Enhancement of the Bioremediation of PCE Contaminated Soil by Rhamnolipid and Two Biological Products

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ABSTRACT

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Tetrachloroethylene (PCE) is a chlorinated solvent used in the dry-cleaning industry. PCE is a toxic chemical and is hazardous to humans and the environment. PCE is a dense non-aqueous phase liquid (DNAPL) and once it leaks into the soil, moves downwards and contaminates the soil and the groundwater. Common remediation techniques like soil washing do not easily remediate the soil contaminated with PCE, unless proper additives like surfactants are added to the washing solution. Surfactants reduce the surface and interfacial tension, and increase the solubility of PCE. Surfactants at concentration above the critical micelle concentration (CMC) form micelles and make PCE partition into the micelles, consequently PCE disperses in the aqueous phase and becomes more bioavailable.

This study evaluates the effectiveness of rhamnolipid and two surfactant-based products (ASAP™ and Degreaser™) on the bioremediation of PCE. These two agents are non-toxic chemicals. Following the USEPA methods, and gas chromatography analysis, diverse 21 day experiments were conducted. ASAP™ and Degreaser™ at a ratio to soil of 1:1 showed removal efficiencies of 45% and 52%, respectively. Addition of rhamnolipid with a ratio

to soil of 1:1 to these products at the same ratios enhanced the remediation up to 55% and 58%. Conducting microbial analysis, a direct correlation was observed between microbial density and PCE removal, which suggests that degradation, had occurred. The removal trend was:

Biological agent + rhamnolipid > biological agent > rhamnolipid > Control.

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At the end of my thesis I wish to sincerely thank my supervisor Dr. Catherine Mulligan, for her guidance, valuable hints, and constant support during this work.

I also would like to express my gratitude to the faculty and staff of the Department of Building, Civil, and Environmental Engineering at Concordia University.

DEDICATION

This work is dedicated to my dear mother, and to my beloved father who is no longer among us and whose absence is sharply felt at this point.

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1. Introduction

1.1. Background

Soil and groundwater contamination is a serious global problem and a big threat to humans and the environment. Hydrocarbon compounds, hazardous waste and heavy metals generated from industrial factories and agricultural enterprises are the greatest sources of soil contamination. These contaminants are either buried directly or are introduced to soil by spillage and leakage. The amount of petroleum products spilled and leaked into soil in the United States has been reported at around 6 million tons per year (Hutchins et al., 1991).

Tetrachloroethylene (PCE) is a chlorinated hydrocarbon widely used as a dry cleaning agent and sometimes is called "dry-cleaning fluid". It is also used as industrial degreaser and cleaner. According to Rossberg et al. (2006) worldwide production of PCE was about 1 megaton in 1985. The major uses of tetrachloroethylene in Canada include use as a dry cleaning solvent, feedstock for the production of fluorocarbons, metal decreasing/cleaning, and textile cleaning (CIS, 1990; Canadian Environmental Quality Guidelines, 1999). Tetrachloroethylene enters the environment through evaporation or through leaking into groundwater and water supplies. PCE is found in almost all Canadian environmental media. However, nation-wide usage of PCE is dispersive and the potential release of PCE in Canada is estimated to be

equal to the Canadian net domestic production (e.g. 19.5 kilo ton in 1989) (Environment Canada, 1996).

1.2. Objectives

The objective of this study was to evaluate the ability of rhamnolipid biosurfactant, and two biological cleaning agents (Biomor ASAP Floor Degreaser™ and Biomor Industrial Degreaser and Concrete Cleaner™) in bioremediation of PCE contaminated soil. Achieving enhancement in PCE degradation by applying different ratios of above additives was another objective of this study.

1.3. Thesis Outline

This study includes 5 chapters followed by an appendix as follows:

Chapter 1 introduces the purpose of the study and describes the problem and objectives of the research.

Chapter 2 covers the related information on the problem, and reviews the previous works and research performed on this issue.

Chapter 3 presents the materials and experimental procedures used in this study.

Chapter 4 exhibits and discusses the results of the experiments.

Chapter 5 develops conclusions and suggests ideas for future work.

The appendix contains some calculations.

2. Litrature Review

2.1. Soil Environment

Soil is generally referred to as the upper most mantel of the earth's surface (Lagrega et al., 2001). The environment beneath the earth is a porous medium which contains water, air, and organic matter. It consists of both consolidated (rock) and unconsolidated (sand, gravel, clay) formations. However, the civil engineers consider all unconsolidated sediments as soil (Lagrega et al., 2001).

The general definition used by engineers for soil indicates a loose material between the ground surface and solid rock. Suspensions and solutions can move through this loose material and due to its highly absorbency it can adsorb particles from the solutions passing through the soil. Soil is also a very suitable habitat for living organisms and contains both organic and inorganic materials (Pierzynski et al., 1994). Soil is formed through a process whereby particles of weathered parental rock or unconsolidated sediment are transported, deposited and accumulated (Lagrega et al., 2001). Mostly decomposed plants or humus create the organic fraction of the soil whereas the inorganic portion of the soil is made of fine minerals such as silicon, aluminium, and iron. Inorganic material is usually subdivided into gravel, sand silt, and clay based on the soil grain size (Lagrega et al., 2001).

2.2. Physical and Chemical Characteristics of Soil

Characteristics of soil vary due to different parent material, climatic conditions (wind, water, temperature), and topography (slope and relief) (Pierzynski et al., 1994; Lagrega et al., 2001).

The inorganic fraction of soil mostly consists of fine material grains which are subdivided into clay, silt, sand, and gravel, based on grain size distribution. The organic part of soil is typically formed by decaying plants and animal residues, a complex mixture of carbon compounds, nitrogen, sulfur, and phosphorus (Pierzynski et al., 1994). The organic part of the soil has a stabilizing role and binds the inorganic part together as aggregates in different sizes and structures. The soil structure varies due to the type of aggregates (Lagrega et al., 2001).

Soil properties like temperature, color, particle size distribution, composition and arrangement of solids, as well as pore volume are some of the most important soil physical properties. On the other hand, pH, cation exchange capacity, buffering capability, and mineral solubility are major chemical properties of soil. Some of the main characteristics of soil are described in more detail below.

2.2.1. Particle Size Distribution

Soil structure depends on the range of soil particle size, which varies from stones to fine clays. Under different conditions over a long period of time

larger particles are weathered and transformed to smaller particles of soil (clay, silt, sand). The texture of soil is indicated by the percentage weight of clay, silt, sand and gravel (Lagrega et al., 2001). Soil particle size distribution has an important role in the amount of water that enters the soil (infiltration) and the amount of gas that moves through the soil. Porosity is a function of soil particle size distribution and soil texture. Soil particle size classification is shown in Table 2.1.

Table 2.1 Size classification of soil particles (Soils and Environmental Quality, 1994)

Fraction	Soil particle	Diameter (mm)	Size comparison
Coarse	Stones	>254	>25.4 cm
	Cobbles	75-254	7.5-25.4 cm
	Gravel	2-75	0.2-7.5 cm
Sand	Very coarse	1.0-2.0	Thickness of a nickel
	Coarse	0.5-1.0	Size of a pencil lead
	Medium	0.25-0.5	Salt crystal
	Fine	0.10-0.25	Thickness of a book page
	Very fine	0.05-0.10	Nearly invisible to the eye
Silt	Coarse	0.02-0.05	Root hair
	Medium	0.01-0.02	Nematode
	Fine	0.002-0.01	Fungi
Clay	Coarse	0.0002-0.002	Bacteria
	fine	< 0.0002	viruses

2.2.2. Soil pH

Soil pH indicates the acidity or alkalinity of the soil and is measured as the negative logarithm of the hydrogen ion concentration in the soil. The acidity of soil increases as pH goes from 7 to 0 and soil alkalinity increases as pH goes

from 7 to 14. Soil chemical reactions, mineral solubility, and the presence of contaminants are dependent on soil pH. Acidic soils mostly contain more minerals, nutrients, and contaminants since these components are more soluble in acid soils than neutral or alkaline soils (Yong et al., 1992; Pierzynski et al., 1994).

Mostly, the best condition for bacterial growth and activity is neutral to slightly alkaline pH. Microbial growth is low at pH 5 or lower. Studies reported that the degradation of hydrocarbon contaminant is faster at pH values above 7 as compared with values of 5 (Cookson, 1995). While acidic soil is a favorable environment for fungal growth, fungi have a low capability for hydrocarbon degradation (Maier et al., 2000).

2.2.3. Organic Matter Content

As mentioned above, soil organic matter is composed of decaying plants and animal remains and is a complex mixture of carbon compounds, nitrogen, sulfur, phosphorus and other biochemical substances (Pierzynski et al., 1994) which influences the behaviour of soil. Organic matter content ranges from less than 1% in coarse textured soils of arid regions to 100% in peat soils (Yong, 2001).

2.2.4. Cation Exchange Capacity

Cation exchange capacity or CEC is one of the most important properties of the soil and indicates the number of exchange sites which are available for the adsorption and release of cations. In general CEC is the total quantity of

positively charged ions which can be held on soil colloid surfaces by relatively weak electrostatic forces; the total negative charge on soil colloids (Aber and Melillo, 2003). It affects the accumulation and transport of the contaminants. Cations with higher charge are substituted with cations of lower charge which forms a permanent charge on the charge site.

2.3. Soil Contaminants

Contaminants in the soil are introduced to soil by man. Hazardous materials on the other hand may be natural or introduced by human activities. There are some hazardous substances in the natural soil like zinc (Zn), nickel (Ni), lead (Pb), chromium (Cr), cadmium (Cd) and copper (Cu). Soil contaminants caused by human activities are released through different industrial processes, transport spills, storage spills, leaks, and treatment effluent (Lagrega et al., 2001).

Common contaminants found in soil are hydrocarbons and heavy metals. The organic fraction of soil has a significant role in adsorbing pollutants such as lead, cadmium and copper; which reduces the chance of surface and ground water contamination. Heavy metals are significant hazards to the environment and humans. Depending on the type of the activity, heavy metals can be present in soil from a few parts per million (ppm) to tens of thousands of parts per million (ppm). Contaminants released to the soil move through the soil and can eventually reach living receptors. The contaminants can be classified into the categories described below.

2.3.1. Hazardous Substances

Organic compounds like fuels and solvents can be harmful to humans or other organisms if not used properly and would have a similar effect as pesticides (Pierzynski et al., 1994). One of the most important characteristics of organic contaminants is their low solubility which makes it difficult to remove the contaminant.

2.3.2. Nutrients

High concentrations of nitrogen and phosphorus can have a negative effect in the site and are considered as contaminants. Nitrogen and phosphorus are mostly introduced to soil through commercial fertilizer, sewage sludge, and municipal solid wastes. Phosphorus in surface water and nitrogen in ground water are major environmental problems (Soil and Environmental Quality, 1994; Pierzynski et al., 1994). The diverse problems nitrogen and phosphorus cause in the environment include toxic algal blooms, loss of oxygen, fish kills, loss of biodiversity (including species important for commerce and recreation), loss of aquatic plant beds and coral reefs, and other problems (Carpenter et al., 1998).

2.3.3. Pesticides

A wide range of organically based chemicals used to control pests, such as insects and weeds, are called pesticides. Consequently, pesticides can be found in sewage sludge and municipal solid waste (Soil and Environmental Quality, 1994; Pierzynski et al., 1994).

2.3.4. Acid Rain

Acid rain is essentially the result of the transformation of sulphur dioxide (SO_2) and nitrogen oxides in the atmosphere into dry or moist secondary pollutants such as sulphuric acid (H_2SO_4), ammonium nitrate (NH_4NO_3) and nitric acid (HNO_3) (Soil and Environmental Quality, 1994; Pierzynski et al., 1994).

2.4. Sources and Practices Contributing to Soil Contamination

Sources of contaminants can be divided into point (direct) sources and non-point (diffuse) sources. This classification is based on the pathways by which the sources release the contaminants to soil. Point sources are localized and their chemical discharge can be measured. Industrial outfall pipes, treated sewage outfalls as well as accidental spills and leaks of contaminants are examples of point sources. Contaminant sources like pesticides and fertilizer runoff are considered as non-point sources (Linz and Nakles, 1996). Some important sources of contaminants at hazardous sites are listed below (Boulding, 1995).

- Contaminant release from disposal-discharge of substances:

These sites are intentionally designed to discharge substances such as sewage, sludge, hazardous waste, nonhazardous waste. Contaminants of concern in these discharges include organics, nitrate, phosphate, and heavy metals.

- Discharge via unplanned release from storage, treatment, and/or disposal of substances:

Sites such as landfills, open dumps, residential disposal, surface impoundments, waste piles and tailings, above ground storage tanks, underground storage tanks, containers and radioactive burial sites are basically designed to store or treat the contaminants. These sites should be designed in a way that no discharge of the contaminants to the subsurface takes place.

- Release of contaminants through transport or delivery of substances:

Contaminants are released through accident or neglect, such as pipeline breakage or traffic accidents which includes raw materials and/or waste releases.

-Discharge of substances as a result of planned activities:

Pesticide and fertilizer applications, mining and mining drainage, urban runoff and irrigation practices might discharge contaminants to the soil and subsurface.

- Natural sources or release of contaminants enhanced by human activities:

In areas near industries and other human activities like metalliferous sites both organics and metal contamination are observed.

2.5. Fate and Transport of Hydrocarbons

The fate and transport of hydrocarbons in the subsurface varies depending on the type and properties of the hydrocarbon and characteristics of the spill site (Alexander, 1999; Xueqing et al., 2001). Soil properties like porosity, particle size distribution, permeability, and organic content have important roles in hydrocarbon fate. Chemical and physical properties of hydrocarbons such as water solubility, bioavailability and vapor pressure are other parameters determining the fate of hydrocarbons in the soil (Williamson et al., 1998; Alexander, 1999; Loehr et al., 2000; Xueqing et al., 2001). Moreover, soil particle size and bulk density affect the characteristics of air and water flow through soil by affecting soil porosity and hydraulic conductivity (Appelo and Postma, 1993).

Determining the fate of hydrocarbons is complicated by hydrodynamic, abiotic and biotic changes that could occur to hydrocarbons upon release to the environment. The analysis of the fate of contaminants becomes even more complicated when the release is exposed to the environment for a long time (Maier et al., 2000; Xuenqing et al., 2001).

Hydrocarbons in vadose zone can exist in pore spaces as vapor, can be adsorbed to soil particles, might dissolve in water or exist as non-aqueous liquid phase (NAPL); however in the saturated zone it can be adsorbed to the soil particles, dissolved in water or a non-aqueous liquid phase (Bekins et al.,

2001). The different pathways known for hydrocarbons in the subsurface are as below.

2.5.1. Adsorption

In this process organic contaminants are adsorbed to the soil particles, so their mobility is limited. The hydrocarbon molecules adhere to soil particles temporarily via van der Waals forces, hydrogen bonding, dipole-dipole interactions, and ion exchange, covalent bonding, etc. (Yong et al., 1992, Alexander, 1999).

2.5.2. Volatilization

Evaporation or volatilization includes transforming the compound from solid to vapor. The volatilization rate depends on contaminant characteristics such as vapor pressure, solubility and half life; soil properties like porosity, water content, density and clay content. Environmental conditions such as temperature, humidity, and wind speed affect the evaporation rate as well (Pierzynski et al., 1994).

2.5.3. Biodegradation

Organic contaminants are transformed to simpler compounds through biodegradation. Microorganisms in the soil like bacteria, fungi, algae and yeast break down the hydrocarbon molecules and transform them to simpler organic compounds (intermediary products) or inorganic products (CO_2, water and mineral salts). Biodegradation can occur under aerobic or anaerobic conditions.

2.5.4. Hydrolysis

Hydrolysis is a chemical process which takes place in the liquid phase. In this process an organic chemical is broken down by reacting with a molecule of water (Hermond and Fechner-Levy, 2001).

2.5.5. Plant Uptake

Organic contaminants can be absorbed and taken up by plant roots. Uptake by plants basically depends on the plant species and the organic chemical properties (Pierzynski et al., 1994). When the chemical is absorbed by the plant, different reactions may occur to transform the chemical into intermediary organic products or inorganic products (Lagrega et al., 2001).

2.6. Remediation Technologies

Several sites in Canada and the US have been contaminated by industrial and waste management activities. The "National Pollution Release Inventory" of Canada reported approximately 13300 metric tons of copper, 9500 metric tons of zinc, 1300 metric tons of lead and 33 metric tons of cadmium are released annually to air, water and soil through industrial operations such as metal processing (NPRI 1995, Environment Canada).

Polluted sites are classified into groups of seriously contaminated sites and lightly contaminated sites. In the first group the contamination is deeply penetrated into the soil thus making the soil heavily contaminated. In the second

group only the surface or top soil layer is contaminated.

Selection of the most efficient and proper remediation technology is highly dependent on the site characteristics and its accessibility as well as concentration, volatility, solubility, and biodegradability of contaminants (Pierzynski, 1994; Mulligan, 1998). Remediation technologies can be subdivided into ex-situ and in-situ processes.

2.6.1. Ex-Situ Processes

The process in which the soil is excavated and then treated by using one or more technologies is considered as ex-situ process. Ex-situ processes are more expensive due to excavation and transport costs. Some of the most important methods are described below.

2.6.1.1. Thermal Treatments

In this method heat is used to remove the contaminants either by evaporation or by incineration so that the contaminant's structure is destroyed by heat. Thermal treatment is used for the soil with organic contamination. High temperature removes the volatile organic compounds (VOCs) by evaporation and converts the rest to carbon dioxide, water, and other combustion products (Lagrega et al., 2001). Consequently the amount of the organics is reduced. This method however does not have an effect on inorganics and sometimes the combustion products are more toxic than the initial contaminants.

2.6.1.2. Biological Processes

Biological treatment or bioremediation is a relatively new and developing method in the treatment of soil contaminated with organic compounds. In this method contaminants are degraded, removed, and/or neutralized by microorganisms such as bacteria, fungi and algae (Lagrega et al., 1994; EPA, 2004). Degrading either transforms the contaminant to simpler organic compounds (byproducts) or mineralizes the contaminant to carbon dioxide, water, and cell mass (Lagrega et al., 1994). Bioremediation can be used in-situ or ex-situ and different parameters such as temperature, soil moisture content, pH, type of electron acceptor, and bioavailability of pollutants have significant roles in providing proper conditions for microorganisms to degrade the contaminants (EPA, 1993; 2000c; Eweis, 1998; Rulknes et al., 1998). It is possible to degrade almost any organic compound if proper conditions for microbial growth are applied and controlled.

2.6.1.3. Soil Washing

Soil washing also referred to as ex-situ soil separation process is used for treatment of soils contaminated with hydrocarbons and heavy metals. This method involves mixing the soil with a suitable wash solution like water, separating the clean soil particles, and then treating the extracted portion of the soil with other remediation methods such as adsorption, air stripping, bioremediation, chemical treatment, filtration, ion exchange or membrane filtration (EPA, 2004). Soil washing is widely used in Northern Europe and America and when applied with other remediation techniques offers promising results.

If the contaminants have low solubility, using additives can improve their removal efficiency (EPA, 1997a). Additives used in soil washing are organic acids (e.g. citric acid), inorganic acids (e.g. hydrochloric acid, bases (sodium hydroxide, etc.), chelating agents (ethylene diaminetetraacetic acid (EDTA), etc.) solvents (methanol, etc.), oxidizing or reducing agents (hydrogen peroxide, etc.) and biosurfactants (EPA, 1991; 1997b, 2000c, 2004).

2.6.2. In-Situ Processes

Ex-situ methods are becoming increasingly less popular due to the high cost of excavation, transport, and disposal in the landfill as well as the lack of available sites to be used as landfill. In addition, excavation and transport of contaminants might be hazardous to people and the environment. Different remediation methods have been developed which do not require excavation. In-situ methods are less costly and can reduce the hazards of transport and excavation to people and the environment. Some important methods are described below.

2.6.2.1. Air Stripping

This process is usually used for treatment of water-soluble organic compounds (such as methanol, ethanol, phenol, etc.). In air stripping the air is injected into water-saturated soil matrix and causes the volatile organic compounds (VOCs) to transfer from the liquid phase (water) into the gas phase (air).In this in-situ technique as the injected air flows upward through the saturated zone, it forms channels through the contaminants, volatilizes the contaminants in the flow channels and transports them into the vadose zone.

Later the transported contaminants would be removed by soil vapor extraction (SVE) or would be biodegraded (Lagrega et al., 2001). Air stripping is most effective for treatment of groundwater with low concentrations of VOC (<200 mg/L) (Lagrega et al., 2001).

2.6.2.2. Soil Vapor Extraction (SVE)

This method is basically used for removing volatile organic compounds (VOCs) like BTEX (benzene, toluene, ethylbenzene, xylene) when the contaminants are in the vadose zone (the unsaturated zone above the ground water table). It involves installing extraction wells or perforated piping in the soil. An air stream is passed through the soil and the contamination in the soil (or soil/water) matrix is transferred to the air stream. Then gas flows containing pollutants are withdrawn from the site (Lagrega et al., 2001).

2.6.2.3. Solidification/Stabilization

In these processes migration rate of contaminants is reduced by applying additives to the medium. These processes often include the use of cement and plastic binding materials. Solidification is slightly different from stabilization as the additive alters the nature of the contaminant and reduces contaminant toxicity along with reducing its mobility (Lagrega et al., 2001). Chemicals like cement, phosphate, fly ash, lime, and sulfur are used as binders and reagents in solidification and stabilization (EPA 2000b).

2.6.2.4. Electrokinetics

Electrokinetics is mostly used for removing heavy metals from the soil espe-

cially from soil containing high amounts of clay and sand. It involves a low intensity electric current which is generated between a cathode and an anode inserted into the soil (Pamukcu et al., 1990). Ions and charged particles transport towards the electrodes of opposite charge and can be collected and removed using different methods such as electroplating, precipitation, and ion exchange (Virkutyte et al., 2002). In saturated soil with a low ground water flow rate or in excavated soil contaminated with heavy metals such as zinc, copper, lead, arsenic, cadmium, chromium, and nickel, electrokinetics can be used (Virkutyte et al., 2002).

2.6.2.5. Ion Exchange

In this process ions in solution are adsorbed to oppositely charged sites on the surface of soil particles. During this process ions of weaker affinity are exchanged by the soil for stronger ions in the solution. Exchange affinity depends on pH, hydrated radius, electric charge, and molecular configuration (Yong, 2001).

2.6.2.6. Vitrification

Vitrification is an expensive method used in soils with high levels of both organic and metal contamination (EPA, 1997b; Martin and Ruby, 2004). In this method electric current at very high temperature (1600°C to 2000°C) is applied to the soil, melts the soil, and converts it into a glass-like solid (EPA, 2004). The organic material is either evaporated or pyrolyzed by the generated heat; and the metals are immobilized within and converted to inert, impermeable, chemically stable, and leach-resistant material (EPA, 1997a).

2.6.2.7. Phytoremediation

In this method contaminants are either stabilized or removed by direct use of plants such as Thlaspi, alfalfa, Indian mustard, and Urticao (EPA, 1998, 1999b; Brown et al., 1994a, b). The plant accumulates or detoxifies the organic and inorganic chemicals. Stabilization is applied through different mechanisms like sorption, precipitation, and contaminant complexation to reduce their mobility and bioavailability while removal involves plant uptake and accumulation of the contaminants in its tissue (Brown et al., 1994a, b; Mulligan, 1998; EPA, 1999b; Pierzynski et al., 2000). The effectiveness of phytoremediation depends on the concentration and type of contaminants, the strength of additives (acid, base, etc.), and plant conditions (EPA, 1998; 1999b). The contaminated plants used in phytoremediation should be collected and disposed of by special techniques such as incineration, drying, acid extraction, or anaerobic digestion (EPA, 1998, 2000c; Bolenz et al., 1990).

2.6.2.8. Soil Flushing

Soil flushing is an in-situ method for removing the contaminants by enhancing contaminant mobilization. Soil flushing is used for removing hydrocarbons, chlorinated hydrocarbons, metals, salts and pesticides (Roote, 1998). In this method, the agent solution is injected into the contaminated zone of soil through an injection well and then the ground water mixed with elutriate is extracted via an extraction well. Elutriate a mix of solution and contaminant, is

discharged and treated above the ground (Roote, 1998). Subsurface transport mechanisms such as advection and molecular diffusion are accelerated through soil flushing. Geochemical dissolution reactions (such as adsorption, desorption) and bioremediation are accelerated as well (Roote, 1998). Flushing agents used in soil flushing are varied; water, dilute acids, and bases, solvents or surfactants are used as flushing agents (Mann et al., 1993). There is no soil displacement in soil flushing thus it has minimal disruption of the ecosystem and consequently reduces the price of the removal process; the workers are also less exposed to contaminants during this process (Mann et al., 1993). Different parameters such as the type of the contaminant, soil structure, and flushing solution affect the efficiency of soil flushing. In general, soil flushing is most effective in permeable, homogeneous soils (e.g. sands, gravels and silty sand with permeability more than 10^{-4} cm/sec). Limitations of this in-situ method are described below (EPA, 2004; Mann et al., 1993).

- When the soil has low hydraulic conductivity or pipes and underground utilities this method is not efficient.

- If the contaminants are insoluble and are tightly bound to soil particles there soil or flushing is not helpful.

- High amounts of organic matter in the soil lead to decreased removal efficiency by causing chemical adsorption of the surfactants on to the soil.

- Hard water reduces the effectiveness of surfactants in soil flushing (EPA, 2004; Mann et al., 1993).

Moreover increasing the contaminant mobility increases the chance of spreading the contaminants in the area (Kommalapati et al., 1998).

2.7. Surfactants

Surfactants or surface-active agents are substances which reduce the surface tension of a liquid and/or interfacial tension between two liquids. Surface tension is described as the net inward attraction force per unit length of the surface (Christofi and Ivshina, 2002).

Surface-active molecules have an amphiphilic nature since they are composed of two portions having opposing character. One is the hydrophilic portion which is polar and the other is the hydrophobic portion that forms the non polar fraction of the molecule. The hydrophilic portion, indicated as the head-group, is water attracting and has a high affinity for the bulk medium; however the hydrophobic portion, designated as the tail-group, has less attraction to the bulk polar medium and is water repelling (Edwards et al., 1992; Mulligan, 2005). Having this amphiphilic nature surfactant tends to gather at interfaces (liquid/vapor, liquid/liquid and liquid/solid) (Zajic and Seffens, 1984), therefore by accumulating at the surface surfactant can replace bulk solution molecules and reduce the surface and interfacial tension. Surface tension reduction facilitates the formation of an emulsion between immiscible liquids, lowering the capillary forces, reducing the contact angle, and increasing the mobility of the contaminant (Mulligan et al., 2001b; Jennings, 2006). In surfactant treatment of contaminated soil in the case of low concentrations of the contaminant, surfactants concentrate at interfaces and replace the bulk molecules

thus reducing the surface and interfacial tension and as mentioned above emulsion formation starts and contaminant mobility increases (Lake, 1989; Mulligan, 1998; Mulligan et al., 2001b).

The reduction in the surface tension has a direct relationship with the concentration of the surfactant but only up to a certain level of concentration which is called the critical micelle concentration (CMC). Increasing the amount of surfactant above the CMC do not reduce the surface tension. However, above the CMC surfactant monomers start to form aggregates known as micelles (Mitchell and Ninhman, 1981; Hiemenz, 1997; Mulligan, 2005). Above the CMC, micelles are formed and not many free monomers are left. The CMC is also referred to as the maximum concentration of surfactant monomers (Mulligan and Gibbs, 2004). The critical micelle concentration is highly influenced by pH, temperature, ionic strength and salinity of the medium (Sabatini et al., 1995; Lin, 1996; Mulligan, 2005).

Micelles can have different structures such as spheres, bilayers, and vesicles. The type and size of the micelles depend on pH, temperature, structure of surfactant monomer, and ionic strength of the solution (Bai et al., 1998). Once micelles are formed, hydrophobic compounds are partitioned into the hydrophobic core of the micelles and consequently are dispersed in the aqueous phase exceeding their solubility limit (Falatko and Novak, 1992; Pennell et al., 1993). By dispersing into the solution, the contaminant's bioavailability increases and biodegradation of organic contaminants is facilitated (Rouse et al., 1994; Miller, 1996; Deshpand et al., 1999). Furthermore, microorganisms

existing in the soil matrix can more easily degrade the organic contaminants.

The ability of surfactants to reduce surface tension is indicated as effectiveness, and the surfactants' potential to obtain low critical micelle concentration is signified as efficiency. These two parameters are used to evaluate surfactants (Mulligan, 2005). Based on this definition a surfactant is considered efficient if it has a low CMC, which means less surfactant is needed to reduce the surface tension of the solution (Mulligan, 2005). In general, desirable characteristics of a surfactant include biodegradability, solubility at ground water temperatures, low adsorption to soil, not high soil dispersion, low surface tension, and low CMC (Kimball, 1992).

2.7.1 Surfactant Types

Classification of surfactants is based on their dissociation in water (Salager, 2002) and hence on the charge type of the surfactants (Bai et al., 1997).

-Anionic Surfactants

Anionic surfactants are negatively-charged and are the most commonly used surfactants. They include alkylbenzene sulfonates (detergents), (fatty acid) soaps, lauryl sulfate (foaming agent), di-alkyl sulfosuccinate (wetting agent), lignosulfonates (dispersants). Anionic surfactants account for approximately 50% of the world's surfactant production (Salager, 2002; Rust and Wildes, 2008). Anionic surfactants exhibit great wetting and emulsifying properties and tend to be higher-foaming materials (Rust and Wildes, 2008).

- Cationic Surfactants

Cationic surfactants are dissociated positively charged chemicals. These surfactants typically have outstanding antibacterial properties, provide good corrosion protection, and can be good demulsifiers (Rust and Wildes, 2008).

- Nonionic Surfactants

Nonionic surfactants do not ionize in aqueous solutions because their hydrophilic head group is nondissociable (Salager, 2002). They are the second largest group of surfactants in industrial production and are desirable due to their low toxicity (Rust and Wildes, 2008).

- Amphoteric Surfactants

These surfactants, depending on the pH of the solution, can behave as a cation or anion. Amphoteric surfactants are "mild" and are progressively used in personal care products (Rust and Wildes, 2008).

2.7.2. Surfactant Uses

The world production of soaps, detergents, and other surfactants was about 18 Mt (million tons) in 1970, 25 Mt in 1990 and increased to 40 Mt in 2000 (Salager, 2002). The largest end use market for surfactants is household cleaning detergents. However, surfactants are also used in industrial & institutional cleaners, personal care, food processing, oilfield chemicals, agricultural chemicals, textiles emulsion polymerization (plastics) as well as paints and coatings (Rust and Wildes, 2008).

To decontaminate the soil or water environment containing hydrophobic pollutants it is required to solubilize the contaminant before it is degraded by

microbial cells. Surfactants increase the surface area of hydrophobic materials and increase their water solubility. Accordingly due to the desired properties of surfactants; such as surface tension reduction, solubility enhancement, and wettability, surfactants are widely used in the soil remediation and petroleum industries.

Biodegradation rates of soil xenobiotics, including alkanes, PAHs, and a variety of other hydrocarbons, can be increased by using surfactants (Aronstein et al., 1991; Burry and Miller, 1993; Bruheim et al., 1999; Margesin and Schinner, 1999). Although surfactants have many advantages they have adverse effects such as toxicity, persistence to degradation, and reduced availability of compounds integrated into micelles which has caused a significant reduction in their usage in soil treatments (Tiehm, 1994; Mulligan et al., 2001b; Cort et al., 2002).

2.8. Biosurfactant

By definition, the surfactants produced by microorganisms, plants, and animals are called biosurfactants (Mulligan et al., 2001b). In other words, biosurfactants are a heterogeneous group of surfactants produced by organisms from various substrates. The substrates biosurfactants are produced from include sugars, alkanes, oils, and wastes (Mulligan et al., 2001b; Rahman and Gakpe, 2008).

Biosurfactants are used as an alternative to synthetic surfactants in a variety of applications because of their many advantages (Hudak and Cassidy,

2004). There are two main parameters for evaluating the biosurfactants effectiveness and efficiency. Their capability to reduce the surface tension of water is called effectiveness. The more the water surface tension is reduced the more effective the biosurfactants is. Critical micelle concentration (CMC) represents the biosurfactant's efficiency. Lower CMCs show more efficiency (Mulligan, 2005).

There is a large range of CMC from 1 to 200 mg/L for different biosurfactants. Various biosurfactants have different surface tension reduction abilities. They are capable of decreasing the surface tension of water from 72 to 27 mN/m (Rosen, 1978; Javaheri et al., 1985; Lang and Wagner, 1987; Persson et al., 1988).

2.8.1. Merits of Biosurfactants

Biosurfactants are biodegradable. Being easily degraded by bacteria and other microorganisms, biosurfactants do not pose much harm to the environment. Biosurfactants can be produced in-situ which saves money and time in the projects. Moreover, the raw material for biosurfactant production is quite cheap and available in large quantities (Kosaric, 1992; Mulligan and Wang, 2006). Biosurfactants have the potential to be used under tough conditions such as extreme temperature, pH, and salinity (Zhang and Miller, 1992). Low toxicity levels are another significant advantage of biosurfactants. The global awareness for the need to protect the ecosystem has led to tightening the environmental regulations and increasing the interest in replacing chemical surfactants with biosurfactants (Banat et al., 2000; Benincasa, 2007).

2.8.2. Biosurfactant Types

Biosurfactants can be classified based on different properties such as their charge type, chemical composition, and molecular weight. The hydrophilic head of the biosurfactant indicates its charge and subdivides the biosurfactants into 4 groups of anionic, cationic, non-ionic, and amphoteric. Common biosurfactants identified so far are either anionic or nonioninc and only a few have cationic properties (Van Ginkel, 1989; Mulligan et al., 2001b).

In classification based on molecular-weight, biosurfactants are divided into low- and high-molecular-weight biosurfactants. Low-molecular-weight biosurfactants are mostly efficient in surface and interfacial tension reduction while high-molecular-weight biosurfactants are generally long chain polymers in best for wetting, coating, and emulsifying immiscible liquids. However, high-molecular-weight biosurfactants do not significantly reduce the surface tension of the solvent (Cooper, 1986; Rosenberg and Ron, 2001).

Based on their molecular structure, glycolipids, lipopeptides, phospholipids, fatty acids, and natural lipids are different groups of biosurfactants (Biermann et al., 1987; Mulligan 2005). The most common type of biosurfactant are glycolipids which are composed of carbohydrate (sugar) head groups and fatty acid tails. Rhamnolipids and sophorolipids are common glycolipids (Desai and Banat, 1997; Mulligan and Gibbs, 2004). Surfactin is one of the best known lipopeptides which basically contains several amino acids as the head group and one or two fatty acids as the tail. Phospholipids include a negatively charged head group and are produced by certain bacteria (such as Acineto-

bacter, Thiobacillus thiooxidans) or yeasts (Cirigliano et al., 1985). Phospholipids form the major components of microbial membranes (Rahman and Gakpe, 2008) and build up a two-layer structure, named the lipid bilayer which is the structural basis of all cell membranes.

Table 2.2 Type and microbial origin of biosurfactants (adapted from Mulligan 2005)

Surfactant Class	Microorganism
Trehalose lipids	*Arthrobacter parafinneus, Corynebacterium spp., Mycobacterium spp., Rhodococcus erythropolis*
Rhamnolipid	*Pseudomonas aeruginosa, Pseudomonas sp.*
Sophorose lipids	*Candida apicola, Candida bombicola, Candida lipolytica, Candida bogoriensis*
Glucose fractose, saccharose lipids	*Arthrobacter sp., Corynebacterium sp., R. erythropolis*
Cellobiose lipids	*Ustilago maydis*
Polyol lipids	*Rhodotorula glutinous, Rhodotorula graminus*
Diglycosyl Diglycerides	*Lactobacillus fermentii*
Lipopolysaccharides	*Acinetobacter sp., calcoaceticus (RAGI), Pseudomonas sp.*
Lipopeptides	*Arthrobacter sp., Bacillus pumilis, Bacillus licheniformis*
Surfactin	*Bacillus subtilis*
Viscosin	*Pseudomonas fluorescens*
Ornithine, lysine peptides	*Thiobacillus thiooxidans, Gluconobacter cerinus*
Phospholipids	*Acinetobacter sp.*
Sulfonylipids	*T. thiooxidans, Corynebacterium alkanolyticum*
Fatty acids (corynomycolic acids, spiculisporic acids etc).	*Capnocytophaga sp., Penicillium spiculisporum, Corynebacterium lepus*

2.8.3. Biosurfactant Removal Mechanism

Biosurfactants have different removal mechanisms depending on the types of contaminants. In the case of an immiscible compound (like non-polar hydrocarbons) as a contaminant, when adding biosurfactant solution below its CMC, the hydrophilic portion of the biosurfactant orients towards the water molecules and interacts with them while the hydrophobic portion of the biosurfactants orients towards the hydrocarbon. Gradually biosurfactants accumulate at the interface and consequently reduce the surface and interfacial tension between the water and the immiscible compound. Surface and interfacial tension reduction results in contaminant mobilization and ultimately makes the contaminant miscible (Bai et al., 1998; Christofi and Ivshina, 2002).

Above the CMC, biosurfactants form micelles which partition into the hydrophobic hydrocarbon. Hence the solubility of the hydrocarbon increases and consequently the bioavailability of the hydrophobic contaminant is enhanced (Bai et al., 1998; Urum and Pekdemir, 2004).

If the biosurfactant is used for metal removal, another mechanism tpredominates. The biosurfactants are present at the solid/liquid interface where metals are bonded to the soil (Christofi and Ivshina, 2002). New bonds start to form between biosurfactant molecules and metals. The metal to soil connection is weaker than the metal to biosurfactant bond so the metals eventually enter the liquid phase and can be removed easily (Miller, 1995; Torrens et al., 1998).

When soil contamination consists of metal and hydrocarbon complexes, the

contamination can be complexed by the micelles and eventually the concentration of metal in the solution is increased (Mulligan, 1998).

2.8.4. Biosurfactants in Soil Remediation

Over the years biosurfactants have demonstrated success in soil remediation applications. In a study conducted by Clifford et al. (2007) rhamnolipid biosurfactant was used to enhance the remediation of tetrachloroethylene (PCE) which is a non-aqueous liquid phase (NAPL) contaminant. It was indicated that the biosurfactant partitioned into the PCE and significantly improved its apparent solubility.

A study by Mulligan and Jalali (2007) showed biosurfactant enhanced bioremediation of a mixed contaminated soil. A soil from Toronto Harbour area, heavily contaminated by heavy metals and hydrocarbons was collected and went through bioremediation tests. 10% TPH and 6% metal content removal were obtained at the end of the experiments.

Also Mulligan et al. (1999) showed heavy metal removal ability for surfactin, rhamnolipids, and sophorolipids. In their experiment with batch washing process, heavy metals, like copper and zinc were removed from the mixed contaminated soil. In research accomplished by Eftekhari (2000) pentachlorophenol (PCP) was removed from soil using rhamnolipids in foam form and showed effective results in soil decontamination. In the review by Banat (1995), biosurfactant ability in soil remediation was indicated. Sophorolipid was used to enhance the remediation of polycyclic aromatic hydrocarbons

(PAHs) in a bilge waste. Satisfactory results were obtained in solubilizing the contaminant and making them more available to the microorganisms.

2.9. Tetrachloroethylene

Tetrachloroethylene, also referred to as tetrachloroethene, perchloroethylene, perchloroethene, perc, and PCE, is a chlorinated hydrocarbon. Tetrachloroethylene has the chemical formula of $Cl_2C=CCl_2$ and is a colourless liquid, volatile, highly stable, non-flammable and has low viscosity. PCE is relatively insoluble in water (Muraoka and Hirata, 1988; ATSDR, 1993). Its sweet odor is recognized by most people if the concentration is over 1 part per million (1ppm) (Agency for Toxic Substances and Disease Registry (ATSDR), 1997; Rossberg et al., 2006).

2.9.1. Tetrachloroethylene Production

The most common method of producing tetracholoroethylene involves using chlorine and 1, 2-dichloroethane. When 1, 2-dichloroethane is heated up to 400 °C with chlorine, chemical reaction results in producing tetrachloroethylene.

$$ClCH_2CH_2Cl + 3\ Cl_2 \rightarrow Cl_2C=CCl_2 + 4\ HCl$$

Byproducts include trichloroethylene, carbon tetrachloride and hydrogen chloride.

Tetrachloroethylene also can be produced by chlorinating light hydrocarbons under high temperature conditions. This production method is related to Fara-

day's discovery in which hexachloroethane was heated until its decomposition into tetrachloroethylene and chlorine (USEPA, 1994; Rossberg et al., 2006; IARC monograph, 2007).

2.9.2. Tetrachloroethylene Uses

Due to its high capability of dissolving organic material, tetrachloroethylene is widely used as a solvent in dry cleaning. Tetrachloroethylene can also be used to degrease metal parts in the automotive and other metalworking industries when mixed with other chlorocarbons (USEPA, 1994; Rossberg et al., 2006; IARC monograph, 2007).

2.9.3. Health and Safety

A high concentration of tetrachloroethylene depresses the central nervous system. Inhaling its vapors (particularly in closed, poorly ventilated areas) might cause dizziness, headache, sleepiness, confusion, nausea, difficulty in speaking and walking, unconsciousness, and even death (Agency for Toxic Substances and Disease Registry (ATSDR), 1997). Also the International Agency for Research on Cancer (IARC) established that there was adequate epidemiological and animal testing data to classify PCE as "probable human carcinogen" (Morrison, 2000).

Severe skin irritation may occur if the skin is repeatedly or extendedly in contact with tetrachloroethylene. Skin irritation happens because tetrachloroethylene dissolves the skin fat and consequently causes skin damage in work environments where people have been exposed to high concentrations of the

chemical for a long period of time. In general, skin contact is not expected as the main route of exposure in work places (The Canadian Centre for Occupational Health and Safety (CCOHS), 1999).

Usually in industry most workers are exposed to levels lower than those causing obvious nervous system effects. The health effects of tetrachloroethylene at levels typically encountered in occupational or environmental exposures have not been well established (IARC monograph, 2007). As shown in one controlled study using volunteers, exposure to 100 ppm for 11 weeks produced decreased coordination. Similar effects were not observed at 20 ppm concentration (The Canadian Centre for Occupational Health and Safety (CCOHS), 1999). The maximum exposure to PCE is limited to 100 ppm for an 8-hour workday over a 40-hour workweek, according to the occupational safety and health administration (OSHA).

Common problems in activities involving tetrachloroethylene are spillage, overfilling, sewer leakage, or the illegal disposal into UIC (Underground Injection Control) wells (e.g. septic systems, drywells) at commercial dry cleaning facilities which cause soil contamination. On the other hand tetrachloroethylene has high mobility and once leaked into the soil, easily moves towards the groundwater and contaminates it. Tetrachloroethylene is very toxic and has density greater than water, so sinks below the ground water and removing it from the site is problematic compared to cleanups of oil spills.

Even small releases of chlorinated hydrocarbons like tetrachloroethylene will produce long-lasting and profound sources of ground water contamination in

Canada (Jackson et al., 1991). Furthermore, depending on the soil characteristics like water content and porosity PCE will contaminate the soil matrix (Anderson et al., 1992a). A soil contaminated with PCE can perform as a long term source for PCE contamination (Lawrence et al., 1990). The soil quality guideline for human health for industrial, commercial and residential use is 0.6 ppm, 0.5 ppm and 0.2 ppm respectively (Canadian Soil Quality Guidelines for the Protection of Environment and Human Health, 1999).

After the Hanshin earthquake in Japan, a vast contamination of ground water and soil was detected. PCE leakage of a dry cleaning shop was the major agent of contamination. This site was decontaminated through nation-wide treatment projects for ground water and soil contamination by VOCs (Toshiaki and Sho, 1999).

In the City of Los Angeles, up to 70 micrograms per liter (µg/L) of tetrachloroethylene (PCE) was detected in dewatering flows associated with a construction project. During construction PCE contamination was detected at six outfall locations. Hydrogeological investigation identified a possible regional source for PCE. Since in the background studies PCE was not identified as a groundwater contaminant, the National Pollutant Discharge Elimination System (NPDES) permit for the Project did not oblige PCE treatment; nor did it specify PCE discharge requirements. Lack of accurate data in the background studies resulted in several million dollars cost for design, construction, and operation of treatment (Craig and Sri Krishnamachari, 1996).

In 1979 the local officials of San Gabriel Valley discovered that drinking water

of this region was contaminated by PCE and TCE at dangerous levels. This contamination took place due to leakage and spillage from the large number of industrial sites located in the region. San Gabriel Valley has over one million residents. The 10 wells are currently contaminated are not usable. Producing 157109661.5 m^3 of water each year, these wells were an important part of the water supply for the San Gabriel Valley. Decontamination costs were estimated to be over $350 million.

The State of Illinois provides another example of PCE contamination. In February 2008 several Illinois towns including Lisle/Woodridge, Naperville, and Downers Grove faced water contamination from PCE and TCE. These two chemicals were used as industrial solvents in degreasing metal parts. To identify the exact source of contamination a six month study and long-lasting decisions would be needed.

Extracting PCE from soil is restricted because of its very low water solubility. Since PCE is a dense non-aqueous phase liquid, it cannot be solubilized in water without additives. Additives used to enhance contaminant solubilization must be of low toxicity and biodegradable (Mulligan et al., 2001). Common additives used in VOC removal include surfactants, organic and inorganic acids, as well as water soluble solvents like methanol.

It was indicated in the study by Clifford et al. (2007) that in PCE removal, biosurfactants at concentrations higher than the CMC, partition significantly into PCE and enhance its apparent solubility. In this study a rhamnolipid biosurfactant produced by *Pseudomonas aeruginosa* ATCC9027 was applied

to verify its ability in PCE mobilization and solubilization. Surfactant flushing is also called surfactant-enhanced aquifer remediation (SEAR). Field demonstrations of surfactant-enhanced solubilization of DNAPL were conducted by Childs et al. (2006). In that study the feasibility of surfactant-enhanced remediation of PCE was demonstrated through surfactant flushing of the contaminated control cell.

In a study performed by Vipulanandan and Harendra (2008) PCE was extracted from contaminated soil using different solvents such as water, methanol, isopropyl alcohol, and different surfactants; and then Fe/Ni nano-bimetalic particles were used to degrade PCE in batch reactors. The results indicated the Fe/Ni particles were effective in degrading PCE. Nanoparticles degrade the PCE to a non-chlorinated chemical such as methane

In other research silicon oil emulsions in water were added to surfactants and used as additives to solubilize the PCE. The results showed oil-based emulsion can be used to treat PCE and other chlorinated solvents (Kwon et al., 2006).

Fenton destruction is yet another approach in PCE removal in which Fe and hydrogen peroxide (H_2O_2) are added to the PCE and destroy the PCE molecule and decrease its concentration in the soil (Kang et al., 2006).

In the present study the effect of biosurfactants, in the form of prepared commercial products, are observed in soil remediation. Commercial cleaning agents used in this study are mixtures of different biosurfactants and pro-

duced in mass quantities. In the case of promising results, application of biosurfactants would be very cost effective and would present a convenient usage of these products. The study also observed and evaluated the effectiveness of biosurfactants used along with nano particles.

3. Materials and Methods

3.1. Materials

3.1.1. Soil Sample

The soil sample was collected from a company in Montreal. The site had been occupied by a dry-cleaner for 25 years and the soil was highly contaminated with dry-cleaning fluid (tetrachloroethylene) also called PCE.

3.1.2. Biomor A.S.A.P Floor Degreaser™

Biomor ASAP Floor Degreaser™, referred to as ASAP™ in this text is a bacteria- based product used as floor degreaser. This product consists of different surfactants, bio-cleaning agents, and lipase enzymes. It penetrates the microscopic pores of the surface and degrades the organic matter in the soil and eliminates the odour. ASAP™ is to be used on hard surfaces, such as quarry tiles, ceramics, concrete floors as well as on resilient tiles. ASAP™ is an environmentally friendly product and does not contain harmful chemicals (Avmor 2005a). In Table 3.1, some of the chemical and physical properties of this product are shown.

3.1.3. Biomor Industrial Degreaser and Concrete Cleaner™

The Biomor Industrial Degreaser and Concrete Cleaner™, designated as Degreaser™ in this text is a biologically-formulated product containing a proprietary microorganism consortium which eliminates petroleum stains and

other organic material from industrial equipment. It is widely used for residual cleaning in parking lots, runways, drive thrus, railway ballasts, gas stations, equipment, and vehicles, as well as in soil bioremediation. Degreaser™ does not contain any solvents, phosphates or petroleum distillates. This product is environmentally friendly and biodegradable (Avmor 2005 b). Some of the chemical and physical characteristics of this product are presented in Table 3.1.

Table 3.1 Properties of Degreaser™ and ASAP™ (Avmor 2005 a, b)

Properties	Degreaser™*	ASAP™*
Physical state and appearance	Opaque white liquid	Opaque blue liquid
Specific gravity	1.0 - 1.01	1.0 - 1.01
Boiling point	100°C (212°F)	100°C (212°F)
pH	10.4 - 10.7	7.5 - 8.5
Solubility	Easily soluble in cold water, hot water	Easily soluble in cold water, hot water
Vapour pressure	Equivalent to water	Equivalent to water
Microbial Population	7.6 billion microbes per 3.78L, 5 types of strains	200 billion microbes per 3.78L, 5 types of strains

*Both products are part of the Domestic Substance List (DSL) with the Canadian government (Avmor 2005a, b).

3.1.4. Rhamnolipid Biosurfactants (JBR 425™)

Rhamnolipids are glycolipid biosurfactants produced by bacteria. Rhamnolipids are mainly composed of one or two rhamnose sugars and fatty acids; their chemical composition depends on their source. The rhamnose sugars compose the hydrophilic portion of the biosurfactant and the fatty acids make the hydrophobic part. Rhamnolipid JBR 425™ is produced from sterilized and centrifuged fermentation broth of *Pseudomonas aeruginosa* soil-borne bacterium (Jeneil Biosurfactants Co. 2002).

Pure rhamnolipid in dry form is a white powder and in an aqueous solution, it range from clear to milky or tan color. Rhamnolipid JBR 425™ has a soapy odor with a specific gravity of 1.05-1.06, pH 6.5 to 7, and is soluble in water at natural pH (Jeneil Biosurfactants Co.2002). JBR 425 is an aqueous solution of rhamnolipids at 25% concentration with molecular formulas of $C_{32}H_{58}O_{13}$ and $C_{26}H_{48}O_9$. Surface tension of rhamnolipid measured by ring method of du Nuoy, was 29 mN/m and interfacial tension measured by pulling ring from water phase was 0.3 mN/m (Jeneil Biosurfactants Co.2002).

Rhamnolipids can act as a natural surfactant, foaming agent, emulsifier or antibiotic. Rhamnolipids have a very low critical micelle concentration in aqueous solution, and show a strong surface tension reduction capability at low concentrations. They act as excellent emulsifiers for a wide range of organic solvents (Ara, 2007). Rhamnolipids also have exhibited promising results in remediation of pentachlorophenol (PCP) contaminated soil (Eftekhari, 2003). They are also used in cleanup processes in areas with oil spills and

contamination. According to the EPA, rhamnolipids are readily biodegradable and have a low impact on the environment.

3.1.5. Zero-Valent Iron

Among many zero-valent metals like zinc, iron, and nickel; zero-valent iron (Fe^0) is the most popular one in DNAPL remediation because of its low cost, efficiency, and nontoxicity (Chang and Cheng, 2006; Urzedo, 2009). Under proper conditions zero-valent iron can reduce chlorinated organic pollutants like PCE, TCE, and DCE (Matheson and Tratnydk, 1994; Gavaskar et al., 2005). The reduction reactions in which zero-valent iron reduces the chlorinated organic contamination are exhibited below.

$$Fe^0 + RCl + H^+ \rightarrow Fe^{2+} + RH + Cl^- \quad (1)$$

$$2\,Fe^{2+} + RCl + H^+ \rightarrow 3Fe^{3+} + RH + Cl^- \quad (2)$$

$$H_2 + RCl \rightarrow RH + H^+ + Cl^- \quad (3)$$

According to equation (1), zero-valent iron oxidizes to Fe (II) ions and degrades the chlorinated organic compound. Subsequently, Fe (II) ions react with other chlorinated organics and are converted to Fe (III). Zero-valent iron is available in granular and nano-particle form. Finer particles are more reactive than the granules and have a greater capacity to degrade the contaminants. Accordingly, at high concentrations of chlorinated organic contamina-

tion nano-particles are used to treat the contamination (Gavaskar et al., 2005). Besides it is easier to inject fine particles into the soil matrix (Gavaskar et al., 2005). The zero-valent iron used in this study was supplied by Nano Scale Corporation.

3.2. Analytical Methods

3.2.1. Particle Size Dirstribution

A Horiba laser scattering particle size distribution analyzer (LA-950V2, ATS Scientific Inc.) was used to measure the particle size distribution of the soil sample. Size distribution from 0.01 to 3000 μm can be measured easily with this instrument and the results are extremely reliable. The measurement unit communicates with a personal computer. The processing normally requires one minute from feeding the sample to the result display. Large soil particles were crushed and particles bigger than 2 mm were removed. Afterwards the sample was fed to the instrument and the distribution of soil particles was analyzed and established by the computer.

3.2.2. Soil pH

Soil pH was measured using a Fisher Scientific pH meter model AR25, dual channel pH/Ion meter. A soil to water weight ratio of 1:10 was used to make the solution; 20 g of soil was added to 200 ml of distilled water in a 400 ml beaker. The solution was placed on an orbital shaker 60 rpm for 60 minutes. Then the solution was left for one hour to reach equilibrium and then the pH

was measured.

3.2.3. Water Content

An oven-drying procedure (ASTM method D2216, 2009) was used to measure the water content of the soil. In this process, 10 g of soil were placed in a clean and dry porcelain dish and left in an oven for 24 hours. The oven was set at 110°C and then the soil was placed in desiccators to reach room temperature and weighed. The difference in weight before and after drying is divided by the weight before drying and multiplied by 100 to obtain the percent water content.

3.2.4. Organic Matter Content

The ignition method (ASTM method D2974, 2009) was used to determine the organic matter content. In this procedure, 5 g of soil were placed in a crucible and dried in an oven at 110°C for 48 hours and placed in desiccators for 30 minutes to reach room temperature. The dried soil was weighed and then placed in furnace at 550°C for 2 hours, left in the furnace overnight and then weighed. The difference in weight divided by the initial soil weight and multiplied by 100 calculates the organic matter content.

3.2.5. Heavy Metal Content

The X ray fluorescence "XRF" Niton XLp 700 series Environmental Analyzer was used to detect the heavy metals in the soil sample. The XRF analyser makes it possible to test the soil for composition and contamination through a

bulk sample test. This instrument can be used both in-situ and ex-situ. Soil samples used for XRF analysis were dry and well homogenized.

Soil was passed through a # 10 (2 mm) mesh sieve and larger particles were separated out. The sample was ground again so particles became finer and more homogenous. Following the manual instructions double ended container was prepared and filled with soil then covered with thin film support. The machine was calibrated and the sample was placed inside the instrument. Three different samples were prepared and tested by the XRF machine.

3.2.6. Cation Exchange Capacity

The Chapman method (1965) was used to measure cation exchange capacity (CEC). The pH-buffered CEC procedure was as follows:

1) 5 g of soil were weighed in a 50 mL polypropylene centrifuge tube.

2) 20 ml of 1M potassium acetate were added to the tube. The tube was placed on an orbital shaker for 5 minutes. This procedure replaces soil bound cations with potassium.

3) The tube cap was removed and distilled water was used to wash down the soil on the sides or cap of the tube.

4) The tube was recapped and placed in a centrifuge and centrifuged for 15 minutes at 1000 rpm. The supernatant was discarded.

5) Steps 2 to 4 were repeated 3 times to replace all soil cations with potassium ions.

6) 20 mL methyl alcohol were added to the tube and put on the shaker for 5 minutes to bring the soil particles to suspension.

7) The tube was centrifuged for 15 minutes at 1000 rpm and the supernatant was discarded.

8) Steps 6 and 7 were repeated 3 times.

9) 25 mL of 1M ammonium acetate were added to the tube and put on the shaker for 5 minutes to re-suspend the soil particles.

10) The tube cap was removed and distilled water was used to wash down the soil on the sides or cap of the tube.

11) The tube was recapped and placed in a centrifuge and centrifuged for 15 minutes at 1000 rpm. The supernatant was poured into a 100 mL beaker.

12) Steps 9 to 11 were repeated 3 times to ensure all adsorbed potassium ions were replaced by ammonium, and the supernatants were poured in to the same beaker.

13) Potassium ions disrobed into solution were measured using Atomic Adsorption Spectrophotometer (AAS). This measurement determines the number of exchange sites that were occupied by potassium. CEC is measured based on milliequivalents of potassium exchanged per unit weight of soil.

3.2.7. PCE Concentration

Following the USEPA's biological effectiveness methods, in order to measure the concentration of PCE in the soil, a gas chromatograph was used. To cali-

brate the GC, 4 solutions of PCE in hexane with different concentrations were made. Then all the solutions were measured by GC and the information for each solution was obtained to generate the calibration curve. All other measurements were calculated based on this calibration curve.

The next step was soil extraction in which 4 g of soil were placed in a 50 mL tube to which 40 mL hexane (organic solvent) were added. The tube was placed on the orbital shaker for 2 hours. Hexane was collected and the PCE concentration was measured with the GC.

Samples were collected from different parts of the main soil and the same instructions were followed. The PCE concentration in the soil was calculated by taking the average of 10 samples.

3.2.8. Experimental Design and Sampling Procedure

The experiment was conducted based on the modified USEPA method of bioremediation agent effectiveness test (USEPA 1996). The tests were performed to measure the effectiveness of two biological products (ASAP™, Degreaser™) and rhamnolipid biosurfactant in removing PCE from contaminated soil. Different 21 day experiments were performed to quantify the removal efficiency of these agents. Experiments involved various individual or combined ratios of additives (rhamnolipid, ASAP™, and Degreaser™). Biosurfactant and biological agents were tested under the hypothesis that addition of these agents would stimulate soil biodegradation.

The setup for the batch experiment was composed of a series of 250 mL

Erlenmeyer flasks used as reactors. Each batch was prepared by adding different soil to solution weight ratios and the solution consisted of different sets of additives, described below.

The flasks were covered with foam stoppers to facilitate aeration while preventing entry of dust and micro-organisms. To maintain the aerobic conditions flasks were located on the New Brunswick scientific INNOVA orbital shaker Model 2000, tightly secured in the flask holders, and then swirled at 150 rpm through the whole experimental period. Any flasks with a considerable amount of soil splashed on their walls were rejected and prepared anew. The shaker was placed inside an incubator (Fisher Scientific Isotemp Model 304) to minimize the temperature variations and the effect of photodegradation. The incubator was set at 22 ± 0.5°C.

Each treatment was sampled at four separate intervals of 3, 7, 14, and 21 days. Each time flasks were completely discharged and followed by PCE extraction. Before extraction 20 mL of the aqueous phase were collected for microbiological analysis.

The experiment involved 2 basic phases:

Phase 1: The effect of individual biosurfactant and biological agents on soil biodegradation

Considering the recommendations of previous works, a soil to solution mass ratio of 1:20 was prepared for each flask (Okoro, 2006; Jalali, 2007). Four different sets of ASAP™ to soil ratios by mass of 1:8, 1:4, 1:2, and 1:1 were

prepared and added to the soil; as well as Degreaser™ to soil ratios of 1:8, 1:4, 1:2, and 1:1 (by mass) were prepared and added to the soil. Table 3.2 exhibits the additive to soil ratios used in phase1.

Table 3.2 Additive ratios in biodegradation experiments

Additive: Soil Ratio (by mass)	Phase 1		
	ASAP™	Degreaser™	Rhamnolipid
	(1:8)	(1:8)	(1:1)
	(1:4)	(1:4)	(2:1)
	(1:2)	(1:2)	Control
	(1:1)	(1:1)	

Phase 2: The effect of the combination of biosurfactant and biological agents on soil biodegradation

After evaluating the results of the phase1, the effect of adding rhamnolipid mixed with the biological cleaning products was assessed. Different additive to soil mass ratios were applied the same way solutions were prepared in phase 1.

A soil to solution mass ratio of 1:20 was prepared and a ASAP™ to soil mass

ratio of 1:1 mixed with rhamnolipid to soil mass ratio of 1:1 was prepared and added to the soil, as well as a ASAP™ to soil mass ratio of 2:1 mixed with rhamnolipid to soil mass ratio of 2:1 was prepared and added to the soil and so on for Degreaser™. The additive ratios in phase 2 are summarized in Table 3.3.

Table 3.3 Additive ratios in biodegradation experiments

Additive: Soil Ratio (by mass)	Phase 2	
	ASAP (1:1) & Rhamnolipid* (1:1)	Degreaser (1:1) & Rhamnolipid (1:1)
	ASAP (2:1) & Rhamnolipid (2:1)	Degreaser (2:1) & Rhamnolipid (2:1)
	ASAP (1:1) & Degreaser (1:1), Rhamnolipid (1:1) & zero-valent iron (1:4)	ASAP (1:1) & Degreaser (1:1) & Rhamnolipid (1:1)
	ASAP (1:1) & Degreaser (1:1), Rhamnolipid (1:1) & zero-valent iron (1:2)	ASAP (1:1) & Degreaser (1:1), Rhamnolipid (1:1) & zero-valent iron (1:1)

*The rhamnolipid concentration used for all treatments was 2%.

3.2.9. Extraction Method

Samples were prepared according to EPA method 8260B for volatile organic compound analysis by gas chromatography, and PCE concentration was measured for each flask.

The Micro scale Solvent Extraction (MSE) EPA method 3570 was used. Sol-

vent extraction and direct injection were performed as below.

For each sample the soil and solution inside the flask were transferred to a 50 mL centrifuge tube, placed in the centrifuge for 10 minutes at 3000 rpm. The supernatant was removed. Four grams of soil were transferred to another centrifuge tube and 40 mL hexane (organic solvent) were added to the sample. The tube was placed on the orbital shaker for 2 hours. Then the hexane was injected into a GC vial using a corning syringe, and a 0.42 µm filter. The extracted PCE concentration was measured by gas chromatography analysis.

3.2.10. Gas Chromatography Analysis

Gas chromatography also called gas-liquid chromatography is a separation technique for organic chemicals which can be volatilized without decomposing or chemically rearranging (EPA 1996).

In the chromatographic process, a mobile phase is passed over a stationary phase and through this, separation is achieved. Because constituents in the mixture have different retention times, they partition differently between the mobile and stationary phase and become separated in this process. Hexane extracts of the samples, blanks, and standards were quantified for tetrachloroethylene (PCE) by EPA standard method 8260B using GC/FID. The gas chromatograph was a Varian 3800 GC with a Varian CP-8400 Auto sampler. The capillary column installed in the system was a 30 meter long column coated with 100% dimethylpolysiloxane. This high resolution J&W Scientific capillary column had an inner diameter of 0.25 mm and 0.25 µm film thickness

capable of withstanding temperatures between -60 and 325°C.

After conducting some preliminary trials based on previous works by Vasefy (2007) and Jalali (2007), the GC conditions were set as follows. The carrier gas was helium with a flow rate of 1 mL/min and a makeup gas flow rate of 28 mL/min, a hydrogen rate of 30 mL/min, and an air flow rate of 300 mL/min. The injector and detector temperatures were kept constant at 250°C. For each experiment run, the initial column oven was set and kept for 1 minute at 40°C and a total run time of 10.46 minutes was obtained by increasing the temperature to 250°C at a rate of 13°C/min, and finally maintained for 6 minutes.

For calibrating the GC, four different concentrations of PCE were prepared and analyzed. A four point calibration curve was obtained and other measurements were calculated based on this calibration curve. The Varian Star Chromatography Workstation Version 5.5 software was used to quantify the GC analysis.

3.2.11. Microbial Analysis

The microbial population was analyzed and measured to evaluate if the PCE removal was accompanied by an increase in the population of the microorganisms. The microbial count kit (IME. Test™ Kool Kount-p, Industrial Municipal Equipment, Inc.) included a snapping cup, test ampoules filled with TTC powder, and microbial count table manual. First the snapping cup was washed carefully. After that, a biochemical IME test ampoule was used to count the concentration of the microorganisms in each flask. The ampoule

contained white powder. The snapping cup was filled with the supernatant from the flask and left to stand for 10 minutes to stabilize. Then the Microbe Hunter ampoule was placed in the cup and snapped inside the water sample. The ampoule was filled by the sample water and the powder was wetted and dissolved. Afterwards the ampoule was placed in the oven at 35°C/95°F and the start time was recorded. The ampoule was examined periodically and when the color changed from grayish-yellow to pink or red, the elapsed time was calculated. The microbial concentration was indicated according to the microbial count table manual provided in the test kit.

4. Results and Discussion

4.1. Soil Particle Size Distribution

The particle size distribution of the soil sample was evaluated using the laser particle size analyzer. According to ASTM method D422 the soil was determined to consist of 25% gravel, 50% sand, 20% silt and 5% clay.

4.2. Soil pH

Soil pH is an important factor in bioremediation since it affects the microbial population existing in the soil. Using the Fisher scientific pH meter, the average pH obtained for this soil sample was 8.5 ± 0.3, i.e. slightly alkaline. Thus, the sample had proper conditions for microorganism and specifically bacterial growth. Bioremediation could potentially then prove to be a suitable remediation technique for this soil sample.

4.3. Water Content and Organic Matter Content

The water content and organic matter content of the soil sample were determined according to the following equations.

$$\text{Water content} = \frac{\text{Weight of soil before drying} - \text{Weight of soil after drying}}{\text{Weight of soil before drying}}$$

$$\text{Organic content} = \frac{\text{Weight of soil before ignition} - \text{Weight of soil after ignition}}{\text{Weight of soil before ignition}}$$

The results are shown in the Table 4.1.

Table 4.1 Water and organic matter content of soil

Parameter	Content (%)
Water content	6.1 ± 0.2
Organic matter content	2.5 ± 0.07

4.4. Heavy Metal Content

Using the XRF, concentrations of all the metals present in the soil were detected. Only concentrations of the certain metals (heavy metals) are presented in Table 4.2 and were compared to the Quebec standards (Quebec Regulations on the Protection and Rehabilitation of Land, 2009).

Table 4.2 Heavy metal contents and threshold according to Quebec regulations

Heavy metal	Concentration in soil (mg/kg)	Regulations	
		Level A (mg/kg)	Level B (mg/kg)
Zn	456.5	500	1500
Ni	< 20	100	500
Cr	< 20	250	800
Pb	33.1	500	1000
Cu	97.5	100	500

Level A indicates the allowable concentration of chemicals for residential areas and level B demonstrates the acceptable concentration for commercial and industrial sites. According to the "Quebec Regulation on the Protection and Rehabilitation of Land, 2009", all the heavy metals detected in the soil sample had concentrations lower than the standard criteria for both residential and industrial sites. Accordingly, the heavy metals were not considered as contaminants of concern for this soil sample.

4.5. Cation Exchange Capacity

Based on milliequivalents of potassium exchanged per unit weight of soil, the cation exchange capacity (CEC) of the soil sample was calculated as below:

CEC = [K (ppm) ÷ 39]

Where K is the concentration of potassium in the solution prepared in section 3.2.6.

The CEC of the soil was calculated to be 7.42 ± 0.10 cmoles/kg, which indicates the soil has a low capacity to exchange cations. Soils with high CEC are capable of exchanging cations and are proper for metal removal. In this study heavy metals were not of concern and thus CEC was not a factor in choosing the removal process.

4.6. PCE Concentration

The initial level of PCE in the soil sample was measured according to the USEPA methods. Using the gas chromatograph (GC), the initial concentration of PCE was indicated to be 460 mg/kg. According to the "Canadian Soil Quality Guidelines for The Protection of Environment and Human Health" the allowable concentration of PCE for agricultural use, residential/parkland, commercial, and industrial use is 0.1, 0.2, 0.5 and 0.6 mg/kg respectively.

4.7. Biodegradation Results

Diverse sets of experiments were performed to measure the effectiveness of two biological products (ASAP™, Degreaser™) and rhamnolipid biosurfactant in removing PCE from the contaminated soil sample. Different individual or combined ratios of additives (rhamnolipid, ASAP™ and Degreaser™) were added to the soil to degrade the contaminants and each agent's efficiency was evaluated via these experiments. The experimental trial was run in two different phases using either individual or mixed cleaning agents. For each treatment analysis of variance (ANOVA) was performed to indicate the significance of the results.

4.7.1. Phase One

The individual effect of each biological agent on soil biodegradation was investigated and analyzed in this part. Phase one included testing rhamnolipid at mass ratios to soil of (1:1) and (2:1), ASAP™ and Degreaser™ each at mass ratios to soils of (1:8), (1:4), (1:2) and (1:1). Table 4.3 shows the results of this phase of the experiment.

4.7.1.1. Effect of ASAP™ on Soil Biodegradation

A. PCE Reduction

The effect of ASAP™ on soil biodegradation was evaluated at 0 (30 minutes), 3, 7, 14 and 21 days through batch tests. The concentration of PCE was measured up to 28 days but after 21 days of experiment, PCE concentration was stable and no more degradation was observed. Accordingly, in the dia-

grams data is not shown after 21 days. Figures 4.1 and 4.2 display the PCE removal over the 21 day period. ASAP™ was used in its original concentration without any dilution or adjustment hence the prepared ratios were precise. Figure 4.1 shows the PCE reduction analysis for additive to soil ratios of 1:4 and 1:8. ASAP™ in the ratios of 1:4 and 1:8 previously exhibited effective results (Vasefy, 2007) for hydrocarbon removal from oil. Each setup consisted of both treatment and control flasks. The control batch contained no additives and when compared to the treatment flasks presented less PCE reduction. The PCE loss in the control batch can be a result of natural volatilization, and also degradation performed by the microorganisms that were naturally present in the soil. The removal efficiency at the ratios of 1:4 and 1:8 were very close (29% and 27%, respectively) and not significant when compared to 19% removal efficiency of the control flask. Considering the low removal efficiency at these ratios, higher concentrations of ASAP™ were selected to achieve better results. Figure 4.2 presents the PCE removal analysis for additive to soil ratios of 1:1 and 1:2 as well as the control batch. ASAP™ was effective at ratios of 1:1 and 1:2 with removal efficiencies of 45% and 31%, respectively.

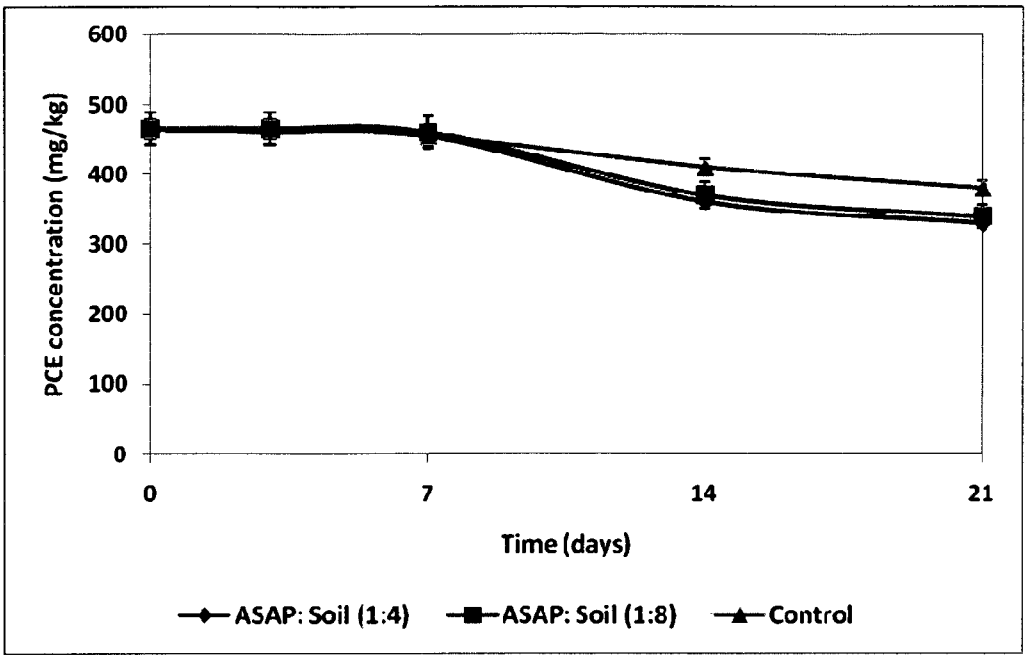

Figure 4.1 Performance of ASAP™ with additive: soil ratios of 1:8, 1:4 and control over time.

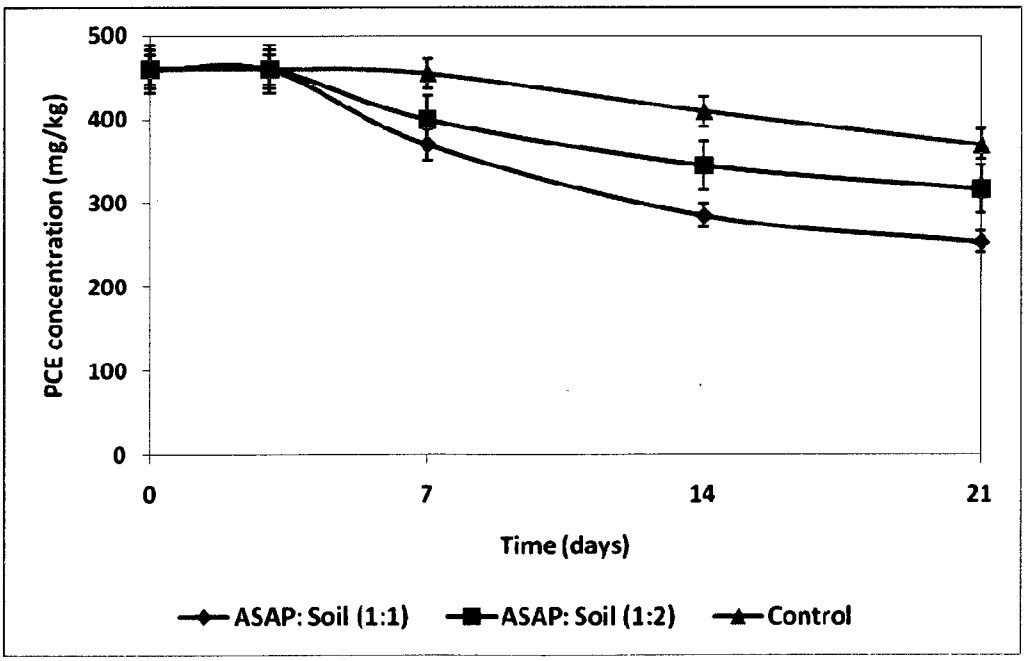

Figure 4.2 Performance of ASAP™ with additive: soil ratios of 1:2, 1:1 and control over time.

B. Microbial Growth

In order to analyze the hydrocarbon removal mechanism some microbiological growth measurements were performed as well. Biosurfactant application to the soil enhances mobilization of the contaminant, consequently improving the contaminant's bioavailability. Hence the microorganisms would be able to use the hydrocarbon contaminant as a substrate and degrade the contaminant. Microbial tests were conducted on the solutions removed from each flask to observe if the PCE removal process was accompanied by any increase in the microbial population. Special microbial kits which detect all types of microorganisms were used to measure the microorganism population. Microbiological analysis of the treatment and control batches are displayed in the semi logarithmic plots in Figures 4.3 and 4.4. Growth curves increased over time and reached their peak at the end of the experimental period.

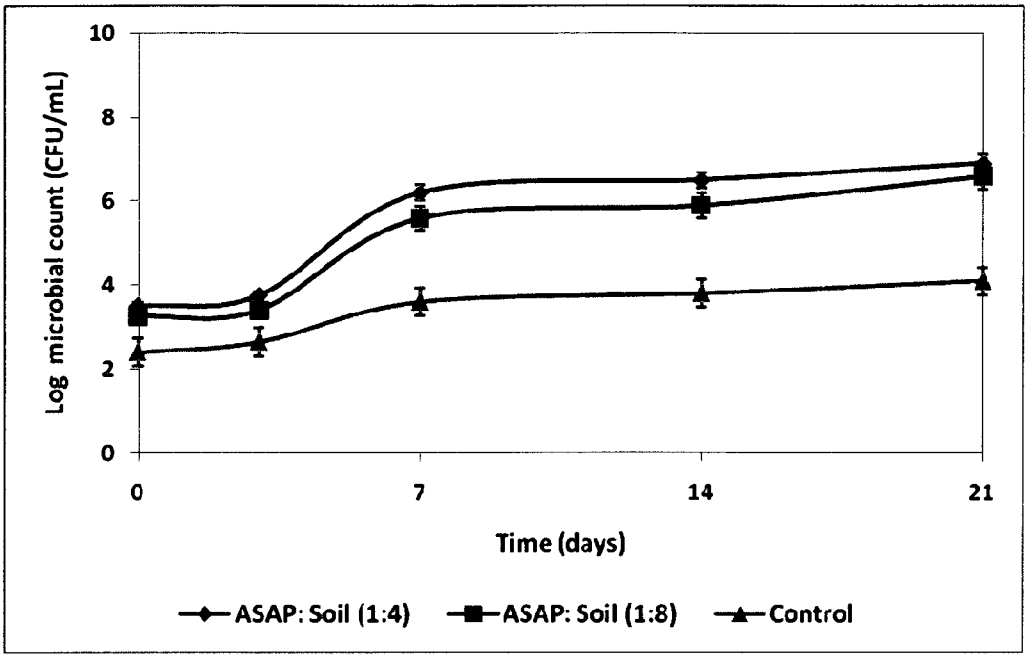

Figure 4.3 Microbial densities for ASAP™ to soil ratios of 1:4, 1:8 and control.

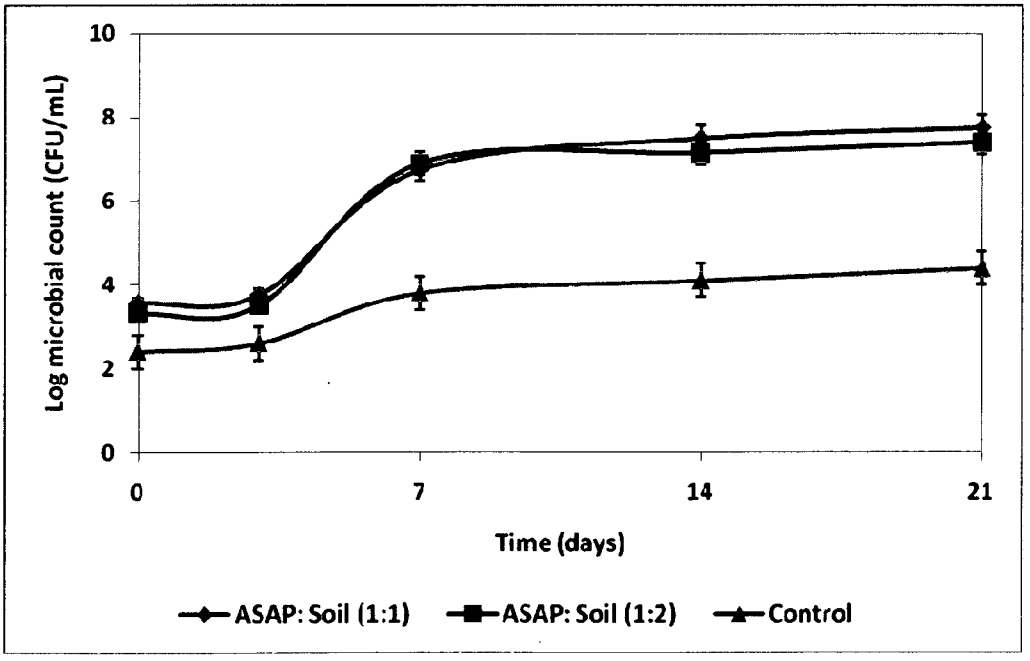

Figure 4.4 Microbial densities for ASAP™ to soil ratios of 1:1, 1:2 and control.

A direct correlation between PCE removal and increasing microbial density was observed in all batches. As seen in the figures, even control batches had microbial growth; however no biosurfactants or any bacteria were added to the flasks. Microorganisms are ever-present in the environment, hence the microbial population found in control flasks are likely to originate from the soil. Since no bacteria were added to the control batches, the microbial populations in these flasks were lower than those in the treatment vessels.

The highest rates of microbial density increase took place within days three and seven of the experiments, for both control and treatment batches. The dramatic increase in the biological population matched with the decrease in hydrocarbon contaminant concentration, supported the idea that degradation had occurred. The typical microbial population curve consists of four major phases: lag, exponential, stationary, and death phase. The exponential phase is the phase in which the microbial population grows at the maximum possible rate. As seen in Figures 4.3 and 4.4, throughout this phase sufficient amounts of the substrate were available for the microorganisms leading to an exponential increase of the microbial population. After 7 days the substrate started to decrease due to the microbial consumption. On the other hand as the microbial population increased a reduction in the growth rate occurred to the point where a steady state microbial density was reached. Once microbial population depletes the substrate, a negative growth rate would be observed.

Different ASAP™ to soil mass ratios as well as control batches exhibited similar patterns. Considering that ASAP™ contains various kinds of biosurfac-

tants, the microbial population of each treatment batch was significantly higher than the ones of the control batches. Accordingly, the ASAP™ to soil mass ratio of 1:1 demonstrated the highest microbial population as compared to mass ratios of 1:2, 1:4 and 1:8. Furthermore the most contaminant removal took place at an ASAP™ to soil mass ratio of 1:1. The fact that a higher concentration of biosurfactant which implies higher microbial density, removed greater amounts of the contaminant, can be an excellent explanation for the biodegradation.

Since PCE is a highly volatile compound, it is necessary to note that there is always contaminant loss due to natural processes like evaporation. The natural PCE loss was observed in the control tests where no additive was added to the soil, and at the end of the experiment the concentration of PCE was less than its initial concentration by 19%.

4.7.1.2. Effect of Degreaser™ on PCE Degradation

A. PCE Reduction

At similar intervals (0, 3, 7, 14 and 21 days) the samples were collected and analyzed to measure the effect of Degreaser™ on soil biodegradation. Figures 4.5 and 4.6 show the PCE reduction throughout the 21 day period. Since no change in PCE concentration was observed after 21 days in the diagrams data is shown for 21 days. Here as well Degreaser™ was used in its original concentration without any dilution or adjustment; thus the prepared ratios were precise. Degreaser™ at the mass ratios of 1:4 and 1:8 have previously displayed effective results for hydrocarbon removal from oil (Vasefy, 2007).

Figure 4.5 shows the PCE reduction analysis for additive to soil mass ratios of 1:4 and 1:8. At the end of the experimental period the concentration of PCE was reduced by up to 34% at a 1:4 Degreaser™: soil ratio, and by up to 29% at a 1:8 ratio. The PCE loss due to natural processes such as evaporation and degradation obtained from the control batch was 18%. Considering the small difference in PCE removal between the control batch and Degreaser™ ratios of 1:8 and 1:4, these ratios were not satisfactory. Accordingly higher concentrations of Degreaser™ were selected to obtain more effective results. Figure 4.6 presents the analysis for additive to soil mass ratios of 1:1 and 1:2, and the control flask.

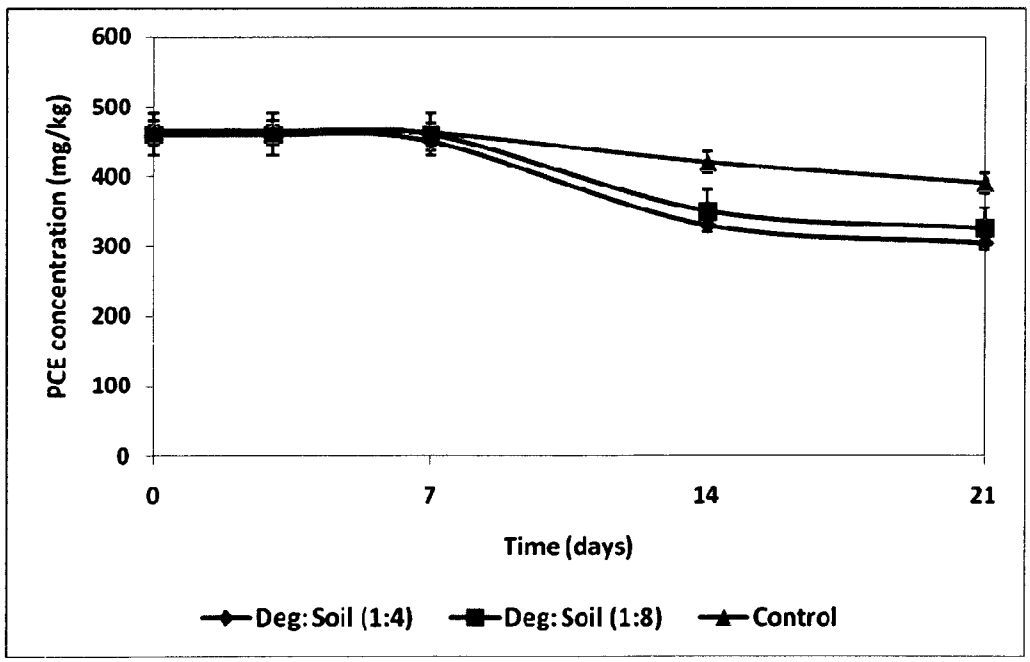

Figure 4.5 Performance of Degreaser™ with additive: soil ratios of 1:8, 1:4 and control over time.

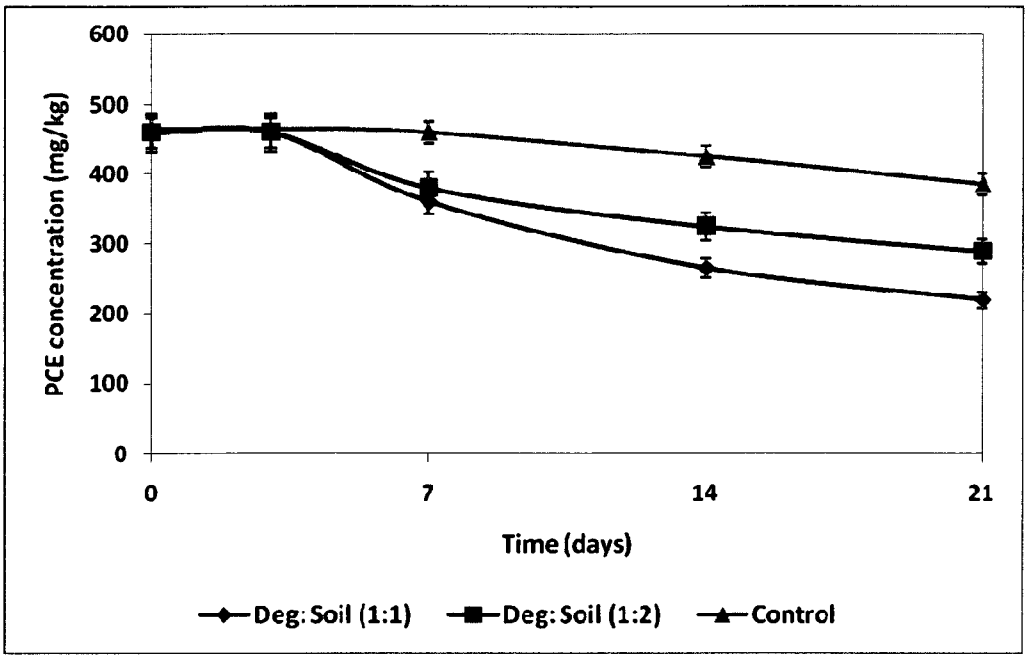

Figure 4.6 Performance of Degreaser™ with additive: soil ratios of 1:2, 1:1 and control over time.

Since a higher concentration of Degreaser™ contains a higher level of biosurfactants, conducting the experiment with higher concentrations of Degreaser™ demonstrated more effective results. The removal efficiency with Degreaser™ at an additive to soil mass ratio of 1:1 and 1:2 was 52% and 37% respectively.

B. Microbial Growth

The semi logarithmic Figures 4.7 and 4.8 show the results of microbial analysis of treatment flasks with Degreaser™ to soil mass ratios of 1:8, 1:4, 1:2, 1:1 and the control flasks over time. As observed for the ASAP™ results, there was also a sudden increase in the microbial population between day 3 and

day 7, known as the exponential phase. The population growth rate was almost negligible by the end of day 21. Higher additive ratios resulted in greater microbial populations. In both cases at the end of the experiment microbial population in treatment batches were 1000 to 10000 times higher than the microbial population in the control flasks. As indicated in Figure 4.7, doubling the concentration of Degreaser™ (additive to soil ratio from 1:8 to 1:4) did not result in any significant microbial population increase.

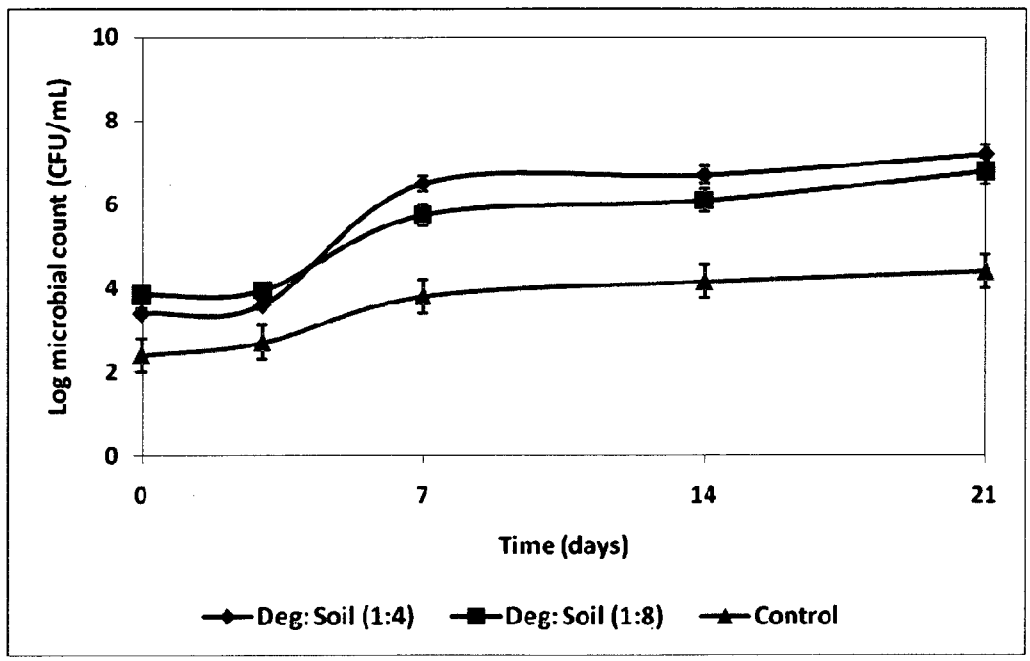

Figure 4.7 Microbial densities for Degreaser™ to soil ratios of 1:4, 1:8 and control.

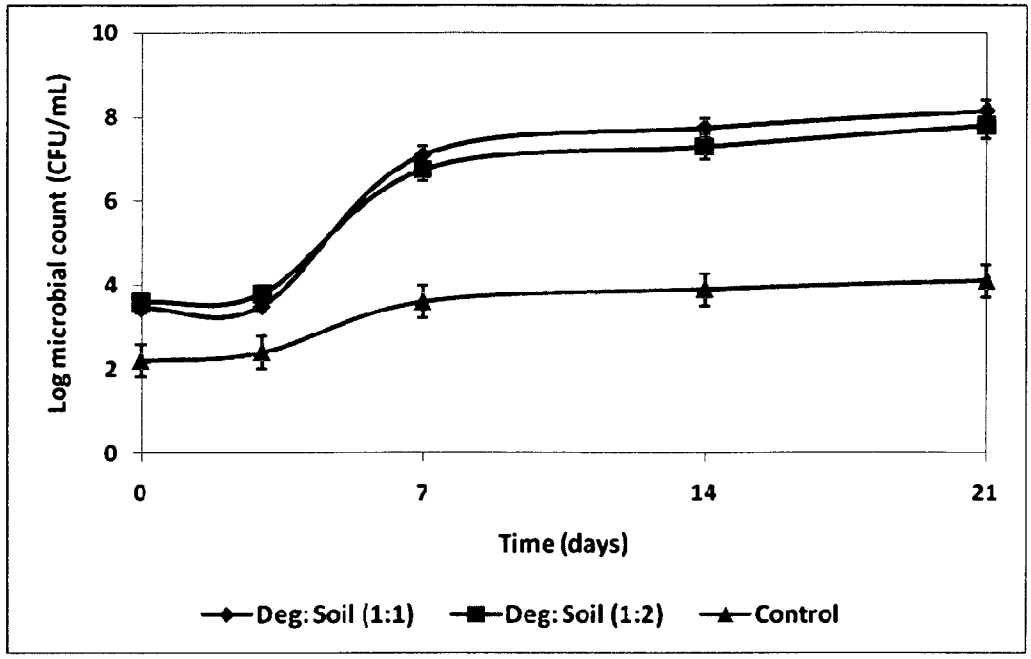

Figure 4.8 Microbial densities for Degreaser™ to soil ratios of 1:4, 1:2 and control.

4.7.1.3. Effect of Rhamnolipid on PCE Degradation

Rhamnolipid biosurfactant as a cleaning agent was applied to the soil through batch tests and was evaluated for PCE removal efficiency. Here as well rhamnolipid was applied to soil with the same procedure as the biological agents. At a low concentration of rhamnolipids, the results for contaminant removal were close to the control batches that contained no additives. Therefore solutions with higher concentrations of rhamnolipid were prepared and added to the soil. Maximum PCE removal (35% and 30%, respectively) took place at a mass ratio additive to soil of 2:1 and 1:1 which still was not satisfactory.

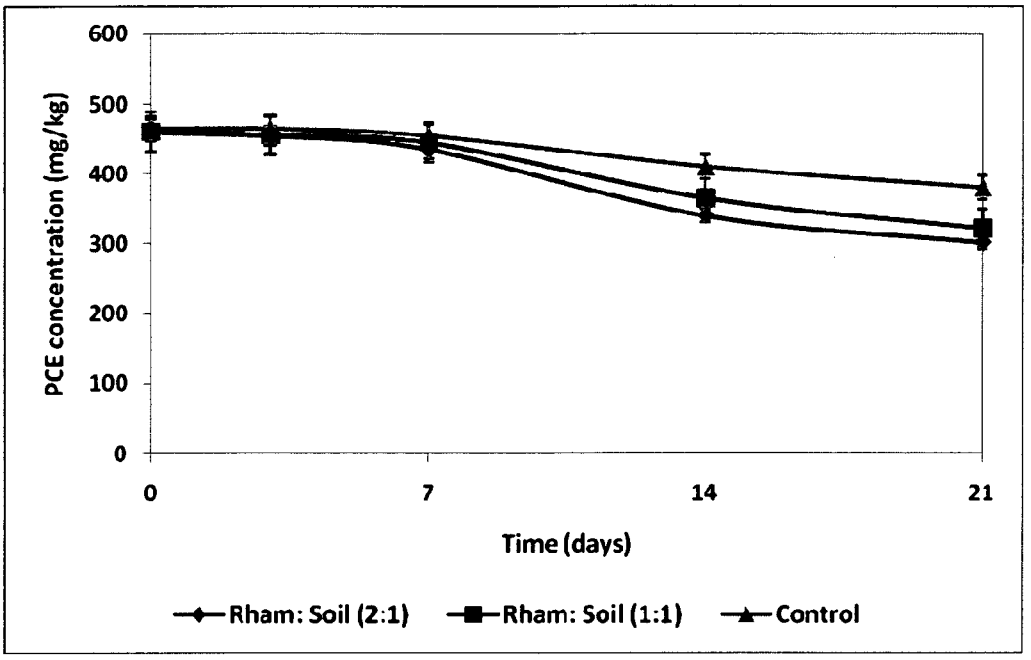

Figure 4.9 Performance of rhamnolipid with additive: soil ratios of 2:1, 1:1 and control over time.

Observing the contaminant reduction over time, it was concluded that rhamnolipid at these concentrations could not degrade the contamination. Probably due to the soil characteristics such as soil pH, rhamnolipid at these concentrations was adsorbed to the soil particles and was not able to reach the contaminants. However applying higher concentrations of rhamnolipid was not cost effective. As described later in this study, in order to evaluate the effectiveness of rhamnolipid, it was added to the biological agents and applied to the soil in the second stage of the experiment.

4.7.1.4. Comparison in Effectiveness of ASAP™ Versus Degreaser™ for PCE Degradation

A. PCE Reduction Efficiency

In general, Degreaser™ and ASAP™ performed similarly for PCE reduction. However, Degreaser™ was slightly more effective than ASAP™ as shown in Figures 4.10 to 4.13. The highest removal efficiency obtained from ASAP™ was 45 % and for Degreaser™ was 52%.

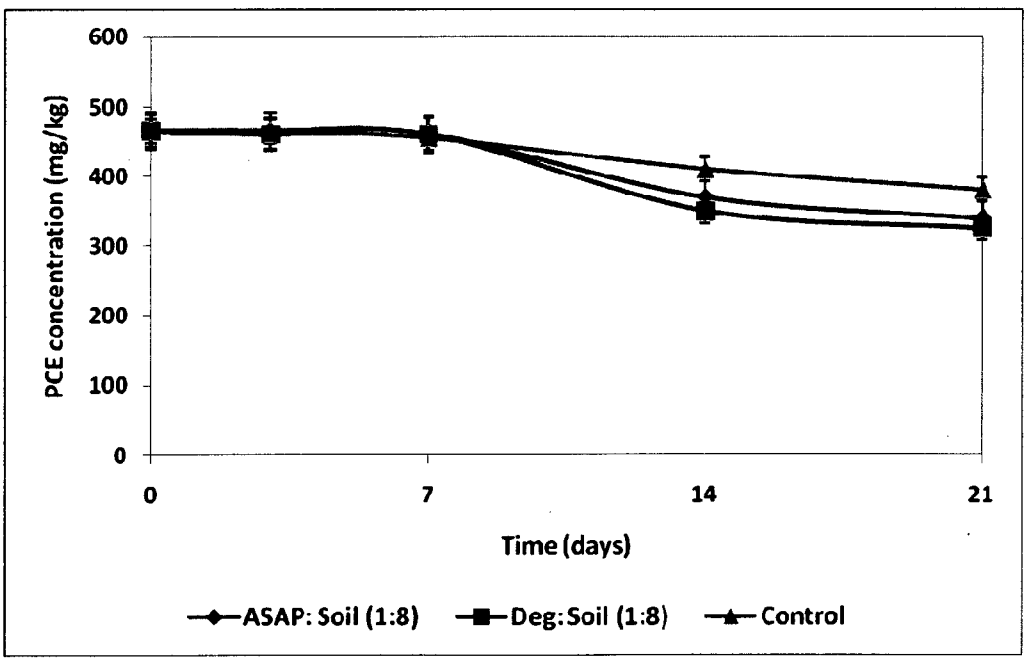

Figure 4.10 Comparison in the effectiveness of ASAP™ vs. Degreaser™ in PCE removal.

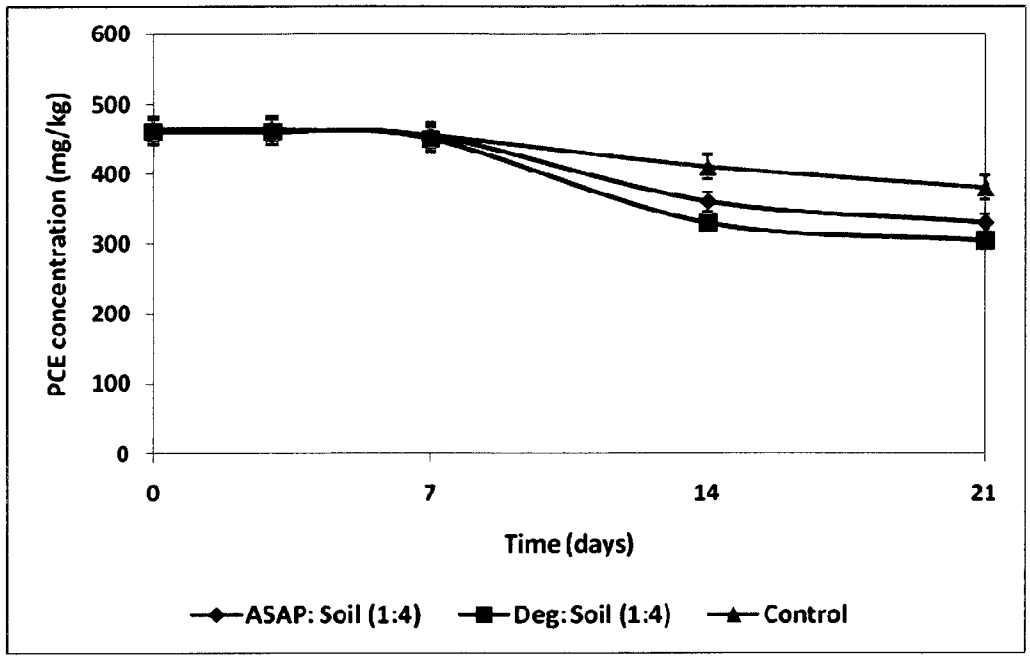

Figure 4.11 Comparison in the effectiveness of ASAP™ vs. Degreaser™ for PCE removal.

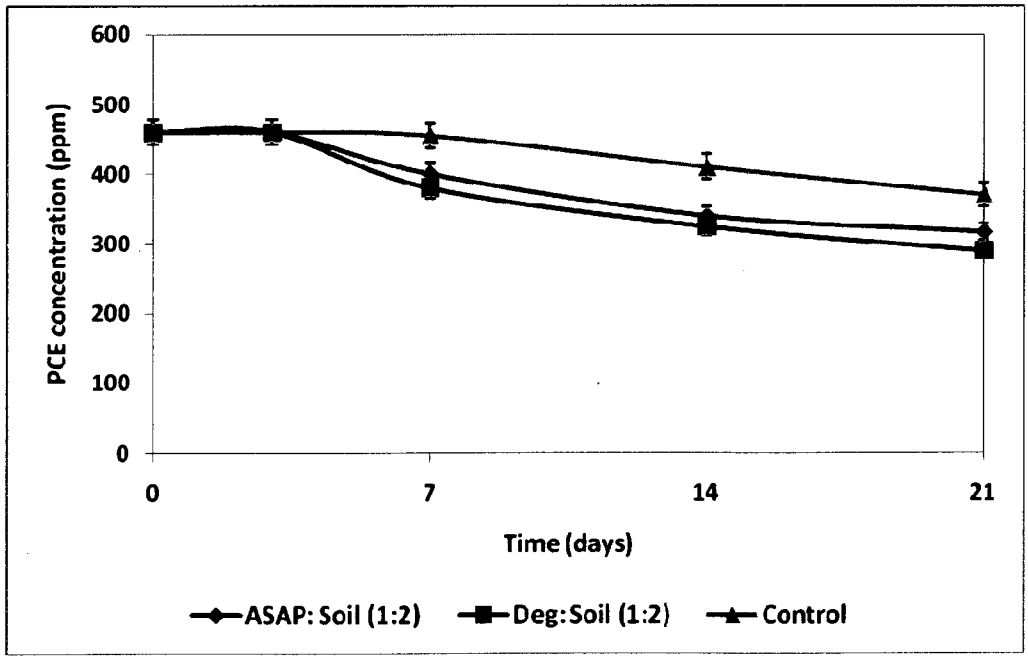

Figure 4.12 Comparison in the effectiveness of ASAP™ vs. Degreaser™ for PCE removal.

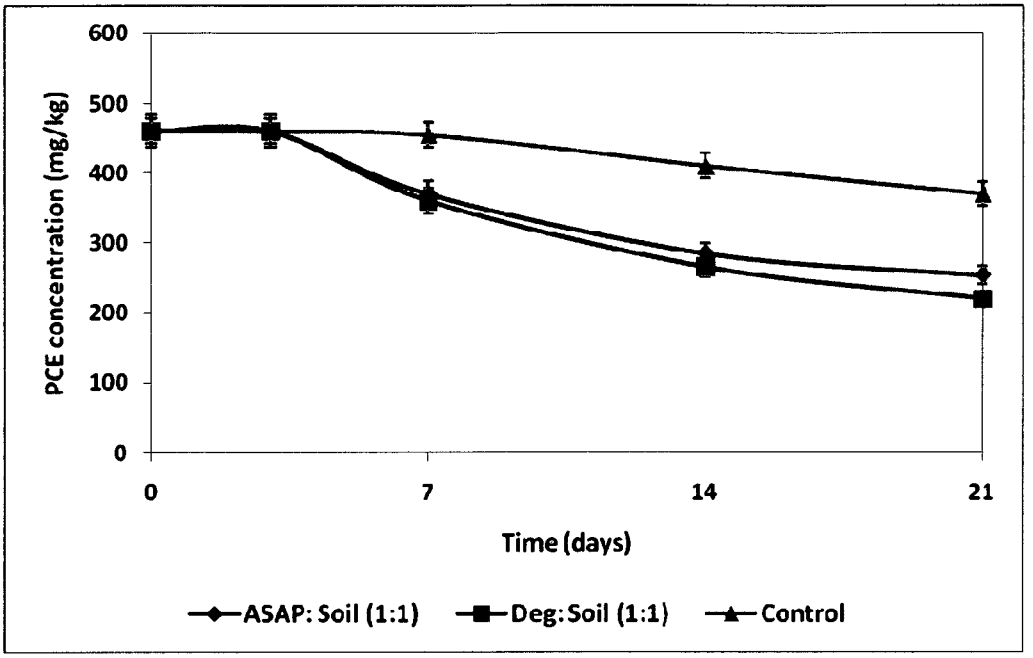

Figure 4.13 Comparison in the effectiveness of ASAP™ vs. Degreaser™ for PCE removal.

B. ASAP™ Versus Degreaser™ in Microbial Population

As shown in Figures 4.14 to 4.17, microbial growth rates of both ASAP™ and Degreaser™ were very close and displayed similar patterns. However, microbial densities in the tests with Degreaser™ were greater than the microbial population in the tests using ASAP™.

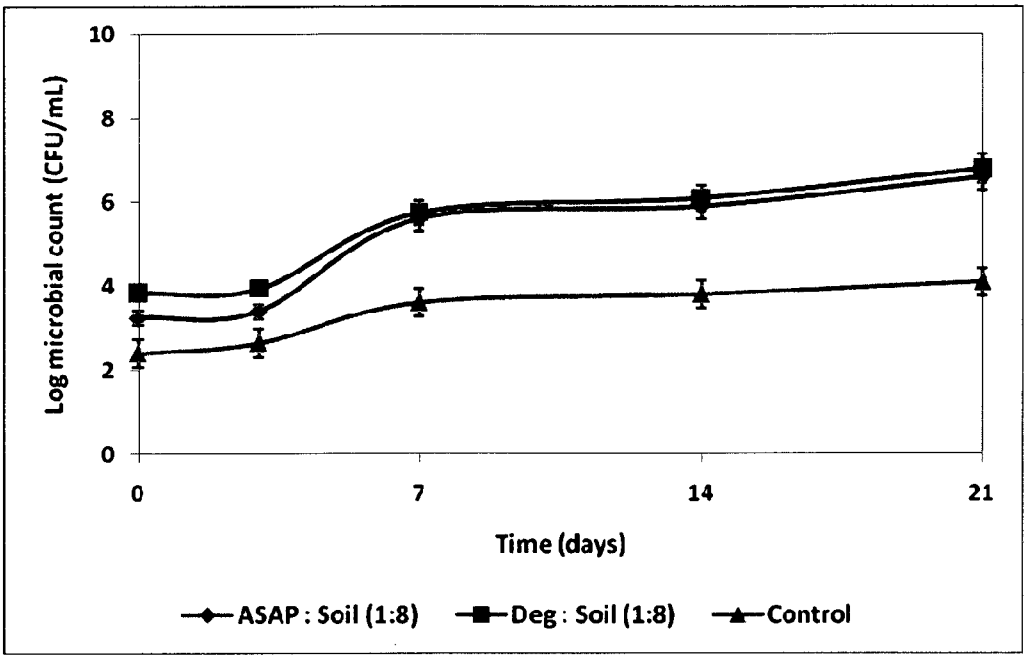

Figure 4.14 ASAP™ vs. Degreaser™ in microbial population at an additive to soil ratio of 1:8.

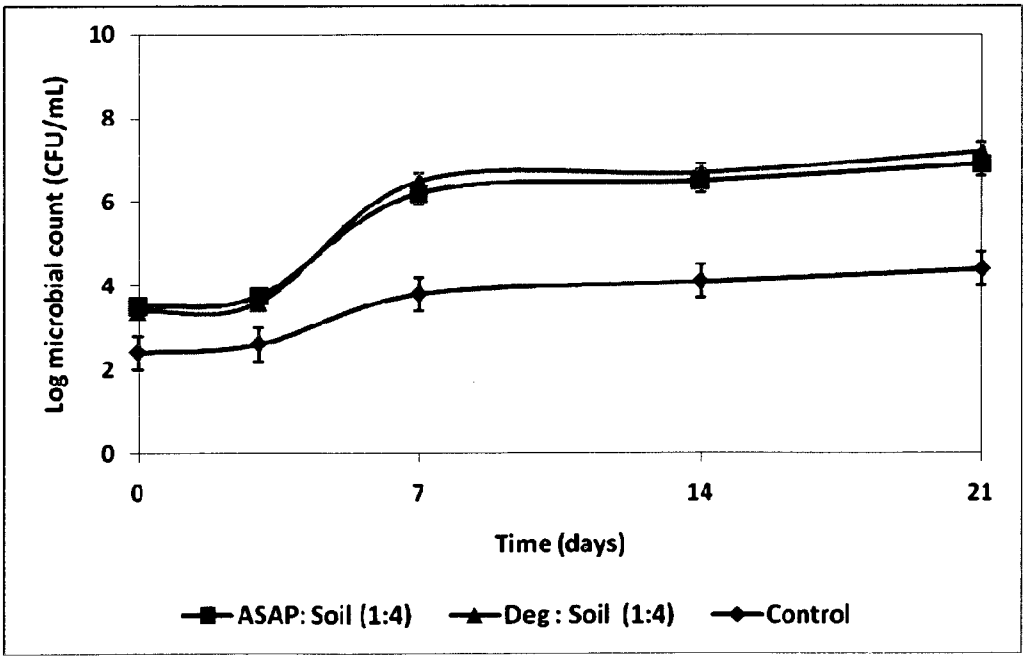

Figure 4.15 ASAP™ vs. Degreaser™ in microbial population at an additive to soil ratio of 1:4.

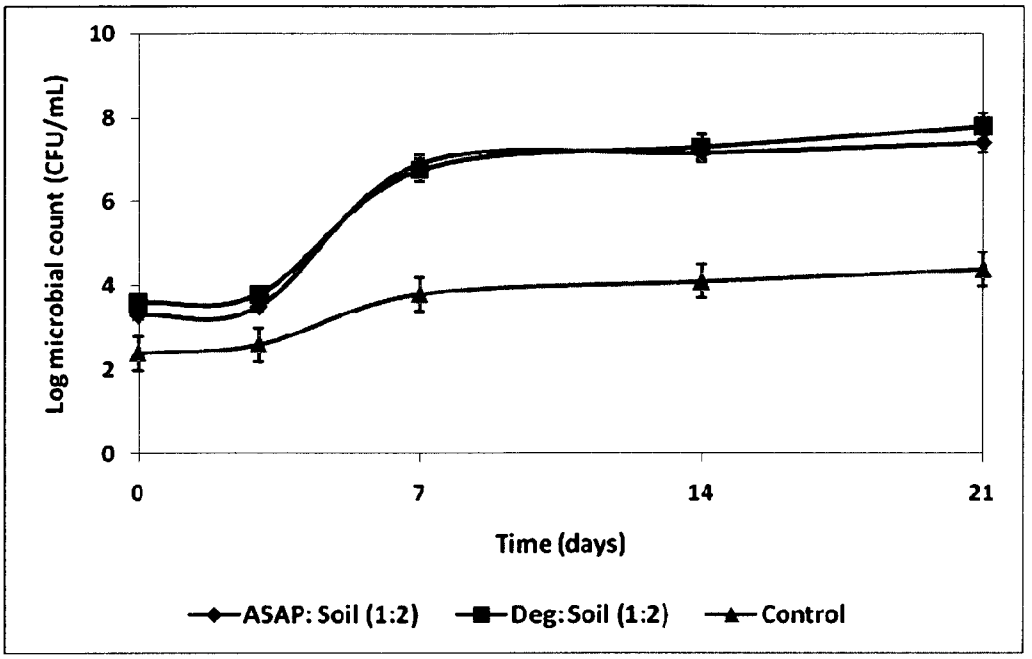

Figure 4.16 ASAP™ vs. Degreaser™ in microbial population at additive to soil ratio of 1:2.

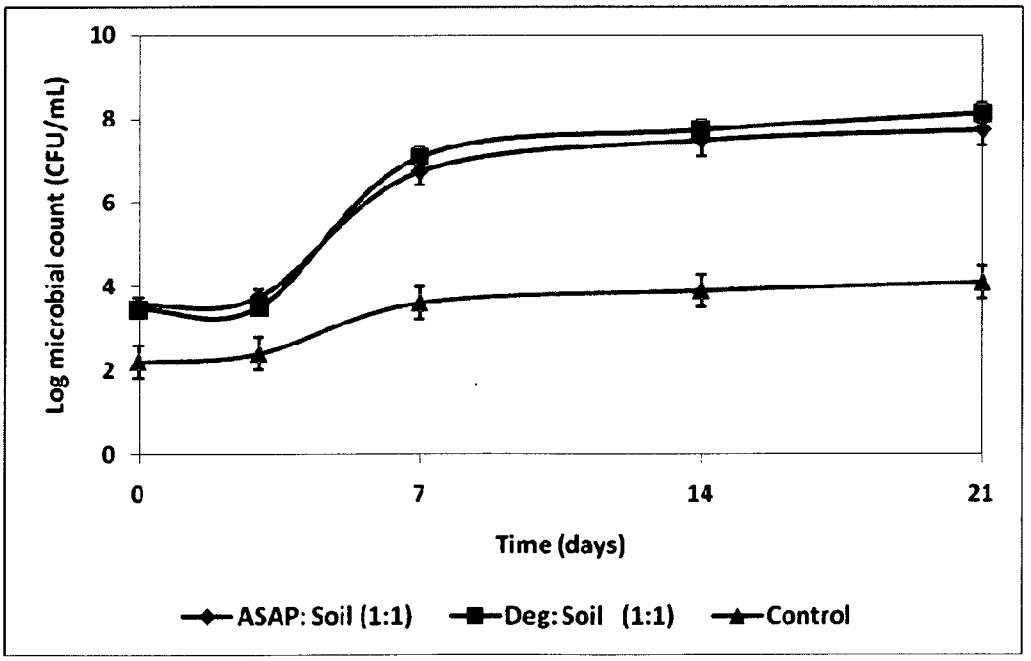

Figure 4.17 ASAP™ vs. Degreaser™ in microbial population at an additive to soil ratio of 1:1.

Table 4.3 PCE removal in phase 1

Treatment	PCE removal (%) in 21 days
Rhamnolipid (1:1)	30 ± 4
Rhamnolipid (2:1)	35 ± 3
ASAP™ (1:8)	27 ± 5
ASAP™ (1:4)	29 ± 3
ASAP™ (1:2)	31 ± 4
ASAP™ (1:1)	45 ± 7
Degreaser™ (1:8)	29 ± 2
Degreaser™ (1:4)	34 ± 3
Degreaser™ (1:2)	37 ± 5
Degreaser™ (1:1)	52 ± 7

4.7.2. Phase Two

In this section, the effect of combining each biological agent with the rhamnolipid biosurfactant and mixing both biological agents with rhamnolipid on soil biodegradation was analyzed. Also in another setup, iron nano-particles were added to the mixture of biological products and rhamnolipid, to evaluate

any possible enhancement in soil remediation. Zero-valent iron in nano-particle form is an effective compound in degrading chlorinated contaminants and in previous studies has been used in soil decontamination (Vipulanandan and Harendra, 2008).

4.7.2.1. Effect of ASAP™ and Rhamnolipid Combination on Soil Bio-degradation

A. PCE Reduction

To determine the effect of adding rhamnolipid biosurfactant to the ASAP™ biological product, the concentration of ASAP™ which resulted in the highest removal in phase one (ratio of 1:1), was selected. A rhamnolipid concentration of 2% with mass ratio to soil of 1:1 was used to prepare the test mixture. Also to obtain even more effective results, a blend containing ASAP™ mixed with rhamnolipid at an additive to soil mass ratio of 2:1 was prepared.

Figure 4.18 shows the reduction in PCE concentration over time for both mixtures and the control (without additive) batch. The removal efficiency of ASAP™ increased from 45% to 58% when rhamnolipid was added. An attractive interaction between rhamnolipid and other surfactant molecules amplified the solubilization. Also similar properties of the biosurfactants present in the solution helped develop synergism. "Synergism in the mixed micelle formation exists when the CMC of a mixture is less than that of individual surfactants among the mixture" (Rosen, 1989). The surface tension reduction of the solution was more effective than each individual biosurfactant. Consequently more contaminants were available for microorganism to be degraded. The removal

efficiency of ASAP™ (2:1) mixed with rhamnolipid (2:1) was satisfactory and as high as 62%.

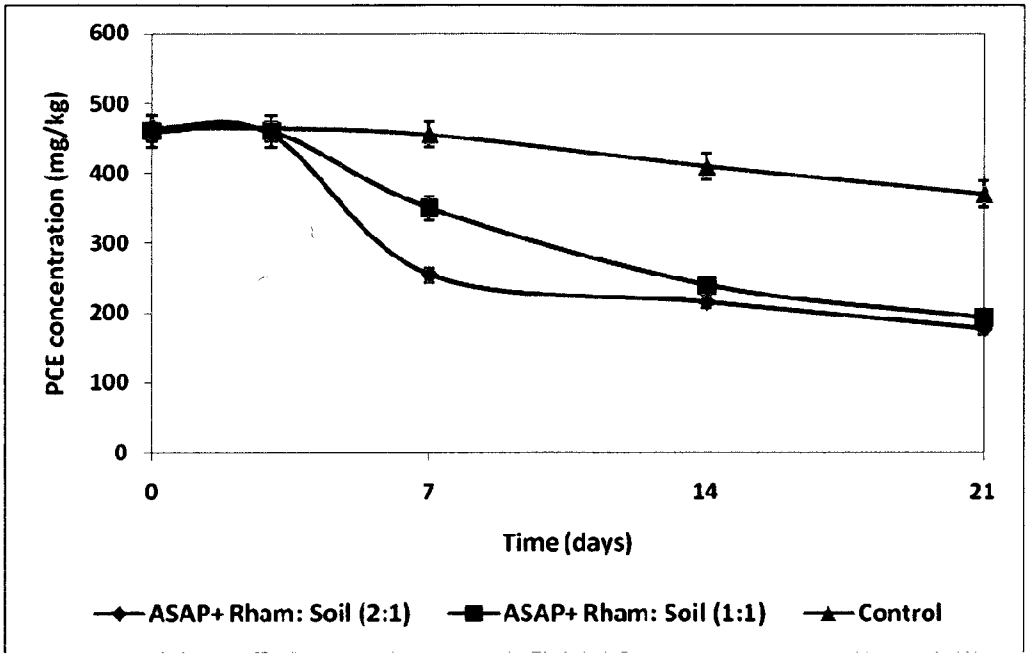

Figure 4.18 PCE reduction of ASAP™ combined with rhamnolipid over time.

It is concluded that rhamnolipid highly increased the rate of degradation and most of the PCE degradation took place in a shorter period of time (14 days).

B. Microbial Growth

Over time the microbial population increased and the growth curve had the same pattern as treatment and control batches in phase 1. On day 21, the microbial population for the ASAP™ with rhamnolipid at a ratio to soil of 2:1 was 10^5 greater than the microbial count in the control batch. Taken together with the fact that the highest removal efficiency was obtained for this additive ratio, the highest microbial density at the end of experiment indicated that biodegradation was the reason for PCE reduction.

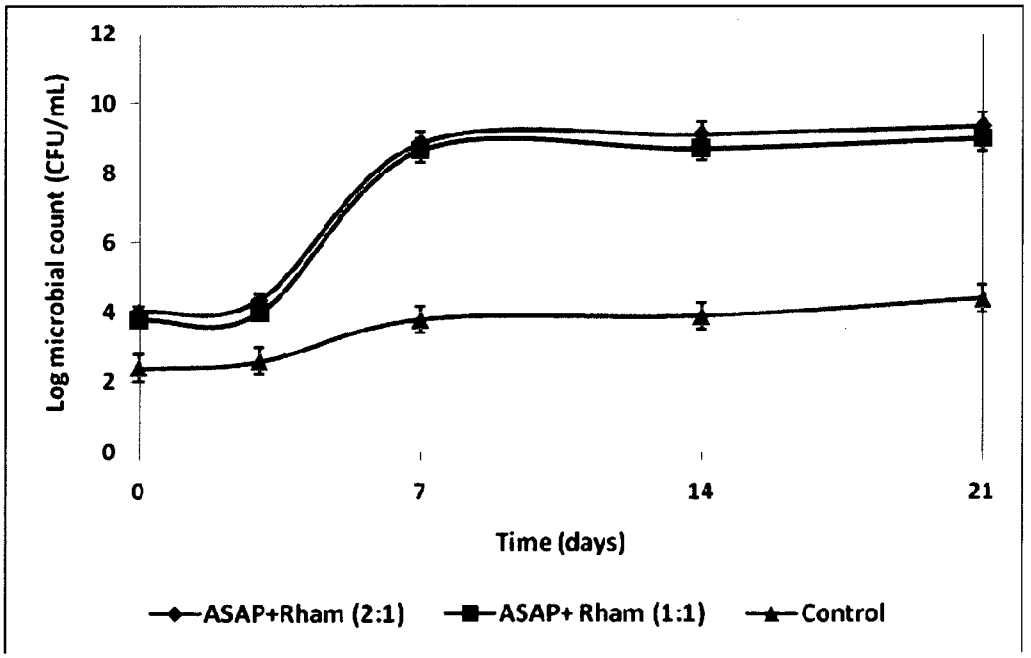

Figure 4.19 Microbial densities for ASAP™ mixed with rhamnolipid, and control.

4.7.2.2. Effect of Degreaser™ and Rhamnolipid Combination on Soil Biodegradation

The Degreaser™ to soil mass ratio of 1:1 which resulted in the highest PCE reduction was used to evaluate the effect of rhamnolipid combined with Degreaser™. Also a higher concentration of rhamnolipid mixed with Degreaser™ was prepared to investigate if the results would significantly improve. As shown in Figure 4.20, applying the mixed solution containing Degreaser™ and rhamnolipid at a mass ratio of 1:1 removed 65% of the initial PCE concentration. The removal efficiency of Degreaser™ was effectively enhanced to 65% by adding rhamnolipid. This was a major efficiency improvement compared to 52% when Degreaser™ was used individually. However, doubling the concentration of Degreaser™ and rhamnolipid resulted in 68% removal which was not a significant increase when compared to 65%.

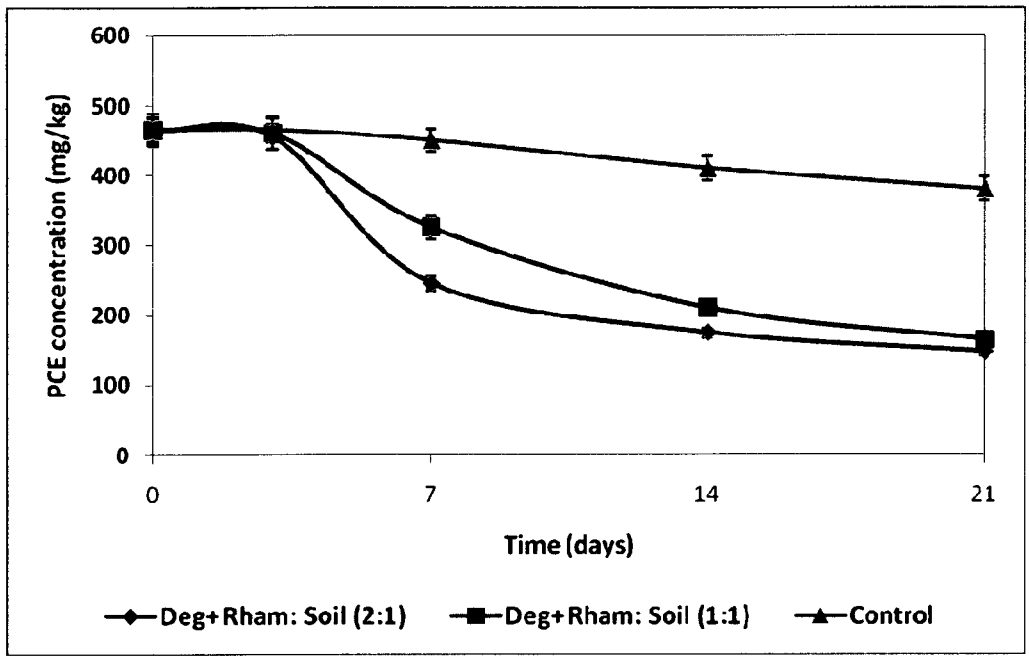

Figure 4.20 PCE reduction of Degreaser™ combined with rhamnolipid over time.

B. Microbial Growth

During the exponential phase the microbial population increased greatly and at the end of the treatment period the population growth rate had almost reached zero. Higher additive ratios resulted in greater microbial populations. As shown in Figure 4.21 the population of the microbes increased up to 10^5 times the initial population and rose from 10^4 to 10^9 (CFU/mL).

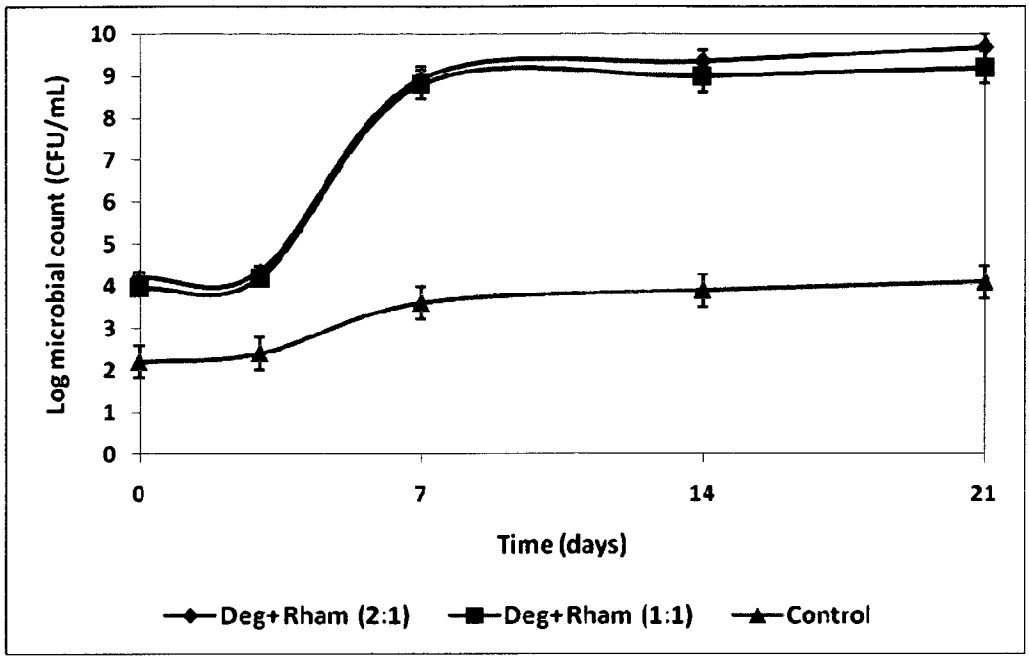

Figure 4.21 Microbial densities for Degreaser™ mixed with rhamnolipid, and control.

4.7.2.3. Effect of ASAP™, Degreaser™ and Rhamnolipid Mixture on Soil Biodegrading

A. PCE Reduction

A solution composed of all additives was prepared and added to the soil in order to observe the PCE biodegradation over time. This solution contained Degreaser™, ASAP™ and rhamnolipid each at the mass ratio to soil of 1:1. Figure 4.22 shows the degradation results over 21 days accompanied by the control batch result. The total PCE removal was 60% which is significant compared to 18% removal in the control flask. Yet compared to the mixture containing just rhamnolipid added to each of biological agents with the same ratio, no noticeable enhancement occurred regarding PCE removal. The re-

moval efficiency obtained from ASAP™ mixed with rhamnolipid was 58%, and the one obtained from the combination of Degreaser™ with rhamnolipid was 65%. Consequently, mixing all three cleaning agents was not a useful procedure.

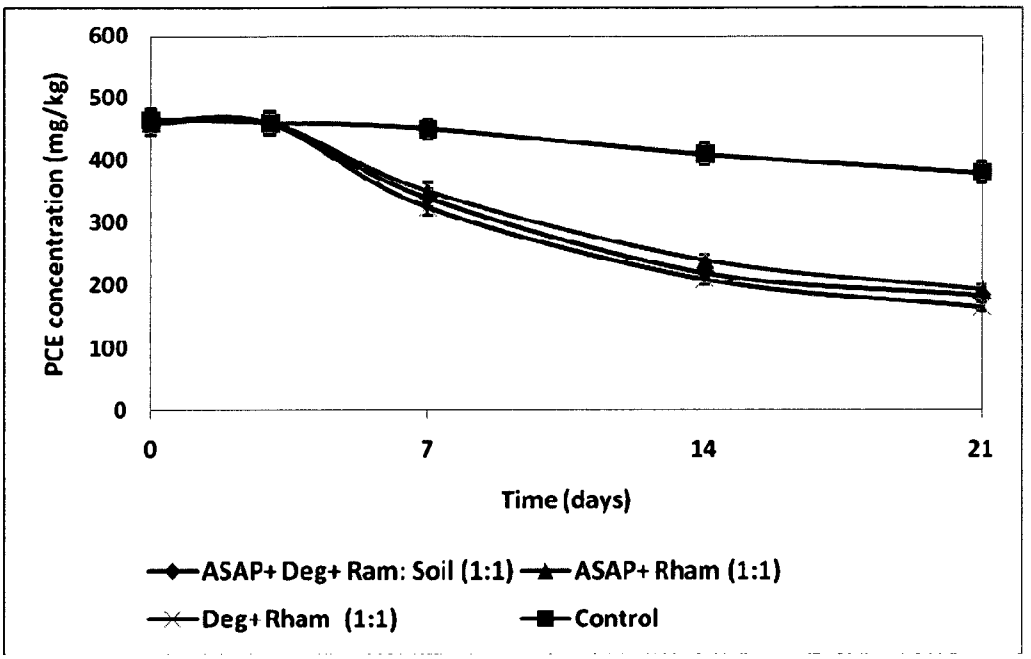

Figure 4.22 PCE reduction of ASAP™ and Degreaser™ combined with rhamnolipid over time.

B. Microbial Growth

Microbial growth in the treatment batch containing the mixture of both biological agents and rhamnolipid biosurfactant was very similar to the microbial population in the batches containing only ASAP™ and rhamnolipid, or Degreaser™ and rhamnolipid. Considering the fact that mixing the three removal

agents did not result in enhancement of PCE removal or a microbial population increase, compared to the mixture of just one agent with rhamnolipid, it is concluded that there is no need to mix all three agents together.

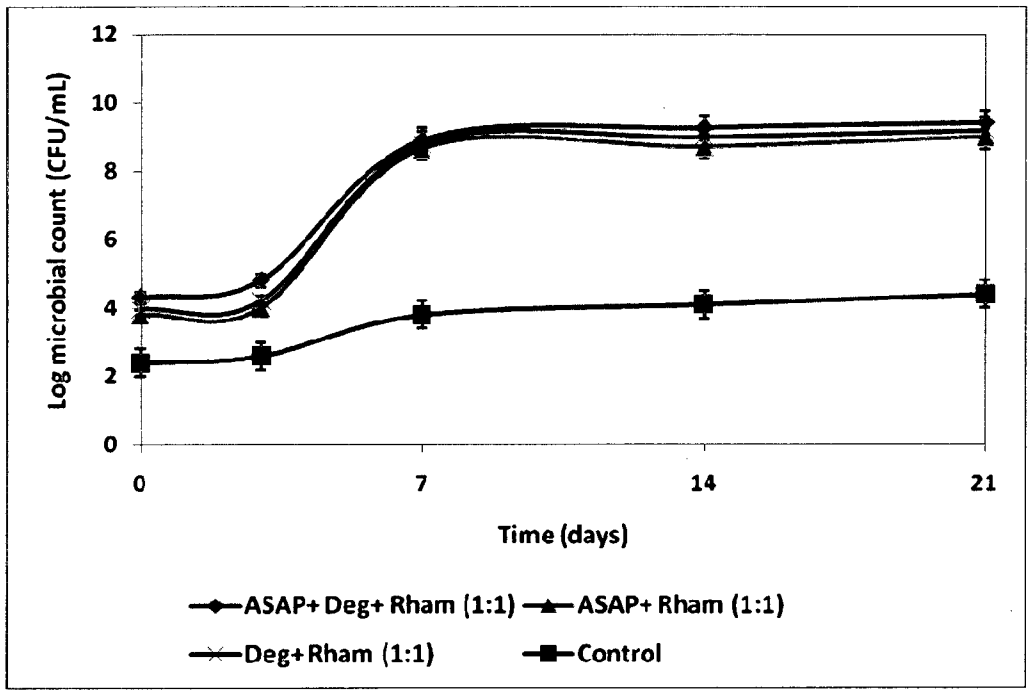

Figure 4.23 Microbial densities for combination of ASAP™, Degreaser™ and rhamnolipid.

4.7.2.4. Effect of ASAP™, Degreaser™, Rhamnolipid and Zero-Valent Iron Nano-Particles Mixture on Soil Biodegradation

Three similar sets of Degreaser™, ASAP™ and rhamnolipid each at the mass ratio to soil of 1:1 (which exhibited satisfactory results in section 4.7.2.3) was prepared, and zero-valent iron at different mass ratios to soil of 1:4, 1:2, and 1:1 was added to each solution. The highest removal obtained through these

experiments was not greater than the mixture of Degreaser and rhamnolipid at the additive to soil ratio in section 4.7.2.3 where no zero-valent iron was added which indicates that zero-valent nano-particles did not cause an extra enhancement in decontamination. The PCE reduction and microbial population diagram exhibited the same pattern as in section 4.7.2.3 so the figures are not repeated here.

Table 4.4 PCE removal in phase 2

Treatment	PCE removal (%) in 21 days
ASAP (1:1) & Rhamnolipid (1:1)	58±4
ASAP (2:1) & Rhamnolipid (2:1)	62±7
Degreaser (1:1) & Rhamnolipid (1:1)	68±5
Degreaser (2:1) & Rhamnolipid (2:1)	65±3
ASAP (1:1) & Degreaser (1:1) & Rhamnolipid (1:1)	60±3
ASAP (1:1) & Degreaser (1:1), Rhamnolipid (1:1) & zero-valent iron (1:4)	61±6
ASAP (1:1) & Degreaser (1:1), Rhamnolipid (1:1) & zero-valent iron (1:2)	60±3
ASAP (1:1) & Degreaser (1:1), Rhamnolipid (1:1) & zero-valent iron (1:1)	64±5

4.7.3. Biodegradation Kinetics

In evaluating the efficiency of bioremediation processes, the kinetics of biodegradation are very important parameters. Analyzing the biodegradation kinetics of the process along with chemical and physical characteristics of a spill, makes it possible to forecast the efficiency or impact of the clean up activity. However, the rate of oil biodegradation is dependent on diverse parameters and is difficult to predict. The type of the contaminant, physical and chemical characteristics of the soil, environmental conditions such as pH and temperature influence the biodegradation kinetics. Assuming that biodegradation was the main procedure in the remediation; the first order biodegradation process was modelled as below:

$$\frac{dC}{dt} = -kC \rightarrow C = C_o \exp^{(-kt)} \quad \text{Equation 4.1}$$

$$\ln \frac{C}{C_0} = -kt \quad \text{Equation 4.2}$$

Where C is the PCE concentration (mg/kg), C_o is the initial PCE concentration (mg/kg), k is the first order biodegradation rate (1/day), and t is time (day). Using Microsoft Excel®, the biodegradation rate coefficient (k) was calculated as the slope of the regression line obtained by plotting ln (C/C_o) versus time (Equation 4.2). The half life ($t_{1/2}$) of PCE was calculated according to the equation 4.3, and determination coefficient (R^2) was determined using Microsoft Excel®.

$$t_{1/2} = \frac{\ln(2/1)}{k}$$

$$t_{1/2} = \frac{0.693}{k} \quad \text{Equation 4.3}$$

Table 4.5 First-order biodegradation rate coefficients and half-lives of PCE

Treatment	K (1/day)	Half-life (days)	R^2
Rhamnolipid (1:1)	0.018	38.5	0.95
Rhamnolipid (2:1)	0.022	31.5	0.96
ASAP™ (1:8)	0.016	43.3	0.92
ASAP™ (1:4)	0.018	38.5	0.93
ASAP™ (1:2)	0.019	36.5	0.97
ASAP™ (1:1)	0.031	22.4	0.96
Degreaser™ (1:8)	0.019	36.5	0.89
Degreaser™ (1:4)	0.022	31.5	0.91
Degreaser™ (1:2)	0.023	30.1	0.96
Degreaser™ (1:1)	0.038	18.2	0.97
ASAP (1:1) & Rhamnolipid (1:1)	0.045	15.4	0.97
ASAP (2:1) & Rhamnolipid (2:1)	0.048	14.4	0.88
Degreaser (1:1) & Rhamnolipid (1:1)	0.053	11.8	0.97
Degreaser (2:1) & Rhamnolipid (2:1)	0.059	13.1	0.90
ASAP (1:1) & Degreaser (1:1) & Rhamnolipid (1:1)	0.048	14.4	0.96
Control	0.010	69.3	0.96

The total degradation time for each treatment can be estimated from the half-life of PCE in each setup. The rate of degradation has an inverse relationship to the half-life; thus the higher the degradation rate, the shorter the half-life. According to the table 4.5, half-lives for PCE followed a trend as shown below.

$t_{1/2}$ Control > $t_{1/2}$ rhamnolipid > $t_{1/2}$ biological agent > $t_{1/2}$ biological agent + rhamnolipid

The coefficient of determination (R^2) measures how well the regression line represents the data and gives some information about the goodness of fit of the model. In general, the range of coefficient of determination (R^2) is between 0 and 1; the closer this parameter is to 1 the better the fit of the regression line. According to Table 4.5, all treatments had a determination coefficient greater than 0.85 and the average determination coefficient for the model was 0.97. These calculations support the initial assumption (first order biodegradation rate) true. Consequently it is concluded that biodegradation rate and PCE concentration are linearly correlated.

The Monod equation represents the growth of the microorganisms.

$N = N_o \exp^{(kt)}$ Equation 4.4

In this equation N is the number of microbial cells per volume, N_o is the initial number of microbial cells, t is time (day), and k is growth rate (1/day). The biodegradation rate coefficient (k) was calculated using Microsoft Excel®. The

slope of the regression line obtained by plotting ln (N/N_o) versus time represents the growth rate.

It is observed the biodegradation rate of PCE is directly related to the growth rate of the microorganisms. Comparing the degradation rate of PCE and microbial growth rate for each treatment, it was observed that the increase of the growth rate of microorganisms resulted in degrading PCE at a higher rate.

Table 4.6 Microbial growth rate

Treatment	K (1/day)
Rhamnolipid (1:1)	0.030
Rhamnolipid (2:1)	0.035
ASAP™ (1:8)	0.048
ASAP™ (1:4)	0.050
ASAP™ (1:2)	0.060
ASAP™ (1:1)	0.062
Degreaser™ (1:8)	0.037
Degreaser™ (1:4)	0.055
Degreaser™ (1:2)	0.058
Degreaser™ (1:1)	0.070
ASAP (1:1) & Rhamnolipid (1:1)	0.067
ASAP (2:1) & Rhamnolipid (2:1)	0.049
Degreaser (1:1) & Rhamnolipid (1:1)	0.066
Degreaser (2:1) & Rhamnolipid (2:1)	0.067
ASAP (1:1) & Degreaser (1:1) & Rhamnolipid (1:1)	0.059
Control	0.033

4.8. Discussion

The percent removal of PCE for all samples during the whole period of treatment is shown in Figure 4.24. The microbial density for each treatment is represented along with the removal percentage. In this figure a strong correlation is observed between microbial density and PCE removal, which shows that the PCE removal, occurred through biological degradation. The general trend in PCE removal as mentioned before was:

Biological agent + rhamnolipid > biological agent > rhamnolipid > control.

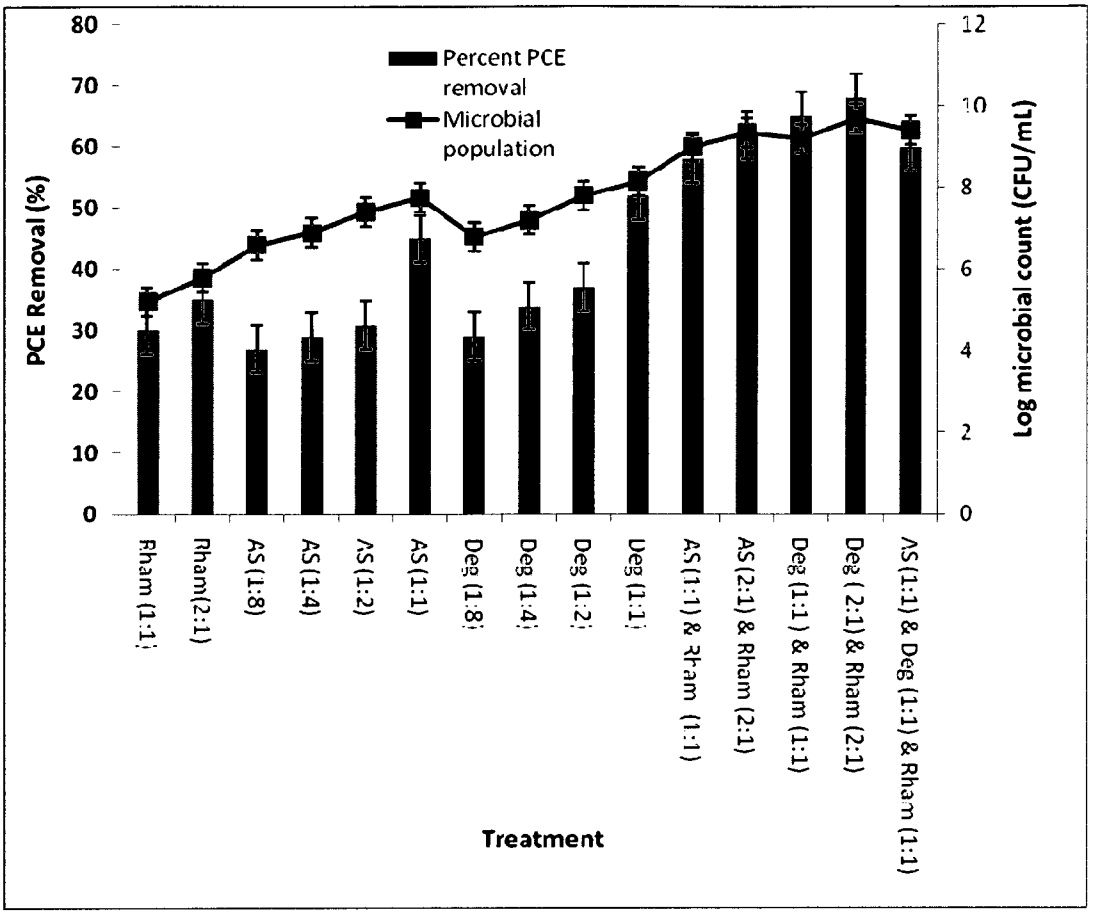

Figure 4.24 Percent PCE removal and microbial densities for various treatments.

In Figure 4.25, the percent removal at each time interval (days 7, 14 and 21) for all of the treatments is demonstrated. As mentioned before, increasing the concentration of biosurfactants resulted in greater contaminant removal. In general, most of the biodegradation occurred in 14 days and up to 21 days, all possible degradation took place. After day 21 no decrease in the PCE concentration was observed.

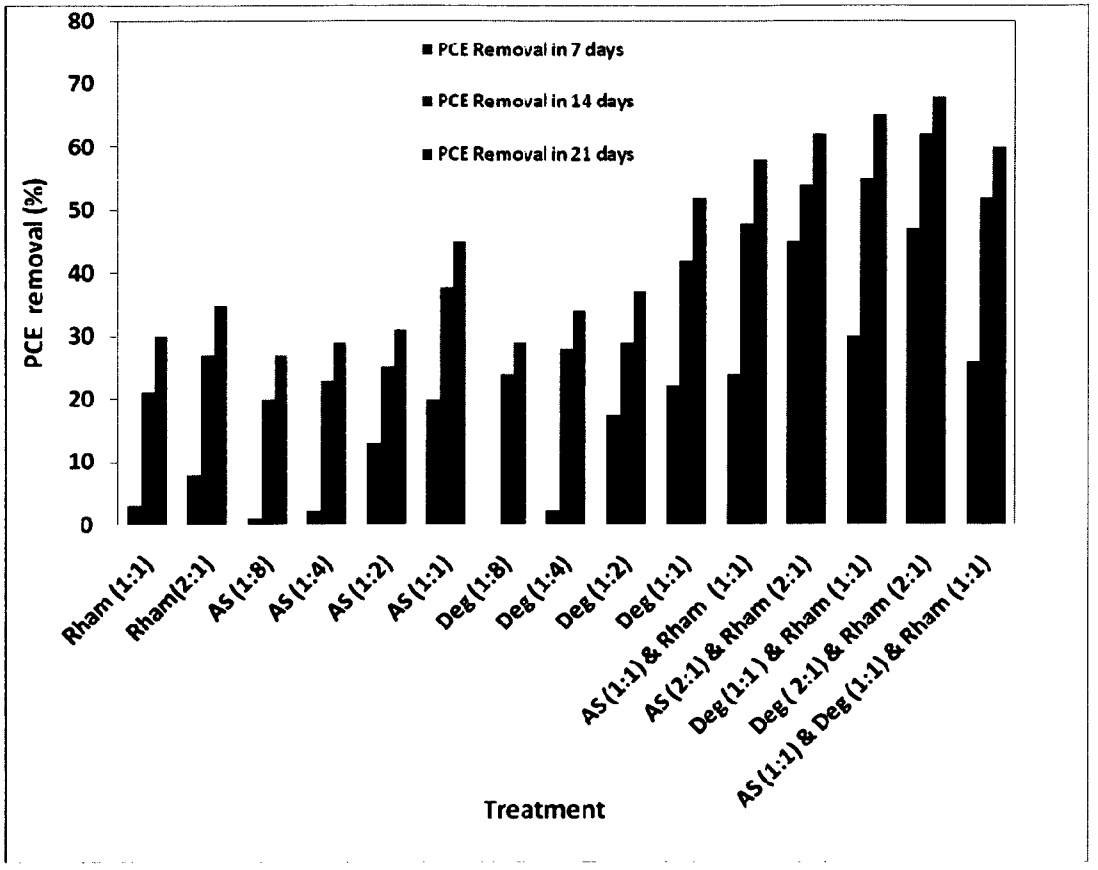

Figure 4.25 Percent PCE removal at different intervals for various treatments substracting the natural losses.

To determine the actual PCE removal by biodegradation, the PCE loss obtained from control batches was subtracted from the total removal. Figure 4.26 illustrates the actual removal performed by biodegradation throughout the entire study period. For almost all treatments there was no or little contaminant reduction in the first three days. It could be interpreted that during this period, mobilization of the contaminants was taking place and the rate of

PCE mobilization into solution was greater than the rate of biodegradation. Becoming mobilized and solubilized, PCE became available and was degraded by microorganisms. In general, an increase in the biosurfactant concentration was associated with an increase in hydrocarbon contaminant removal and an increase in microbial density. The mechanism of decontamination biosurfactants reduced the surface tension of the PCE and made it more available for the microorganisms. The increase in the population of the microorganisms indicates that PCE has been used as a substrate for the microorganisms. Consequently being degraded by the microorganisms, the PCE level decreased.

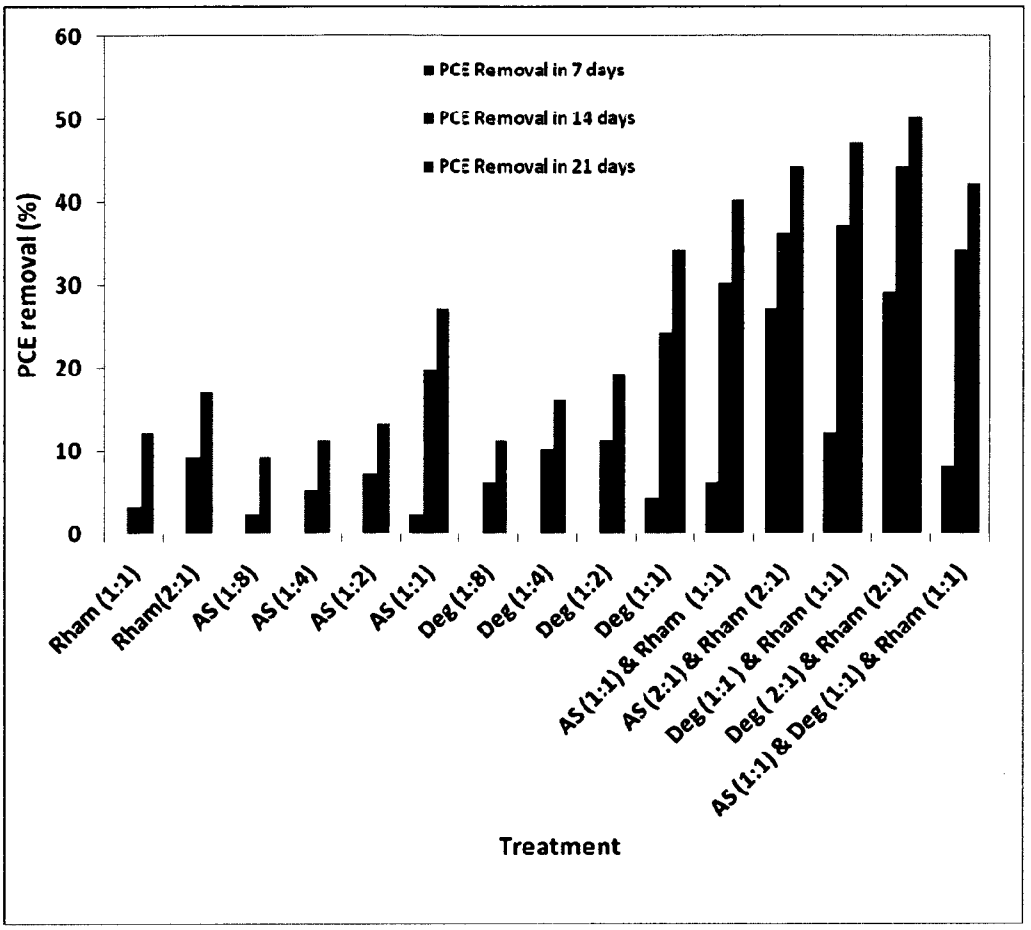

Figure 4.26 Percent PCE biodegradation at different intervals for various treatments.

5. Conclusions and future work

5.1. Conclusions

The results obtained from this study showed that adding biosurfactant to the washing solution slightly increased the rate of PCE removal in soil, compared to applying water alone as the washing solution (control batch). Rhamnolipid biosurfactant at mass ratio to soil of 1:1 was able to increase the PCE removal up to 30%. Considering the fact that rhamnolipid was used at a concentration of 2%, the ratio of actual rhamnolipid to soil is relatively low: 1:200, and at this ratio the removal efficiency of 30% is considerable.

Two biological products (ASAP™ and Degreaser™) tested in this study also highly enhanced the bioremediation of the soil. Individual application of ASAP™ at mass ratios of 1:1 and 1:2 enhanced the remediation to 45% and 31% respectively. Degreaser™ increased the removal efficiency of PCE up to 52% and 37% at mass ratios of 1:1 and 1:2 respectively.

Treatments performed by combination of rhamnolipid biosurfactant and biological agents exhibited the most efficient results. Combination of ASAP™ and rhamnolipid enhanced the remediation of PCE up to 58% at ratio to soil of 1:1. With respect to the results obtained from the individual use of rhamnolipid which showed 30% removal at this ratio, and ASAP™ which showed 45%, the enhancement obtained by combining these two agents is promising. Also the combination of Degreaser™ and rhamnolipid substantially improved the remediation of PCE. A mixture of a mass ratio of 1:1 Degreaser™ and

rhamnolipid had an efficiency of 65%, which when compared to the efficiency of either of these two agents alone is very high. However, mixing all three additives did not cause a remarkable enhancement in PCE removal. Therefore, it can be summarized that it is best not to mix all additives but to combine either of the biological products with rhamnolipid. In both phases Degrease™ performed slightly better than ASAP™ in the degradation of PCE.

The microbial analysis showed that removal of PCE from soil was directly related to the microbial density. In the treatments using rhamnolipid, PCE was dispersed into the aqueous phase and became more bioavailable, consequently biodegradation was enhanced. In the treatments with the biological agent, the same mechanism is believed to have occurred but since these agents have a complex nature the exact process is not known. Moreover, these products contain added nutrients which may have increased the solubilization of PCE.

The first order biodegradation rate was followed in the treatments and the average determination coefficient of 97% proved this idea. The general trend in PCE removal was seen as:

Biological agent + rhamnolipid > biological agent > rhamnolipid > Control batch.

This thesis exhibited the possibility of remediation of a chlorinated organic compound from soil by addition of biosurfactant with biological cleaning agents, which are environmentally friendly, non-toxic, and present minimal harm to humans and the environment.

5.2. Future Work

The following recommendations for future work can be made:

- Evaluating the effect of other biosurfactants such as surfactin and sophorolipids on PCE biodegradation.
- Performing the biological treatment in continuous mode with multiple washing.
- Using column tests instead of batch treatments in order to observe the possibility of in situ remediation by soil flushing.
- In this study iron nano particles were applied to the soil at the same time with biosurfactant and biological cleaning agents. In the continuation of this study to assess the efficiency of nano particles on decontamination of PCE, it is desirable to use nanoparticles following the application of biosurfactant or to evaluate other types of nanoparticles.
- Pre-treating the contaminated soil by applying techniques such as air-stripping prior to biological treatment.
- Performing multistage treatments to achieve more efficient results. This could include performing the same batch treatment after the solution is extracted from soil, or it could be presented as conducting the biological treatment followed by other remediation techniques.

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7. Appendix

Water Content

Weight of soil before drying: 10 g

Weight of soil after drying: 9.63 g

$$\text{Water content} = \frac{\text{Weight of soil before drying} - \text{Weight of soil after drying}}{\text{Weight of soil before drying}}$$

$$\text{Water content} = \frac{(10-9.63)}{10} \times 100 = 6.1\%$$

Organic Matter Content

Weight of soil before ignition: 9.63 g

Weight of soil after ignition: 9.39 g

$$\text{Organic content} = \frac{\text{Weight of soil before ignition} - \text{Weight of soil after ignition}}{\text{Weight of soil before ignition}}$$

$$\text{Organic matter content} = \frac{(9.63 - 9.32)}{9.63} \times 100 = 2.5\%$$

Cation exchange capacity

Based on milliequivallents of potassium exchanged per unit weight of soil, the cation exchange capacity (CEC) of the soil sample was calculated as below:

CEC = [K (ppm) ÷ 39]

Sample 1 CEC = [290 ÷ 39]= 7.44 (cmoles / kg)

Sample 2 CEC = [284 ÷ 39]= 7.28 (cmoles / kg)

Sample 3 CEC = [295 ÷ 39]= 7.56 (cmoles / kg)

The CEC of the soil is average of the three CECs.

CEC = 7.42 ± 0.10 (cmoles / kg)