samples were gathered downstream of the known area of impacts, adjacent to the area of impacts, from within the plume area and up gradient of the plume area.

Currently there are no guidelines or standards established for BTEX/TPH in sediment; however detections of TPH were noted in seven of the 10 sampling locations. Most of the concentrations of TPH were low, however at two locations: 10-ST133-N1E1 and –ST136 the TPH concentrations were quite substantial at 7310 and 8640 mg/kg respectively. Sampling station 10-ST133-N1E1 is located at a considerable distance (downgradient) of the known

PHC plume at the Site and sampling station 10-ST136-N1E1 is located northwest and cross-gradient of the inferred PHC groundwater plume extent.

At each of the sampling locations, sediment samples were collected and submitted for laboratory analysis of PAHs. Out of the 10 samples, one sample (10-ST136-N1E1) was found to contain PAH exceedances above the CCME ISQG. As indicated above, sampling station 10-ST136-N1E1is located northwest and cross-gradient of the inferred PHC groundwater plume extent at Site E. Based on the current sediment sampling results, PAHs in sediment do not appear to be widespread across the Site, however further assessment in and around the area of 10-ST136-N1E1 would be warranted to determine the full extent of PAH impacts in sediment in this section of the creek.

Metal concentrations were detected in each of the sediment samples taken from Site E, however exceedances of the CCME PEL and/or ISQG were only noted at one sampling location; 10-ST136-N1E1. At this station cadmium, copper, lead, mercury and zinc were found in exceedance of either the CCME PEL and/or ISQG. Based on the current sediment sampling results, metals in sediment do not appear to be widespread across the Site, however further assessment in and around the area of 10-ST136-N1E1would be warranted to determine the full extent of metal impacts in sediment in this section of the creek.

Evidence suggests that impacts exist in the sediment from the area where 10-ST136-N1E1 is located, based on the fact that an elevated TPH concentration was noted as well as the identified PAH and metal exceedances. Possible impacts may also exist at sample location 10-ST133-N1E1 where an elevated TPH concentration was noted. The impacts at 10-ST136-N1E1 may or may not be associated with the noted soil and groundwater impacts at the Site. This station is located cross-gradient from both soil and groundwater impacts and is not located within a stream that runs through the impacted area of the Site. Other sediment stations (e.g. 10-ST137-N1E1) that are located in and around both soil and groundwater impacts did not show any apparent impacts. This being said, the impacts noted at 10-ST136-N1E1 may be originating from alternative upgradient areas of the LTF. Furthermore, the elevated TPH concentration in sediment at 10-ST133-N1E1 do not appear to be linked to the apparent soil and groundwater impacts at Site E (i.e. north of Tank Lot 1526) due to the distance downstream. The elevated TPH concentration at this location may be associated with another source or be attributed to the same source that has contributed to the impacts at 10-ST136-N1E1. Additional sampling upstream (between 10-ST133-N1E1and –ST136) and downstream of 10-ST133-N1E1 would be required to determine if the elevated TPH concentration noted at 10-ST133-N1E1 is an isolated occurrence.

3.2.5.2 Impact of Site L

1) Soil

Soil analytical data from current investigation as well as the data from previous investigations has been considered in order to delineate soil impacts across the Site. Note, historical data has

been compared against current applicable criteria or standards (e.g. CCME CSQG or CWS) and used to delineate and/or define the inferred extents of soil impacts at the Site.

BTEX/PHCs: As indicated above, one of the ten soil samples collected during this investigation was found to exceed the applicable standards for both PHC F1 and PHC F2. This sample was collected from within the former tank footprint. There appears to be one large area of BTEX/PHC impacted soils that extends from the former tank footprint north and northeast of the tank lot. It should be noted that BTEX/PHC impacted soils outside of the tank berm are suspected to be related to the impacted soil within the tank berm. After comparing ground elevations between borehole's LTF2030-2007-MW90S and LTF2030-2007-MW86S, soils were found to be impacted within the same depth elevations. As such and as depicted on the Figure L5, the areal extent of BTEX/PHC impacted soil is delineated to the north, south, and west, but not fully delineated to the northeast and east. It should also be noted that the laboratory detection limits for the BTEX results of the soil from T-17 and T-18 are above current CCME guidelines. As such, there may be impacts existing at these former test locations which were not previously detected.

PAHs: As indicated above, one of the ten soil samples collected during this investigation was found to exceed the applicable guideline for naphthalene. This sample was collected from within the former tank footprint. The inferred PAH impacted soil extent based on data collected from the current and previous investigations is shown on Figure L6 (Attachments). There appears to be one large area of PAH impacted soils within former tank footprint that extends north of the tank lot. The aerial extent of PAH impacted soil is delineated to the north, south, and southwest, but not fully delineated to the northeast and northwest.

2) Groundwater

Groundwater analytical data from current investigation as well as the data from previous investigations have been considered to delineate the groundwater impacts at the Site. Furthermore, all historical data pertaining to the Site has been compared against the current applicable criteria (i.e., CCME CWQG-FWAL or GBIAC) so that analytical exceedances could be used to define/delineate the current inferred extent of groundwater impacts at the Site.

BTEX/PHC: Groundwater samples from four of the 13 monitoring wells sampled during this investigation contained concentrations in excess of the applicable criteria for one or more parameters of BTE and/or PHCs. There is one dissolved BTE/PHC impacted plume at the Site. The plume extends from the footprint of the former tank to north of the tank lot. Based on the current and previous investigations, this plume has not been delineated in all directions, apart from south of the footprint of the former tank and north of 10-MW290-N1E1.

PAHs: Groundwater samples from four of the 12 monitoring wells sampled during this investigation contained concentrations of naphthalene in excess of the applicable CCME guidelines. There are three PAH plumes identified at the Site: one large plume and two

smaller and somewhat isolated plumes represented by single sampling points (i.e. wells). The largest plume is located in the vicinity of the former tank footprint and extends north and northeast beyond the tank lot berm along the LTF access road leading to the tank lot. To the east of this plume is the former fuel recovery pond used during the remedial efforts employed at the Shell Spill Site (Site S). This plume has been partially delineated to the south; south of the former tank footprint, and northeast; north of 10-MW290-N1E1. The predominant PAH impact in this plume is naphthalene, however along the western portion of the plume there is also observed exceedances of anthracene, fluoranthene, phenanathrene and pyrene. In addition to the larger plume, there are two smaller PAH plumes that exist along the former remedial trench (also used during the Shell Spill Site recovery efforts); these plumes exist northeast and southeast of the of the tank lot. Furthermore, since both of these impacts were observed within monitoring well installed within (or near too) the former remedial trench area, the PAH exceedances are most likely stemming from residual impacts existing in these sections of the former remedial trench. The impacts observed at LTF2030-2007-MW94 have been broadly delineated to the northeast, southeast and south. Impacts in the groundwater from LTF2030-2007-MW94 included anthracene, benzo(a)antrhacene, benzo(a)pyrene, fluoranthene, phenanthrene and pyrene.

The impacts at LTF2030-2007-MW92 are delineated to the north and south and impacts at this location include pyrene.

Metals: Metals in groundwater appear to be widespread across the Site. Groundwater samples from each of the newly installed monitoring wells (10) were found to exceed applicable CCME guidelines for one or more metal parameters analyzed. Aaluminum exceeded at seven locations, cadmium at two locations, chromium at three locations and mercury at three locations.

3) Sediment

During the current investigation, sediment samples were collected from eight locations at the Site. All eight of the sediment samples were analyzed for BTEX/TPH (ARBCA methodology), PAHs and metals.

Currently there are no criteria established for BTEX/TPH in sediment. Modified TPH concentrations are notably high within two samples: 10-ST121-N1E1 (1180 mg/kg) and 10-ST122-N1E0 (1750 mg/kg). Detectable concentrations of benzene and ethylbenzene were found at sample locations 10-ST117-N1E0 and 10-ST121-N1E0, respectively. PAH exceedances were also noted at these two locations. The parameters exceeding the applicable guidelines are benzo(a)anthracene and pyrene.

There was one sediment sample collected for PDB analysis (i.e. 10-ST124-N2E0) with the reported value of 9100 cfu/g.

CHAPTER 4
DEVELOPMENT OF THE PILOT-SCALE PHYSICAL MODEL

Based on the site investigation and the recommendations from the literature, sites E and L were together chosen as the original site for the pilot-scale biosurfactant-enhanced in-situ bioremediation due to its complex subsurface and geological conditions. According to the locations of the available boreholes, a rectangular study area was selected and cut from the original site for the pilot-scale experiment (Figure 12). The study area has a length of 810 m and a width of 270 m, with 20 boreholes spreading over the left and center. The right part of the study area lacks borehole but is still included because seawater intrusion is of important concern. Each borehole has its own soil profiles as shown in Figure 13. Three main types of soil can be distinguished: silt, sand, and clay. The next section will focus on introducing how to downsize the study area into the pilot-scale vessel and how to design its subsurface soil profile using the borehole properties.

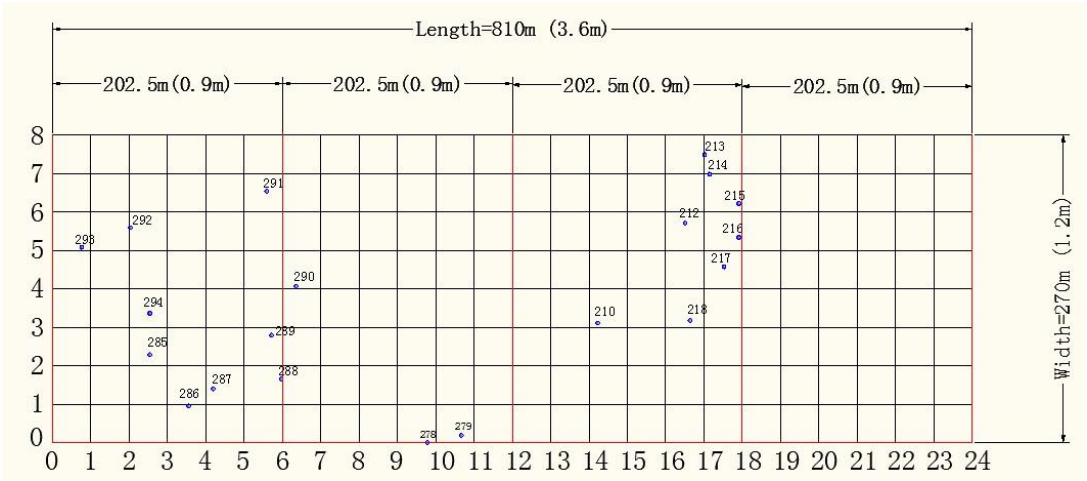


Figure 12 Overview of the study area

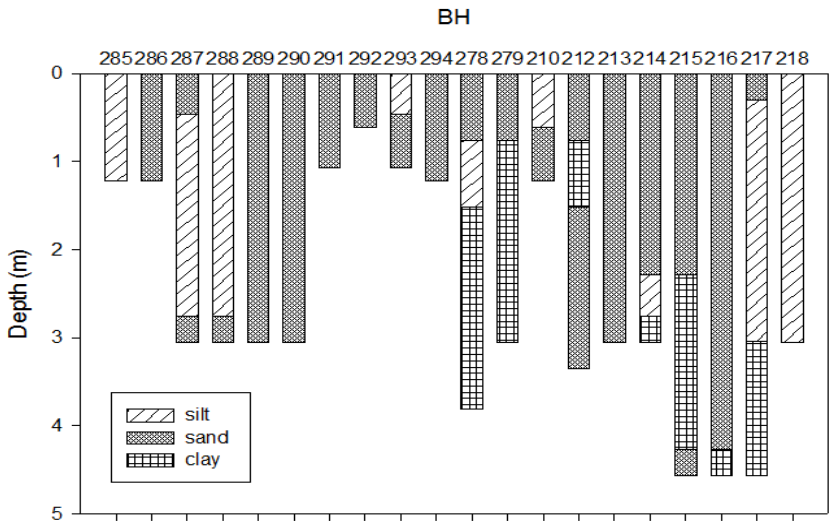


Figure 13 Borehole data

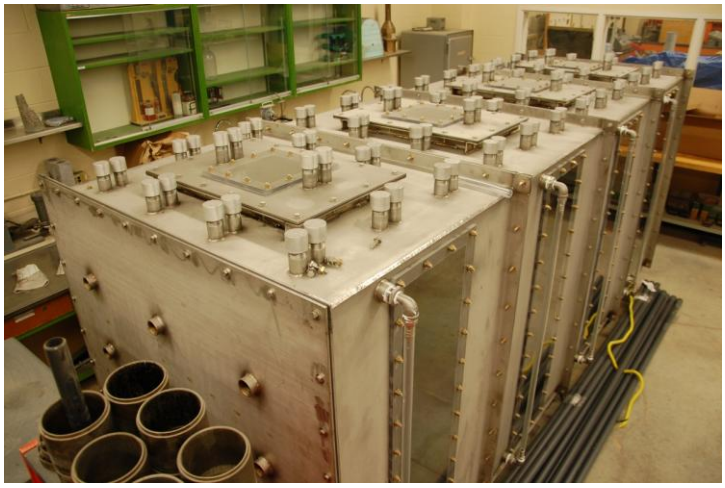
4.1 Design and Construction of a Pilot-Scaled Vessel

In the soil lab at Memorial University of Newfoundland, St. John's, a pilot scale vessel with 3.6 meters long, 1.2 meters wide, and 1.4 meters high (~ 5 m^3) was fabricated (

Figure 14). In order to provide support to the pressure generated from soil and water, this vessel was made of 6-mm thick stainless steel and 8-mm thick double-layer toughened glass. This design can also prevent the vessel from the potential corrosion caused by contaminants. The total weight can reach up to approximately 20 tonnes when fully loaded with soil and water. The vessel is mainly comprised by four individual sections as shown in Figure 15 (a). Four observation windows with a transparent water level gauge are built on the front of each section of the vessel to illuminate the water level and observe the soil profiles (Figure 15 (b)). A total of 12 instrumental holes on the top of each section are assigned for insertion of detecting instruments, such as dissolved oxygen sensor, thermo-sensor and pH sensor, into different depths. A manhole is also fabricated on the top of each section for the purposes of soil loading and vessel maintenance, etc. Besides the instrumental holes on the top, there are additional 12 sampling holes on the back of each section, which are used to collect soil and water samples from different depths inside the vessel. Flanges with 44 bolts on them were used to connect the four sections (Figure 15 (c)). In order to prevent the leakage of water or air, anti-organic solvent and anti-high temperature rubber made pads were placed between the flanges. Each end of the vessel was equipped with a stainless steel end boards with 12 (four rows, three columns) water inlets and outlets, respectively (Figure 15 (d)).

The water inlets are connected with a water container through a centrifugal pump, a pressure reduction valve, and a flow meter. Each row of the inlets has an individual valve to control water flow patterns. The water outlets are connected with a drainage basin and sump pump to continuously discharge effluent to a sink. Petroleum hydrocarbons are managed to leak into the system through a contaminant container. In order to determine the performance of biosurfactant-enhanced soil remediation under various conditions, the pilot-scale system is connected with an air pump, which was an essential instrument for pneumatic remediation methods, and a steam generator, a humidity adjustment instrument which were connected with the vessel through a set of piping.

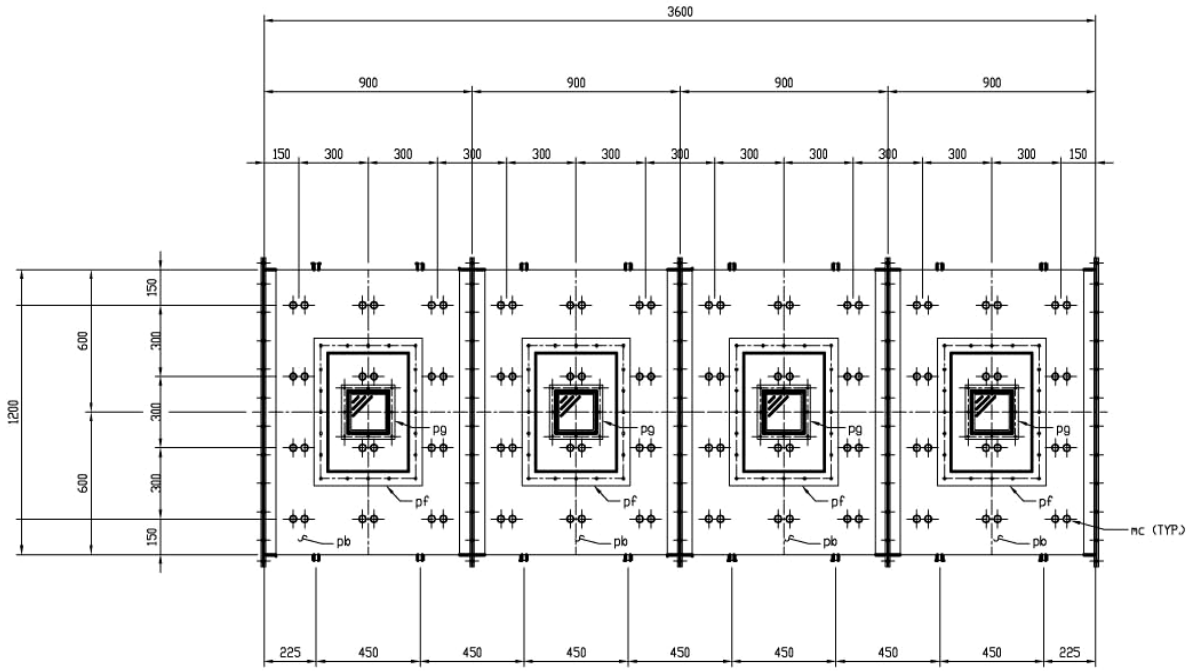
(a) Front view of the pilot-scale vessel



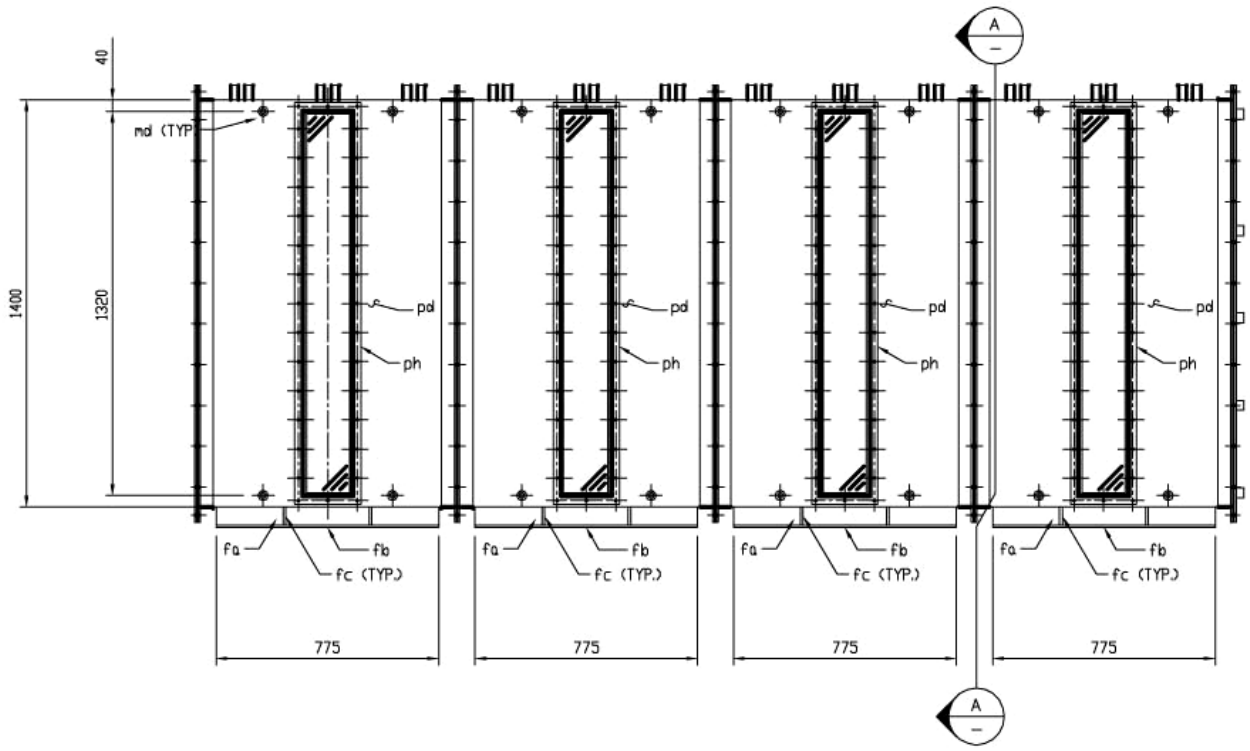
(b) Plan view of the pilot-scale vessel

(c) End view of the pilot-scale vessel

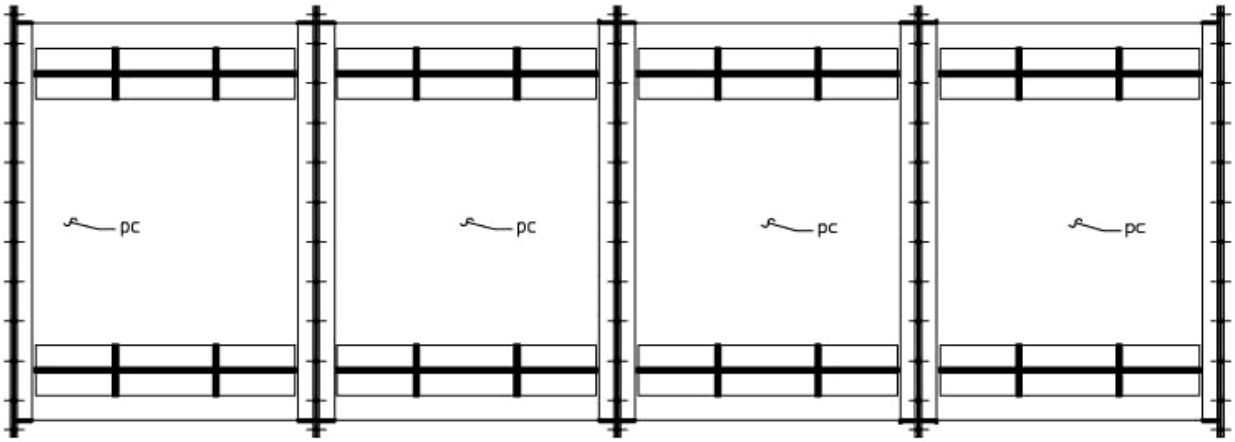
Figure 14 Image of pilot scale vessel



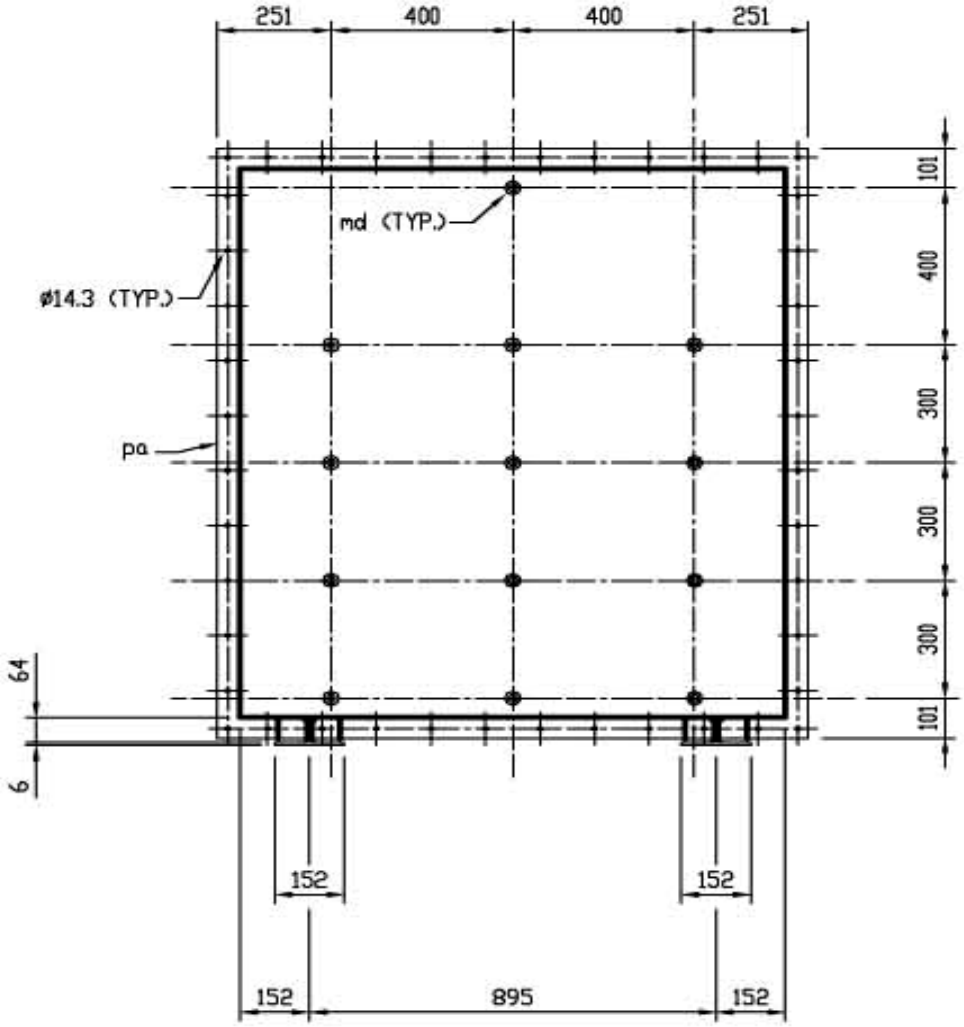
(a) Plan view of the pilot-scale vessel



(b) Front view of the pilot-scale vessel



(c) Bottom view of the pilot-scale vessel



(d) End view of the pilot-scale vessel

Figure 15 General layout plan of the pilot-scale vessel

4.2 Design of Subsurface Soil Profile

To scale down the site conditions to the pilot-scale experimental system, the development of the subsurface soil profile of the site is important before the design and setup of the physical vessel. To better mimic how soil profiles and groundwater flow may affect the bioremediation of organic contaminants in the study area (810 m * 270 m), the study area was proportionally scaled down according to the size of the vessel (3.6 m * 1.2 m) as shown in Figure 12. The horizontal plane of the vessel was further evenly divided into 24*8 cells, which were 0.15 m in both length and width (Figure 12). Meanwhile, the borehole depth was also scaled down from 4.57 m (deepest point of the boreholes in the study area) to 1.2 m (pilot-scale vessel).

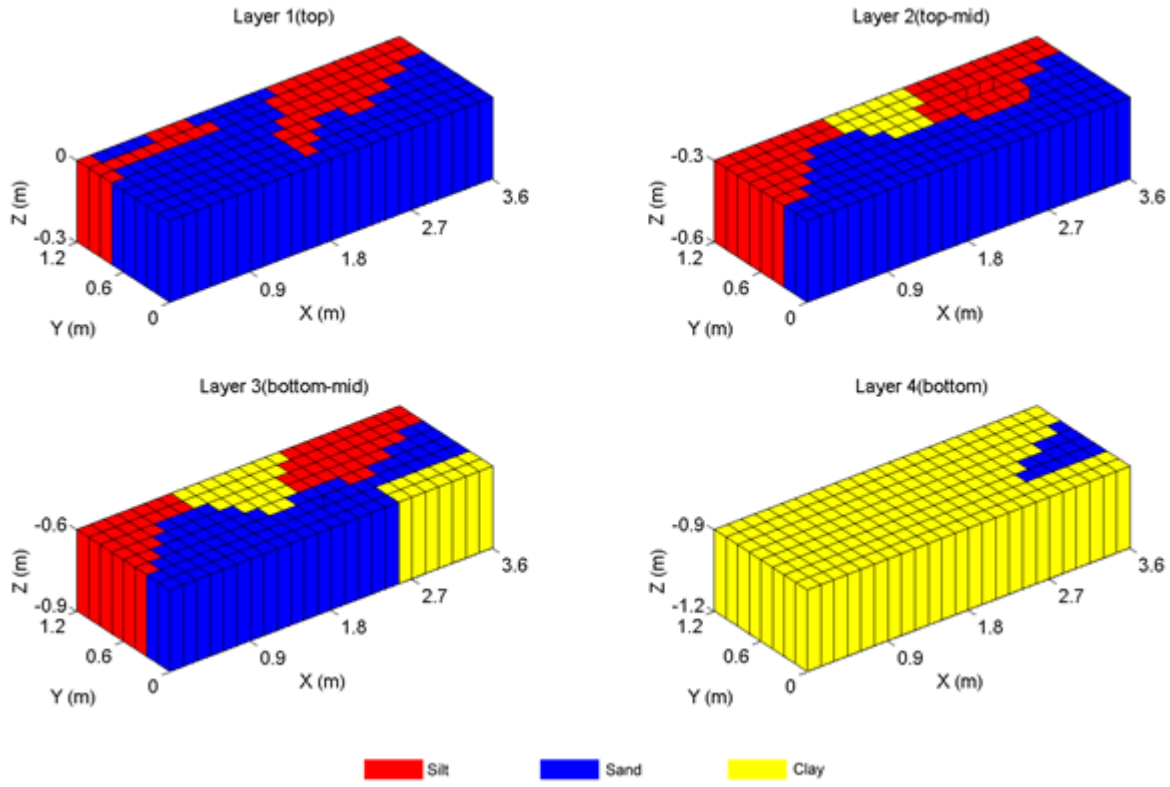
The depth of 1.2 m in the vessel was further divided into 24 planes. Each planes 0.05 m in depth and should have 24*8 cells as aforementioned. The soil characteristics of the cells on each plane were determined by referring to the soil data of the boreholes at that specific plane. The percentages of all the three types of soil (i.e., silt, sand, and clay) at each borehole were used as initial input to employ the nearest interpolation technique in Matlab. Then all the 24*8 cells on this plane were given interpolated values for the normalized composition of silt, sand, and clay in percentage. For the ease of soil loading and experiments, the vessel will only have four major soil layers (i.e., top, top-mid, bottom-mid, and bottom) with 0.3 m depth of each, which means each layer will contain six of the aforementioned planes. All the percentages of soil, sand, and clay of each cell on those six planes were added up and further compared to see which type of soil had the largest value. The type of soil had the largest value was assigned to the cell on the major layer. This procedure was repeated for each cell on the four major soil layers and the results were shown in Figure 16. It shows that sand mainly dominated the top three layers while clay concentrated in the bottom layer. Silt can be found at the top left and top right corners of the top three layers.

4.3 Loading of Soils into the Pilot-scale Physical Model

Uncontaminated soils taken from 5 Wing Goose Bay was pre-selected to ensure its inside conditions were in accordance with the target site. Then soils will be filled into the vessel to simulate the real conditions of the target site following the previous site investigation results.

Based on the above analyses of soil properties, three types of soils were selected to fill the vessel, including clay, silt and fine sand. The clay soil was transported in sealed 45-gallon drums with a minimal upper space inside and it was retained at room temperature for no longer than 10 days. The silt and fine sand soils were transported by in an open air truck bed, stored outdoors and loaded into the vessel within 2 days. Soil properties were analysed with samples collected from different locations and depths of the target site. Considering the site subsurface was mainly consisted of sand, a wet-sieving procedure was applied to determine the properties of the soil. The end section of the vessel was filled with clay collected from the base to prevent the leakage of spilled oil from the vessel during the bioremediation tests. Soils transported from 5 Wing Goose Bay were stored outdoor and loaded into the vessel within two days. The inner surfaced of the vessel was marked into several grids based on the generated subsurface model. Soils then were loaded into the vessel type by type. When all

soils were dumped into the vessel, tap water was poured into the soil surface at every 100 mm of depth. More soils were filled in the grids with overnight deposition.



Legend:

Real site size: 810*270*5(m)

Tank size: Length*Width*Depth= 3.6*1.2*1.4(m) ➔24*8* 4layer

Scale: (Tank: Site)= Horizontal: 1:225; Vertical:1:3.57

Each grid size: (Length* Width) = 33.75*33.75 (site)

Figure 16 Conceptual model of the site subsurface

4.4 Design and Manufacture of Sampling Apparatus

Selection of equipment for collecting or processing water-quality samples depends on the physical constraints and safe operation of the equipment and on its suitability with respect to achievement of study objectives. Criteria for selecting equipment for water sampling depend on (1) the mechanical constraints of the equipment to perform adequately under given environmental conditions, (2) the adequacy of equipment operation to obtain water-quality samples that represent the environmental conditions of the sample source, and (3) the adequacy of the equipment materials and construction to maintain sample integrity and not be

a source of leaching and sorption of chemical substances (F.D.Wilde). Regarding groundwater sampling, the system selection is mainly based on the type of well, depth to water from land surface, physical characteristics of the well, groundwater chemistry and the objectives for study. Selecting the appropriate equipment for collecting groundwater samples is important in order to meet the requirements for study objectives and data quality. Either pumps designed specifically for water sampling from monitoring wells or a bailer or thief-type sampler could be applied to collect groundwater samples. The sampling equipment could either be purchased from commercial sources or designed and manufactured individually.

Principally groundwater sampler could be categorized into pumps, bailers and specialized thief samplers. Submersible pumps (positive pressure or other types of positive-displacement pumps) designed specifically for collection of water samples from monitoring wells generally is preferred because they do not create a vacuum. Examples of pumps suitable for sample collection from monitoring wells are shown in the figures as below. Type A is peristaltic suction-lift pump showing detailed pump head; B is electrical gear or rotor pump; C is electrical centrifugal impeller pump showing detailed impeller assembly. We mainly use type A pump in the lab (Figure 17).

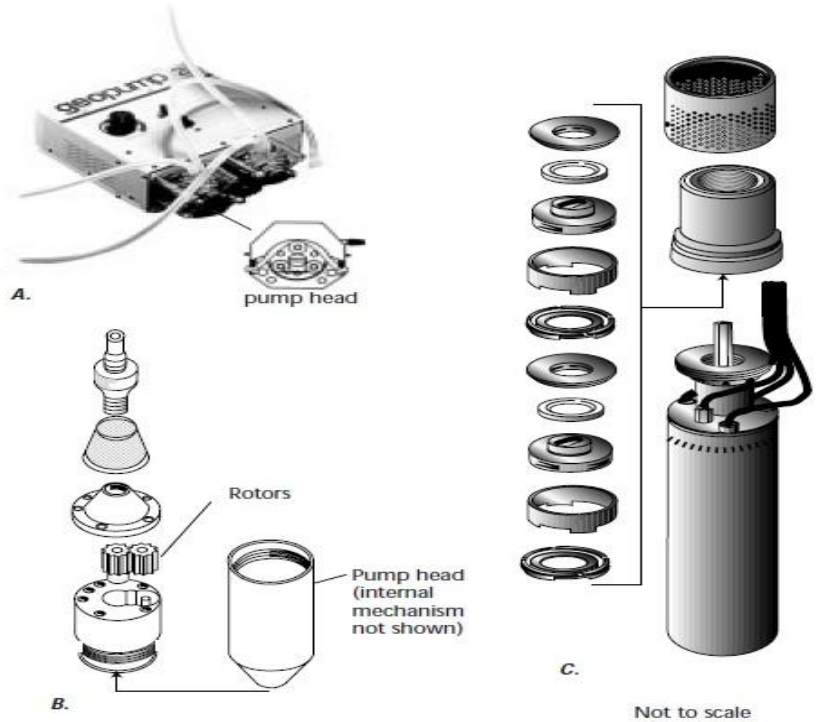


Figure 17 Pumps typically used for withdrawal of water samples from monitoring wells (Wilde et al., 2005)

The other major category for groundwater sampler collection is bailers and specialized thief samplers. Specialized sealed down-hole samplers are designed to capture and preserve In-situ groundwater conditions by precluding sample aeration and pressure changes from sample degassing or outgassing. Such sampling equipment includes syringe samplers, true thief

samplers, samplers using hermetic isolation methods and chlorofluorocarbon samplers. Neither of these two types of samplers is recommended in practice, due to the fact that it could easily cause disturbs while withdrawing water repeatedly. The disturbance could result in stirring up or mobilizing particulates, including colloidal matter or mineral precipitates that are artifacts of well construction and are not part of the ambient groundwater flow. Hence, in this project, pump based options would be applied for water sampling.

In addition to the main component for groundwater sampling as mentioned previously, a certain types of support equipment are also required in order to maintain an efficient and clean deployment of the sample line. Commonly used support equipment is listed in the table below.

Table 9 Support equipment for groundwater sampling

Handline or manual/power reel with line
Tripod assembly with manual or power reel
Wellhead guide for flexible sample line to pump
Wheeled carts to transport portable sampling equipment
Energy source for reels and pumps (batteries, compressor, or generator)
Other

Water samples must be processed as quickly as possible after collection. The equipment most commonly used for sample processing includes samples splitters, filtration units or assemblies, and chambers in which samples are processed and treated with chemical preservatives.

A groundwater sample generally is not composited, instead the samples is pumped directly into separate bottles for designated analyses. The exception is when the sample is collected using a non-pumping method such as bailer or thief sampler. In our case, a pump based sampling method would be deployed. Hence, it will not go into detail regarding the types of sample splitters.

After the collection, the samples should be filtered for analysis of inorganic constituents, organic compounds, and biological materials to help determine the environmental fate and quantify the transport of these target analytes. Membrane filters commonly used to filter inorganic samples generally are made of cellulose nitrate, polycarbonate polymers, or polyethersulfone-based media. There filter media are not suitable filtering samples to be analyzed for organics; glass microfiber is the media used for filtering most organic samples, and silver filters are used for dissolved-organic compound samples. A stainless steel or fluorocarbon polymer pressure filter assembly fitted with a 47 mm, 0.45µm pore size silver membrane filter is used to separate dissolved from suspended phases of organic carbon. The filter is as shown in the Figure 18 below.

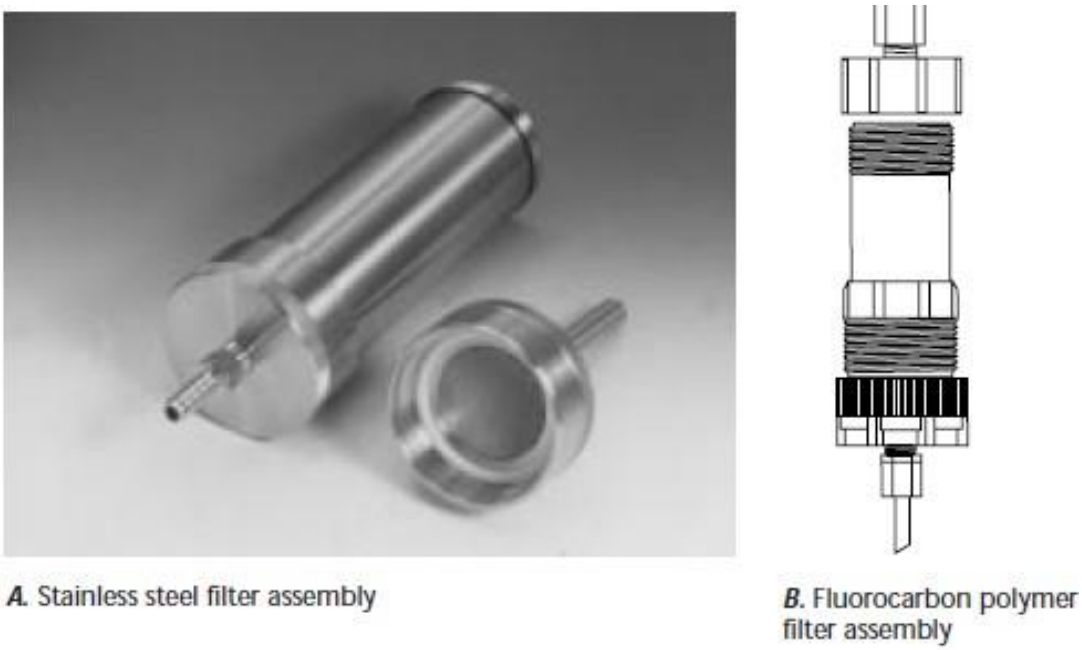


Figure 18 Apparatus for filtering samples for analysis of dissolved/suspended organic carbon (Wilde et al., 2005)

Processing and preservation chambers could reduce the possibility of random atmospheric contamination during sample splitting, filtration, and preservation. These chambers are required for samples for trace-element determinations. The processing chamber can also serve as a collection chamber from pumped samples. There is no standard design for either fixed or portable chambers. However, to prevent contamination of inorganic samples with metals, the materials used in their construction should be either non-metallic or completely covered by or embedded in non-metallic material. An example of a polyvinyl chloride frame of a processing or preservation chamber is shown in Figure 19.

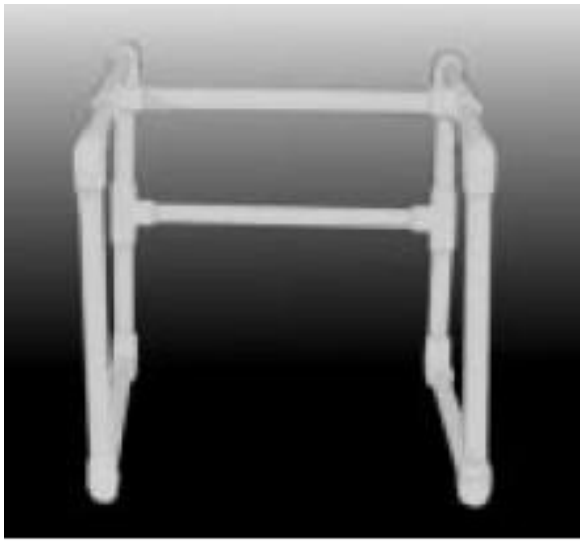


Figure 19 A polyvinyl chloride frame of a processing or preservation chamber (Wilde et al., 2005)

CHAPTER 5
PILOT-SCALE BIOSURFACTANT-ENHANCED BIOREMEDIATION

5.1 The Pilot-Scale Experimental System

Physical conditions: The pilot-scale system was set up to physically simulate the on-site Conditions. The pilot-scale system and the accessories were assembled in the Northern Region Persistent Organic Pollution Control (NRPOP) Laboratory at MUN, which is founded by the Canada Foundation for Innovation (CFI) and the Industrial Research and Innovation Funds (IRIF) of Newfoundland and Labrador Government.. The water and drainage containers were connected to the upstream inlet and downstream outlet, respectively. A contaminant container was also used to facilitate the leakage of petroleum into the system.

The water level gauges were installed to show the depth of the water table in the pilot vessel. The tap water stored in water containers was pumped into the vessel through six water inlets on the inlet-end board as upstream groundwater inflow through a peristaltic pump. Before the start of the experiment, water in the container was kept still overnight to reach room temperature (10 ℃). A 7-day buffering time preceded all experiments in the vessel so that the temperature at every location could reach equilibrium.

Monitoring, injection and extraction wells: The monitoring wells were set up to facilitate access to the groundwater so that a "representative" view of the subsurface hydrogeology could be obtained, either through the collection of water samples or the measurement of physical and hydraulic parameters. In this study, four monitoring wells were also used for injection and extraction purposes during the remediation processes. In total, 20 wells were allocated in four sections of the pilot system. Soil in the system was stratified into four layers. Due to the high water table at LTF, the second, third and fourth layers being saturated with water. Each layer was set 30 cm deep. Among the wells, 10 of them were installed to reach the third soil layer; the other 10 wells could reach the fourth layer. The wells were sealed by rubber caps at the top. For each well, a plastic hose was installed that passed through the caps and reached its bottom. The outside of the hose was clamped so that the air and groundwater in the well were isolated from outside conditions.

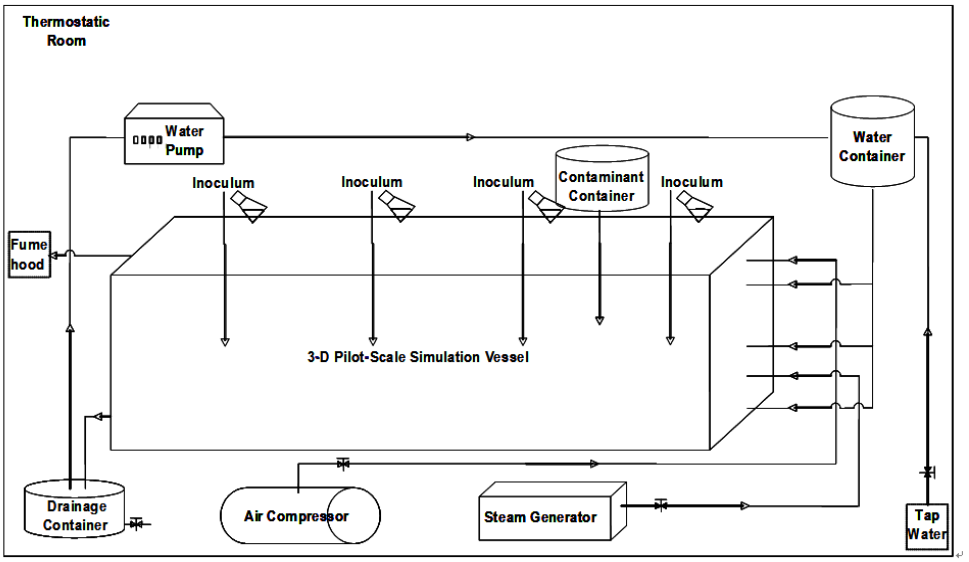


Figure 20 Pilot scale remediation simulation process

5.2 Experimental Materials

5.2.1 Selection of Contaminations

Organic Contaminants: According to site investigation, the major organic contaminant at site E was ethylbenzene, xylenes, and other petroleum hydrocarbons. Thus regular gasoline (#87), purchased from a commercial gas station, was used for microbial screening and bioremediation experiments. Considering the monoaromatic hydrocarbons such as ethylbenzene and xylene represent an important class of environmental contaminants because of their recognized toxicity to different organisms' high concentration, ethylbenzene and xylene will be selected as the representative contaminants.

Heavy metals: Lead was selected as the target heavy metal contaminants in this study due to its wide spread in groundwater/sediment/surface water samples in site E as well as its high toxicity. The $(CH_3COO)_2Pb \cdot 3H_2O)$ was dissolved in distilled water to simulate the contamination of heavy metal.

5.2.2 Production of Biosurfactant

5.2.2.1 Production of Biosurfactant

Collection of samples for screening: Produced water samples from oil and gas platforms, sediment samples from coastal line near a refinery company, and water samples from local harbours were collected for screening novel biosurfactant producing microbes. The produced water samples were collected by Centre for Offshore Oil, Gas and Energy Research (COOGER), Fisheries and Oceans Canada, and the basic information of each produced water sample was also provided by COOGER. Other samples were collected by NRPOP laboratory, before the collection of sediment samples, the *In-situ* testing of temperature, salinity,

conductivity and total dissolved solid (TDS) were measured with Thermo Scientific Orion Star A222 advanced portable conductivity meter (Fisher Scientific Limited, Nepean, Canada). The GPS data at each sampling site were also recorded. Samples were shipped with ice bags and stored in amber bottles at 4^{o}C.

The basic information of the sampling sites and physiochemical properties of the samples were summarized in Table 10. Some of the sampling photos were shown in **Error! Reference source not found.**. All samples were taken within N43.9^{o} to N47.8^{o}

Isolation and preliminary screening: Each collected samples were first enriched with two different recipes of medium in 125 mL conical flasks. The first one composed of $(NH_4)_2SO_4$, 10 g; NaCl, 2.2 g; $FeSO_4$ $7H_2O$, 2.8×10^{-4} g; KH_2PO_4, 3.4 g; K_2HPO_4 $3H_2O$, 4.4 g; $MgSO_4$, 0.5 g; yeast extract, 0.5 g and trace element solution, 0.5 ml L^{-1} of distilled water, with 2% (v/v) n-hexadecane as the sole carbon source which was adopted and modified from Peng et al. (2007). The trace element solution contained $ZnSO_4$, 0.29 g; $CaCl_2$, 0.24 g; $CuSO_4$, 0.25 g; $MnSO_4$, 0.17 g L^{-1} and was filter-sterilized. The second recipe is a modified Atlas oil agar medium which composed of $MgSO_4$, 0.2 g; $CaCl_2$ $2H_2O$, 0.05 g; KH_2PO_4, 1 g; K_2HPO_4, 1 g; $(NH_4)_2NO_3$, 1 g; $FeCl_3$, 0.05 g; NaCl, 28 g; standard nutrient broth, 2 g L^{-1} of distilled water, with 1% (v/v) n-hexadecane and 1% (v/v) clear diesel fuel. The chemicals used were analytical grade, unless otherwise specified. Enrichment was conducted at 30^{o}C, 200 rpm for 3-5 days until observable turbidity occurred.

After enrichment, the consortia were serial diluted to 10^{8} and spread on agar plate with the same composition of the enrichment medium. The resulting plates were incubated at 30^{o}C for 3-5 days until slow growers form clear colonies. Each colony was transferred to 1 mL liquid medium containing the same medium in a 2 mL Eppendorf® tube and incubated at 30^{o}C, 200 rpm for 2 days. The resulting culture were be used for the Drop collapsing test (Bodour and Miller-Maier, 1998). Two µL of mineral oil was added to each well of a 96-well microtiter plate lid, and the lid was equilibrated for 1 h at room temperature, and then 5 µl of each culture and one control (distilled water) was added to the surface of oil. After 1 min, the flat of the water droplet were observed and recorded as "+". Those cultures that forms the same droplet as the control were scored as "-". The cultures gave positive responses were subjected to purification with streak plate technique for 3 times. As a results, biosurfactant producers were isolated from sample CBS, NA1, NA2, NA3, P1, P4, P6.

Table 10 Basic information of the sampling sites and physiochemical properties of the samples

Sample ID	Conductivity (ms/cm)	TDS (ppt)	Salinity (ppt)	Temperature (^{o}C)
P1	47.9	>200	29.76	N/A[a]
P2	74.2	>200	46.43	N/A
P3	232.4	>200	>80	N/A
P4	75.8	>200	47.37	≈60
P5	78.5	>200	48.98	N/A
P6	22.68	11.11	12.57	N/A
P7	75.5	>200	47.33	≈60
P8	74.8	>200	47.36	≈60
P9	75.4	>200	47.16	≈60
P10	74.8	>200	46.82	≈60
SJ	44.5	>200	26.76	N/A
CBS	49.9	>200	30.46	N/A
NA1	37.6	18.4	23.63	19
NA2	95.6	5.13	5.984	25.3
NA3	33.4	16.4	20.85	22.2
NA4	588	>200	0.331	11.8

[a] not available

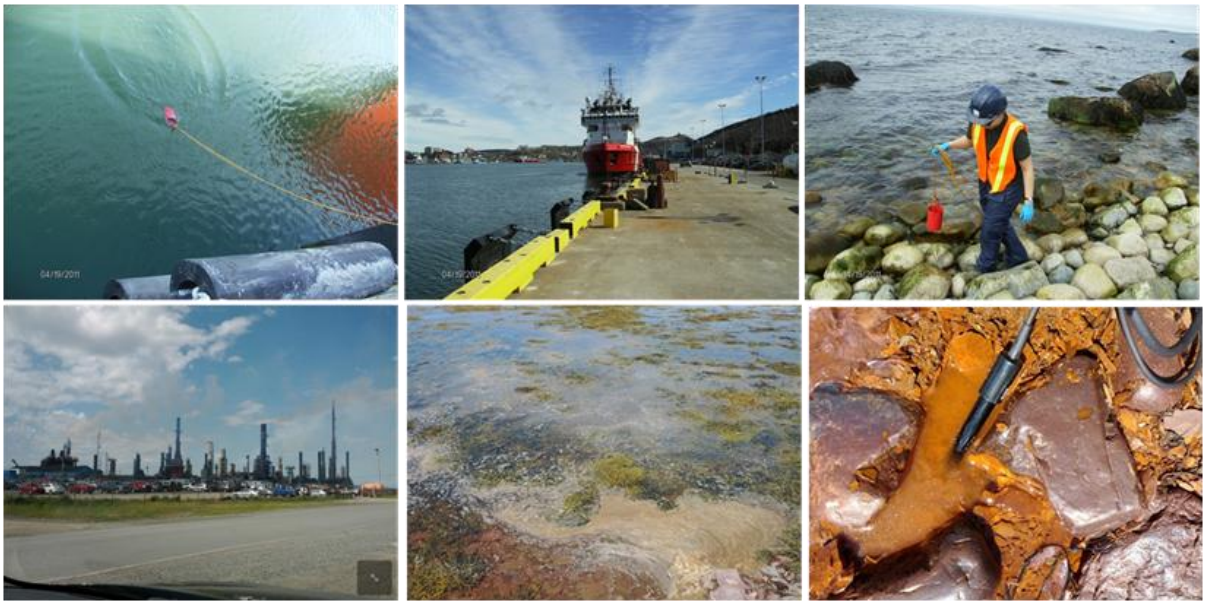


Figure 21 Photos of sampling trips

The purified isolates were then subjected to 16S ribosome DNA sequencing using universal bacterial primers F27 and R926 (position in *Escherichia Coli* 8-27 and 926-907, respectively). Aliquot of the each culture was used as DNA template in a PCR reaction using the primer pair. After gel electrophoresis comfirmation of successful PCR reaction, PCR products were subjected to a clean-up process and measured by a NanoDrop spectrophotometer to determine the concentrations. Lastly, sequencing reactions with the last PCR products were conducted and measured with Applied Biosystems 3130 and/or 3730 system in Creait Network of Memorial University of Newfoundland. The obtained DNA sequence was matched with BLAST database. The DNA sequencing results were shown in Table 11.

Table 11 Identification of the isolated biosurfactant producer

Sample ID	No.of isolates	Isolate ID	Letters (bp)[a]	Species	Max identity%
CBS	1	CBS1	910	*Bacillus Thuringiensis*	99
NA1	1	NA1-2	719	*Bacillus subtilis*	100
NA2	3	NA2-3	754	*Bacillus subtilis*	100
		NA2-6	622	*Bacillus subtilis*	99
		NA2-7	240	*Bacillus subtilis*	98
NA3	10	NA3-1	251	*Bacillus amyloliquefaciens*	100
		NA3-2	791	*Rhodococcus opacus*	98
		NA3-3	795	*Rhodococcus wratislaviensis*	98
		NA3-4	780	*Bacillus sonorensis*	99
		NA3-5	482	*Rhodococcus phenolicus*	98
		NA3-6	870	*Pseudomonas peli*	99
		NA3-7	825	*Bacillus flexus*	99
		NA3-8	791	*Bacillus mycoides*	100
		NA3-9	320	*Bacillus subtilis*	99
		NA3-11	376	*Bacillus subtilis*	96
P1	2	P1-2	475	*Bacillus subtilis*	99
		P1-6	722	*Bacillus subtilis*	96
P4	1	P4-1	655	*Rhodococcus equi*	99
P6	6	P6-2	793	*Bacillus subtilis*	100
		P6-3	733	*Bacillus subtilis*	98
		P6-4	688	*Rhodococcus erythropolis*	99
		P6-5	648	*Bacillus subtilis*	99
		P6-7	708	*Bacillus thuringiensis*	100
		P6-9	783	*Rhodococus erythropolis*	99

[a] length of sequence subjected to matching with Blast database

Generation of substrates: Low-cost industrial waste streams as unconventional substrates (e.g., refinery oil) were selected for biousrfactant production. Refinery oil waste was passed through a vacuum funnel with filter to remove settleable solids. The pretreated samples were stored at 4℃ until needed for biosurfactant production.

Microorganisms: both the newly-lab-obtained biosurfactant producers (part 1) and a commercial biosurfactant-producing microorganism (Pseudomonas aeruginosa ATCC 9027 strain) were selected. Each microbial strain was enriched and the cells then were collected by centrifugation and washed with filter-sterilized hexane and saline, and resuspended in saline to adjust OD of 0.7 at 660 nm, was used as inoculum.

Media and cultivation conditions: For biosurfactant production a mineral salt medium with the following composition(g/L) was utilized: Na_2HPO_4 (2.2), KH_2PO_4 (1.4), $MgSO_4 \cdot 7H_2O$ (0.6), $FeSO_4 \cdot 7H_2O$ (0.01), NaCl (0.05), $CaCl_2$ (0.02), yeast extract (0.02) and 0.1 mL of trace element solution containing (g/L): 2.32g $ZnSO_4 \cdot 7H_2O$, 1.78g $MnSO_4 \cdot 4H_2O$, 0.56g H_3BO_3, 1.0g $CuSO_4 \cdot 5H_2O$, 0.39g $Na_2MoO_4 \cdot 2H_2O$, 0.42g $CoCl_2 \cdot 6H_2O$, 1.0g EDTA, 0.004g $NiCl_2 \cdot 6H_2O$ and 0.66g KI. Cultivations were performed in 250mL flasks containing 50ml medium at room temperature, and stirred in a rotary shaker for 3 days.

The medium and cultivation optimization were conducted in a series of experiments changing one variable at a time, keeping the other factors fixed at specific sets of conditions. Five factors were chosen aiming to obtain higher productivity of the biosurfactant: carbon source (C), nitrogen source (N), agitation speed, temperature and pH. Biosurfactant productivity was evaluated by the surface tension measurement (using a tensiometer) and emulsification index (E24) determination.

Biosurfactant isolation and purification: The culture broth was centrifuged to remove the cells and thereafter sterilized with millipore membrane filter. The clear sterile supernatant can serve as the source of the crude biosurfactants. The biosurfactants were recovered from the cell free culture supernatant by cold acetone precipitation as described by Pruthi and Cameotra (Pruthi and Cameotra, 1995). Three volumes of chilled acetone were added and allowed to stand for 10h at 4 ℃. The precipitate was collected by centrifugation and evaporated to dryness to remove residual acetone after which it was re-dissolved in sterile water.

5.2.2.2 Characterization of Newly Produced Biosurfactant

Composition and structure of the extracted biosurfactants: The purified extracts were separated first on a high performance thin layer chromatography (TLC) with different color indicators showing fractions of fatty acids, phospholipids, saccharide and lipopeptides. The bands appeared on the TLC was eluted separately in 4 different groups for further characterization with a Gas chromatography–mass spectrometer (GC-MS). Compounds with no match in the MS library would be subject to Nuclear magnetic resonance (NMR), infrared (IR) spectroscopy analysis for the deduction of the molecular structures.

Functionality of the extracted biosurfactants: emulsification capability assay and surface tension were determined with different concentrations of biosurfactants to generate two response curves. Reduction of surface tension at pH, temperature and salinity conditions were plotted to illustrate their performances under extreme environmental conditions.

Evaluation of toxicity: The EPA authorized acute toxicity tests with Menidia beryllina and Mysidopsis bahia with or without No. 2 fuel oil were conducted to exiamine the newly developed biosurfactants in lab.

5.3 Experimental Process

Two experimental runs were conducted to examine the performance of the enhanced bioremediation process through the newly-developed biosurfactants. Run #1 was performed with four stages: (I) contaminant leakage to the pilot-scale vessel, (II) contamination and natural attenuation in the subsurface, (III) enhanced in-situ biodegradation through the newly developed biosurfactants and a pump-and-treat technique with the existence of hydrocarbon contaminants only, and (IV) enhanced in-situ biodegradation through the newly developed biosurfactants with the coexistence of heavy metal (Pb). Run #2 was designed as a comparison run, which followed the same stages (I) and (II) as those in run #1 but without the addition of biosurfactants during the pump-and-treat remediation process in stage (III) and (IV). The pilot-scale process is stated in detail as follows.

5.3.1 Contaminant Introduction and Loops Formation Stage

Removal of hydrocarbon only and co-existence of heavy metal was determined in round (III) and (IV) respectively. Thus two kind of contaminants need to be prepared and injected at different stage.

Leakage of hydrocarbon: To simulate hydrocarbon leakage, 15 liters of gasoline was injected into the bottom of the second soil layer at the upstream location during a 1-day period. At the same time, tap water from a water container was pumped into the system at a rate of 20 liter/day through a peristaltic pump. The water levels in the upstream and downstream gauges were kept at 55 and 45 cm high, respectively.

Preparation of co-contaminated soil: The $(CH_3COO)_2 Pb \cdot 3H_2O$) was dissolved in distilled water to achieve concentrations, 1000 mg/L of Pb, and injected into the bottom of the second soil layer at the upstream location during a 1-day period together with 15 liters of gasoline (without adjusting the pH value). At the same time, tap water from a water container was pumped into the system at a rate of 20 liter/day through a peristaltic pump. The water levels in the upstream and downstream gauges were kept at 55 and 45 cm high, respectively.

5.3.2 Natural Attenuation Stage

After the leak, the same flow conditions were maintained for 26 days to simulate the process of contamination and natural attenuation in the subsurface. The enhanced in-situ biodegradation was then started right after the 26-day period.

5.3.3 Biosurfactant-enhanced Bioremediation Stage

Biosurfactants: The biosurfactant solution was injected into the treatment zone through injection wells along with oxygen and nutrients. The concentrations were 0.2 % and 200 mg/L for the biosurfactant solution and oxygen, respectively. The injection lasted for 18 days with an injection flow of 10 liter/day in run #1 (water was injected with the same rate and duration in run #2). The extraction flow rates in extraction wells were both maintained at a speed of 15 liter/day. The temperature of the injected water was maintained at 10 ℃.

5.4 Experimental Sampling and Analysis

For each experimental process listed above, duplicate samples were selected and the related duplicate analytical treatments were conducted. The final result of each sample came from the average of the experimental results.

5.4.1 Collection and Analysis of Groundwater Sample

Water samples from different well locations in the pilot-scale vessel were collected every other day. A peristaltic pump was used to obtain groundwater samples through pre-installed monitoring wells. For each monitoring well, a groundwater sample was collected into a 22-mL standard glass bottle and immediately sealed by a serum cap.

Collected groundwater samples were analyzed of organic compounds and geochemical indicators including BTEX, methane (CH_4), CO_2, inorganic nutrients, anions, ferrous iron [Fe(II)], pH, DO, and total organic carbon (TOC). TPH and BTEX concentrations were quantified through a Gas Chromatograph-Mass Spectrometry (GC-MS). Methane was analyzed on a Shimadzu GC-9A GC using headspace techniques. Ion chromatography was used for inorganic nutrients and anions (NO_3^-, NO_2^-, SO_3^{2-}, and $SO42^-$) analyses. Perkin-Elmer plasma II inductively coupled plasma–argon emission spectrometer (ICP–AES) was used for Fe(II) analyses following standard methods. TOC was analyzed by a total carbon analyzer. DO, Eh, pH, CO_2, and temperature were measured in the field. A pH/Eh meter was used for pH and Eh measurements, a DO meter was used for DO and temperature measurements, and a Hach digital titrator cartridge was used for CO2 measurements.

5.4.2 Collection and Analysis of Soil Samples

In the laboratory experiments, soil samples (2-3 g) were taken from sample holes using a stainless steel auger at every 20 days. The holes were sealed immediately after sampling. In the field experiment, two replicate samples taken from each layer were composited and used for measuring TPH and BTEX concentrations. Soil pH, moisture contents, and residual N and P also were determined. In this study, TPH losses during the soil remediation are negligible considering the tank is concealed during the whole process of experiment.

Soil gas was determined by sensors installed at various depths and lateral locations in the pilot-scale tank and analyzed for O_2 and CO_2 using infrared/electrochemical sensors. The data on O_2-CO_2 soil gas concentrations were used for calculation of respiratory quotient (RQ) and temperature coefficient (Q10).

5.4.3 Determination of Bacterial Activities

Bacterial concentration determination: Microbial cell numbers were estimated using the most probable number (MPN) method (Braddock and Catterall, 1999). Briefly, 1g soil samples was added to 10 mL of distilled water and vortexed for 30 min and serially diluted to 10^{-10}. Sterile Tryptic Soy Broth was dispensed (250µl) into 96-well microtitre plates and the wells were incubated (five replicates) with 10µl of the respective dilutions of soil samples for total heterotrophic microorganisms. Bacterial growth was determined by turbidity. For diesel fuel-degrading microorganisms, a Bushnell–Hass medium and a tetrazolium chloride (TTC) solution as the indicator were used. After inoculation with dilutions of the soil samples, microtitre plates were inoculated with 10µl of diesel oil (sterilized through 0.2 µm membrane). The static cultures were incubated without agitation at room temperature (27°C) for 10–14 days. At the end of this period, each plate was scored visually by violet color development (indicating reduction of the tetrazolium dye via respiration) for the diesel oil-degraders (Braddock and Catterall, 1999). Microbial population was then determined using statistical tables found in Standard Methods of Soil Analysis (Lorch et al., 1995).

Biochemical assays: Dehydrogenase activity (DHA) was determined by the reduction of 2-*p*-iodo-nitrophenyl-phenyltetrazolium chloride (INT) to iodo-nitrophenyl formazan (INTF) using 1g of soil at 60% of field capacity, exposed to 0.2 mL of 0.4% INT in distilled water for 20h at 22°C in darkness. The INTF formed was extracted with7.0 mL of a mixture of 1:1.5 tetrachloroethylene/acetone by shaking vigorously for 1min. INTF was measured spectrophotometrically at 490 nm (García et al., 1992)

Traditional enzyme assays such as those for glucosidase, phosphatase and urease were used to test natural system with high amounts of complex organic compounds (e.g. cellulose). In our system, the main existed organic compounds would be the PHCs and

BTEX. Their corresponding degrading enzymes are still studied at molecular bases using genetic tools such as qPCR.

For the enzyme assay, controls were included with each soil analyzed. The same procedure, as for the enzyme assay, was followed for the controls but the substrate was added to the soil after incubation prior to the analysis of the reaction product. All data were expressed based on the oven dry weight of the soil.

5.5 Experimental Results

5.5.1 Contaminant Loops Formed at the Natural Attenuation Stage

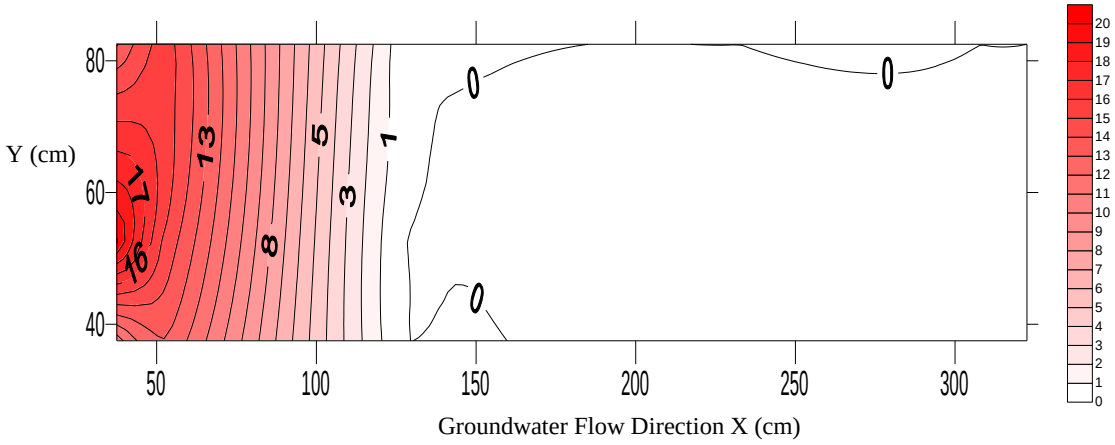
The pilot-scale vessel was used to physically simulate the transport and fate of contaminants in the subsurface and further examine the performance of the newly developed biosurfactants during a bioremediation process. The natural attenuation experiment lasted 26 days after the gasoline leak, followed by an 18 day enhanced biodegradation action in each experimental run.

The benzene concentrations were monitored in the 26 day natural attenuation and 18 day enhanced-bioremediation phases of run #1. Well 4, the closest one to the leak source, encountered the highest benzene concentrations during the natural-attenuation phase. The relatively high concentrations were also observed in wells 2 and 6, which were placed in the third layer. The contaminant could easily reach the wells along with the groundwater flow since the leak occurred at the top of the third layer. Moreover, relatively high concentrations occurred in wells 1, 3, and 5 because the wells were located in the sand zone and only 15 cm away (in X direction) from the source of the leak. Due to low porosity and permeability of the silt and clay, benzene was not observed in the down gradient domain of the pilot-scale system until day 20 (e.g., the contaminant reached well 16 on day 20).

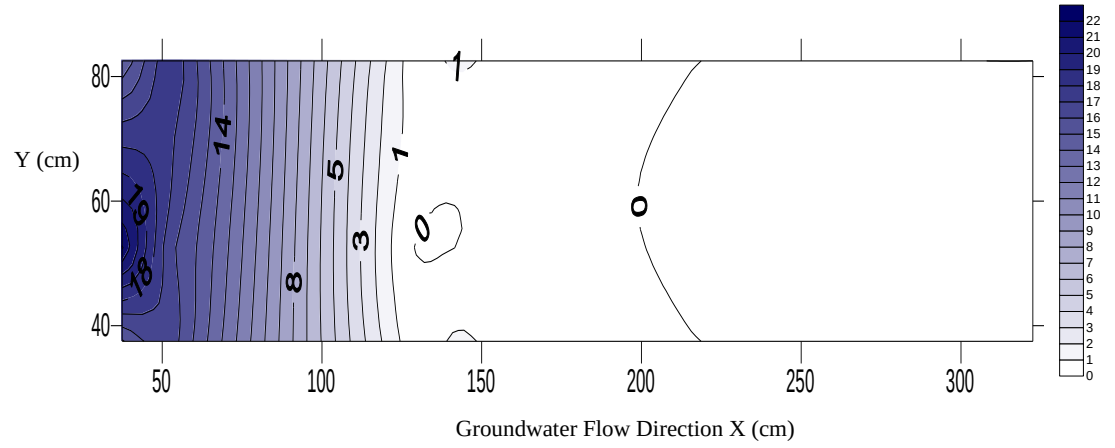
Figures 22 to 25 present the profile of benzene concentration on days 4, 10, 18 and 26 after the leak in run #1. Through the four figures, the movement of benzene in the subsurface could be clearly identified. Benzene moved together with the groundwater flow for about 1.3 m in the first 4 days (Figure 22a) and over 2.0 m on day 10 (Figure 23a). Being located in the sand zone, benzene was found to have accumulated around wells 7 and 11 gradually. On day 18 after the leak, three contaminated loops were obviously formed and the loop centers were around wells 4, 7 and 11, respectively (Figure 24a). Twenty six days after the leak, benzene moved about 2.3 m in the X direction of the pilot-scale vessel and reached the clay zone in well 16 (Figure 25a). Within the 26-day period, benzene spread in the vessel, which indicated that variations in the benzene concentrations were consistent with the subsurface soil profile. Wells 17 to

20 were located downstream in tile and clay zones and far away from the leak source, thus it had no contact with contaminants in this study.

Figures 22 to 25 also present the variation of toluene concentrations during the natural attenuation stage in run #1. After 26 days of transport, toluene concentrations around wells 7 and 11 were about 10 times that of the benzene concentrations at the same locations. Toluene concentrations were discovered to be significantly higher than benzene concentrations in all the wells on day 26. This is because of the higher content of toluene (2.73 - 21.8 % w/w) in gasoline than that of benzene (0.12 - 3.5% w/w), even the solubility of toluene is relatively low (0.05 g/100mL for toluene but 0.18 g/100mL for benzene at 25 ℃).

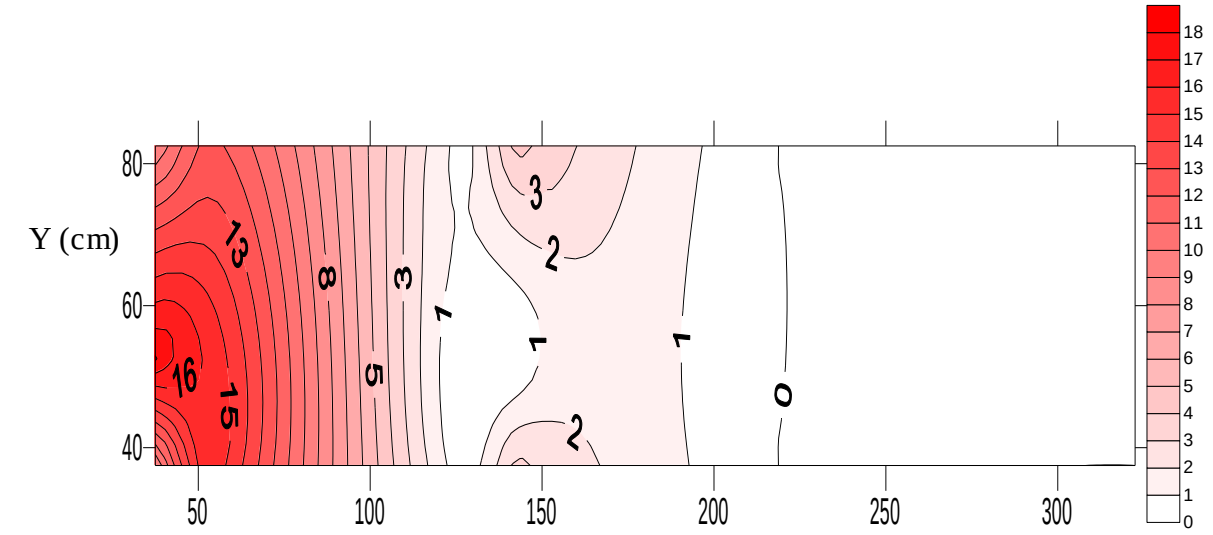


(a) Benzene concentrations (mg/L)



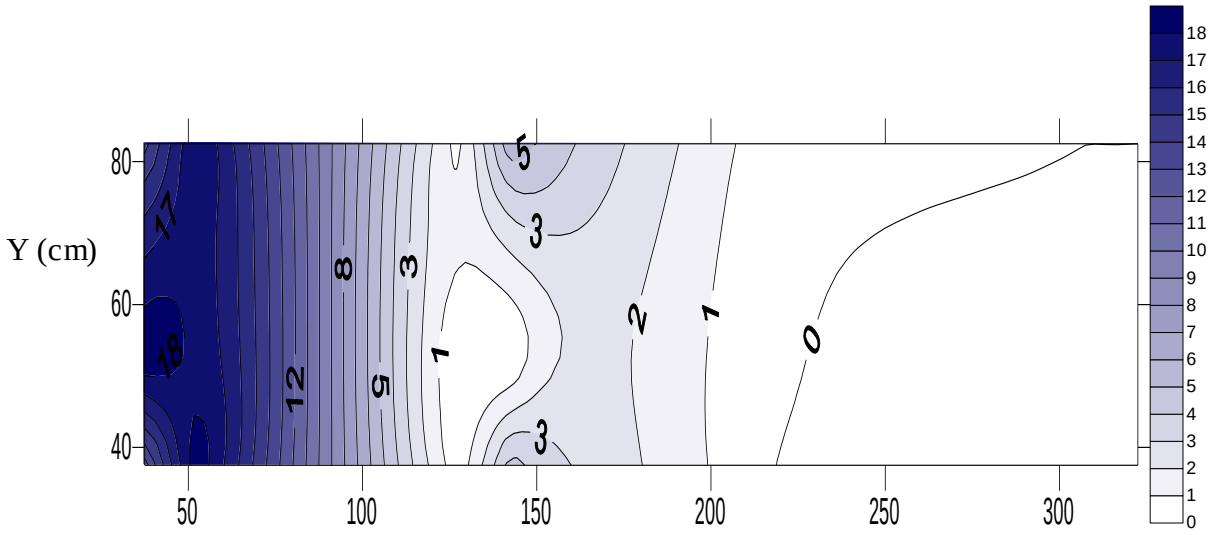
(b) Toluene concentrations (mg/L)

Figure 22 Contaminant concentrations in run #1 on day 4 after the leakage



Groundwater Flow Direction X (cm)

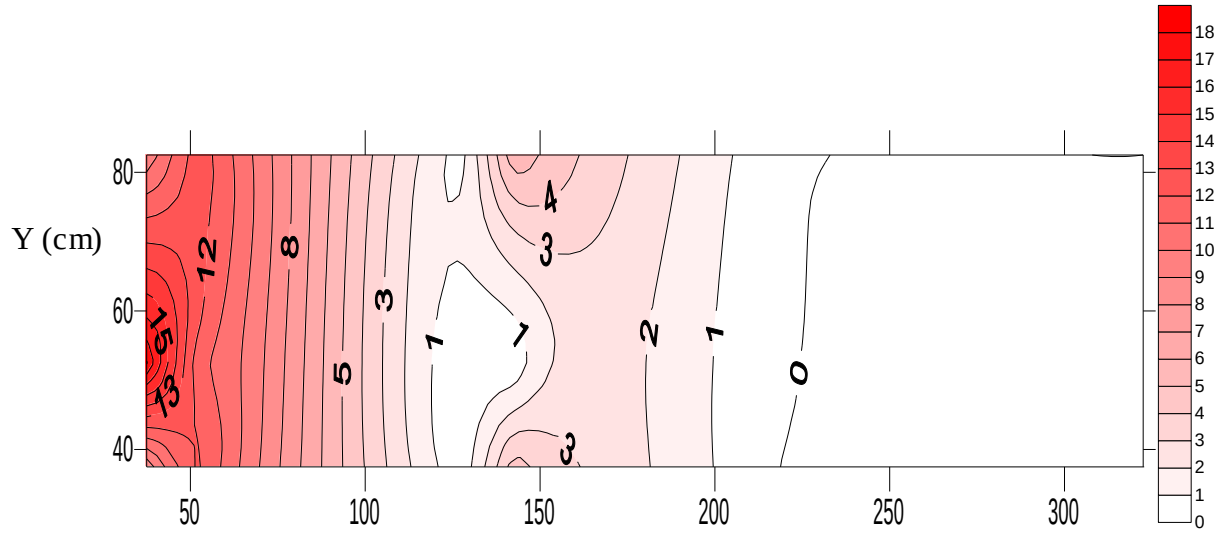
(a) Benzene concentrations (mg/L)



Groundwater Flow Direction X (cm)

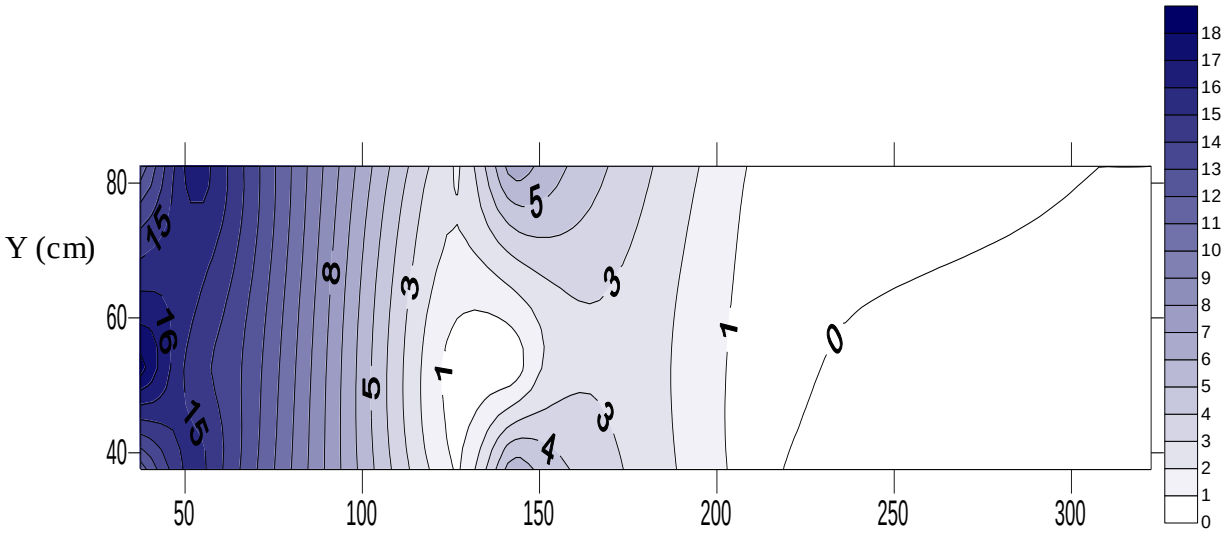
(b) Toluene concentrations (mg/L)

Figure 23 Contaminant concentrations in run #1 on day 10 after the leakage



Groundwater Flow Direction X (cm)

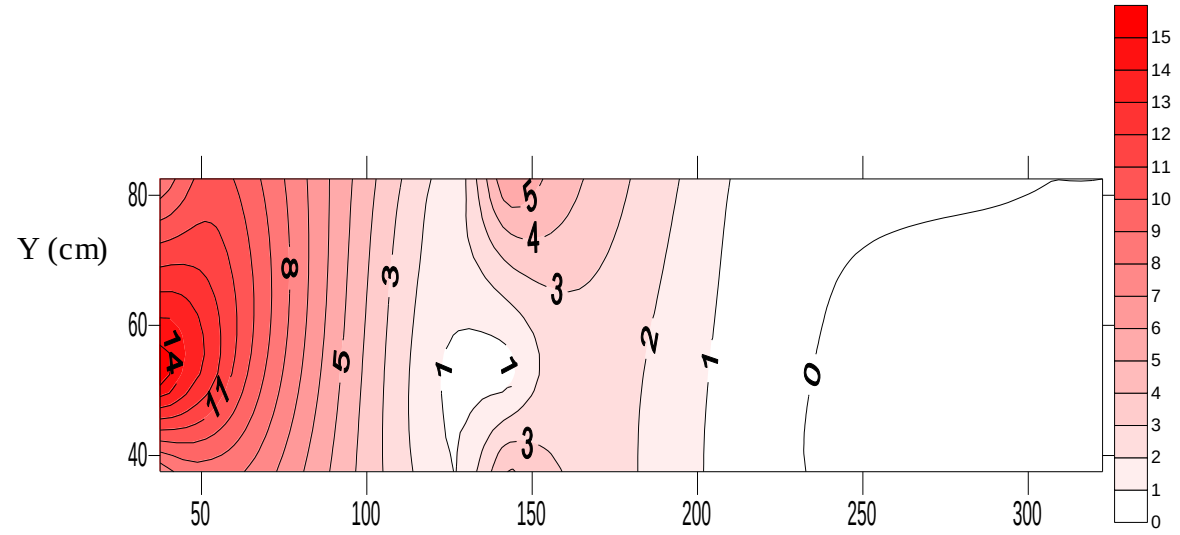
(a) Benzene concentrations (mg/L)



Groundwater Flow Direction X (cm)

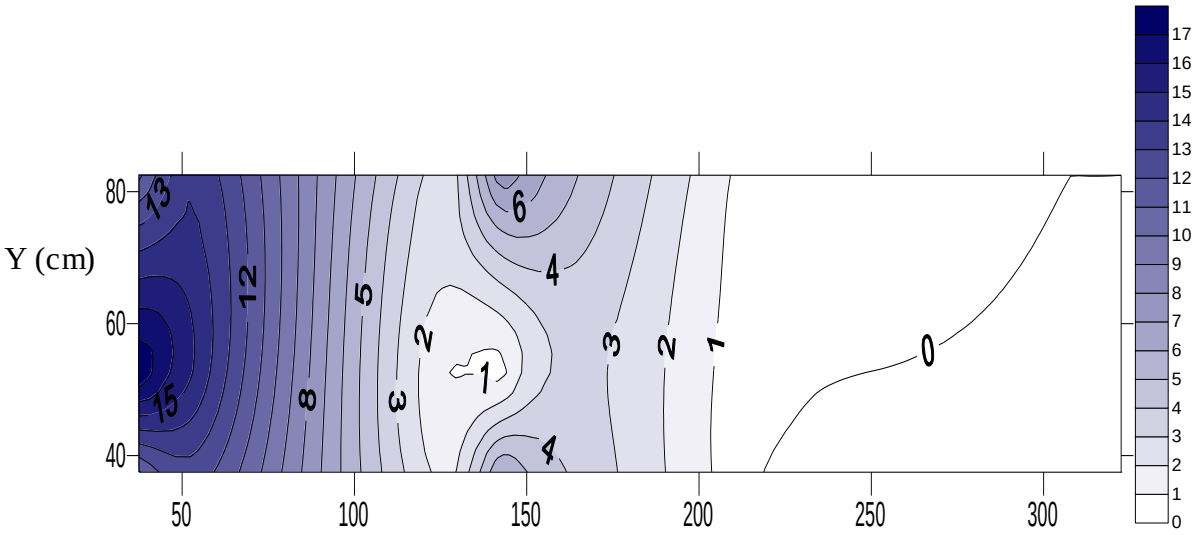
(b) Toluene concentrations (mg/L)

Figure 24 Contaminant concentrations in run #1 on day 18 after the leakage



Groundwater Flow Direction X (cm)

(a) Benzene concentrations (mg/L)



Groundwater Flow Direction X (cm)

(b) Toluene concentrations (mg/L)

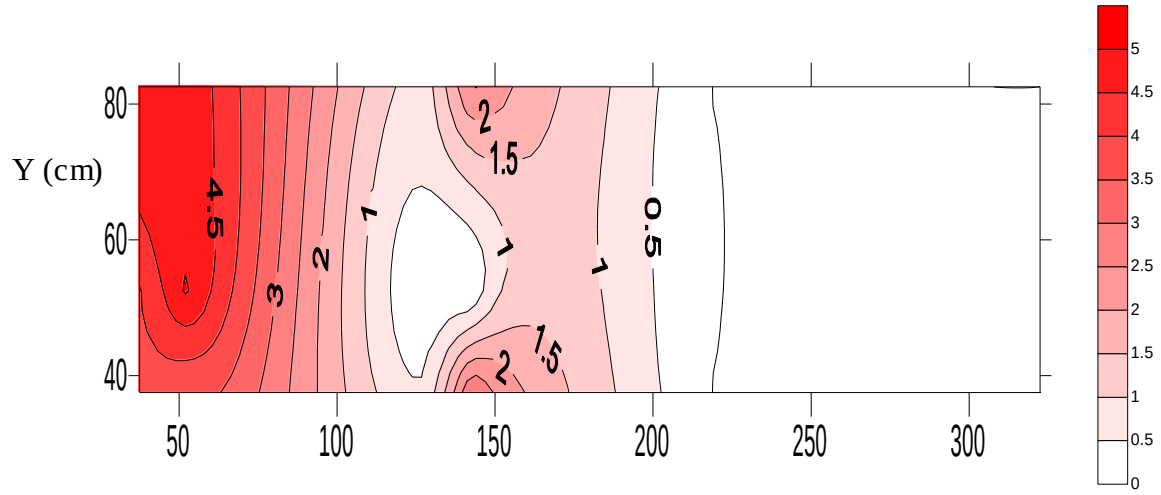
Figure 25 Contaminant concentrations in run #1 on day 26 after the leakage

5.5.2 Contaminant Attenuation at the Enhanced-bioremediation Stage

On day 28, an enhanced in-situ biodegradation action was undertaken. Experimental results indicated that the benzene concentration in groundwater varied greatly due to the injection and extraction actions in run #1. The location of the peak concentration moved towards the downstream. The benzene concentration in groundwater decreased greatly in comparison with those in the earlier periods. The peak benzene concentration decreased from 13.69 mg/L at the beginning of stage (3) in well 4 to 3.96 mg/L on day 8 after the biosurfactant-enhanced bioremediation action started and finally reached 0.72 mg/L on day 18. The removal rate was calculated as 94.7 % due to the biosurfactant-enhanced bioremediation within 18 days. Except for well 4, the benzene concentration in all wells in the pilot-scale vessel were below 0.5 mg/L under the CCME standard for subsurface soil based on future land use as residential or parkland after the 10-day bioremediation treatment. Figures 26 and 27 show benzene and toluene concentrations on days 8 and 16 after remediation action started in run #1.

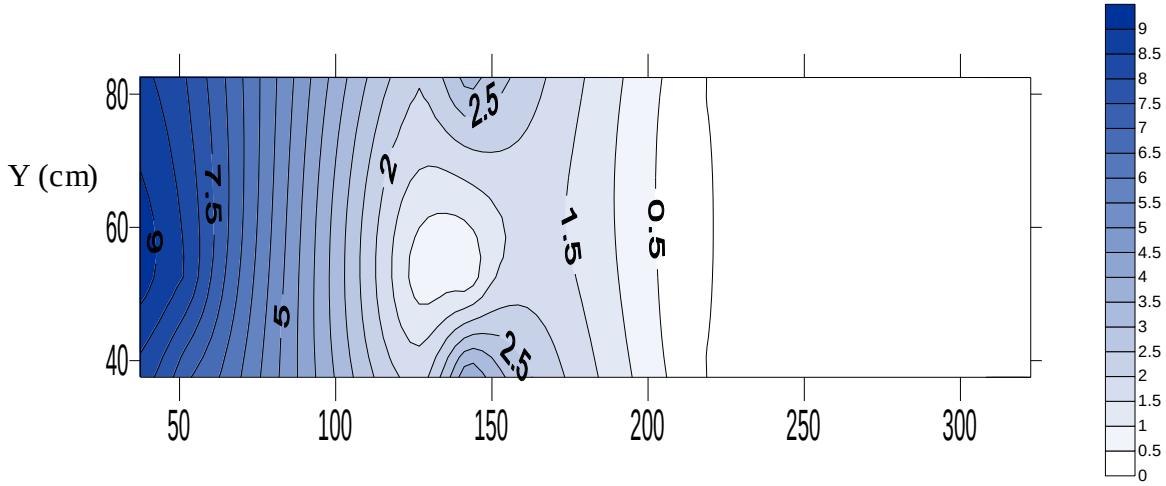
Figure 28 presents the variation of in benzene concentration from the extraction wells 7 and 11 during run #1. This shows that the concentrations increased first and then decreased continuously. From days 1 to 26, the increase in benzene concentration resulted from the contaminant movement together with the groundwater flow downstream in the vessel. Benzene concentration was observed to have a sharp increase right after stage (3) started (on day 27) due to the contaminant-plume through the enhanced injection and extraction flows. The benzene concentrations in the extraction flows of wells 7 and 11 started to decrease because of the enhanced bioremediation within the 18-day period.

A variation in benzene concentration in the groundwater at the pump-and-treat stage in run #2 was also observed. The injection and extraction of water also had an effect on bioremediation enhancement. The peak benzene concentration decreased from 13.38 mg/L at the beginning of stage 3 in well 4 to 7.40 mg/L on day 8 after the pump-and-treat action started and finally reached 2.68 mg/L on day 18. The removal rate was calculated as 79.9 % due to the pump-and-treat action within the 18 day period. In comparison with the benzene removal rate of 94.7 % due to the biosurfactant-enhanced bioremediation, the injection of newly developed biosurfactant solution was proven to be able to significantly improve the biodegradation of subsurface contaminants. A similar conclusion could be made based on the comparative analysis of benzene concentrations of almost all wells in runs #1 and #2.



Groundwater Flow Direction X (cm)

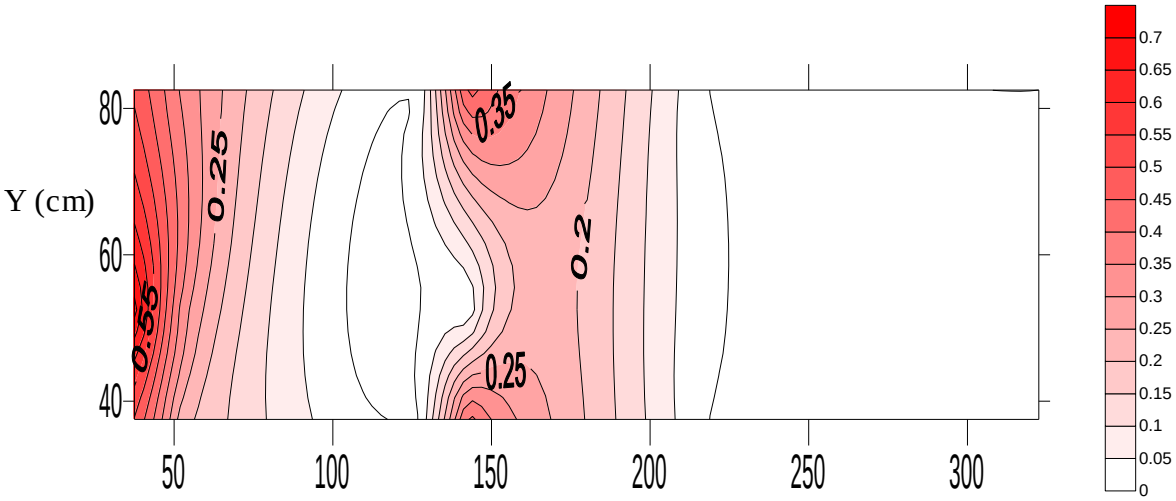
(a) Benzene concentrations (mg/L)



Groundwater Flow Direction X (cm)

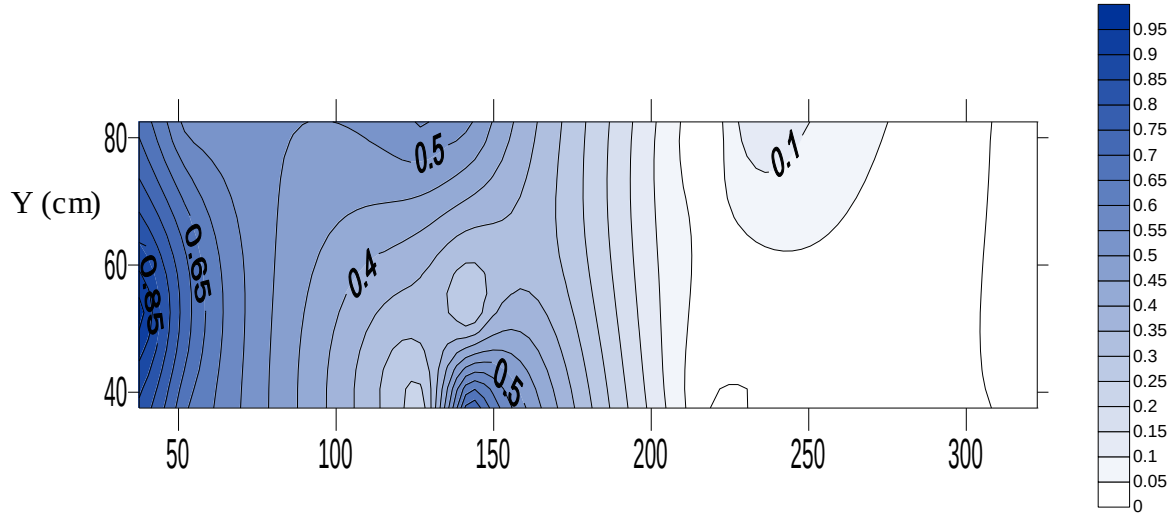
(b) Toluene concentrations (mg/L)

Figure 26 Contaminant concentrations in run #1 on day 9 after the remediation started



Groundwater Flow Direction X (cm)

(a) Benzene concentrations (mg/L)



Groundwater Flow Direction X (cm)

(b) Toluene concentrations (mg/L)

Figure 27 Contaminant concentrations in run #1 on day 18 after the remediation started

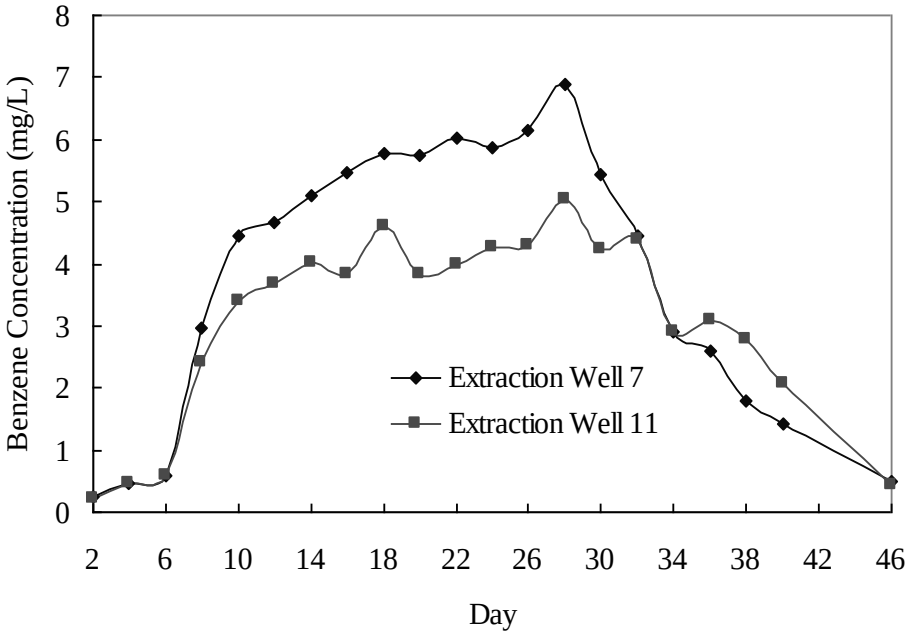


Figure 28 Benzene concentrations (mg/L) in extraction wells 7 and 11 in run #1

5.5.3 Influence of Soil Types on Efficiency of Bioremediation Enhancement

Biosurfactants have the potential to enhance bioremediation processes through both media and biological improvement. When biosurfactants are added to the environment with microorganisms growing on water-insoluble substrates, they have been revealed to improve biodegradation by increasing the surface area of hydrophobic substrates, increasing the bioavailability of hydrophobic substrates and regulating microbial attachment-detachment to/from surfaces (Rosenberg et al., 1999). Biosurfactants have been proven to effectively enhance the contaminant removal from subsurfaces in this study. However, the efficiency of bioremediation enhancement is not identical at all well locations due to the influence of subsurface soil types, which can be disclosed by the following discussion.

Figure 29 shows benzene concentrations vs. bacterial concentration in wells 3 and 4 during run #1. The decrease in benzene concentration corresponds significantly to the increase in bacteria in both wells, which showed that the removal of benzene was indeed partially or mainly due to the microbial biodegradation. During both stages 2 and 3, bacterial concentration in the sand zone (well 3) was much higher than in the silt zone (well 4), even both of the wells had similar contaminant concentrations. The results indicated that it was hard for inherent PHC-degrading bacteria to grow in a relatively low-permeable site and the enhanced-bioremediation actions were meaningful to such a subsurface environment.

Figure 30 shows benzene concentrations in three different wells during stage 3 in Runs #1 and #2. The wells 5, 10 and 16 were located in the sand, silt and clay zones, respectively, with the subsurface hydraulic conductivity in the range from 10^{-5} to 10^{-7} m/s. The addition of

the biosurfactant showed a superior effect on benzene removal in wells 16 (Figure 30c) and 10 (Figure 30b) in run #1, in comparison with the performance of the pump-and-treat action in run #2. The media enhancement of biosurfactants in clay (well 16) and silt (well 10) was confirmed with obvious evidence. However, such evidence was not discovered in well 5 (located in a sand zone), where runs #1 and #2 showed a similar efficiency on bioremediation enhancement (Figure 30a). The results indicated that the addition of biosurfactants resulted in a higher efficiency of bioremediation enhancement in the clay or silt zone (wells 16 and 10, respectively) than that in the sand zone (well 5). Thus, the newly developed biosurfactants would have a greater potential of application to western Canadian sites where low-permeable subsurface extensively exists. Furthermore, the results from both runs #1 and 2 indicated that the developed pilot-scale system could effectively facilitate simulation for natural attenuation and the enhanced remediation processes.

The QC/QA of the pilot-scale system was assessed through analysis of the duplicated samples in each run. The results indicate that, for 79 % of the experimental data, the deviations are less than 6 %; for over 90 % of the experimental data, the deviations are less than 10 %.

Data analysis of the pilot-scale examination has not been completed yet. More experimental results shall be released through journal publications and conference presentations.

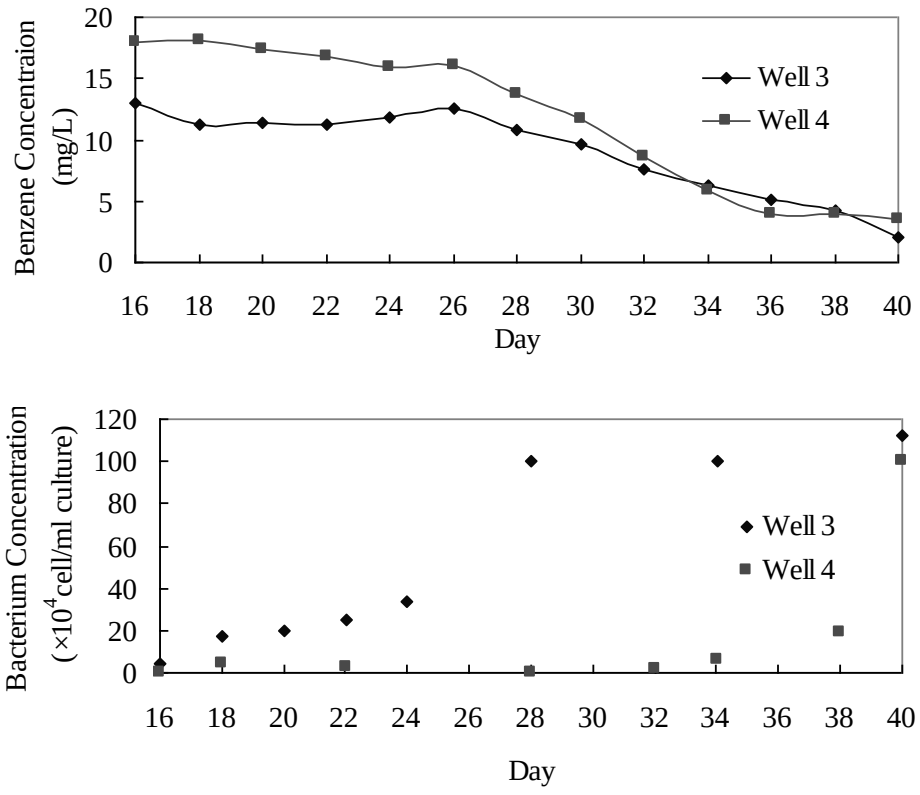


Figure 29 Benzene concentrations vs. bacterium concentrations in wells 3 and 4 during run #1

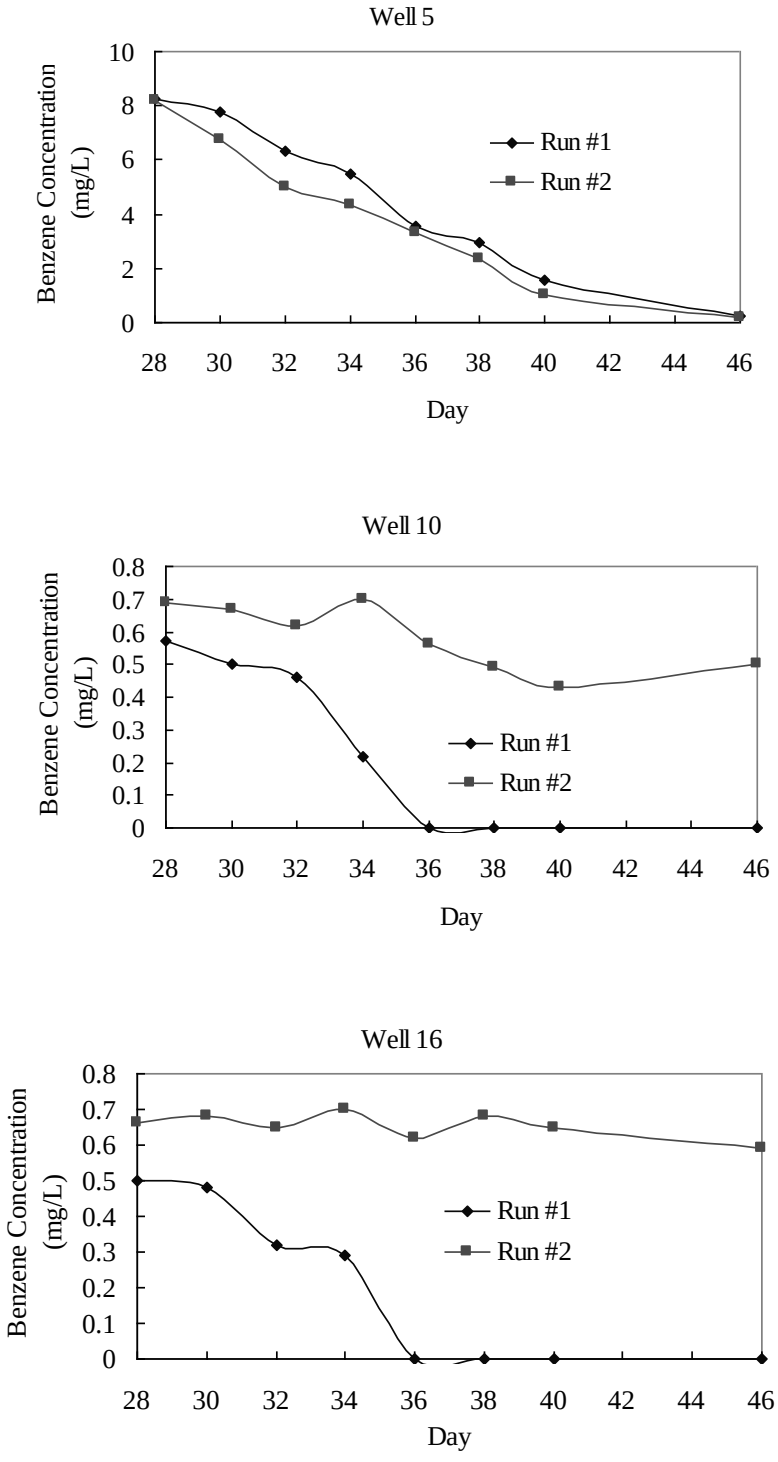


Figure 30 Benzene concentrations (mg/L) in three different wells during stage 3 in runs #1 and #2

CHAPTER 6
CONCLUSIONS

This research tackled a pilot-scale demonstration of the promising biosurfactant-enhanced In-situ bioremediation of a PHC and heavy metal co-contaminated site in NL to address a wide range of challenges facing local site remediation actions. In-depth investigation of the effects of physicochemical, hydrological and biological factors on bioremediation performance was conducted, which plays an ever-increasing role in the implementation of the advanced bioremediation measures.

In-situ bioremediation technique offers a relatively low cost approach for managing petroleum-contaminated soils in cold regions, with the potential to achieve reasonable environmental outcomes in a timely manner. The challenge for scientists, engineers, and environmental managers is to derive or refine a range of remedial strategies that are well suited or optimized for cold region conditions. This report conducted an overview of petroleum contamination, regulation, and remediation in NL. A number of factors, including the properties and fate of oil spilled in cold environments, the major microbial and environmental limitations of bioremediation were discussed in this research. The microbial factors include bioavailability of hydrocarbons, mass transfer through the cell membrane, and metabolic limitations. As for the environmental limitations in the cold regions, the emphasis is on soil temperatures, freeze-thaw processes, oxygen and nutrients availability, toxicity, and electron acceptors. Recent advances in environmental applications of biosurfactants were included. Effects of the spatial heterogeneity, advective-dispersive transport and harsh environmental conditions on bioremediation actions, especially in large environmental systems were also discussed.

A NL contaminated site was selected in this research, followed by a detailed site characterization. The target contaminated site was selected within the Lower Tank Farm (LTF) at 5 Wing Goose Bay, Labrador. The Goose Bay Remediation Project (GBRP) has officially begun in 2010 and is estimated to be completed by 2020. Of the many contaminated sites in the Goose Bay, the five most severe ones were identified as the main legacy contaminated sites, and the LTF is one of them. The majority of environmental contamination at the site can be attributed to past storage and handling practices of a broad range of environmental contaminants, particularly hydrocarbons and heavy metals. Before conducting the pilot-scale bioremediation, a comprehensive site investigation was performed to facilitate the experimental design. The key factors achieved by site investigation though literature review and site visit include: (a) contaminant types and their physical and chemical characteristics (e.g., concentration, solubility, density and volatility); (b) subsurface conditions, such as soil type, hydrological/geological characteristics, homogeneity in vadose and saturated zones and soil permeability; (c) groundwater conditions, such as depth of perched water, depth of saturated groundwater and hydraulic conductivity; (d) potential extent of contamination, such as residual-phase and gaseous-phase hydrocarbons in the vadose zone, free-phase and dissolved-phase hydrocarbons in the saturated zone and the area of contamination; (e) adjacent surface conditions, such as conditions of operating property above the contaminated zone (e.g., open

space, tanks, pipes, paving and structures) and open space available for treatment; and (f) related standards including clear-up criteria.

To scale down the site conditions to the pilot-scale experimental system, the development of the subsurface soil profile of the site was conducted. Soil and groundwater conditions around and within boreholes were the inputs of this process. The Minitab software package was employed to interpolate and extrapolate the missing data and graphically represent the results. Given the heterogeneity that exists in nature, it is simply not feasible to completely define subsurface conditions at a given site. Attempting to do so will require an infinite number of borings, monitoring wells, samples and analyses. Therefore, it is feasible and necessary to make assumptions accompanied by sensitivity analysis when designing subsurface soil profile. The potential assumptions in this research include: (a) each cell or grid represents a single type of soil, either clay or silt or sand; (b) if two or more types of soil exist within a cell, then the soil with the highest proportion in weight is chosen; (c) the level of groundwater table is horizontal within the modeling domain; and (d) fluctuation of the groundwater table is minor and can be ignored. Based on the available data and assumptions, a conceptual model of the site subsurface was generated.

A pilot-scale experimental system was set up in the lab. A customized pilot-scale physical vessel used to provide controlled environmental conditions for simulating site remediation was designed and manufactured. It is located in the Northern Region Persistent Organic Pollution Control (NRPOP) Laboratory at MUN, which is founded by the Canada Foundation for Innovation (CFI) and the Industrial Research and Innovation Funds (IRIF) of Newfoundland and Labrador Government. The vessel consists of a 3.6m L × 1.5m W × 1.2m D stainless steel basin. The sealed vessel, equipped with flow controller, drainage collectors and sensors, help mimic various site conditions. In this report, the vessel was filled with pre-selected soils (sand, till, clay) according to the conceptual model to ensure the inside conditions are similar to those at the study site with a specific scaling-down ratio. The sampling outlets and monitoring/injection/extraction wells were settled within the pilot-scale experimental system to facilitate the bioremediation treatment and water/soil sample collection during the experiments.

In the past few years, a biosurfactant enhanced in-situ bioremediation approach through biosufactant production, purification, and characterization has been developed by Dr. Zhang's research group. In this research, the newly-developed cold-adapt biosurfactants were applied to the pilot-scale system as the washing agent through injection/extraction to improve removal of the co-contaminants, and as the additive in the mixing tank to enhance subsurface media conditions and microbial activities. Environmental factors (e.g., temperature, pH, nutrients, and oxygen supply) influencing behaviors of biosurfactants were examined during the processes for system optimization. Concentrations of biosurfactants, heavy metals, benzene, toluene, ethylbenzene, xylenes (BTEX) and data of total petroleum hydrocarbons (TPH) were obtained after the lab analysis through using the tensiometer, Flame Atomic

Absorption Spectrometer (FAAS) and Gas Chromatograph-Mass Spectrometer (GC-MS). Oxygen uptake rates through microbial activities and biosurfactant recovery rates were also monitored. The pilot-scale performance of biosurfactants and the associated bioremediation technologies were examined. Through a number of experimental studies as well as systematic consideration of factors related to source and site conditions, the research outputs are expected to help generate an environmental friendly and economical/technical feasible alternative to solve the challenging site contamination problems in NL.

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