BIOREMEDIATION OF USED ENGINE OIL CONTAMINATED SOIL BY *Bacillus salmalaya* 139I

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INSTITUTE OF BIOLOGICAL SCIENCES
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Field of Study: Master's of Technology (Environmental Management)

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ABSTRACT

This study was carried out to test the effectiveness of *Bacillus salmalaya strain* 139I and its biosurfactant to decontaminate soil using an *ex-situ* washing technique. The experimental variables include temperature (25, 37, 42 and 55°C), concentration of mixed liquor (0.01, 0.1, 1 and 10%-v/v), volume of mixed liquor (5, 10, 15 and 20 ml), shaking speed (80, 120, 160 and 200 strokes/min) and washing time (5, 10, 15 and 20 min). To determine the most optimal environmental conditions for the treatment, Taguchi Experimental Design Method was applied based on the Orthogonal Array L16' 4^5. Results showed that the percentage removal of used engine oil ranged from 15.48% to 47.36%. The highest removal of 47.36% can be obtained when washing temperature was at 25°C, with 20 ml of 10% v/v mixed liquor, 200 strokes min^{-1} shaking speed and 20 min washing time. However, removal equilibrium cannot attained at these maximum points, except shaking speed which indicated maximum removal efficiency at third level (160 stroke/min). Washing time was found to be the most influential variable for the treatment. C/N ratio of 23:1 of contaminated soil sample is within range required to degrade hydrocarbon contaminated soils mentioned in literature. However, after 30 days of treatment the degradation of used engine indicated the need of providing nutrients such as carbon, nitrogen and sulphur, oxygen and water in order to guarantee bacterial growth and acceleration in the degradation rate of contaminants. As 83% of used engine oil degradation achieved in period of only 2 months of treatment, *Bacillus salmalaya 139I* can be considered as an effective microbe to decontaminate soil.

ABSTRAK

Kajian ini telah dijalankan untuk menguji keberkesanan *biosurfactant* berasal dari *strain Bacillus salmalaya* 139I untuk menyah-pencemaran tanah menggunakan teknik basuhan *Anex-situ*. Parameter eksperimen termasuk suhu (25, 37, 42 dan 55 ° C), kepekatan *biosurfactant* (0.01, 0.1, 1 dan 10% -v / v), jumlah *biosurfactant* (5, 10, 15 dan 20 ml), kelajuan goncangan (80, 120, 160 dan 200 strok / min) dan masa basuhan (5, 10, 15 dan 20 min). Kaedah Design Eksperimen Taguchi telah digunakan berdasarkan *Array ortogon L16 '45* bagi menentukan keadaan persekitaran yang paling optimum untuk rawatan,. Hasil kajian menunjukkan bahawa peratusan penyingkiran minyak enjin terpakai adalah dari 15.48% kepada 47.36%. Penyingkiran tertinggi 47,36% diperolehi apabila suhu membasuh adalah pada 25 ° C, dengan 20ml 10% v / v *biosurfactant*, kelajuan goncangan 200 stroke min-1 dan masa basuhan 20 min. Berdasarkan prestasi statistik keluk parameter, kecekapan penyingkiran yang paling tinggi boleh dicapai pada 55 ° C, 20 ml, 160 strok / min, 2% -v / v dan 20 minit. Walau bagaimanapun, keseimbangan penyingkiran tidak boleh dicapai pada titik maksimum, kecuali kelajuan goncangan yang menunjukkan kecekapan penyingkiran maksimum pada tahap ketiga (160 strok / min). Parameter masa membasuh didapati paling berpengaruh untuk rawatan ($\Delta = 4.77$). 83% daripada degradasi minyak enjin terpakai telah dicapai dalam tempoh dua bulan berdasarkan gabungan teknik basuhan dan teknik pemulihan persekitaran. Sampel tanah yang tercemar dengan nisbah C / N 23: 1 adalah dalam julat yang diperlukan untuk degardasi hidrokarbon tanah tercemar seperti yang disebut dalam sorotan kajian. Walau bagaimanapun, selepas 30 hari rawatan degradasi minyak enjin terpakai telah menunjukkan keperluan untuk menyediakan nutrien seperti karbon, nitrogen dan sulfur, oksigen dan air bagi menjamin pertumbuhan bakteria dan pencepatan dalam kadar degradasi bahan cemar.

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LIST OF SYMBOLS AND ABBREVIATIONS

A. Hybridus : Amaranthus hybridus

ATP : Adenosine tri-phosphate

BTEX : benzene, toluene, ethylbenzene, and xylenes

$CaCO_3$: Calcium carbonate

CCME : Canadian Council of Ministers for the Environment

CHNS : Carbon, Hydrogen, Nitrogen and Sulfur

CMC : Critical Micelle Concentration

GC : Gas chromatography

HCl : Hydrochloric acid

ICCT : International Council on Clean Transportation

LPG : Liquefied Petroleum Gas

$MgCO_3$: Magnesium carbonate

ML : Mixed liquor

MS : Mass spectrometry

NaCl : Sodium chloride

Na_2SO4 : Sodium sulfate

NO : Nitric oxide

PAHs : polycyclic Aromatic Hydrocarbons

Pb : lead

PCB : Polychlorinated biphenyl

PCDD : Polychlorinated dibenzodioxin

PCDF : Polychlorinated dibenzofuran

PCP : Phencyclidin

RCOC : Remaining Crude Oil Contaminant

RDX : Cyclotrimethylenetrinitramine

TPH	: Total petroleum hydrocarbon
USEPA	: United States Environmental Protection Agency
pH	: potential hydrogen
Zn	: Zinc
FCCs	: Fluidized Catalytic Crackers
VTB	: Vacuum Tower Bottoms
UNIDO	: United Industrial Development Organization
TNT	: Trinitrotoluene
USA	: United States of America
SDS	: Sodium Dodecyl Sulfate
SN	: signal-to-noise
SO_2	: Sulfur dioxide
SPSS	: Statistical Package for the Social Sciences
SVOCs	: Semi-volatile organic compounds
TX100	: Triton X-100
TW80	: Tween 80
UCM	: unresolved complex mixtures
USDA	: United States Department of Agriculture
V/V	: Volume/Volume

LIST OF EQUATIONS

CHAPTER ONE

INTRODUCTION

1.1 Background of study

Engine oil and other oil by-products play a great role in the current economy of most developed and underdeveloped countries, cities and rural areas (Obayori *et al*., 2014). All sectors including the agriculture industry uses these products to maintain and run engines and machines to produce more products and supply more goods in the competitive market (Lopes & Bidoia, 2009). However, release and spillage of these components, especially after use, to the environment lead to negative human and environmental effects (Dominguez & Pichtel, 2003). Furthermore, the production of oil and its by-product continues and so does the consumption and leaks will as long as researchers do not find better alternatives. As a result, it is necessary to create a suitable environmental management system plans and find the better alternatives for the treatment.

The presence of lubrication additives and heavy metals in the used engine oil makes it highly toxic compared with crude oil, it can lead to serious disturbance for microorganisms communities and soil characteristics, which effects on biomass production, moreover, used engine oil affect on kidneys, heart, lungs and nervous system for many terrestrial and aquatic animals, and this is the reason why it is necessary to consider used engine oil pollution and crude oil pollution separately. Furthermore, crude oil contaminated soil and sea has resulted in extensive research on hydrocarbon biodegradation and effects on the environment, which have been reviewed many times. However, pollution due to used engine oil has not received the same attention proved by lack of previous research. Therefore, numerous researches discovered the high danger and toxicity of used engine oils compared with crude oil (Vazquez, 1989; Obayori *et al*., 2014).

The uses of engine oils are such as lubricants for vehicles and the majority of the agricultural and industrial machines in all cities and rural areas for developed and underdeveloped countries, thus, causing pollution to occur in all parts of the ecosystem such as soil, water and air, caused in catastrophic effects to human health, plants and animals. For example, prolonged exposure and high hydrocarbon concentrations released by the used engine oil, may cause the development of liver and kidney disease, possible damage to the bone marrow and increased risk of cancer (Obayori *et al*., 2014). Moreover, spillage of used engine oil on soil can produce important changes in the microorganism communities that participate in the nitrogen cycle, caused by shortage in oxygen quantity, as well as, the development of anaerobic microzones, which have adverse effects on soil organisms such as bacteria, fungi, and other multicellular organisms (Vazquez, 1989).

Generally, PAHs and heavy metals in used engine oils are the main toxic compounds that are present in high concentrations, which as a result lead to many researchers conducting research on the effects of PAHs and heavy metals on all parts of the ecosystem (Nwite & Alu, 2015). Odjegba and Atebe (2007) conducted a study and found that because of the reduction in pH and lack of available nutrients caused by used engine oil contamination, plants that are exposed to high concentrations of used engine oil showed lower carbohydrate content, lead to considerable reduction in the total biomass quantity. Also it was found that used engine oil affected the time of germination, percentage germination, plant height, leaf production and biomass (Adedokun & Ataga, 2007; Nwite & Alu, 2015). Moreover, the spillage of used engine oils onto the aquatic system can affect the kidneys, heart, lungs and nervous system; it can affect unbelievable amounts of aquatic organisms such as the phytoplankton species, crustaceans, mollusks and fish populations (Vazquez, 1989; Samuel, 2011).

Used engine oil can be released into the soil and aquatic system under four major ways: escape and loss during engine operation (Figure 1.1); during application on rural roads for dust control; during asphalting; and, finally, when it is disposed directly in a landfill (Vazquez, 1989). Thus, imposes on the environmental institutions of the use of large sums of money in order to reduce or eliminate the pollutants and restoring all parts of the site to a useful purpose.

Figure 1.1 Spillage of used engine oil in residential areas

In order to remediate and eliminate the pollution caused by used engine oil, many researchers proposed different techniques and methods, which is mostly divided into two main categories (*in-situ* remediation techniques and *ex-situ* remediation techniques). Generally, *in-situ* remediation techniques treat the contaminants at the site, which causes limitations such as time constraints and change of environmental conditions. However, *ex-situ* remediation techniques are based on excavation, transport and treated contaminants at specific controllable conditions, where the contaminant removal efficiency can reach high levels. However, these techniques also showed limitations such as high cost of contaminants treatment, excavation and transport (Mohammed, 2004; Williams, 2006).

The washing technique is one of the *ex-situ* physical and chemical techniques based on the use of surfactants such as washing solutions. Generally, different environmental variab were applied based on previous laboratory researches, for optimal environmental conditions (Balaji *et al.*, 2014). The washing method is an *ex-situ* technique that is applied when the amount of contaminated soil is small or the contamination occurred at the residential areas where the *in-situ* treatment is difficult to apply (Figure 1.2). Moreover, the washing method can lead to high degrees of pollutant removal efficiency due to the controllability of many factors such as, moisture, temperature, agitation, salinity, and pH (Mohammed, 2004). In some cases when the amount of contaminated soil in residential areas is big, an *ex-stiu* treatment will be more appropriate.

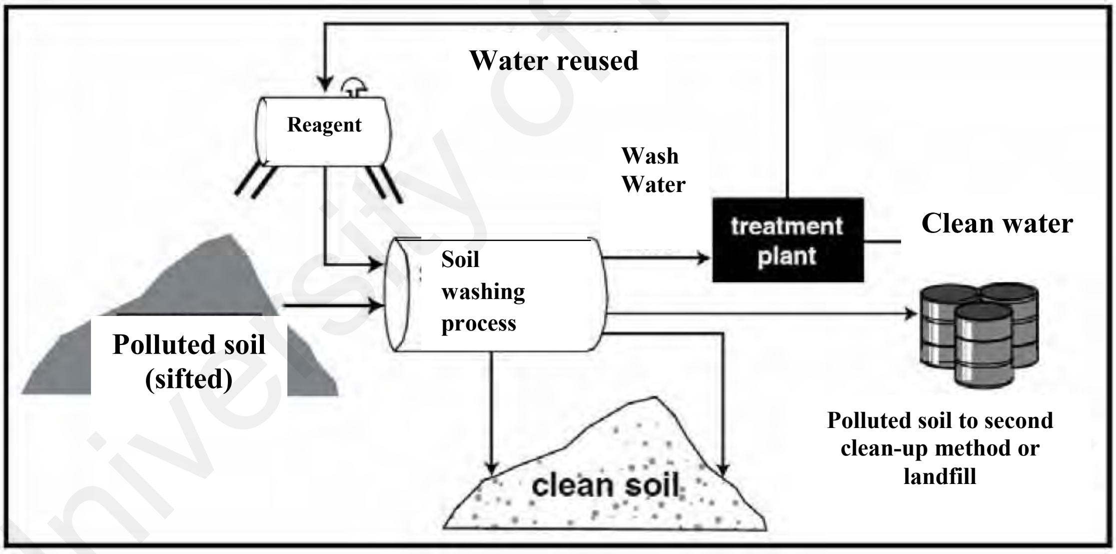


Figure 1.2 Schematic of *ex-situ* Soil Washing Process (USEPA. www.epa.gov)

Many researchers conducted research based on the removal of contaminants from weathered and freshly contaminated soils using the washing technique, and received good results, such as Urum et al. (2003) and Urum (2004) who had used different types of soils and surfactant using different washing techniques which included test tubes, stirred tank reactors, packed column, and an air bubble assisted stirred tank reactor. Han & Ni (2009) also conducted a research using weathered contaminated soil treated by a class of green non-ionic surfactant (alkyl polyglucosides) mixed with inorganic salts (NaCl and Na_2SO4). Xing & Jia (2000), Peng *et al*. (2011), Urum (2004) and others are researchers who had obtained very interesting results in their research, which is mostly based on the remediation of waste oils using the washing technique and surfactants, proved the high performance and effectiveness of washing techniques in the remediation of numerous types of hydrocarbon contaminated soils.

The majority of researchers suggested the remediation of the used engine oil contaminated soils at the early stages of spillage, because the weathering process will be a big obstacle faced the remediation procedure, as volatile and semi-volatile elements are released to the air and soluble compounds affected the water system (Han and Ni, 2009). However, at the end of the weathering process, only concentrates and heavy metal compounds will bind to the soil particles and inhibit most of the remediation techniques. According to Han and Ni (2009) and Peng *et al*. (2011) results, it is clear that combination of two remediation techniques and use of more than two type of surfactants is the only way to enhance the degree of contaminant removal efficiency to the desired level. Nevertheless, the physical and chemical characteristics of the contaminated soil such as porosity, moisture, grain size, nutrients and contaminant itself play important roles during the remediation process (Urum, 2004).

1.2 Statement of problem

Most accidents and leakage of used engine oil onto soil often occurs at residential areas. Since the contamination is very small as compared to serious spillage of crude oil, it is not economical to apply effective *in-situ* techniques (Mohammed, 2004). However, applying the *ex-situ* technique, the contaminated soil can be removed and transported where treatments can then be applied under controlled conditions for optimal temperature, volume and concentration of mixed liquor including bacteria and its biosurfactant, agitation, and washing time. Furthermore, remediation based on the use of soil washing treatment has been proposed by many researchers as a promising innovative remediation technology due to its potential in treating a huge range of contaminants. Also, the washing technique is less time consuming as compared to phytoremediation and bioremediation which is largely affected by climatic factors (Urum *et al*., 2004; Mohammed, 2004).

In order to test the effectiveness of the soil washing technique and evaluate the optimum conditions for the treatment, this study focused on Taguchi Experimental Design Method and using *bacillus salmalaya strain* 139I with its biosurfactant as a solution of washing, at different concentrations. Solution volume, washing temperature, washing time and shaking speed are the other variables to be tested (Urum, 2004; Urum *et al*., 2004). Weathered contaminated soil will be treated because the results will be more realistic compared with non-weathered contaminated soil (Han and Ni, 2009), which although have better removal efficiency but not realistically possible conditions for the treatment.

1.3 Aim of research and objectives

Aim of this study is to find effective method to decontaminate used engine oil contaminated soil without harm its physical and chemical properties.

This research has the following specific objectives dealing soil contaminated with used engine oil:

I. To characterize the soil contaminated used engine oil.
II. To test the effectiveness of washing technique using *Bacillus salmalaya strain* 139I.

III. To investigate the optimal conditions and to test the most influential variables of the washing technique.

IV. To determine the residual compounds after the application of washing technique.

1.4 Project Summary

The project was carried out in five stages as indicated in Figure1.3.

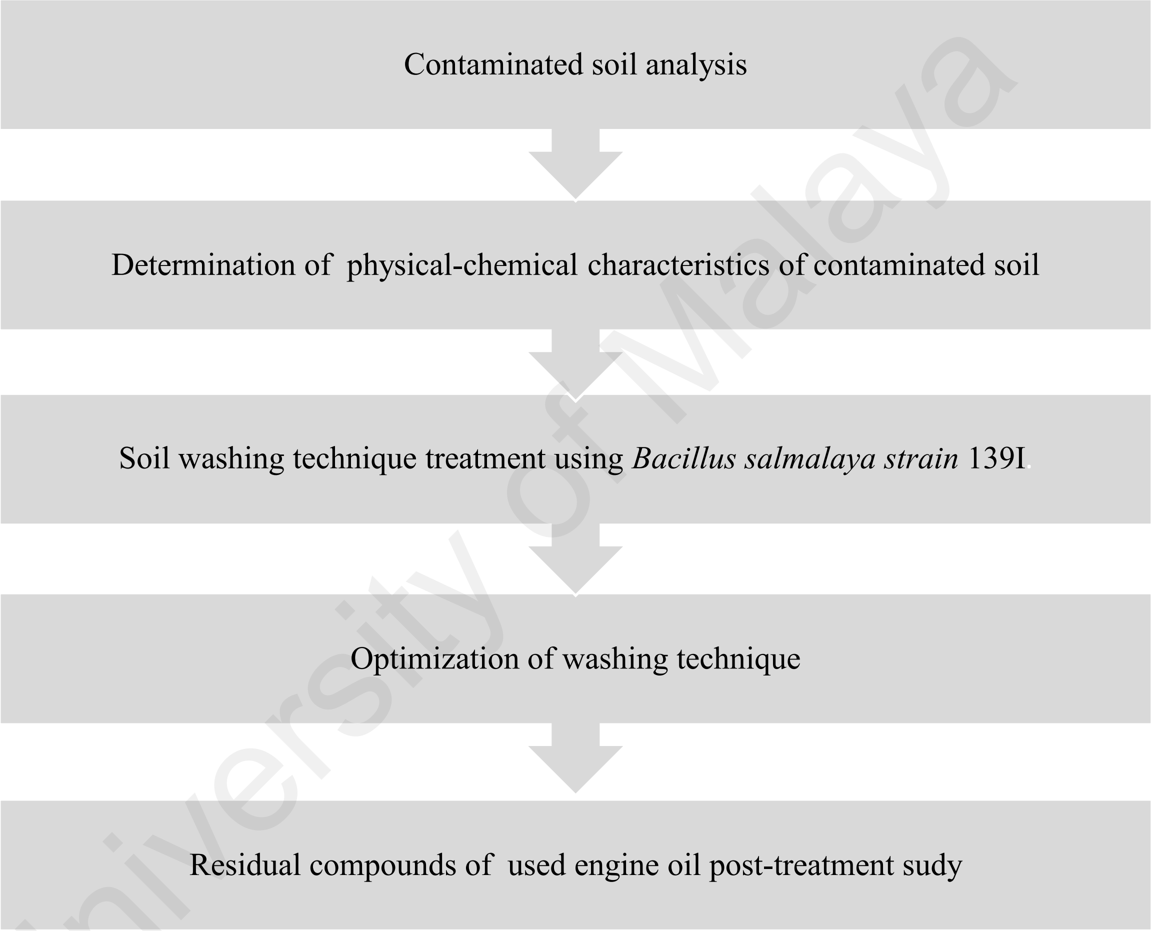


Figure 1.3 Simplified research flow

CHAPTER TWO

LITERATURE REVIEW

2.1 Overview on the environmental pollution and impacts from used engine oil

Uncontrolled disposal of waste oil into gutters, water drains, open vacant plots and farmlands can have numerous short and long-term impacts and consequences for human and ecosystems (Nixon & Saphores, 2007; Achuba & Peretiemo, 2008). Due to the increase in the rate of engine oil consumption from the upsurge in the number of vehicles and generator mechanics, chronic pollution as a result of used engine oil is more widespread than crude oil pollution (Achuba & Peretiemo, 2008).

The lungs and skin are parts of human anatomy that is majorly affected by the toxicity of used engine oil and other lubricating oils (Obayori *et al.*, 2014). Inhalation, aspiration or ingestion of these materials produces lipid pneumonia and lipid granuloma of the lung (Vazquez, 1989). Besides that, the effects that were observed on the skin were such as: eczematous dermatitis, contact dermatitis, folliculitis, oil acne, lipid granuloma and melanosis (Nwite & Alu, 2015). In addition, a number of reports associated with occupational exposure to waste oils reported squamous-cell cancers of the skin, scrotal cancer, bladder cancer and lung cancer (Vazquez, 1989). Likewise, numerous studies reported the serious effects of used and waste oil on microbial activities, germination of seeds, seedling growth, chlorophyll production, protein content, leaf production and biomass in the plants (Vazquez, 1989; Odjegba & Atebe, 2007; Adedokun & Ataga, 2007; Samuel, 2011; Obayori *et al.*, 2014; Nwite & Alu, 2015).

2.1.1 Used engine oil - air pollution and its impacts

Used engine oil is not liberated into the environment only by spillages or accidents but also through volatile and semi-volatile compounds that can easily spread to the air immediately after spillage on the soil or aquatic bodies. Moreover, it can be released into the environment from the exhaust system during engine function and also from engine leaks (Anoliefo & Edegbai, 2000). The burning of used engine oil in inadequate installations and conditions, when employed as an auxiliary fuel in some special burners also carried serious air pollution effects (Figure 2. 1). Metal emissions released can be in particle form (dust) and in gas form, which can be represented by 0.81 – 1.29 % Pb and 0.23 – 0.29 % Zn. SO_2, NO_x and HCl which are very toxic gases derived from high amounts of sulphur, nitro-compounds, solid particles and chloride that can emit to the air during combustion operations (Vazquez, 1989).

Figure 2.1 The use of used engine oil as auxiliary fuel in some special burners

Particles and gas emissions can affect (in percentage) the reduction of cellular ATP in the rabbit alveolar macrophage cytotoxicity assay and also among the most toxic for the mouse bacterial infectivity assay (Vazquez, 1989; Nixon & Saphores, 2003). Moreover, oil vapours are toxic to some species - such as mammals and birds - as toxic gases can easily damage the central nervous system of these animals and cause reproductive problems, particularly for birds where dust emissions coat eggs and cause the inhibition of gas exchange by the sealing of pores; the fact that leads to abnormal embryo development (Nixon & Saphores, 2003).

2.1.2 Used engine oil – water pollution and its impacts

Spilled used engine oil and synthetic lubricants in the streets, road and in car parks may enter stormwater runoff and directly affect the nearest water bodies such as rivers, bays, oceans and lakes (Figure 2.2). It can disperse more readily into water and be absorbed more easily into soft tissues and distort an unbelievable amount of aquatic organisms such as phytoplankton species, crustaceans, mollusks and fish populations (Vazquez, 1989; Samuel, 2011). On the other hand, some individual species of yeast and bacteria which are not pollution-sensitive may not be affected.

Figure 2.2 Used engine oil transportation through stormwater runoff to water bodies

Ayoola & Alajabo (2012) reported a histopathology study on the effects of engine oil in vital processes such as growth, reproduction, detecting early effects in cells, tissues and organs. The tested organism was *sarotherodon melanotheron*, which is known under the name of black jaw tilapia, which, is one of the most popular brackish water fish species in West Africa. The toxicity tests were based on histopathological alterations of kidney, gill and muscle on exposure to engine oil (Ayoola & Alajabo, 2012). Results show that severe congestion was observed on the gill tissues exposed to high concentrations of engine oil, with calcification and inflammation of the gills. The decrease in quantity of dissolved oxygen in the water due to the oxidative biodegradation of engine oil compounds could also lead to significant respiratory distress. Moreover, cytoplasmic vacuolations, which is probably caused by glycolysis leading to microsomal and mitochondrial dysfunctions, was observed on the kidney tissues at high concentrations of engine oil (Ayoola & Alajabo, 2012). Thus, large quantities of toxic pollutants can accumulate in fish organs, and can cause the peripheral sense organs to malfunction for people living in coastal areas and rely on fish for their consumption animal protein (Ayoola & Ajani, 2007).

2.1.3 Used engine oil – soil pollution and impacts

Environmental sustainability depends largely on a sustainable soil ecosystem (Adriano *et al*., 1998). The ecosystem is altered and agricultural activities are affected once the soil is exposed and affected by the waste oil pollution (Adedokun & Ataga, 2007). Once used engine oil is released to the soil as shown in Figure 2.3, it can create a serious changes in all soil parts, including, aeration, immobilization of soil nutrients and the lowering of pH (Achuba & Peretiemo, 2008). The reduction in soil porosity due to hydrocarbons leads to oxygen deficiency and the increase in the number of anaerobic nitrogen-fixing microorganisms, such as *Clostridium (*Vazquez, 1989). As a result, it was found that the number of nitrogen -fixing, denitrifying and ammonifying microorganisms in hydrocarbon-contaminated soils are greater than in uncontaminated soils (Ismailov, 1983). Thus, auto-regulation of the nitrogen cycle due to the presence of hydrocarbons reduces the effects of pollution in this cycle, but it does not occur in all soil biological cycles.

Figure 2.3 Used engine oil spillage onto soil

Odjegba and Atebe (2007) conducted a study aimed at investigating the effects of used engine oil on carbohydrate, mineral elements and nitrate reductase activity of *A. Hybridus* (leafy vegetable). After eight weeks of treatment, they found that the hydrocarbon degradation by microorganisms lead to the liberation of carbon atoms, which react with water and cause reduction in pH. As a result, the pH showed a decreasing trend in used engine oil treated soil according to the concentration of used engine oil treatment (Odjegba & Atebe, 2007). It was also observed that the soil treated with high used engine oil concentrations (more than 2%) had no significant decrease in Electricity Conductivity due to the hydrophobic nature of used engine oil, which limits and inhibits the availability of nutritional elements for plant absorption. Thus, plants exposed to sub-lethal doses of used engine oil showed lower carbohydrate content compared with those not treated indicated a negative impact on total plant biomass (Odjegba & Atebe, 2007).

Milala *et al*. (2015) tested pH and Electricity Conductivity in used engine oil contaminated soil samples (0.25% to 2%) and uncontaminated soil samples. They found that not much difference were shown in the soil pH between the control and the contaminated soil samples; pH was found to be pH 7.0 for control and from pH 6.9 to 6.0 for used engine oil contaminated samples, according to the used engine oil concentrations (Milala *et al*., 2015). Achuba and Peretiemo (2008) obtained similar results for pH, where they tested the activity of two enzymes; Dehydrogenase (EC 1.1.1.1) and Catalase (EC 1.11.1.6) in soils contaminated with different used engine oil concentrations (0.25%, 0.5, 1, 1.5, and 2%). The results showed a slight decrease for pH but no significant difference between pH of control and pH of treated samples (Achuba & Peretiemo, 2008).

Used engine oil had effects on germination time, percentage, plant height, leaf production and biomass. This fact is proven by many researches; such as Adedokun &

Ataga (2007), who had found that growth parameters of cowpea on soil polluted with spent engine oil were comparatively low compared to the control. However, soil amended with waste cotton and saw dust showed improvement in plant growth (Adedokun & Ataga, 2007). Similar results were obtained by Nwite and Alu (2015), who had tested the effects of different levels of spent engine oil on grain yield of maize. Maize seed germination was delayed and inhibited. Besides that, the heavy metals of lead and cadmium were found to have been uptaken by the maize grain (Nwite & Alu, 2015). Results confirmed the fact that hydrocarbons inhibited seed germination and plant growth (Adedokun & Ataga, 2007; Nwite & Alu, 2015). As a result, it was clear that amendment of contaminated soils using organics and wastes can improve the soil properties, leads to more plant growth and production.

2.2 Overview on chemical constituents of crude oil

An overview on the chemical constituents of crude oil and different refining processes will help to more clarify the origin and chemical composition of used engine oil, wich present our contaminant. Actually, each crude oil is unique and is a complex mixture of thousands of compounds. Most of the components in crude oil are hydrocarbons (organic components composed of carbon and hydrogen atoms) with additions of small amounts of other hetero-elements such as sulfur, as well as nitrogen and certain metals (e.g., nickel, vanadium) (ICCT, 2011).

The physical and chemical properties of crude oil depend not only on the number of carbon atoms in the molecule (Table 2.1), but also on the nature of the chemical bonds between carbon atoms and hydrogen, and hetero-atoms in various ways; single bonds, double bonds, and triple bonds in order to form different classes of hydrocarbons, such as, paraffins, aromatics and naphthenes (Figure 2.4). However, Olifins usually are not present in crude oil but they are produced in certain refinery operations, which is dedicated to gasoline production (ICCT, 2011). Aromatic compounds contain benzene rings in their chemical structure, which affects their evaporation rate, and solubility in water (Urum, 2004). Moreover, asphaltens do not appreciably evaporate, disperse or degrade but can stabilize water-in-oil emulsions when they are present in quantities greater than 3%-mass (Urum, 2004). Based on crude oil composition, it is clear that used engine oil compositions are more complicate and dagerous, due to presence of heavy metals and high doses of PAHs.

Table 2.1 The general properties of different hydrocarbon ranges (adapted from CCME, 2008a).

Number of Carbon Atoms	Properties
< 10	Highly volatile, soluble, mobile, easy biodegraded.
10-16	Volatile, lower solubility, somewhat less mobile, easy biodegraded.
16-34	No – volatile, limited solubility, less biodegradable than C_{10} - C_{16}
> 34	No-volatile, limited solubility, not easily biodegradable.

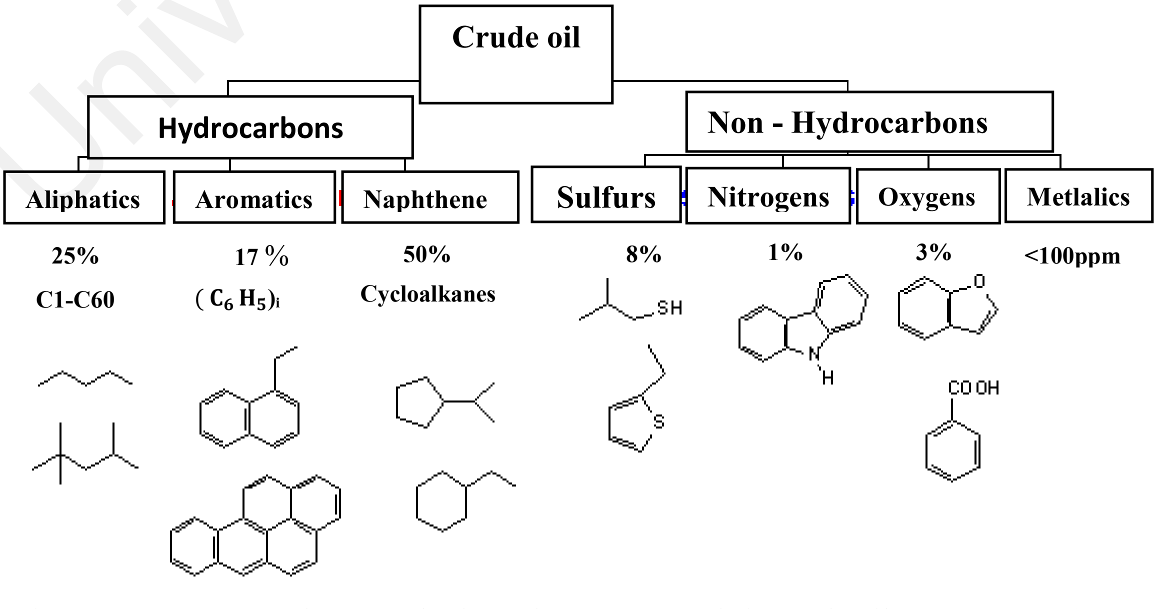
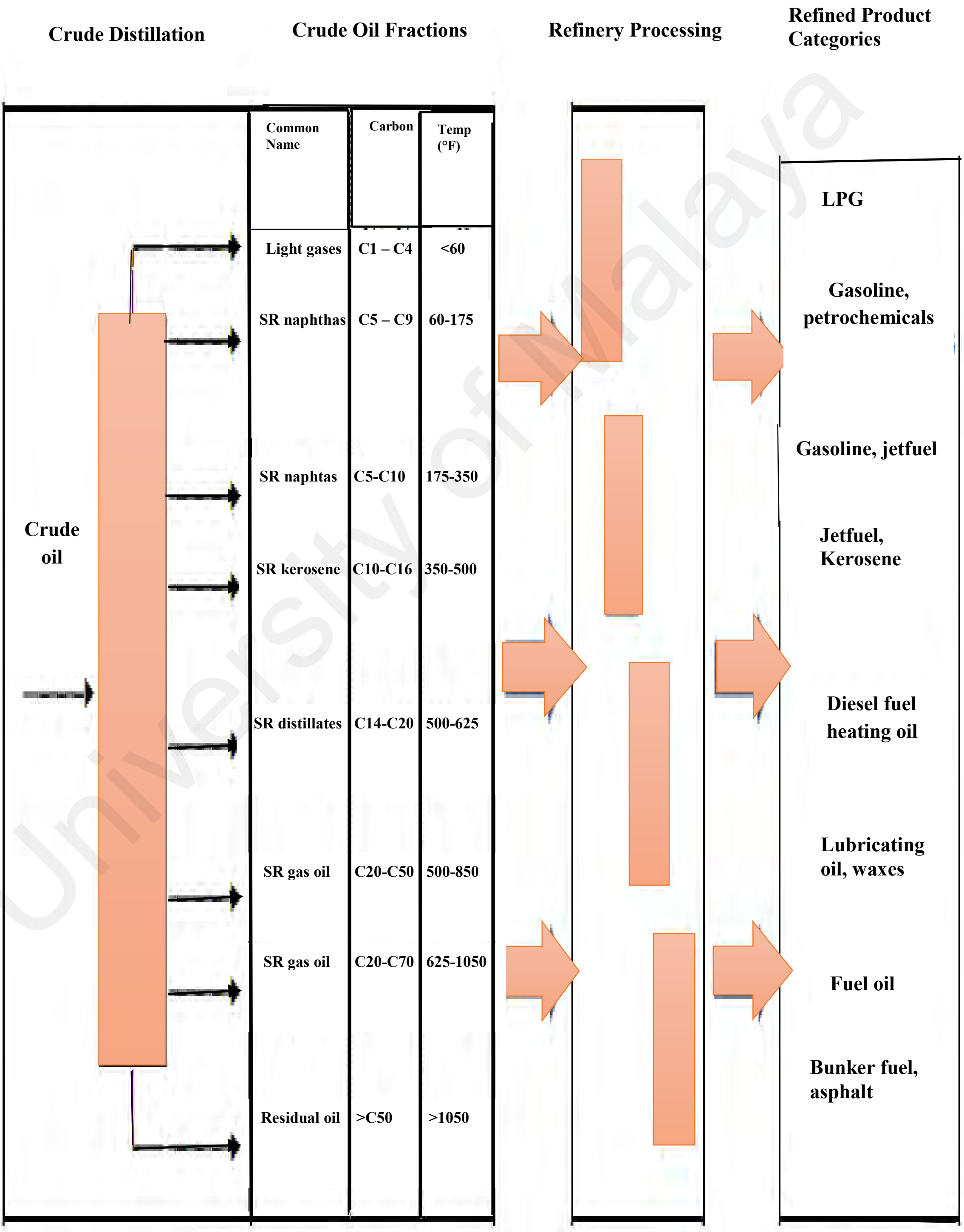


Figure 2.4 Important classes of hydrocarbon compounds in crude oil (Harraz, 2015)

2.3 Crude oil to engine oil and other by-products (refinering process)

Once discovered, drilled and brought to the earth's surface, crude oil is transported to a refinery by pipeline, ship or both. At the refinery, it is treated and converted into consumer and industrial products (Figure 2.5). Three major refinery processes change crude oil into finished products (Table 2.2) (ICCT, 2011; Colwell, 2009).



Common Name	Carbon	Temp (°F)
Light gases	C1 – C4	<60
SR naphthas	C5 – C9	60-175
SR naphtas	C5-C10	175-350
SR kerosene	C10-C16	350-500
SR distillates	C14-C20	500-625
SR gas oil	C20-C50	500-850
SR gas oil	C20-C70	625-1050
Residual oil	>C50	>1050

Figure 2.5 Schematic view of crude oil distillation and downstream processing (ICCT, 2011)

Table 2.2 Refinery classes and characteristic yield patterns (Hart Energy Consulting, 2011)

Refinery Category	Characteristic Processes	Product Yield Profile (Vol%)		Comments
		Gasoline	Diesel and Jet	
Topping	Crude distilation	31	30	• Product Sulfur levels same as crude fraction sulfur levels • Product yields and quality determined solely by crude properties • Gasoline has low octane
Hydroskimming	Crude distilation Reforming Hydrotreating	28	30	• Product Sulfur levels controllable by hydrotreating • Some capability to improve product yields and quality • Gasoline octane improved by reforming
Conversion	Crude distilation FCC and/or hydrocracking Reforming Alkylation and other upgrading Hydrotreating	44	32	• Product sulfur levels controllable by hydrotreating • Substantial capability for yield and quality improvement
Deep Conversion	Crude distilation Coking FCC and/or hydrocracking Reforming Alkylation and other upgrading Hydrotreating	47	42	• Product sulfur levels controllable by hydrotreating • Maximum yields of high-value refined products • Maximum capability for quality improvement • Essentially all residual oil (destroyed)

Note: Gasoline and distillate fuel yields are nominal estimates, based on the processing of an average quality crude oil

2.3.1 Separation

The first step is to separate the crude oil into its naturally occurring components. This is known as separation and is accomplished by applying heat through a process called distillation (Figure 2.5). Separation is performed in a series of distillation towers, with the product from the bottom of each tower feeding the next. Product streams from the distillation tower range from gases at the top to very heavy, viscous liquids at the bottom. However, in all cases, these product streams are still considered 'unfinished' and require further processing to become useful products (Colwell, 2009; Aitani, 2016).

2.3.2 Conversion

The process found that the existence of gasoline was too little with too much heavy oil in the crude oil, even after distillation process, hence, indicating why the conversion processes are so important. Their primary purpose is to convert low valued heavy oil into high valued gasoline. All products in the refinery are based on the same building blocks; carbon and hydrogen chains, where the longer the carbon chain, the heavier the product will be. Fluidized Catalytic Crackers (FCCs), delayed coking and Hydrocracking are processes used to convert heavy material into more valued products with the use of high temperature and a catalyst, which promotes the reaction that breaks the heavier chains to lighter chains such as Liquefied Petroleum Gas (LPG), gasoline and diesel, and jet fuel and diesel (Colwell, 2009). Although, by-products at this stage became less viscous and heavier, a next series, to remove the sulfur and breakdown the hydrocarbon chains to minimum, are needed. The light liquid products are sent directly for Catalytic Reforming and the other liquid products are blended directly into jet fuel and diesel (ICCT, 2011). On the other hand, there are other hydro-carbon chains that are too short; butane is produced as a by-product by combining *n*- butane isomer into a long chain using catalysts. At high temperatures and in the presence of hydrogen, the catalysts will reform paraffins into aromatics and the conversion process is known as Catalytic Reforming (Aitani, 2016). Some of the aromatics produced are sent to petro-chemical manufacturers, where they are converted to plastics and fabrics. The heaviest material in the refinery is the Vacuum Tower Bottoms (VTB) (Aitani, 2016).

2.3.3 Purification

Generally, purification is sulfur removal through the hydrotreating process, a process similar to hydrocracking but without converting heavy molecules into lighter ones. Unfinished products are contacted with hydrogen under heat and high pressure in the presence of a catalyst, resulting in hydrogen sulfide and desulfurized products. Finally, the hydrogen sulfide converts to elemental sulfur and water. The sulfur is sold as a refinery by-product (ICCT, 2011; Colwell, 2009).

2.4 Fresh engine oil to used engine oil transformation process and effects

Better understanding of transformation process of engine oil to used engine oil will clarify and illustrates the main differences in chemical compositions between them and the main causes in that.

2.4.1 Fresh engine oil

Engine oil is one of the several refined products of crude oil (Obayori *et al.*, 2014). It is widely used in automobile engines, hydraulic systems and industrial machines (Lopes & Bidoia, 2009). Fresh engine oil contains a higher percentage of fresh and lighter hydrocarbons; the base oil contains C16 - C36 hydrocarbons and more than 75% of cyclic-alkanes. Most c-alkanes in the base oil have 1 to 3 rings and long alkyl side chains. Ordinarily, it contains very small amounts of polycyclic aromatics hydrocarbons (PAHs) (Obayori *et al.*, 2014).

2.4.2 Transformation process and effects

Used engine oil is produced when fresh engine oil is subjected to high temperatures and high mechanical strain when running the vehicle or machinery for a stipulated time (Bhattacharya & Biswas, 2014). It is a brawn-to-black liquid mixture (Figure 2.6), consisting of low to high molecular weight (C_{16} to C_{36}) aliphatic and aromatic hydrocarbons, polychlorinated biphenyls, chlorodi benzofurans, lubricative additives, and decomposition products along with heavy metal contaminants, such as, zinc, lead and chromium, coming from engine (Abdulsalam *et al*., 2013). Thus, metals can build up in plants, animals, soil, sediments and non-flowing surfaces of water, and absorbed into various tissues of human, plants and animals by their movement in the environment, which can result in serious health problems such as anaemia, tremor and consequently, resulting in death (Obayori *et al*., 2014). Other health hazards include mutagenicity and carcinogenicity (Abdulsalam *et al*., 2013).

Figure 2.6 Sample of used engine oil sample (Indiamart, 2015)

A thousand million gallons of used engine oil is generated annually from mechanical workshops, which is not recycled but spilled and dumped by automobile and generator mechanics into runoff, gutters, water drains and open vacant plots and farmlands (Bhattacharya & Biswas, 2014). The fact require a serious decisions and adjustments in some environmental legalisations and policies, moreover, improvements of tools and techniques of treatment. Out of this, 1L is enough to contaminate 1 million gallons of fresh water (Bhattacharya & Biswas, 2014).

2.5 Contamination mechanisms

As aforementioned, different pollutants such as used engine oil can enter the environment directly as a result of accidents, spills during transportation, leakage from waste disposal or storage sites, and also from industrial facilities (Riser, 1992) (Figure 2.7). The difficulties associated with the clean-up of petroleum-contaminated sites have demonstrated that there is a need to develop remediation technologies which are feasible, quick and deployable in a wide range of physical settings.

Figure 2.7 Used engine oil spill during transportation (Alan, 2013)

2.6 Remediation technologies

Various treatment technologies have been proposed for treating contaminated sites. Generally, the methods were divided into three main categories; biological treatment, physicochemical treatment and thermal treatment (Figure 2.8). According to the areas where treatment can be applied, researchers divided it into two basic processes namely *In-situ* and *Ex-situ* remediations (UNIDO, 2014). As our research is based on soil washing techniques, which is under physico-chemical treatment (*Ex- Situ* treatment), it is not necessary to go through all soil remediation technologies, but suffice to mention the overview on *In-situ* remediation technique with examples of the technique explanation (bioremediation and phytoremediation) and examples for the technique of Ex-situ remediation techniques (Landfarming and thermal process). Despite, many techniques proposed as an appropriate technique for the treatment of contaminated soils, nevertheless, each technique has a numerous of limitations.

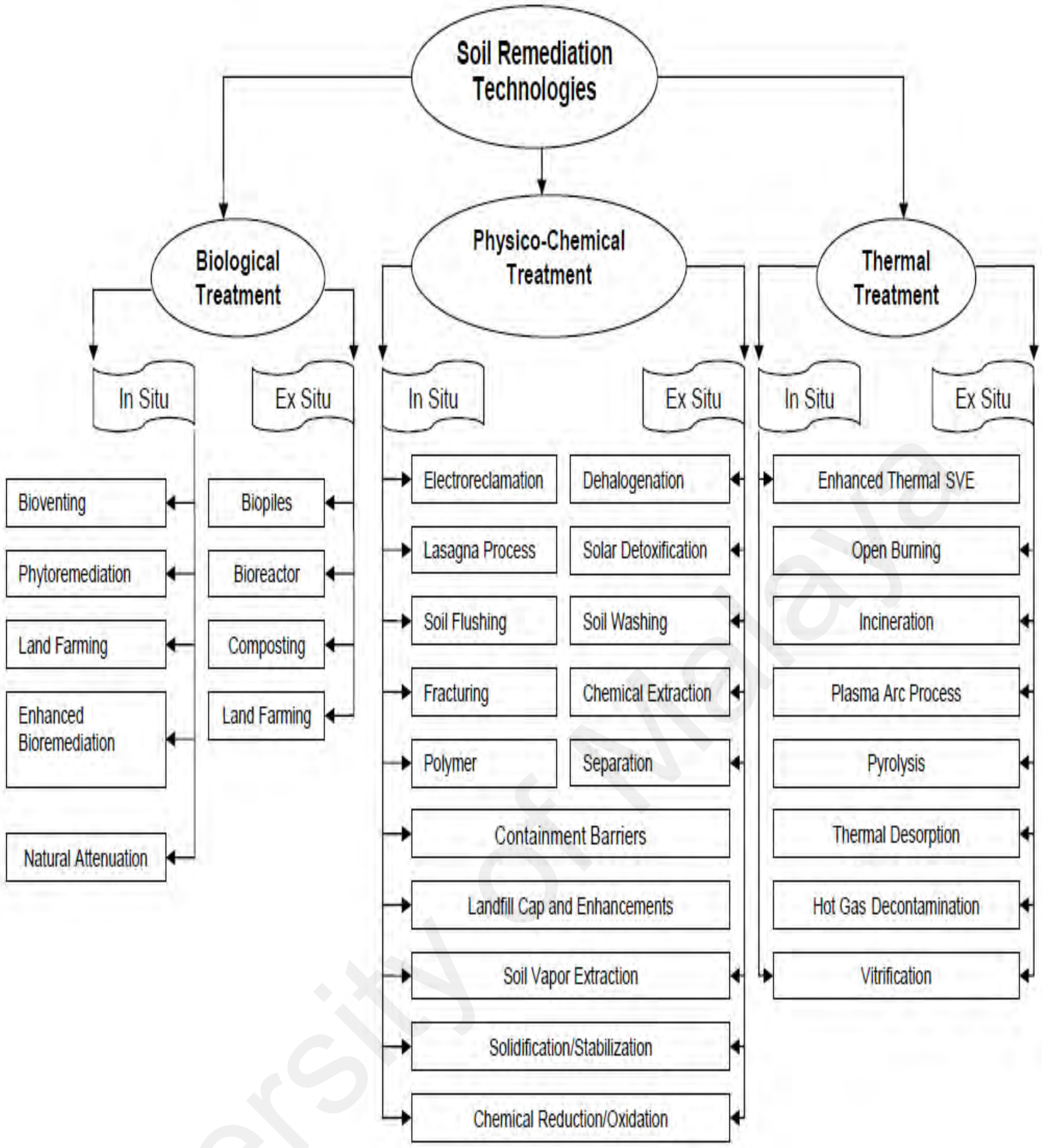


Figure 2.8 Soil remediation technologies (UNIDO, 2014)

2.6.1 *In-situ* treatment methods

Treatment was carried out in the subsoil without any excavation or scrap operations. Treatment includes techniques such as bioremediation, phytoremediation along with physical, chemical, and thermal processes (Table 2.3). *In-situ* remediations are less costly compared with *ex-situ* remediations, due to the high cost of excavation and transportation. However, these techniques are less controllable and less effective (Mohammed, 2004; Williams, 2006). Two most used and effective techniques, which are the *in-situ* bioremediation and phytoremediation, will be explained.

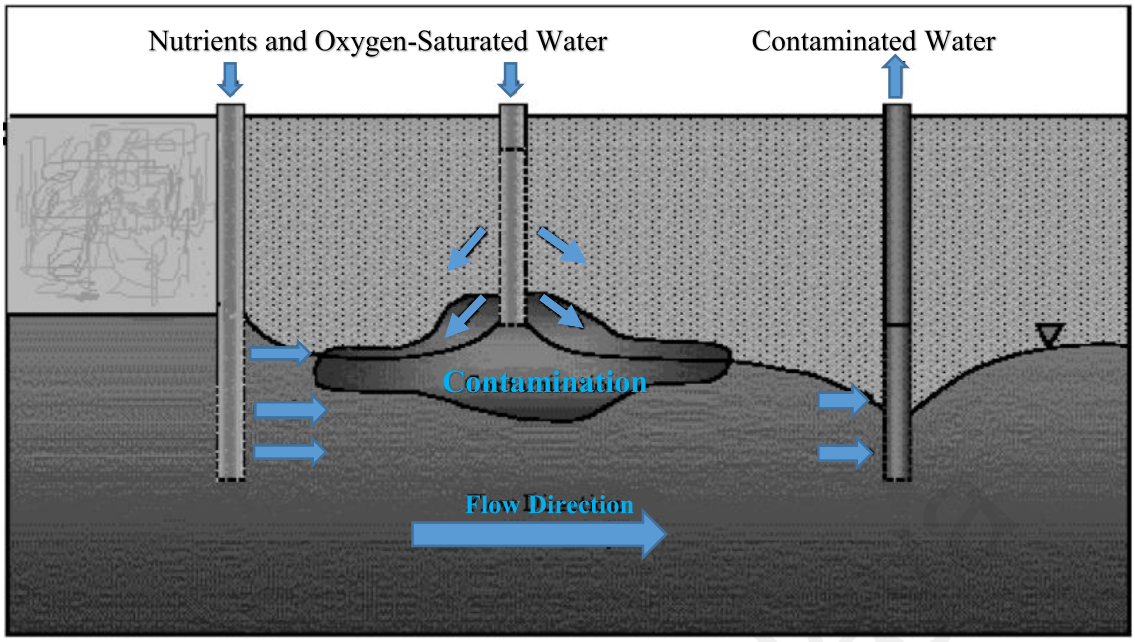


Figure 2.9 Stimulation of indigenous bacteria by injection of air and nutrients (Mohammed, 2004)

Table 2.3 Compound removal and soil constraints for *in- situ* remediation techniques (Williams, 2006)

In- Situ Techniques	Effectively Removed Compounds	Soil constraints
Thermal Remediation	BTEX	Must be homogeneous, have high permeability and low organic content
Chemical Oxidation	PAH, TCE	Must be permeable
Soil Vapor Extraction	BTEX	Must have low percent fines and correct moisture content
Bioventing	PAH, nonchlorinated solvents	Must be homogenous, may be unsaturated
Biosparging	PAH, nonchlorinated solvents	Must be homogeneous and saturated
Bioslurping	Free Product (Petroleum)	Must be homogeneous and saturated
Phytoremediation	TPH, BTEX, PAH, TNT, RDX	Must have contamination in shallow soil

2.6.1.1 *In-situ* bioremediation

Generally, bioremediation is a technique of acceptance to remediate surface and subsurface contaminated soils and aquatic systems, based on enhancing and motivating the sets of microorganisms to break down contaminants by adding or injecting oxygen and nutrients such as carbon, nitrogen and phosphorous (Figure 2.9). The bioremediation technique does not environmentally damaging process. However, it has limitations such as: space requirements, monitoring difficulties and extended treatment time (Mohammed, 2004; Dadrasnia *et al*., 2013).

2.6.1.2 Phytoremediation

Phytoremediation is an *in-situ* technique which is a relatively new concept and has not yet been proven in the marketplace (Dadrasnia *et al*., 2013). It is based on the usage of grasses, legumes, and aquatic plants, and deep rooted trees (Figure 2.10). It has been used to remove hydrocarbons such as Total Petroleum Hydrocarbons, benzene, toluence, ethylbenzene, xylenes, Polycyclic Aromatic Hydrocarbons, Trinitrotoluene, and radio nuclides (Dadrasnia *et al*., 2013). Plants can remove pollutants from ground water and soil. Roots help to support a wide variety of microbes by providing an organic carbon source (Williams, 2006). Other applications of phytoremediation include landfill caps, buffer zones for agricultural runoff, industrial wastewater treatment and sometimes used as a final polishing step in conjunction with other techniques. Nevertheless, successful phytoremediation is limited by many factors such as time consumed, soil characteristics, adequate water and nutrients, and suitable climate conditions to support plant growth (Williams, 2006; Dadrasnia *et al*., 2013). Moreover, deeper contamination may require assistance from other techniques (Dadrasnia *et al*., 2013). Plants can remediate the contaminated soil under several mechanisms such as; direct uptake and integration of contaminant into plant biomass, phytostabilization of contaminant in the subsurface, releasing of enzymes and stimulation of microbial activities in the rhizosphere (Dadrasnia *et al*., 2013).

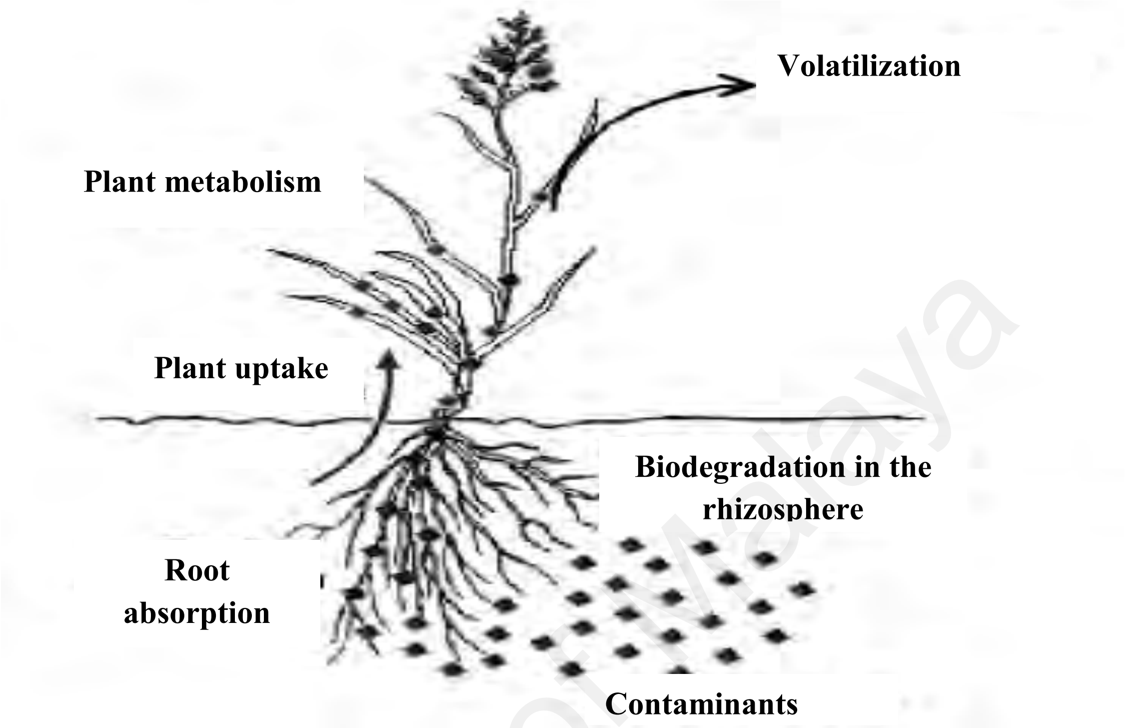


Figure 2.10 Schematic of different mechanisms of contaminant removal by plant (Kamath et al., 2004).

2.6.2 *Ex-situ* treatment methods

2.6.2.1 Overview on general *ex-situ* treatment methods

With the ex-situ processes, the contaminated soil must be removed to an off site remediation facility. This method can be applied if the amount of contaminated soil is small or if the oil contamination occurred at the surface of residential areas or industrial estates, making an *in-situ* treatment impossible. *Ex-situ* treatment includes techniques such as landfarming, biopiling, and processing by bioreactors along with thermal, chemical, and physical processes (Table 2.4). Ex-situ treatments of contaminated sites lead, in many cases, to a greater degree of remediation as compared to *in-situ* treatment due to the controllability of many factors such as, moisture, temperature, salinity, and pH.

However, many people are looking towards *in-situ* remediation techniques due to the costs of excavation and transportation of soil used in *ex-situ* remediations (Mohammed, 2004; Williams, 2006). Landfarming and thermal techniques are the most applicable techniques to remediate a wide range of contaminants including hydrocarbons

Table 2.4 Compound removal and soil constraints for *ex-situ* remediation techniques (Williams, 2006)

Ex Situ Techniques	Effectively Removed Compounds	Soil constraints
Thermal remediation	TPH, PAH, BTEX, PCB, PCP, PCDD, PCDF	No specific constraints
Soil washing	TPH, BTEX, PAH, PCB, Heavy metals, dioxins	Must be made homogeneous to treat
landfarming	PAH, PCP	No specific constraints
Biopiling	BTEX, PAH, TNT, RDX	Must be made homogeneous to treat
Bioreactors	PAH, PCB	Must be separated by particle size in order to treat

2.6.2.2 *Ex-situ* landfarming remediation

Landfarming is one of the most practical *ex-situ* biological processes used to remove pollutants such as PAH and PCP using microorganisms, such as bacteria and fungi. The process is based on the spreading of excavated contaminated soils in layers no more than 0.4 m thickness and covered by concrete or clay membrane (Figure 2.11). The pH will be adjusted to approximately pH 7, by adding crushed limestones, and moisture and nutrients, which are added to enhance the remediation process by stimulating aerobic microbial activity (Williams, 2006). Prior tested bacteria will be added to soil to achieve speedy degradation. Before covering the contaminated soil, it is necessary to mix the contaminated soil to increase the contact between hydrocarbons and microorganisms and also to increase the amount of oxygen in the soil (Khan *et al.*, 2004). However, some factors may limit the applicability and effectiveness of the process include; the need of large amount of space and the requirement of suitable climatic conditions.

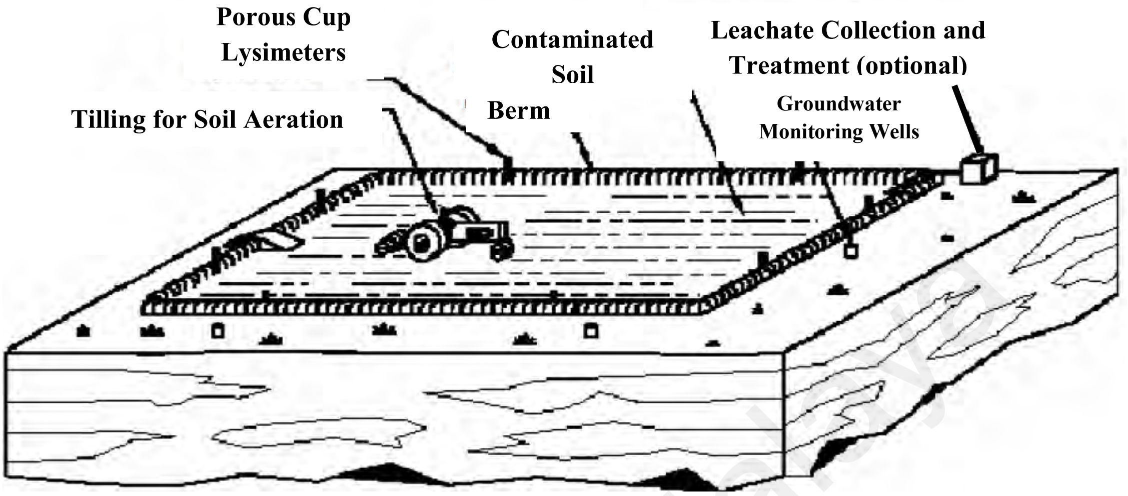


Figure 2.11 Landfarming Technique (United States Environmental Agency, 2004)

Landfarming techniques have been practiced by the petroleum industry for more than two decades (Riser, 1992). However, according to Khan *et al*. (2004), Paval and Gavrilescu (2008) the performance of landfarming technology is influenced by the requirement of certain factors such as:

- Volatile compounds must be pre-treated.
- Not efficient to degrade heavy components.
- Possibility of pollutant transfer to surroundings.
- Optimum temperature at 25-40°C.
- Optimum moisture to get highest degradation is at 18% to 33%.
- Adding Nitrogen to reduce the ratio of C: N to 9: 1 will ensure high efficiency of process.
- pH need to be maintained within the range of 6.5 to 7.5.

2.6.2.3 *Ex-situ* thermal remediation

According to Van *et al.* (2002), *ex-situ* thermal remediation consists of transferring pollutants such as TPH, PAH, BTEX, PCB, PCP, PCDD and PCDF from the soil to a gas phase following three major steps: soil conditioning, thermal treatment, and exhaust gas purification (Figure 2.12). Soil is usually heated to a low temperature range of 350°C - 550°C. However, gases are not destroyed but burnt in an after-burner chamber at approximately 1200°C so dioxins are destroyed (Koning *et al*., 2000). The efficiency of desorption can be greater than 99% but the presence of water and clay reduces its effectiveness since more than the contaminant water is being vaporized and air generated from silt and clay will interfere with pollutant air emissions (Khan *et al*., 2004).

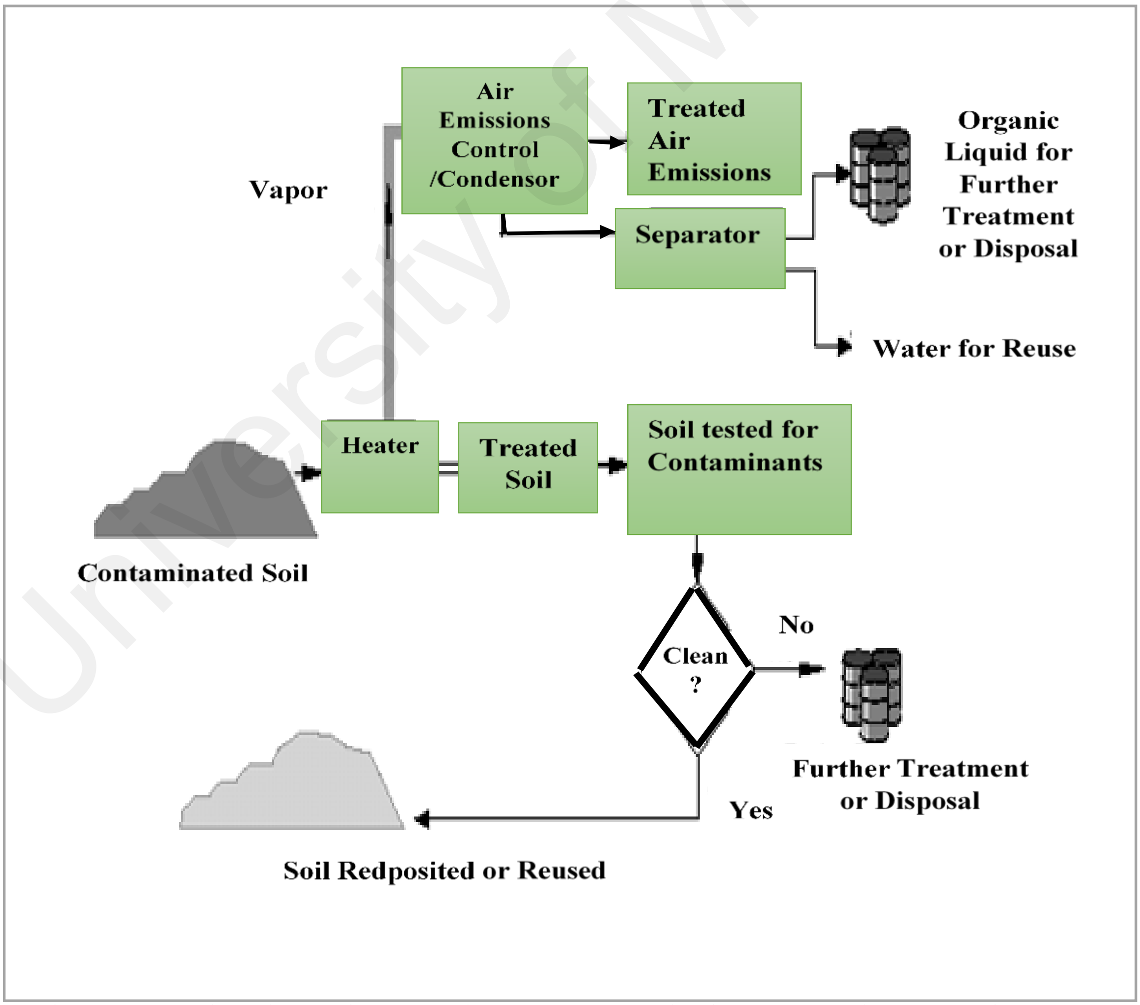


Figure 2.12 The Thermal Desorption Process (United States Environmental Agency, 1996)

2.6.2.4 Contaminated soil washing technique

Traditional soil washing in the mid-1980s referred to scrubbed fine particles, which contain the most portion of contaminants from larger particles based on water solution (Figure 2.13). The USA, the Netherlands and Germany were among the countries that provided facilities and frameworks for the soil washing technique (Griffiths, 1995). However, many researchers have developed new techniques, based on mixing water with additives such as alkaline, acids and surfactants to separate contaminants from fine particles by mobilization and solubilization mechanisms. Biosurfactants are selected based on their high capacity to mobilize and solubilize a major range of contaminants and also their environmental health effects (Griffiths, 1995; Urum *et al*., 2004). Semi-volatile organic compounds (SVOCs), petroleum and fuel residuals, heavy metals, PAHs, and pesticides are contaminant groups which can be treated using soil washing technique (Semer & Reddy, 1996). Soil washing is cost-effective because it reduces the quantity of material that would require further treatment by other technologies (Khan *et al*., 2004; Semer & Reddy, 1996; Griffiths, 1995).

Figure 2.13 Soil washing process (Griffiths, 1995).

2.6.2.4.1 Soil washing steps

Excavation of contaminated soil, which is the process of removing contaminated surface or subsurface materials from contaminated zones, is the quick and surest initial method to reduce human and environmental risks at patch treatment. Before beginning the excavation of contaminated soil, it is necessary to determine contaminated soil borders by soil sample testing. Efficient excavation requires powered equipments such as backhoes and excavator trackhoes. Moreover, it is necessary to stock contaminated soil in plastic tarps or containers to prevent the contaminated soil from blowing or washing away (USDA, 2002; USEPA, 2012) excavation will be completed when the remaining soil test results around the holes meet the established clean-up levels (Figure 2.14).

Figure 2.14 Samples need to be collected to confirm that soil left onside is clean (USEPA, 2012)

The washing of contaminated soil was carried out in a small and transportable reactor (Figure 2.15) to ensure proper mixing and settling of contaminated soil (USDA, 2002). It is necessary to remove soil particles that are coarser than 2 inches before the contaminated oil goes through the washing process. Sand particles need only initial rinsing treatment through a soil scrubbing unit. Whereas silt and clay fractions that contain almost the total quantity of contaminants due to the strong adherence of contaminant to clay and silt particles, were treated using additives such as biosurfactant washing solutions or other detergents; only after this stage the contaminants were isolated from the soil in wastewater solution (Semer & Reddy, 1996). Finally, the contaminated wastewater is treated and recycled using a conventional wastewater treatment system, and the treated soil is placed in an appropriate location (Figure 2.16).

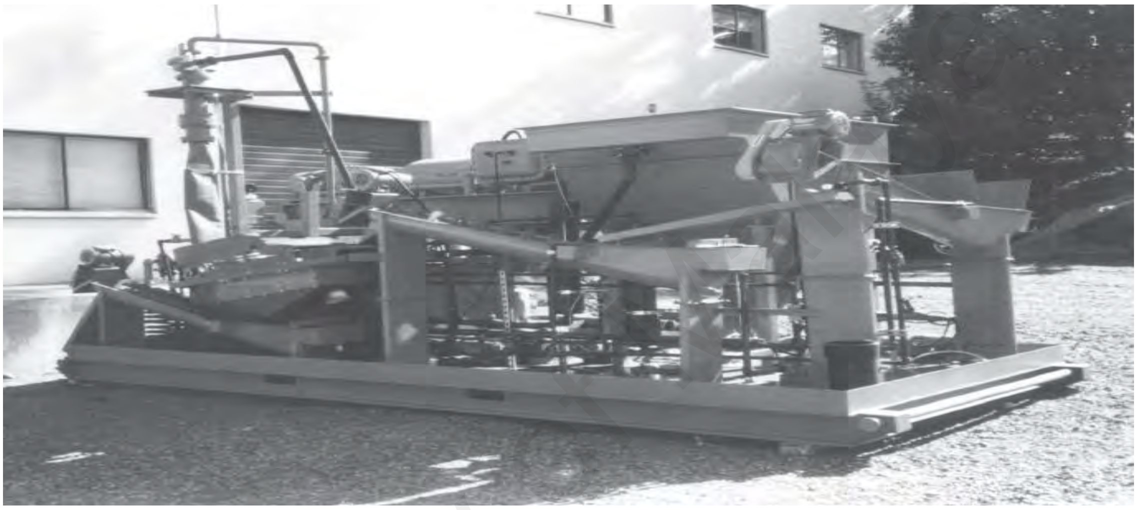


Figure 2.15 Typical soil washing unit (USDA, 2002)

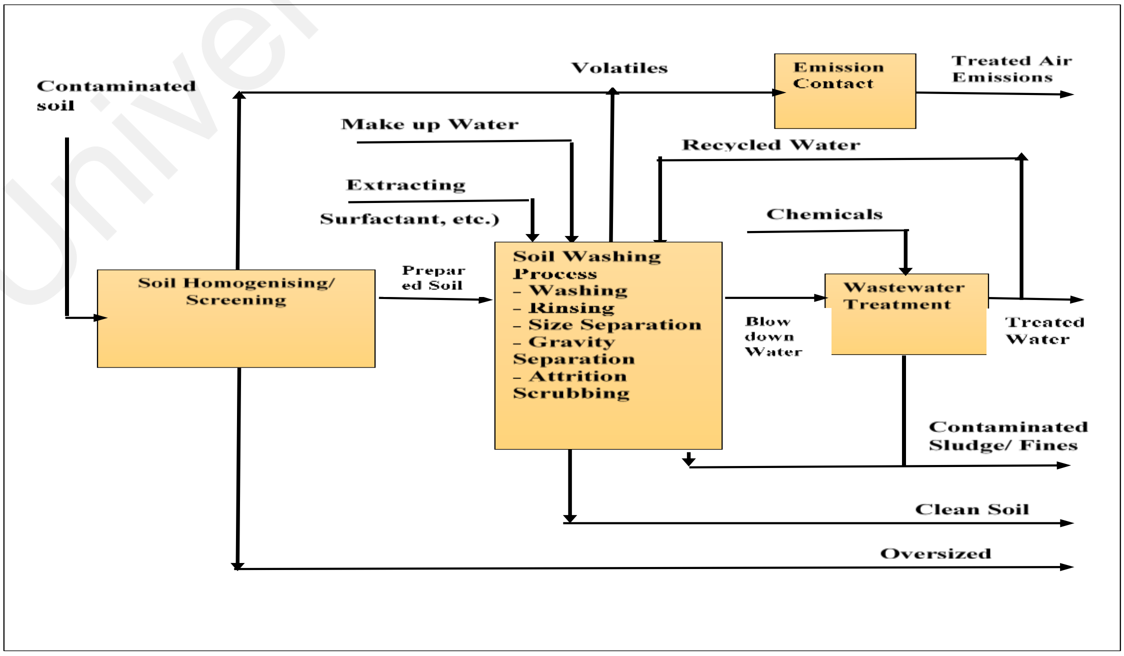


Figure 2.16 Flow-sheet of soil washing process (not including excavation and transport) (UNIDO, 2014)

2.6.2.4.2 Soil washing advantages and limitations

The washing process has advantages of being a close system which permits controlled conditions of treatment, while also has wide applications for varied groups, such as hydrocarbons and heavy metals (Semer & Reddy, 1996). Also, the contaminant mass is reduced to a significant level. Moreover, soil washing is relatively cost effective compared to landfilling and other technologies (Semer & Reddy, 1996). However, soil washing techniques have many disadvantages and limitations; soil washing techniques are considered ineffective for contaminated soil with clay-size particles as high degree of contaminants adsorbed onto clay and silt particles can hamper the washing process. Additionally, complex waste mixtures make formulating the washing fluid difficult (Pavel and Gavrilescu, 2008). Besides that, washing wastewater need additional treatments to isolate and recycle the contaminant (USEPA, 2012). Generally, soil washing is used in combination with other technologies: bioremediation, incineration and solidification/stabilization (Pavel and Gavrilescu, 2008).

2.7 Surfactants and remediation applications

2.7.1 Surfactants and contaminant removal mechanisms

2.7.1.1 Surfactants

Surfactants are amphiphilic molecules having two major components; a hydrophilic group or moiety (polar, water soluble) and a hydrophobic group, or moiety (apolar, water fearing). This dual nature causes surfactants to adsorb at interfaces thereby reducing the interfacial energies (Rosen, 1989; Pandey *et al.*, 2014). Depending on the surfactant head group, they are classified as anionic, cationic, or zwitterionic (cationic and anionic groups) (Deshpande *et al.*, 1999) (Figure 2.17).

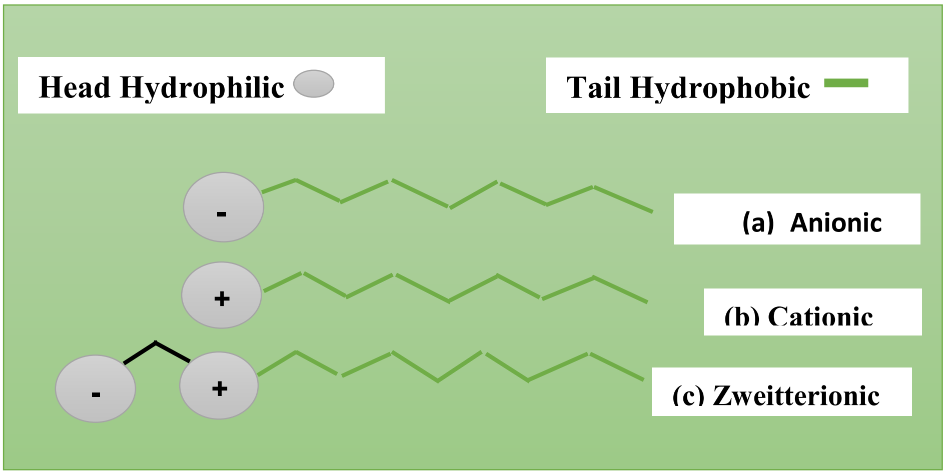


Figure 2.17 Surfactant classification according to the composition of their head: (a) anionic (negatively charged), (b) cationic (positively charged) and (c) zweitterionic (two oppositely charged groups) (Deshpande et al., 1999).

Surfactant molecules are surfactant monomers that exist as a single unit. However, when the water surface does not have enough area for large concentrations of surfactants, the surfactant will then start to cluster together in conglomerates called micelles (Figure 2.18). The minimum concentration where micelles start to form is called the Critical Micelle Concentration (CMC) (Urum, 2003). Surfactants with biological-based materials are named Biosurfactants, and have been classified and proposed for different environmental applications as an alternative to synthetic surfactants. Biosurfactants can be more effective, cost-effective and low in toxicity (Urum *et al*., 2003).

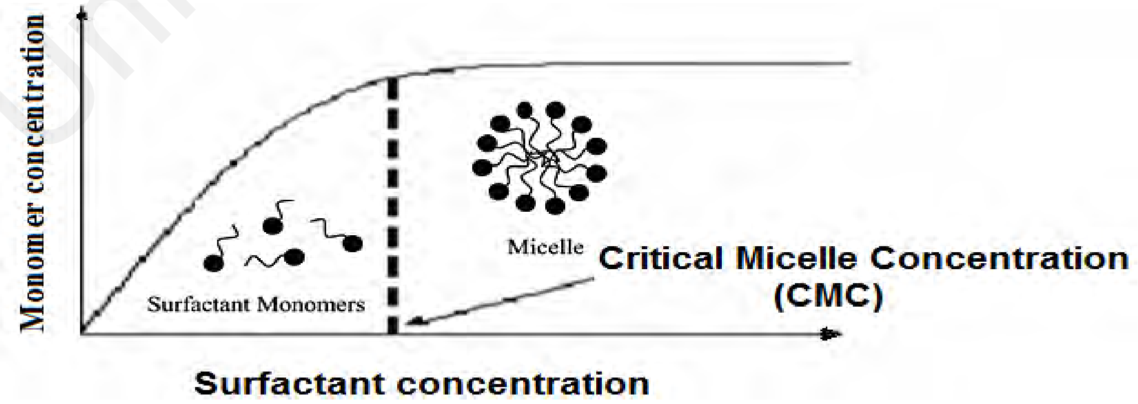


Figure 2.18 Formation of micelles at critical micelle concentration. Adapted from AATDF (1997).

2.7.1.2 Contaminant removal mechanisms

Generally, surfactant washing solutions can extract and remove the contaminants from the contaminated area (water or soil) under two main mechanisms. The first mechanism is based on the displacement of the oil, whereas the second is based on the dispersion and the solubilization of the oil (Figure 2.19) (Ang & Abdul, 1991).

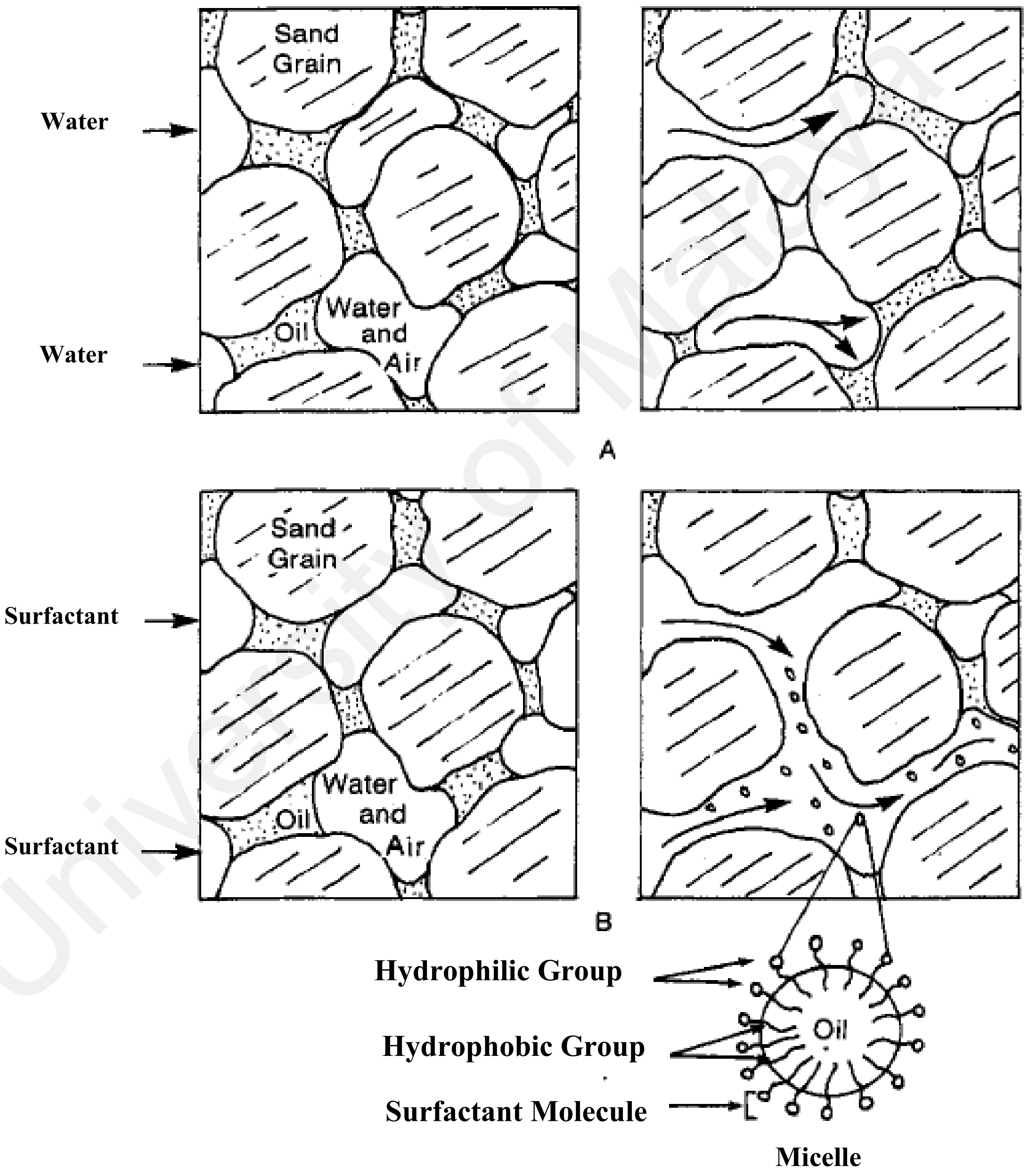


Figure 2.19 The displacement and solubilization of oil. (A) the oil displaced by water from large pores can again be trapped in smaller pore spaces. (B) the oil displaced by surfactant solution is dispersed and solubilized in the surfactant micelles and carried through the pores by the flowing water (Ang & Abdul, 1991).

2.7.1.2.1 Mobilization mechanism

The mobilization mechanism occurs at concentrations below the Critical Micelle Concentration (CMC), where the surfactants exist in the monomeric state. Surfactants reduce the surface and interfacial tension between air/water, oil/water, and soil/water systems. Surfactants in contact with the soil/oil system increase the contact angle and reduce the capillary force holding oil and soil together due to the reduction of the interfacial force. As a result, the residual oil saturation in the presence of surfactants is appreciably lower, and more oil is mobilized than with simple water floods alone (Urum *et al*., 2004; Ang & Abdul, 1991).

2.7.1.2.2 Solubilization mechanism

The second mechanism for enhanced soil washing is solubilization. The solubility of oil increases dramatically due to the aggregation of surfactant micelles. The hydrophobic end of the surfactant molecules clusters together inside to form the micelle structure with the hydrophilic end exposed to the aqueous phase on the exterior (Urum, 2004). The dispersed and dissolved oil trapped in the micelles could easily be washed from the porous medium with the surfactant macromolecules (Ang & Abdul, 1991).

2.8 Bacteria application in contaminated soil washing technique

As previously mentioned, surfactants are used widely to remove different contaminants from water bodies and soils, but the results obtained from contaminated soil washing technique using a single surfactant clearly showed that they were not at the desired points (Urum *et al*., 2003). However, adding some additives such as mineral salts or combining between two surfactants enhanced the contaminant removal efficiency at acceptable points (Han & Ni, 2009). Numerous researches were conducted in order to determine the optimal environmental conditions for the treatment process, and the influence of different environmental factors such as washing time, washing temperature, volume of surfactant, surfactant concentration and shaking speed on the output of treatment (Urum *et al*., 2003). These results from previous researches were used as reference as a comparison in the current study.

Previous researches were focused on remediation of weathered and non-weathered contaminated soils, high clay and silt content effects, two or more surfactant combination effects; physicochemical soil properties and hydrocarbon effects (Han *et al*., 2009). Through these researches, it is clear that researchers were in accordance for some results and in contrast for some others. Han *et al*. (2009) conducted a research on the treatment of real weathered contaminated soil (over one year). He argued with most of the researchers such as Urum (2004), Kuyukina *et al*. (2005) and Santa *et al*. (2007). He considered their methods as less effective in practical applications, because their researches were mainly carried out on artificially contaminated soils produced in the laboratory, as real contaminated soils tend to show complexities during remediation.

2.9 Cases study

2.9.1 Extraction of Heavy oil blends from weathered and non-weathered contaminated soils using single type surfactant.

Urum *et al*. (2003) and Urum (2004) used in their research weathered and non-weathered contaminated soils with different sizes and different techniques which include test tubes, stirred tank reactors, packed columns, and an air bubble assisted stirred tank reactors, using five types of biosurfactants and a type of chemical surfactant (aescin, lecithin, rhamnolipid, saponin and sodium dodecyle sulphate). A maximum removal efficiency of 43% and 48% for lecithin and tannin were obtained at the lowest concentration solution of 0.004%-mass (Urum, 2004). This indicated that removal occurred below the Critical Micelle Concentration under the mobilization mechanism, because of the reduction of interfacial tension in the system water-oil-soil (Urum, 2004). These results agreed with Abdul *et al*. (1990) and Deshpande *et al*. (1999), but contradict Ang and Abdul, (1991). However, SDS and rhamnolipid were obtained from the highest removal efficiency beyond the Critical Micelle Concentration through the solubilization mechanism.

Urum (2004) had used the Taguchi experimental design plan, and chose the Performance Statistic as the optimization criterion. It was found that the increase in the volume of surfactant solutions from 5 to 20 ml enhanced oil removal efficiency by using rhamnolipid biosurfactant and Soduim Dodyecyl Sulfate, hence an increase in volume enhances the interaction between the crude oil and washing media (Urum, 2004). Similarly, increasing the shake speed agitation enhanced the oil removal efficiency due to the exposure of the surfactant solution to larger surface areas across the varying shake speeds (80-120 stroke/min) (Urum, 2004). Also, increasing the washing time enhances the oil removal for both weathered and non-weathered contaminated soils (Urum, 2004). Urum (2004) also found that the increase of washing temperature and surfactant concentrations using rhamnolipids increased oil removal efficiency significantly. However, oil removal from non-weathered samples had shown a constant degree when it was above 20°C.

The quantity of crude oil removed from the weathered soil after washing with the surfactant solution (rhamnolipid, saponin and SDS) in the test tube revealed that SDS exhibited the maximum crude oil removal (46.09%), followed by rhamnolipids (43.93%), and saponins (26.83%). Such low removal efficiency proved the negative effects of the weathering process in the soil washing technique (Urum *et al*., 2003). At the end of the research, it was found that the temperature and concentration of biosurfactant solution were the most influential parameters on the treatment of non-weathered contaminated soils, due to the existence of volatile and semi-volatile compounds. However, the washing time and volume of biosurfactant had the most influential impact on the treatment of weathered contaminated soils (Han *et al*., 2009).

2.9.2 Extraction of crude oil from real weathered sandy contaminated soil using surfactant mixed with inorganic salt

Han *et al*. (2009) conducted a research on the treatment of real weathered contaminated soil (over one year) using a class of green non-ionic surfactant (alkyl polyglucosides), which are surfactant with an uncharged polar head, mixed with inorganic salt (NaCl and Na_2SO4).

Han *et al*. (2009) found that increase in temperature can enhance the oil removal efficiency because it can decrease the viscosity of the oil while increasing the negative charge potential of the soil silica, which reduced the re-adsorption of contaminants through electrostatic repulsion (Balzer, 1993). Therefore, the results agreed with those reported by Zhang *et al*. (2001) and Urum *et al*. (2003). As for the washing time, 85% of oil removal was attained within the first 5 minutes. However, equilibrium was not attained even after 1 hour of removal and this result agreed with those reported by Urum et al. (2004), where the comparison of the washing of weathered and non-weathered contaminated soils indicated that washing of weathered contaminated soil needs considerable time.

Increasing the volume of surfactant enhances oil removal by providing more surfactant monomers that mobilizes and solubilizes contaminants in micelles. This result is consistent with those found by Urum *et al*. (2004). Besides that, increasing the agitation speed would enhance the attrition between soil particles and the flushing force of washing solution. This can accelerate the diffusion of the desorbed contaminants and their interaction with the surfactant micelles in the solution. Similarly, Bernardez and Ghoshal (2008) found that oil decreased from 11 mg g^{-1} to 8 mg g^{-1}dry soil, when the agitation speed was increased from 130 to 370 rpm; further increase on agitation speed cannot improve the removal efficiency (Han *et al*., 2009).

2.9.3 Mixture of two surfactants benefits and negative impacts of clay in the contaminated soil washing treatment process

Xing *et al*. (2000) reported the benefits of nonionic and anionic biosurfactants in the soil washing treatment, and the negative impacts of clay content in the soil. Surfactants used in the study were Alcohol polythoxylate (AEO-9, nonionic) and Sodium alcohol polyethoxylated ethers sulfate (AES, anionic). When clay was 20.80%, the removal efficiency using mixed surfactants was 75.10%, while removal efficiency was only 12.70% when the clay content was 90% (Xing *et al*., 2000). Furthermore, when the mixture of two surfactants (0.1% AES and 0.5% AEO-9) were used, the removal efficiency was much higher (Xing *et al*., 2000).

2.9.4 PHAs Removal from sandy contaminated soils and environmental condition effects

Peng *et al*. (2011) conducted a research using two types of surfactants (Tween-80 and Triton X-100) in order to test the effectiveness and best removal environmental conditions to remove PHAs from contaminated soil using the washing technique. Five factors were tested namely, stirring speed, washing time, surfactant concentration, volume of biosurfactant, and washing temperature. The process of washing was done with a fixed value of all the factors and only the value of the tested factor was changed each time.

Results showed that increases in stirring speed enhanced removal efficiency up to 250 rpm, and later dropped at 300 rpm. It was clear that further increase in stirring speed caused the slurry to have less relative movement, which leads to lower removal (Peng *et al*., 2011). Removal efficiency quickly increased in the first 30 and 60 minutes for TX100 and TW80, respectively, and later approached a stable level, indicating that the washing-off process is time-dependent. Although the removal efficiency increased with the increase of surfactant concentrations for both surfactants, there is an inflection point for effectiveness due to the surfactant characteristics, such as their critical micelle concentration points and ability to adsorb at interfaces. It is clear that the increase in washing solution volume enhanced removal efficiency (Peng *et al*., 2011). However, after a certain volume, the removal rate slows down and decreases. This leads researchers to take intermediate volume ratios such as optimal conditions in order to reduce wastewater generation post-treatment (Peng *et al*., 2011). It was found that the removal efficiency was not affected by the increase in temperature, and the separation of incrustation or soil-trapped PAHs - which plays a more important role than desorption and dissolution - was determined mostly by mechanical conditions (Peng *et al*., 2011).

2.9.5 Enhancing contaminant bioavailability for microbial population activities using biosurfactants

Moldes *et al*. (2011) tested the effectiveness of biosurfactants from *Lactobacillus pentosus* in soil charged with 700 and 70000 mg/kg of octane and incubated at 25°C without shaking. The biosurfactants were applied at its critical micelle concentration points insuring superabundant of biosurfactants in the washing solution. After 15 days, the results showed that the biosurfactant accelerated the biodegradation of octane to 58.6% and 62.8% for soil charged with 700 and 70000 mg/kg octane, respectively, whereas after 30 days reached 76% in both cases (Moldes *et al*., 2011). Thus, the use of biosurfactants from *Lactobacillus pentosus* enhanced the microbial population activities by reduction in soil, contaminant and washing solution interfaces, which causes in mobilizing of contaminants from the soil to make them more available. Additionally, the results showed that the octane concentration was kept very high in soils, which was sterilized and a very small amount degraded probably due to the loss of sterile conditions by time or evaporation of octane, indicating importance of microorganisms to metabolize contaminants. However, in the presence of biosurfactants, the results showed improvement in the solubilization of octane to be metabolized by the microbial biomass of soil (Moldes *et al*., 2011). It can reduce octane concentration from 700 mg/kg to 297.8 mg/kg with 15 days in incubation, a value which is achieved only after 30 days in the absence of biosurfactants (Moldes *et al*., 2011).

2.10 Weathering phenomena and their effects on freshly contaminated soil and GC/MS analysis

2.10.1 Weathering phenomena effects on freshly contaminated soil

In order to observe the effects of weathering phenomena on lighter oil component in contaminated soil, Urum (2004) used the weathering method, where different particle size contaminated soils are subjected to heating, using a fan-assisted oven (Genlab Widnes) at 50°C for 14 days, and the mass reduction of the contaminant determined through n-hexane extraction and using GC/MS analysis.

The loss of different fractions of oils is based on their chemical and physical properties and on the time of exposure to different environmental factors. Generally, within the first 24 hours, the more volatile components are the first fractions lost in the weathering process, due to evaporation (Urum, 2004). The evaporation rate was found to be higher in contaminated soil with smaller particle sizes (clay and silt) compared to sand, coarse and gravels (Urum, 2004). After 14 days, the rate of evaporation decreases and the proportion of higher molecular weight components increased, which makes the remediation process, at this stage, more complicated and difficult (Butt et al., 1986). Han *et al*. (2009) and Urum et al. (2004) results proved that weathered crude oil is much more difficult to remediate as compared with those freshly contaminated soil.

2.10.2 GC/MS Analysis

GC-MS analysis was used to evaluate which compounds were lost after the weathering phenomena or after remediation. Urum (2004) conducted GC/MS analysis for three samples namely fresh oil, oil extracted from non-weathered contaminated soil, and oil extracted from weathered contaminated soil.

Results shows that hydrocarbon fractions less than C_9 were reduced before the contamination process. Moreover, there was more loss of hydrocarbon fractions during the contamination process. It was clear that weathering process reduced most of the hydrocarbon fractions that were less than C_{16}, leading to the formation of unresolved complex mixtures (UCM), which are materials that cannot be detected by the GC/MS (Urum, 2004). Similarly, Garcia and Brebbia (1988) found that most aliphatic smaller than C_{16} were reduced to trace levels, after heating for 7 days.

CHAPTER THREE

RESEARCH METHODS AND MATERIALS

3.1 Introduction

This chapter describes the methods used in this study as well as the most important components of research; soil preparation and its physico-chemical characteristics, and analysis of contaminants in the soil system prior and post treatment. The soil washing technique, which is a physico-chemical treatment, was chosen in order to evaluate and estimate the suitable environmental conditions for the treatment to enhance used engine oil removal efficiency from contaminated soil. Finally, the bioremediation technique, which comes as a second treatment, is combined with the washing technique. To get realistic results, real weathered contaminated soil was selected for this study. *Bacillus salmalaya 139 SI,* which is used in our study, was isolated from an agricultural soil in the Serdang agriculture centre, Malaysia. Within 60 days. It showed a high degree of performance in degradation of waste crude oil polluted soil and treatment of heavy metals (Dadrasnia and Salmah, 2014; Mohammad *et al.*, 2016).

3.2 Soil characteristics

3.2.1 Soil preparation and size distributions

The soil sample used in this study was collected from the surface soil of a weathered contaminated site at Chiank Auto Electrical and Mechanical SDN BHD, Gombak, in Kuala Lumpur. The soil was prepared using standard method, it was air-dried indoors at ambient temperature (27°C ± 2) for one week, screened through a 2 mm sieve to remove coarse fragments, and then mechanically mixed to ensure homogeneity (Han *et al.*, 2009). The sieved soil sample was stored in airtight containers at 4°C, and 1 kg of the contaminated soil was used to determine the size distribution using the Octagon Test sieve shaker following the methods of Arora (1989).

3.2.2 C, H, N and S determination

CHNS Elemental Analyser (Perkin Elmer Model: CHNS/O 2400 Series II) was used to determine the percentage of carbon, hydrogen, nitrogen and sulfur components in the weathered contaminated soil, where 2 g of the dry solid sample was prepared and analysed. Generally, the process is done to determine the elemental composition of samples such as carbon, hydrogen, nitrogen and sulfur based on its uses in the combustion process, in an excess of oxygen, to break down the substances into simple compounds, which are then measured. The masses of the combustion products were used to calculate the composition of unknown samples and the result analysis were based on the ratios between different compounds and working with specific chemical formula. The accepted deviation of elemental analysis results was 0.4% (Sansoni & Iyengar, 1980).

3.2.3 Soil pH

Soil pH was determined in distilled water at a solid/liquid ratio of $1{:}2g\ mL^{-1}$, using the STARTER300 pH Meter (OHAUS Corporation, USA). Ten grams of air-dried weathered contaminated soil in 20 ml distilled water was mixed and shaken for 5 minutes, and then allowed to settle for 30 minutes. After shaking the content again, the pH was measured (Rowell, 2014). The measurement was carried out in triplicates to ensure accuracy.

3.2.4 Soil water content by mass and volume

A soil sample (150.06 g) was deposited onto a moisture box (50g) and subjected to drying at 105° C to a constant mass (Singh, 2014). The water content with respect to mass (%) and volume (%) were calculated using the following equations:

Water (%) by mass = (wet mass – dry mass/ dry mass x 100) (1)

Water (%) by volume = (volume of water / bulk volume of soil) x 100 (2)

3.2.5 Porosity and Permeability measurements

In order to determine the soil porosity and permeability, the following materials were used: two caps (one without hole and one with hole at the bottom), graduated cylinder, large beaker, soil sample, spoon and stopwatch. Similar steps as reported in previous studies were adopted for the measurements (Terzaghi *et al*., 1996). Analysis will be in triplicate and the standard error will be determine following the formula:

$$SE_{x^-} = \frac{S}{\sqrt{N}}$$

Where:

SE_{x^-} : Standard error of the mean

S : Standard deviation of the mean

N: Number of observations of the sample

3.2.6 Presence of Inorganic carbonate ($CaCO_3$ and $MgCO_3$) in the soil

According to McLean (1982), inorganic carbonates are mostly formed in soils with pH range between 7.8 and 8.2. A confirmatory tests to check for the presence of inorganic carbonates was done by adding a few drops of HCl acid to sample before and after heating at 600°C for 4 hours using the Muffle Furnace JSR Model.

3.3 Preparation of mixed liquor as the washing solution

Bacillus salmalaya strain 139SI, which is used in the study, has been discovered and isolated at Molecular Bacteriology and Toxicology Laboratory (MBTL), Institute of Biological Sciences (ISB), Faculty of Science, University of Malaya. It showed good activity on onsite trial of its biosurfactant in degrading oil surface layer, flocculating & emulsifying activity and glassware cleaning (unpublished, 2013). Currently the research focusing on the mass production of *Bacillus salmalaya* strain 139SI cell bacteria and its cell-free supernatant containing the biosurfactant compounds for applications in oil biodegradation, agricultural systems, biocontrol diseases, and environmental treatment in oil recovery/water drinking system/wastewater /leachate.

The solution used in the study is a mixed liquor of bacteria with its biosurfactant and nutrients (ML). As a control, distilled water was prepared. However, mixed liquor will play their role during the second part of treatment, which is bioremediation). Taguchi experimental design is a suitable design for optimization of soil washing technique for efficient removal of used engine oil (Urum, 2004). In the present study, the influence of five factors, (as listed in Table 3.1), on the used engine oil removal efficiency were tested in 16 runs. It was done by, 5 grams of prepared contaminated soil and introduced into 45 mL centrifuge test tube. The test tubes were placed horizontally in a temperature-regulated water bath and shaken laterally following the experimental plan and conditions as shown in Table 3.2, while the range of values is listed in Table 3.1.

Table 3.1: Experimental variables and levels

Variable	level			
	Level 1	Level 2	Level 3	Level 4
(A)Washing temperature (˚C)	25	37	42	55
(B)Volume of ML solution (mL)	5	10	15	20
(C)Concentration of ML % v/v	0.01	0.1	1	10
(D)Shaking speed (strokes min^{-1})	80	120	160	200
(E)Washing time (min)	5	10	15	20

Table 3.2: Coding used for variables during optimization experiment

Experiments numbers	Experimental variables and their levels				
	A Washing temperature	B Volume of ML solution	C Concentration of ML	D Shaking speed	E Washing time
1	1	1	1	1	1
2	1	2	2	2	2
3	1	3	3	3	3
4	1	4	4	4	4
5	2	1	2	3	4
6	2	2	1	4	3
7	2	3	4	1	2
8	2	4	3	2	1
9	3	1	3	4	2
10	3	2	4	3	1
11	3	3	1	2	4
12	3	4	2	1	3
13	4	1	4	2	3
14	4	2	3	1	4
15	4	3	2	4	1
16	4	4	1	3	2

At the end of each washing, the sedimentation of the contents and the solution were separated for different extractions and analyses. The washed soil was rinsed with 5 mL of distilled water at the corresponding temperature before being shaken laterally for 3 minutes prior to the disposal of the waste water. This allowed the removal of the remaining ML and used engine oil from the walls of the tube. Thus, and as such, the emulsion process was prevented (Urum *et al*., 2003).

3.3.1 Used engine oil removal efficiency range

10 mL of n-hexane was add to 5g rinsed soil, and then shaken laterally for 5 minutes on an orbital shaker (Model N-Biotek). The n-hexane-used engine oil extract was filtered using Whatman number 4 filter paper. The process was repeated four times; the fourth extract gave the same absorbance reading as a pure n-hexane (zero absorbance).

The n-hexane-used engine oil extract was collected in one volumetric flask and made up to 50 mL with n-hexane. The extract was placed in a beaker of known weight and the solvent was evaporated under vacuum (70°C water bath on rotary evaporation) using rotary evaporation (Model Eyela, N-1100) (Urum *et al.*, 2003; Ijah & Ukpe, 1992).

The initial and remaining used engine oil were extracted and the percentage of quantity removed were calculated using the formula (Ijah & Ukpe, 1992);

Used engine oil removed, (%) = $\frac{o_i - o_r}{o_i} \times 100$ (3)

Where:

o_i: is the initial weight of the used engine oil

o_r: is the weight of the remaining used engine oil.

3.3.2 Used engine oil removal percentage through 16 trials

Used engine oil were extracted through 16 trials using ML such a washing solution, and distilled water such as a control. Results will be obtained based on formula number 3 (section 3.4).

Based on SPSS Software, and in order to find the nature of relationship between control and ML, results obtained using control and ML were compared through all 16 trials. Based on temperature variables (Table 3.2) results were divided on four groups.

3.3.2.1 First group

Group contains runs number 1, 2, 3 and 4 with fixing temperature of 25°C

3.3.2.2 Second group

Group contains runs number 5, 6, 7 and 8 with fixing temperature of 37°C

3.3.2.3 Third group

Group contains runs number 9, 10, 11 and 12 with fixing temperature of 42°C

3.3.2.4 Fourth group

Group contains runs number 13, 14, 15 and 16 with fixing temperature of 55°C

3.4 Effects of different variables on used engine oil removal efficiency

According to Pignatiello (1988), the performance statistic was chosen as the optimization criterion. This was evaluated using the equation:

$$Z = -10 \log \left(\frac{1}{n} \sum_{n_i=1}^{n} \frac{1}{y_i{}^2}\right) \qquad (4)$$

Where:

Z: is the performance statistic

n: is the number of repetitions done for an experimental combination

y_i: the performance value of the *i*th experiment

Based on the Minitab Software, in order to determine the effects of different variables on used engine oil removal efficiency and the most influential variables, the response table for the performance statistic means was evaluated based on equation 4.

3.4.1 Effects of temperature on used engine oil removal efficiency

In order to see the effects of washing temperature on the used engine oil removal efficiency, the temperature was altered according to the arrangement illustrated in Table 3.1. with 25°C, 37°C, 42°C and 55°C. To understand the nature of the relationship between temperature and used engine oil removal efficiency, it is important to establish the performance statistic Plot for washing temperature based on equation number 4. The variation trends were compared with temperature effects on non-weathered contaminated soil treatments.

3.4.2 Effects of volume washing solution on used engine oil removal efficiency

The effect of ML volume washing solutions were determined based on the performance statistic plot of volume solution. 5ml, 10ml, 15ml and 20ml were the washing solution levels applied in the treatment (Urum *et al*., 2003). From the performance statistics plot of the solution volume, it will be easier to read performance statistics results and determine the relationship between volume solution and degree of used engine oil removal.

3.4.3 Effects of concentration solution on used engine oil removal efficiency

The increase in the concentration of ML from 0.01% (v/v) to 10% (v/v) (Table 3.1), and based on the washing concentration performance on the statistic plot, the trend of changes in the degree of used engine oil removal efficiency can be observed. Trend changes in the curve will explain the removal mechanisms, mobilization or solubilisation of used engine oil, occurred during the treatment processes.

3.4.4 Effects of shaking speed on used engine oil removal efficiency

Shaking speed performance statistic plot will gave a clear idea on the effect of physical variables in the treatment process and to understand relationship between soil particle sizes and agitation speed, which makes used engine oil more available to bacteria and its biosurfactant (Urum *et al*., 2003). Shaking speed was increased from 80 to 200 strokes/ min using orbital shaker (Table 3.1).

3.4.5 Effects of washing time on used engine oil removal efficiency

The washing time performance statistic plot provides very interesting data to understand the role of time through the treatment process for weathered contaminated soils, which experienced a loss of the majority part of their volatile and semi-volatile compounds. The treatment process was done in 5, 10, 15 and 20 minutes (Urum *et al*., 2003).

3.5 Determination of optimum environmental conditions for the treatment

According to Urum *et al*. (2003) maximum points on the different curves obtained from our research were considered as optimum environmental conditions for the treatment.

3.6 Determination of most influential variables for the treatment

Generally, based on Minitab Statistical Software, the most influential variable on the output of treatment were determined based on the response table for performance statistic means, by calculating the Sigma factor (Δ), for each environmental variable. The most influential variable will be the one with the highest value for sigma:

Δ = Maximum performance statistic mean value – Minimum performance statistic mean value

Where:

Z_{max} = -10 log $(\frac{1}{n}\sum_{n_i=1}^{n}\frac{1}{{y_{max}}^2})$ (see equation 4 in page 48)

Z_{min} = -10 log $(\frac{1}{n}\sum_{n_i=1}^{n}\frac{1}{{y_{min}}^2})$

3.7 Determination of Signal-to-noise ratio effect

In order to observe the effect of noise source during the washing process, each experiment was triplicated under the same conditions at different times, and the effect for each variable was also determined (Urum *et al*., 2003; Box, 1988). The signal-to-noise or SN number for each experiment was calculated with the following equation:

SN_i= 10 log $\frac{\bar{y}_i^2}{s_i^2}$ (5)

Where:

$$\bar{y}_i = \frac{1}{N_i}\sum_{u=1}^{N_i} y_{i,u}$$

$$s_i^2 = \frac{1}{N_i - 1} \sum_{u=1}^{N_i} (y_{i,u} - \overline{y_i})$$

y_i: removal efficiency mean at *ith* experiment.

s_i: standard deviation at *ith* experiment.

i: experiment number

u: trial number

N_i: number of trials for experiment i

After evaluation of the response table for SN means, the noise effect of each variable on the output of treatment was calculated based on the sigma factor (Δ), following the formula:

$$\Delta = \text{SN Maximum} - \text{SN Minimum} \qquad (6)$$

Where:

$$\text{SN}_{max} = 10 \log \frac{{y_{SN\,max}}^2}{{s_{SN\,max}}^2} \qquad \text{(see equation 5 in page 50)}$$

$$\text{SN}_{min} = 10 \log \frac{{y_{SN\,min}}^2}{{s_{SN\,min}}^2}$$

3.8 Evaluation of bioremediation technique

Existence of bacteria in the mixed liquor, which is used such a washing solution, instigate to add a set of experiments based on time variable, which is the most influential variable for washing treatment process. To test the effectiveness of bacteria to remove banded remaining used engine oil from the washed contaminated soil, the research is based on the combination between experiment 4 conditions, where removal percentage of used engine oil was found at maximum point, and bioremediation technique, but with increase in the amount of contaminated soil to 20 g in order to get more clear and accurate results.

At the end of experiment 4, the washed contaminated soil was left at room temperature (37°C±2). 10 ml of water was added twice a week with moving contaminated soil to ensure good aeration. However, control was conducted without adding water and aeration. The washed contaminated soil was treated without any addition of nutrients because contaminated soil has previously been indicated to have a C/N ratio with a value of 23:1. This value is relatively high compared with that mentioned in literature as researchers estimated that the accepted C/N ratio in the soil is around 10:1, which is the same value required to degrade hydrocarbon contaminated soils (Thapa et al., 2012). Table 3.3 shows bioremediation plan, where samples are divided in two set-ups: set-up without water and agitation (-), and with water and agitation (+), respectively. The experiments will be conducted in triplicates.

Table 3.3 Experimental design for bioremediation experiment

Days	0	15	30	45	60
without water and agitation	-	-	-	-	-
with water and agitation	-	-	-	-	-

3.9 Detailed analysis of the used engine oil break down after treatment

GC/MS analysis study the detailed hydrocarbon compositions before and after the treatment. It will enhance the selection of mixed liquor for contaminated soils treatment once chemical-physical characteristics of the contaminants are known (Urum *et al.*, 2004). Used engine oil was extracted from the contaminated soil samples using n-hexane and diluted (one drop oil in 100 ml hexane), and treated using Shimadzu Model: GC QP2010 Ultra. The injection value was 1ɥL, whereas the column temperature was first held at 55°C for 2 min, and subsequently increased from 55 to 150°C at 15°C min^{-1}, 150°C to 165°C at 1°C min^{-1}, and 165°C to 300°C at 5°C min^{-1}, respectively (Han et al., 2009).

At the end of the GC-MS analysis, different contaminant components were determined and compared together prior and after washing process based on GC-MS standard library and Chem Draw Ultra 7.0 Software. Thus, the mixed liquor reaction mechanism with contaminated soils and output of treatment were evaluated.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Introduction

The soil used in this study was collected from weathered contaminated site, contains remaining and banded heavier hydrocarbons compounds, and this made treatments more difficult and complicate compared with those in freshly contaminated soils (Han *et al*., 2009). The general properties of contaminated soil are summarized in Table 4.1.

Table 4.1 Major properties of the contaminated soil sample

Properties	Value			
Soil texture (v %)	gravel > 2mm	40 %		
	sand 2mm to 0.05mm	56 %		
	silt +clay Under 0.05 mm	04 %		
pH	6.90			
Water (%) by mass	3.30			
Water (%) by volume	0.53			
Porosity (%)	40			
CHNS (%)	Carbon %	Hydrogen %	Nitrogen %	Sulfur %
	4.42	1.23	0.19	1.82

4.2 Soil characteristics

4.2.1 Soil particles size analysis

The particle size analysis showed that soil is sandy soil with 56% of sand and only 4% of clay and silt. This result gave an overview on the future of contaminated soil washing treatment results. Removal efficiency of hydrocarbons in sandy soil was greater than that found in clay soils, where the porosity was very low and the hydrocarbons bind strongly to the soil. The concepts were demonstrated by Xing *et al*. (2000) where the removal efficiency for oil decreased with increase in clay content of the soil. With 20.8% of clay, the removal efficiency using mixed of two surfactants was 75.1%, but it was 12.7% when the clay content was 90 %. Therefore, it is more difficult to remove oil trapped by clay than on sandy soil (Xing *et al*., 2000). Moreover, Urum (2004) demonstrated in his research that the evaporation rate of volatile and semi-volatile compounds in freshly contaminated soils was found to be higher in contaminated soils with small particle sizes compared with those that have big sized particles, which proved the presence of high concentration of contaminants in clay and silty soils as compared to that in sandy soils. Thus, based on the type of contaminated soil used, it is possible to predict future washing process results. However, other soil properties and the used engine oil itself need to be more clear and understandable.

4.2.2 C, H, N and S determination

The percentage of the carbon, hydrogen, nitrogen and sulfur components in the contaminated soil was 4.42%, 1.23%, 0.19% and 1.82%, respectively. Comparing the percentage of carbon presented in the tested sample (4.42%) with the carbon content in uncontaminated soils (1% to 3%), it was clear that the carbon percentage in this study was slightly higher than that reported in Mesic *et al*. (2012), and probably this excess carbon is caused by the spillage of used engine oil into the soil (Mesić *et al*., 2012).

Moreover, based on table 4.1, it was found that the C/N ratio with a value of 23:1 is relatively high, but still in the range mentioned in literature, where researchers estimated that the accepted C/N ratio in the soil is around 10:1, which is the same required to degrade hydrocarbon contaminated soils reported by Thapa *et al*. (2012). C/N ratio over 30:1, which is reported by researchers such as Thapa *et al* (2012), is considered high and results in soil nitrogen deficiencies as the, microbes in the soil need nitrogen in their food, will use available nitrogen, which cause in depletion of soluble N in different forms (Mesić et al., 2012). As for C: N: S ratio of 23: 1: 2.4 indicated higher carbon and sulfur compared with constant soil organic matter ratio suggested by Rice (2002) 10: 1: 0.1 and Stevenson (1982) 14: 1: 0.13. Excess of carbon and sulfur in our contaminated soil probably caused by the release of used engine oil (Murphy, 2014).

4.2.3 Soil pH

Generally, the best degradation of hydrocarbons occurred at soils with pH values ranging between pH 4.5 and pH 7.4, but at pH 8.5 the rate of degradation starts to reduce (McLean, 1982). The soil sample in this study has a value pH of 6.9 (Table 4.1), which is within suitable pH range. Soil organic matter (C, H, N, S) buffers the soil against major swings in pH by either taking up or releasing H+ into the soil solution, making the concentration of soil solution H+ more constant, resulting in a pH almost close to neutral which is suitable for the specific crop to be grown (Cooperband, 2002). Therefore, the pH was within the acceptable limit, and a value of pH 6.9 indicated optimum nutrient balance (Thapa *et al*., 2012). The acceptable value of pH in this study is probably due to the weak concentration of the used engine oil in the soil samples with only 27.64 mg g^{-1} of dry soil, which is lower than those reported in previous research. The results of pH is agreeable to the results reported by Odjegba and Atebe (2007) that indicated a decrease pH value as the used engine oil concentration increases.

As mentioned by many researchers; such as Thapa *et al* (2012) and Cooperband (2002), applying some type of surfactants in contaminated soil remediation can change their pH and affect soil properties. The washed contaminated soil samples were measured for their pH value and results are shows in Table 4.2.

Table 4.2 Mean pH results before and after washing treatment comparison for 16 trials

Experiment	Initial pH	Final pH	Standard error for final pH
1	6.90	6.93	0.44
2	6.90	7.00	0.12
3	6.90	6.98	0.82
4	6.90	7.30	0.33
5	6.90	7.20	0.11
6	6.90	7.06	0.74
7	6.90	6.95	0.59
8	6.90	6.96	0.41
9	6.90	7.10	0.55
10	6.90	6.94	0.78
11	6.90	7.03	0.81
12	6.90	6.95	0.59
13	6.90	6.99	0.23
14	6.90	7.20	0.22
15	6.90	6.95	0.35
16	6.90	7.10	0.66

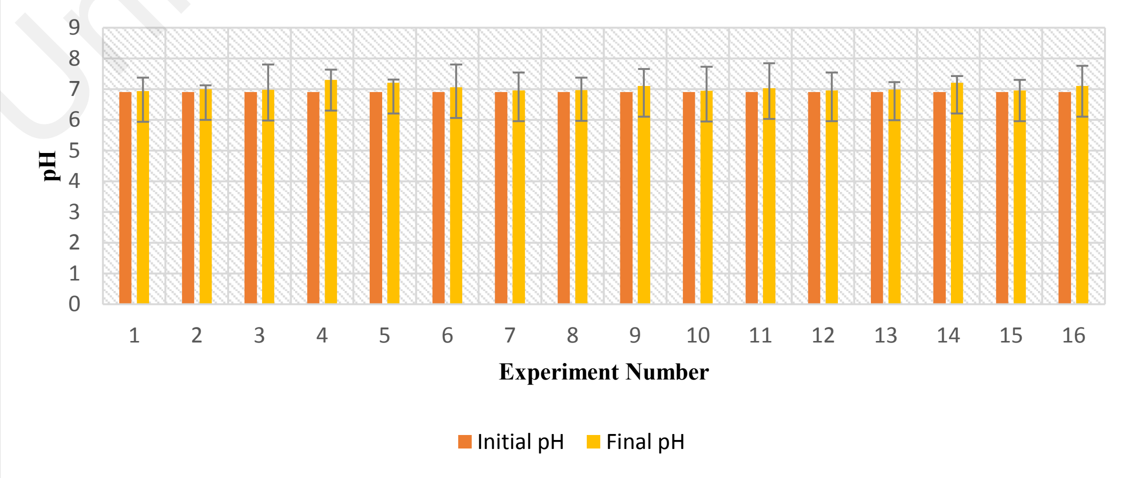


Figure 4.1 Initial and Final pH comparison (Bars indicate standard error ($S = 0.48$)

The results showed no significant difference between untreated contaminated soil pH and treated contaminated soils pH through 16 runs. The pH value in all the experiments remained close to pH 7 (Figure 4.1). Thus, remediation of contaminated soils using Mixed liquor of *Bacillus salmalaya* 139I with its biosurfactant and nutrients do not have any negative effects on the pH of the contaminated soil indicating that no pH adjustment is required.

4.2.4 Soil water content

Moisture of the soil contaminated with used engine oil was calculated based on Equation 1 and 2 (section 3.2.4). Water (%) by mass and volume were found to be only 3.30% and 0.53%, respectively. 3.30% is a small amount which is out of range and incomparable with the optimum soil moisture value required for degradation of contaminants, which is 30% to 90%, get require some soil irrigation to achieve the optimal moisture level (Thapa *et al.*, 2012). Thus, the best future treatment require an increase in water content by water irrigation or increasing Soil Organic Carbon, which will lead to improved physical properties of soil and lead to more water retention (Krull *et al.*, 2004). Wolf and Snyder (2003) found that the increase of 1% Soil Organic Carbon can add 1.5% additional moisture by volume to the treated soil, lead to increase in degree of contaminants degradation.

4.2.5 Porosity and permeability

The porosity of the soil was measured after the treatment process in all 16 trials and compared with the initial porosity and engine oil removal percentages (Table 4.3). Porosity which is the percentage of void volume to the total volume of soil plays a big role to deliver water, air and nutrients (McLean, 1982; Arora, 1989). The results showed that soil porosity was 40% (not including used engine oil banded in the soil). Although moderate permeability is associated with this percentage, it is easier to get a high contaminant removal efficiency as compared with that associated with clay and silty soils, where the remediation technique becomes more difficult and ineffective because of reduction in total void volume (Urum, 2004). Used engine oil decreases soil porosity and permeability depending on their concentration in the soil.

The results show that the porosity percentages and used engine oil removal percentages had the same trends. It was clear that the highest porosity percentage of 58% was obtained at experiment 4 where removal percentage of used engine oil was found to be the highest (47.36%). The smallest porosity percentage of 46% was found associated with the lowest removal percentage, which was obtained in experiment 1 where all environmental variables were applied at their lowest levels. The results indicated a parallel relationship between the porosity percentages and removal percentages of used engine oil (Figure 4.2).

Table 4.3 Relationship of removal used engine oil and porosity.

Experiment	Initial porosity (%)	Final porosity (%)	Porosity standard error	Used engine oil removal degree using ML	Used engine oil removal standard error
1	40	46	1.20	15.84	0.93
2	40	50	0.56	26.26	0.60
3	40	50	0.77	26.24	0.27
4	40	58	1.50	47.36	1.30
5	40	56	2.10	40.32	1.11
6	40	53	1.54	31.32	0.11
7	40	50	0.52	25.29	0.48
8	40	50	0.62	26.38	0.81
9	40	53	0.15	34.07	1.35
10	40	51	1.30	28.76	1.09
11	40	52	1.11	32.05	1.14
12	40	53	0.87	33.66	1.45
13	40	52	0.44	30.16	0.60
14	40	53	1.43	34.08	1.00
15	40	48	2.03	22.61	0.65
16	40	21	4.67	40.20	0.15

The use of a Mixed Liquor as the washing solution for the treatment lead to the enhancement of contaminated soil porosity by removing banded used engine oil in the soil, which was proven by the results obtained through 16 runs. Thus, the use of ML leads to enhanced soil properties by enhancing the contaminated soil porosity. Adedokun and Ataga (2007) results showed improvement in porosity enhancement using waste cotton and saw dust such as soil amendments.

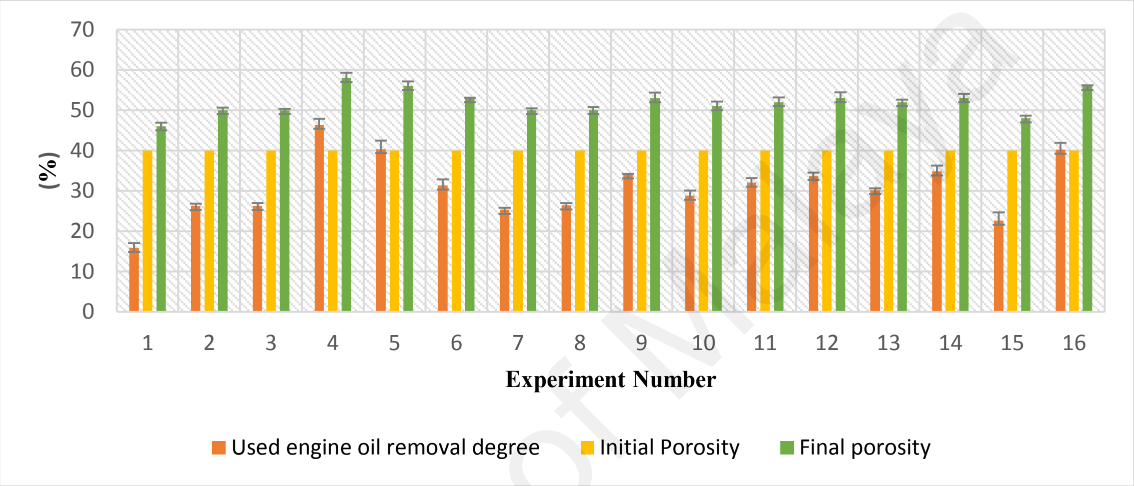


Figure 4.2 Relationship between initial porosity, final porosity and removal percentage of used engine oil (Bars indicate standard error ($S = 0.82$ and $S = 1.11$) for removal percentage of used engine oil and final porosity, respectively).

4.2.6 Inorganic carbonate ($CaCO_3$ and $MgCO_3$) testing

In order to test the existence of inorganic carbon in the soil, a few drops of HCl acid were added into the sample. Insignificant effervescence occurred, which normally indicates the presence of small amounts of inorganic carbon such as calcite and dolomite. However, according to McLean (1982), inorganic carbonates are mostly in soils with pH values ranging between 7.8 and 8.2. Soil samples used in this study had a pH value of 6.9 (out of range). Moreover, after 4 hours of soil combustion using the Muffle Furnace, no effervescence was observed using HCl. Thus, the first effervescence was due to the reaction of acid with organic carbon existing in plant or animal debris, indicating the absence of inorganic carbon, such as ($CaCO_3$ and $MgCO_3$), in the soil sample.

4.3 Results of used engine oil analysis in the soil system

The initial extracted used engine oil concentration was 27.64 mg g^{-1} of dry soil. This is lower than those reported in literature on soil washing, especially Urum et al. (2003) who had applied the washing method on non-weathered crude oil contaminated soil with 54.4 mg g^{-1} of dry soil. However, Han *et al*. (2009) applied the method using weathered soil over a year and attained 123 mg g^{-1} of dry soil.

4.4 Removal efficiency for the contaminated soil using washing process

4.4.1 Results based 16 trials

The percentage of used engine oil removal was determined based on equation 3. The results in 16 runs (Table 4.4) for the five variables, namely, the washing temperature, volume of ML, concentration of ML, shaking speed and washing time for optimization of washing efficiency showed that the removal efficiency percentage ranged from 15.48% to 47.36%. The least level of removal was recorded at run 1 where the prevailing variables were washing temperature (25°C), volume of ML (5mL), concentration of ML (0.01%-v/v), shaking speed (80 strokes min^{-1}) and washing time (5 min). Meanwhile, the highest degree of removal at 47.36% was observed in run 4 with a combination of washing temperature (25°C), volume of ML (20mL), concentration of ML (10%-v/v), shaking speed (200 strokes min^{-1}) and washing time (20 min).

The contaminated soil porosity of 40%, and the small amounts of clay and silt (only 4%) that exist in the soil sample were probably the main variables that affect the results of the treatment. This fact was proven by Xing *et al*. (2000) with results that showed the removal percentage of 75.1% when clay content was 20.8%. However, when clay content was 90%, the removal percentage was only 12.7%.

Using mixed liquor (including bacteria with its biosurfactant, and nutrients) negatively affected the washing technique results indicated maximum removal efficiency of only 47.36%. However, bacteria will be the most affected variable on bioremediation process, which built the second part of the treatment in this study plan. The use ML, without adding any additives such as inorganic salts, was the main reason for the moderate results obtained. Although Han *et al*. (2009) used weathered contaminated soil (over one year) mixing a single surfactant (alkyl polyglucosides) with inorganic salts (Nacl and Na_2SO4) led to more than 85% of the crude oil removal percentage. Moreover, a combination between two or more surfactants leads to a more efficient removal. Xing *et al*, (2000) proved that mixing the two surfactants (alcohol polythoxylate and sodium alcohol polythoxylated) led to more than 75% removal percentage. Thus, combining of two biosurfactants or adding of additives to the treatment process lead to more efficient removal of contaminants (Xing *et al*., 2000; Han *et al*., 2009).

4.4.2 Statistical analysis of the removal efficiency

As the treatment carried out based on Taguchi Method Design, which is part of Minitab Software (Urum *et al*., 2003), it is important to mention that the used engine oil removed by any variable had an effect on the other variables. Thus, the results are explained based on the same method. As shown in Table 3.2, to achieve optimal conditions for the experimental design and on temperature variable, 16 runs were divided into 4 groups: the first group contains Runs 1, 2, 3 and 4 with a fixed temperature of 25°C; the second group contains Runs 5, 6, 7 and 8 with a fixed temperature of 37°C; the third group contains Runs 9, 10, 11 and 12 with a fixed temperature of 42°C; the fourth group contains Runs 13, 14, 15 and 16 with a fixed temperature of 55°C. Using SPSS Software, a comparison between water and ML removal efficiency was realized. The mean, standard deviation, standard error mean and independent T-test were the variables calculated for all 16 trials and the results are as illustrated in Table 4.4

Table 4.4 Comparative statistical results of the 16 experimental trials for mixed liquor and control

Run	Efficiency Removal Group 1 biosurfactant 2 control	.Number of repetition	Mean (y_i)	Std. Deviation (s_i)	Std.Error Mean	T-test for Equality of Means Sig.(2-tailed) (P value)
1	1	3	15.84	1.61	0.93	0.00
	2	3	4.34	0.28	0.16	
2	1	3	26.26	1.13	0.65	0.00
	2	3	18.57	1.33	0.77	
3	1	3	26.24	0.48	0.27	0.67
	2	3	26.95	2.71	1.56	
4	1	3	47.36	2.27	1.31	0.00
	2	3	32.38	3.62	2.09	
5	1	3	40.32	1.93	1.11	0.00
	2	3	23.33	1.53	0.88	
6	1	3	31.32	0.19	0.11	0.00
	2	3	15.55	1.89	1.09	
7	1	3	25.29	0.83	0.48	0.00
	2	3	20.58	1.08	0.62	
8	1	3	26.38	1.41	0.81	0.01
	2	3	19.39	2.31	1.33	
9	1	3	34.07	2.34	1.3	0.00
	2	3	18.98	3.00	1.73	
10	1	3	28.76	1.90	1.09	0.00
	2	3	15.12	3.86	2.23	
11	1	3	32.05	1.99	1.14	0.00
	2	3	24.77	1.48	0.85	
12	1	3	33.66	2.52	1.45	0.23
	2	3	28.43	1.05	0.60	
13	1	3	30.16	1.05	0.60	0.01
	2	3	21.09	3.30	1.90	
14	1	3	34.80	1.74	1.00	0.00
	2	3	24.42	2.25	1.30	
15	1	3	22.61	1.13	0.65	0.41
	2	3	24.64	3.73	2.15	
16	1	3	40.20	0.2	0.15	0.00
	2	3	23.12	2.45	1.46	

4.4.2.1 First group: Run 1, 2, 3 and 4 with temperature fixed at 25°C

Figure 4.3 shows removal percentage of used engine oil for washing treatment in Run 1. Under the conditions of washing temperature (25°C), volume of ML (2mL), concentration of ML (0.01%-v/v), shaking speed (80 strokes min^{-1}) and washing time (5 min), the used engine oil decreased from an initial value of 27.64 mg g^{-1}, which is 15.84%, against control which showed only a slight removal percentage of 4.34%. Variables applied at their lowest levels were the main reason to get the lowest removal percentage among all runs.

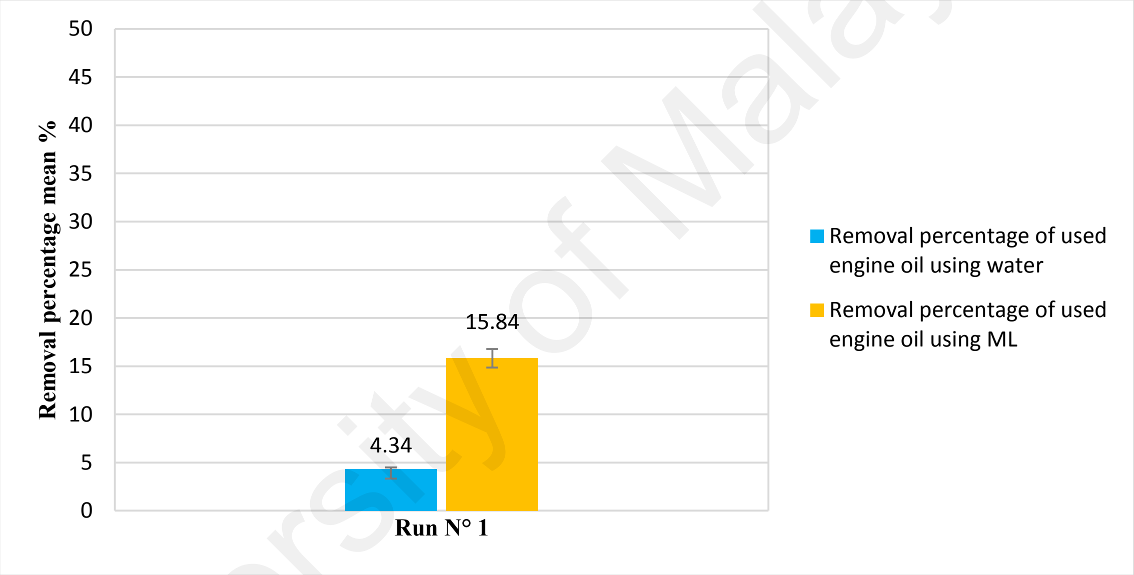


Figure 4.3 Percentage of the removal of used engine oil in Run 1. (Bars indicate standard error (*S* = 0.93 and *S* = 0.16) for removal percentage using ML and water, respectively)

Figure 4.4 shows the washing treatment results for Run 2 under the condition of; washing temperature (25°C), volume of ML (10mL), concentration of ML (0.1%-v/v), shaking speed (120 strokes min^{-1}) and washing time (10 min). The results showed that fixing the temperature at 25°C and increasing all variables values to their second level led to an enhanced removal percentage of used engine oil 18.57% and 26.26% as compared to 4.34% and 15.84% obtained in Run 1 using water and ML, respectively.

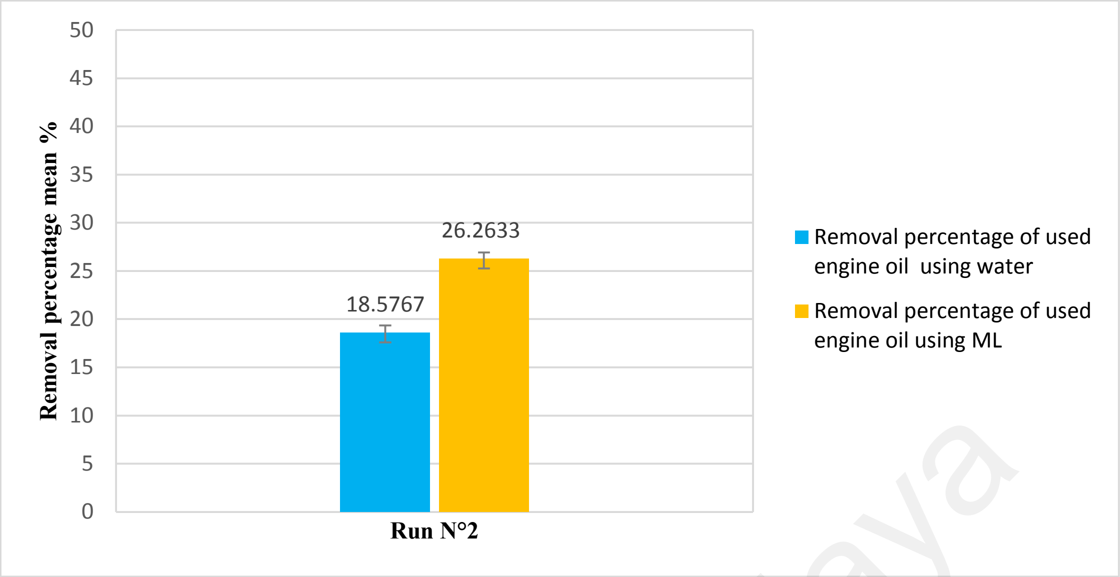


Figure 4.4 Percentage of the removal of used engine oil in Run 2. (Bars indicate standard error ($S = 0.65$ and $S = 0.77$) for removal percentage using ML and water, respectively).

In Figure 4.5, the treatment of used engine oil shows that the temperature was fixed at 25°C and all variables values were increased from level 2 to level 3 (Table 3.1), and based on the combination of washing temperature (25°C), volume of ML (15mL), concentration of ML (1%-v/v), shaking speed (160 strokes min^{-1}) and washing time (15 min), the removal percentage of used engine oil increased to 26.95% using water. However, the use of ML showed almost similar removal efficiency as that obtained in Run 2. A *p*-value of 0.067, which is the level of marginal significance between two variables, indicated a resemblance between water and ML results.

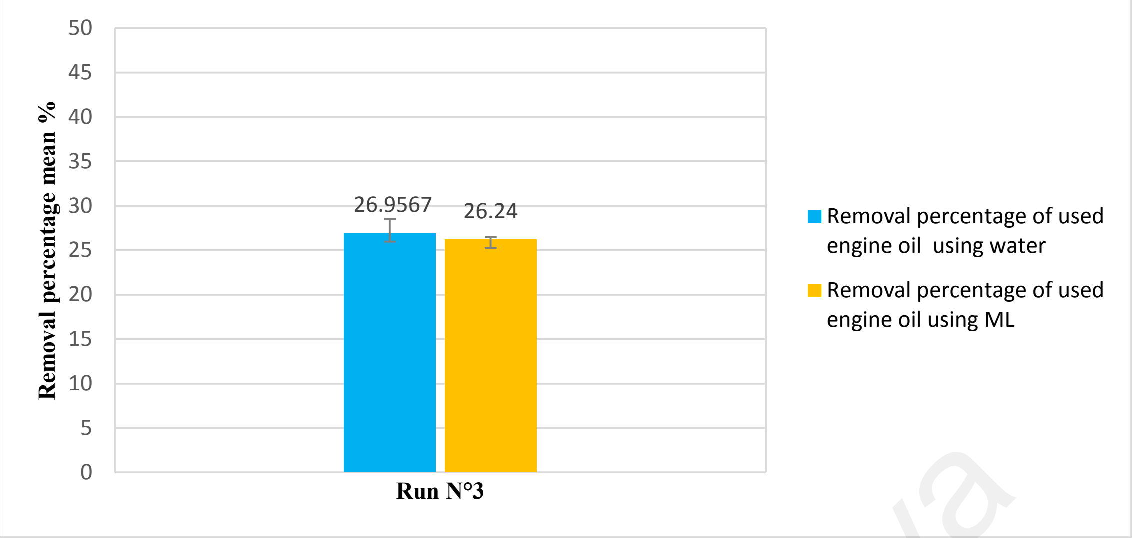


Figure 4.5 Percentage of the removal of used engine oil in Run 3. (Bars indicate standard error ($S = 0.27$ and $S = 1.56$) for removal percentage using ML and water, respectively).

As shown in Run 4, 32.38% and 47.36% were found to be the maximum removal percentage using water and ML, respectively (Figure 4.6). Values for all variables were increased to their maximum level 4: volume of ML (20mL), concentration of ML (10%-v/v), shaking speed (200 strokes min^{-1}) and washing time (20 min). Temperature was fixed at 25°C.

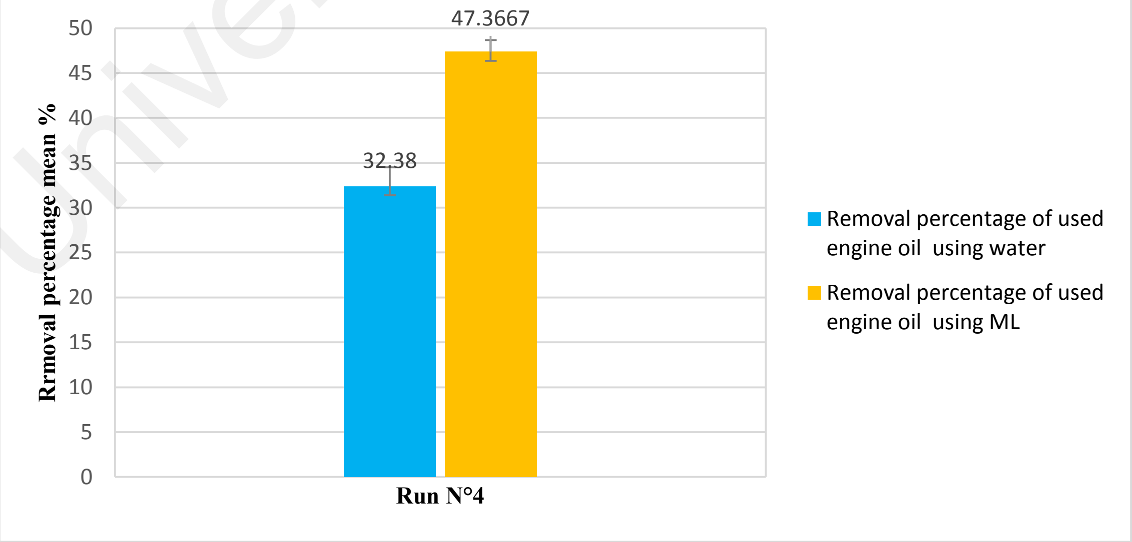


Figure 4.6 Percentage of the removal of used engine oil in Run 4. (Bars indicate standard error ($S = 1.31$ and $S = 2.9$) for removal percentage using ML and water, respectively)

From Table 3.2 and the results obtained in Table 4.4, the results showed that fixing of temperature at 25°C for Run 1, 2, 3 and 4, and gradually increasing all variables values led to a gradual increase in the removal of used engine oil (Figure 4.7). Removal of used engine oil increased from 4.34% to 32.38% in contaminated soil treated using water; and from 15.84% to 47.36% using ML. The results obtained in Run 1 were found to be the lowest for both ML and control. However, the results obtained in Run 4 were found to be the highest overall run. The *p*-value was found to be lower than 0.05, indicating a significant difference between the removal of used engine oil using water and ML, except for Run 3, which had a *p*-value of 0.67. The standard error, ranged from 0.27 to 1.31, was at acceptable values for all experiments (Table 4.4).

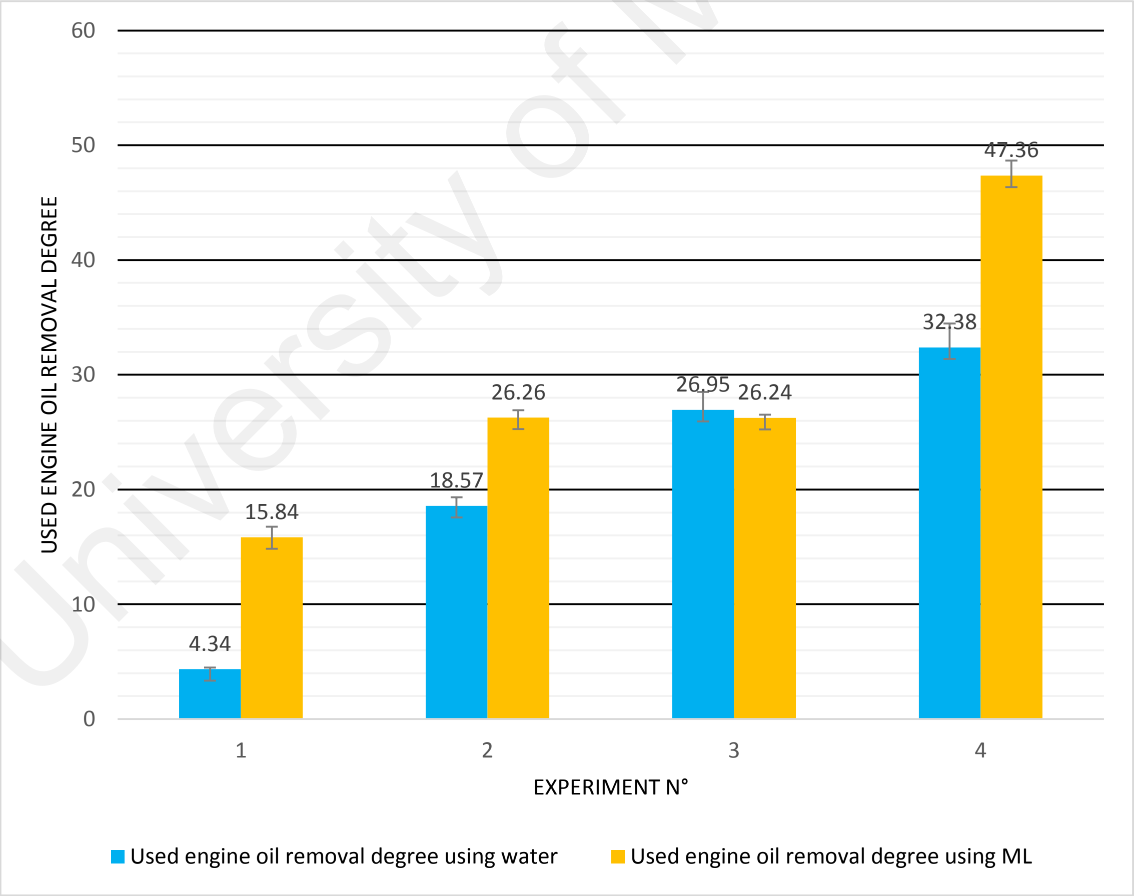


Figure 4.7: Percentage of the removal of used engine oil comparison for Runs 1, 2, 3 and 4 (temperature fixed at 25°C).

4.4.2.2 Second group: Run 5, 6, 7 and 8 with temperature fixed at 37°C

As mentioned before, the used engine oil that was removed by any variable had an effect on other variables. Temperature was fixed at 37°C for Run 5, 6, 7 and 8 as shown in Table 3.2. Washing time, shaking speed, concentration and volume of ML were applied at the maximum values for Run 5, 6, 7 and 8, respectively. However, volume of ML, concentration of ML, shaking speed and washing time were at the minimum for Run 5, 6, 7 and 8, respectively.

Figure 4.8 shows that the temperature was fixed at 37°C and minimum level values were applied for volume of ML (5ml), but the washing process continued to run at maximum time (20 min) leading to 40.32% removal of used engine oil; 40.32% as compared to 23.33% in the control. Other variables were applied at 0.1%-v/v and 160 strokes min^{-1} for concentration of ML and shaking speed, respectively. The lowest volume of ML applied during Run 5 was probably the main reason for the negative effects on the treatment output.

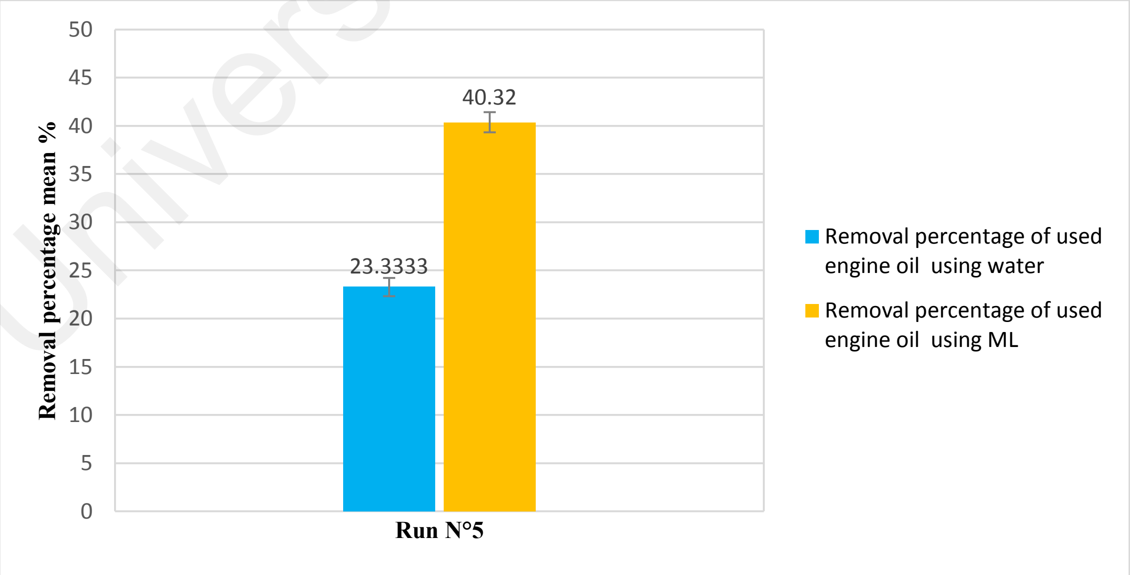


Figure 4.8 Percentage of the removal of used engine oil in Run 5. (Bars indicate standard error ($S = 1.11$ and $S = 0.88$) for removal percentage using ML and water, respectively)

Referring to Figure 4.9, the removal of used engine oil reached 31.32% and 15.55% using ML and water, respectively. The results were obtained at maximum shaking speed of 200 strokes min^{-1} and concentration of ML at the lowest level value of 0.01%-v/v. The volume of ML applied was 10 ml, and the washing process was conducted for 15 minutes. The ML was applied at the lowest concentration level of 0.01%-v/v and was possibly the main reason for the decrease in the efficiency of used engine oil removal.

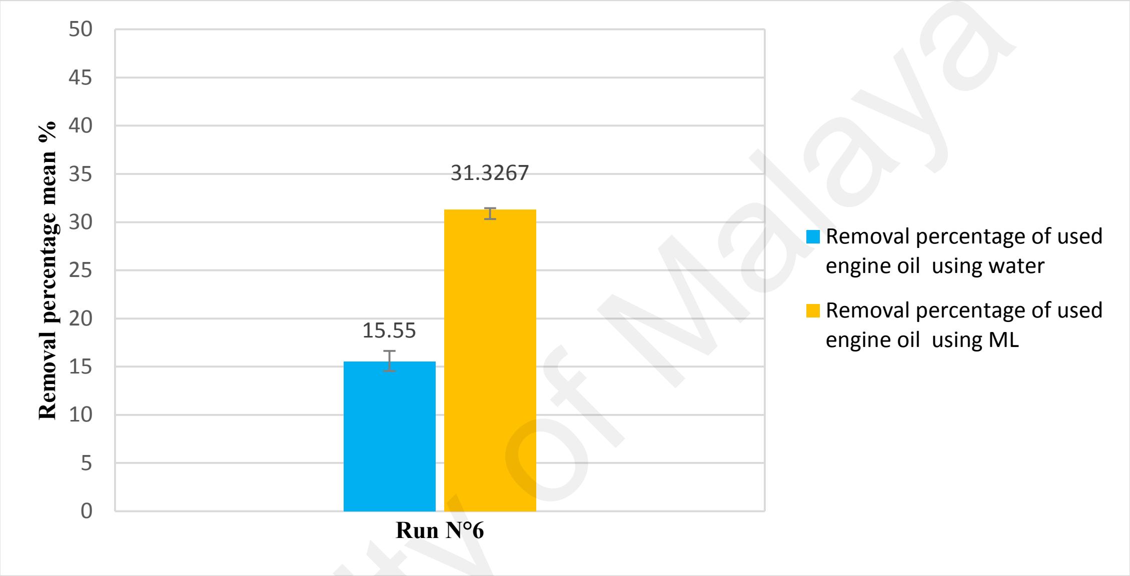


Figure 4.9 Percentage of the removal of used engine oil in Run 6. (Bars indicate standard error ($S = 0.11$ and $S = 1.09$) for removal percentage using ML and water, respectively).

Run 7 was applied with ML at highest concentration of 10%-v/v and the lowest shaking speed of 80 strokes min^{-1}. The volume of ML was 15 ml and the washing time of 10 minutes. The results were below average with only 20.58% and 25.29% of the removal of used engine oil using water and ML, respectively (Figure 4.10). This is due to the application of lowest shaking speed, and insufficient washing time that the removal efficiency was low.

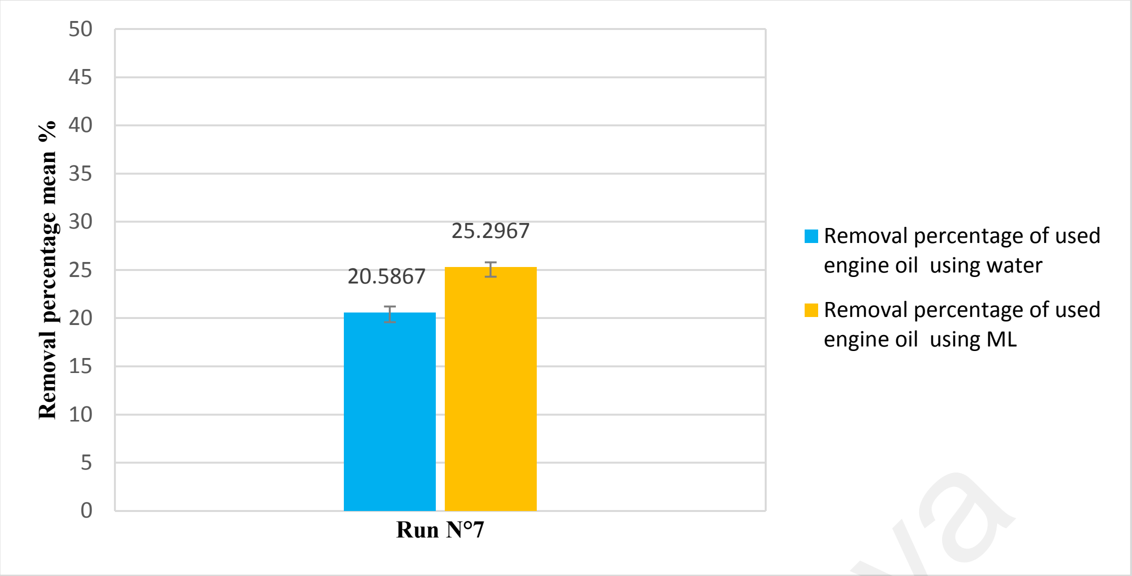


Figure 4.10 Percentage of the removal of used engine oil in Run 7. (Bars indicate standard error (S = 0.48 and S = 0.62) for removal percentage using ML and water, respectively).

The application of the lowest washing time of 5 minutes, and shaking speed of 120 strokes min^{-1} during the washing treatment process were the main reason for the decrease in the removal of used engine oil (Figure 4.11). Only 19.39% and 26.28% were obtained using water and ML, respectively.

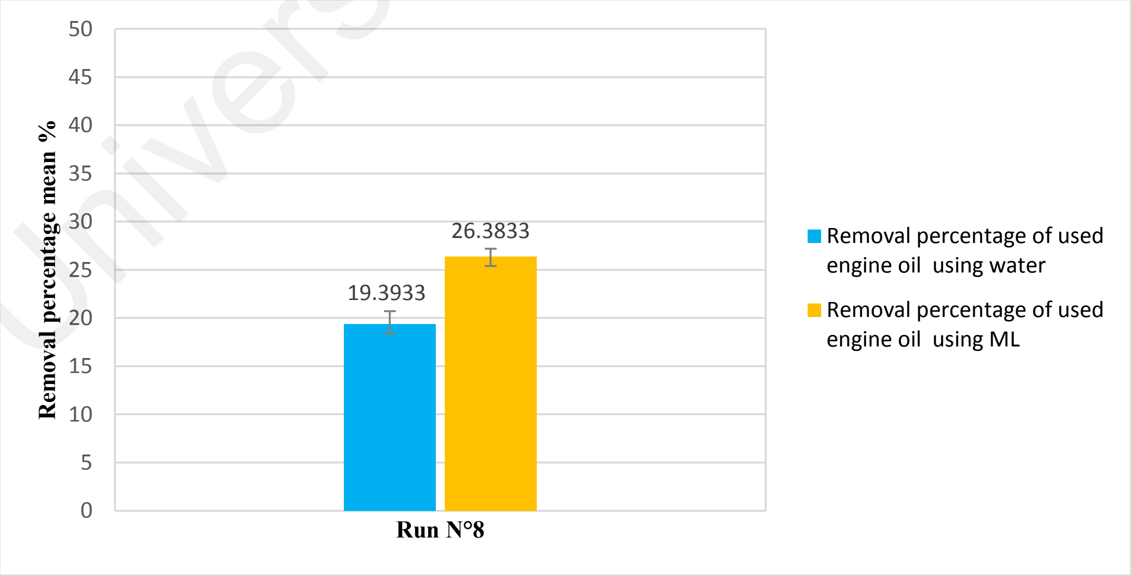


Figure 4.11 Percentage of the removal of used engine oil in Run 8. (Bars indicate standard error (S = 0.81 and S = 1.33) for removal percentage using ML and water, respectively)

4.4.2.3 Third group: Run 9, 10, 11 and 12 with temperature fixed at 42°C

The temperature was fixed at 42°C for Run 9, 10, 11 and 12. However, other variables were changed according to the experimental design. Generally, the results showed a decrease in the removal of used engine oil for 34.07%, 28.7%, 32.05% and 33.66% by ML in Run 9, 10, 11 and 12, respectively. Using water as the washing solution showed lower results as compared to those obtained using the ML solution with only 18.98%, 15.12%, 24.77% and 28.43% for Run 9, 10, 11 and 12, respectively (Figure 4.12 to Figure 4.15). The *p*-value was lower than 0.05 for all experiments, except for Run 12 (*p*-value = 0.23), indicating no significant difference in the obtained results. Thus, the experimental design plan for the treatment that applied the lowest level value for one of the variables during each run was the main reason for the weak results, because each variable will affect the efficiency of other variables.

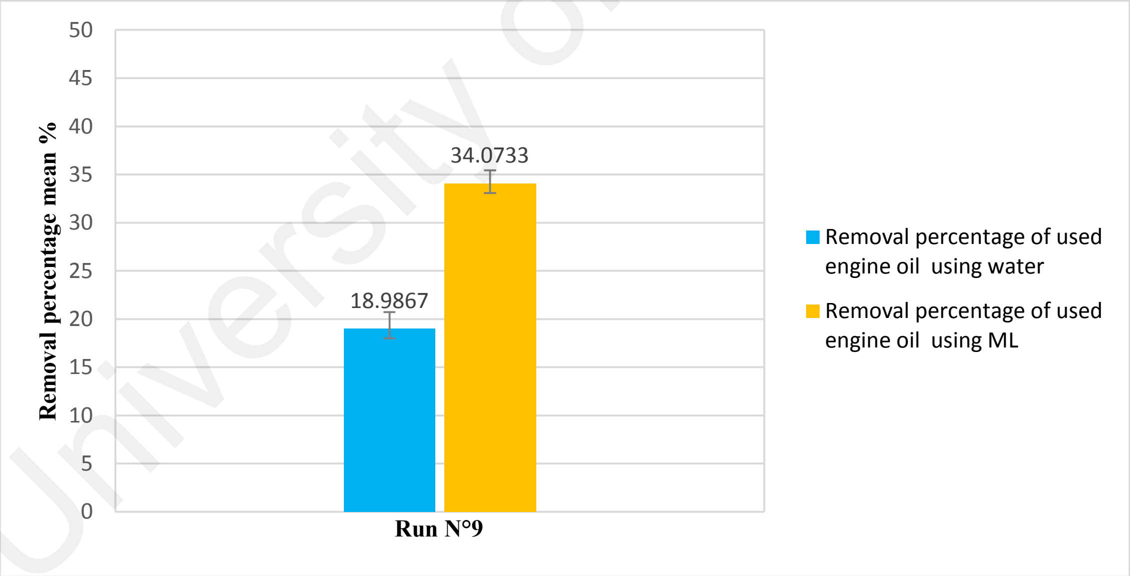


Figure 4.12 Percentage of the removal of used engine oil in Run 9. (Bars indicate standard error ($S = 1.35$ and $S = 1.73$) for removal percentage using ML and water, respectively

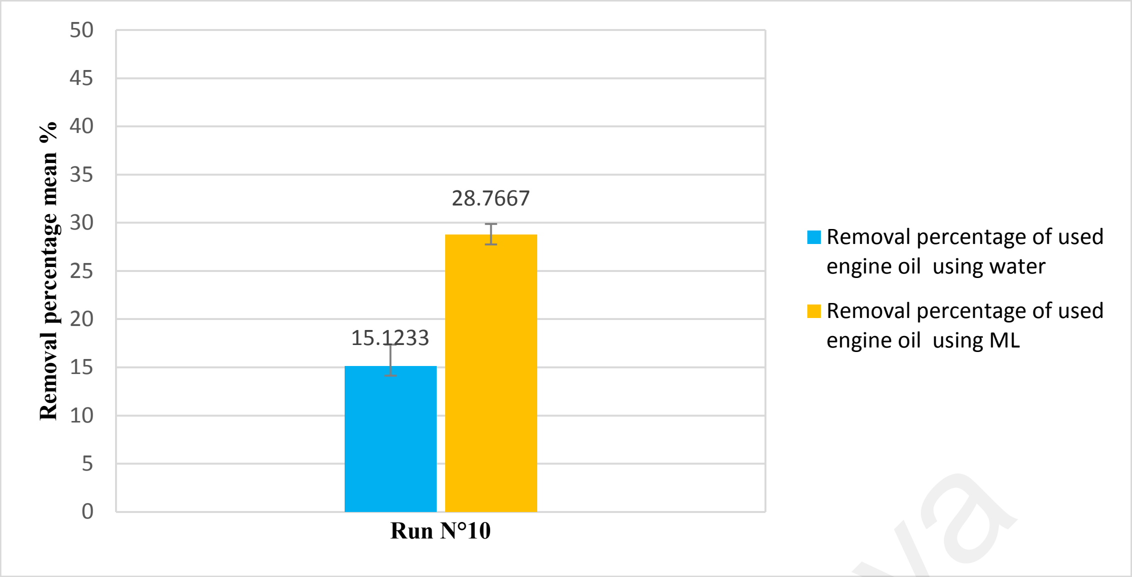


Figure 4.13 Percentage of the removal of used engine oil in Run10. (Bars indicate standard error (S = 1.09 and S = 2.23) for removal percentage using ML and water, respectively)

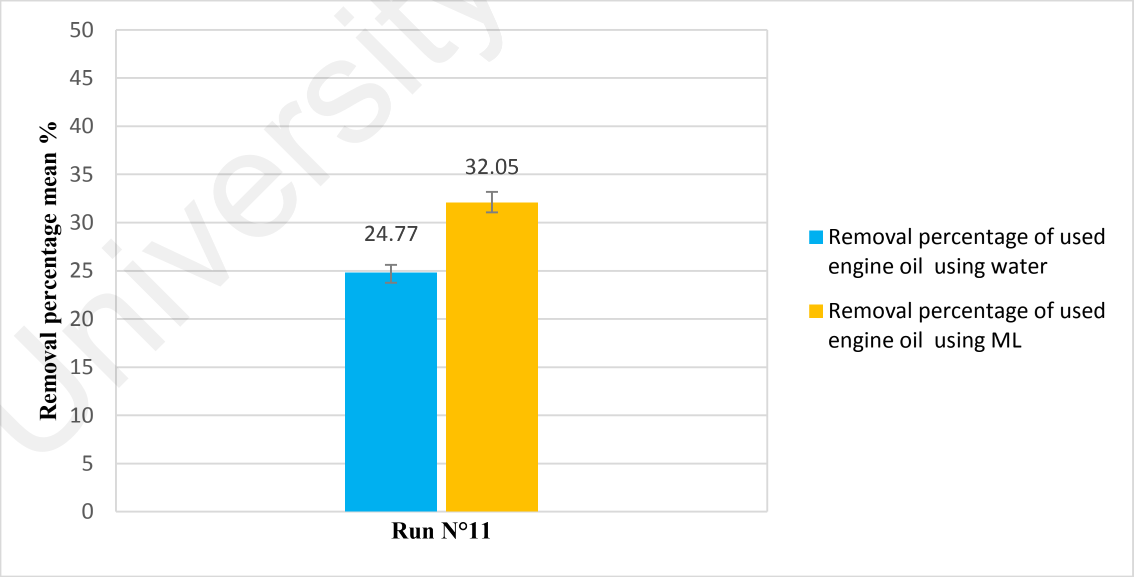


Figure 4.14 Percentage of the removal of used engine oil in Run11. (Bars indicate standard error (S = 1.14 and S = 0.85) for removal percentage using ML and water, respectively)

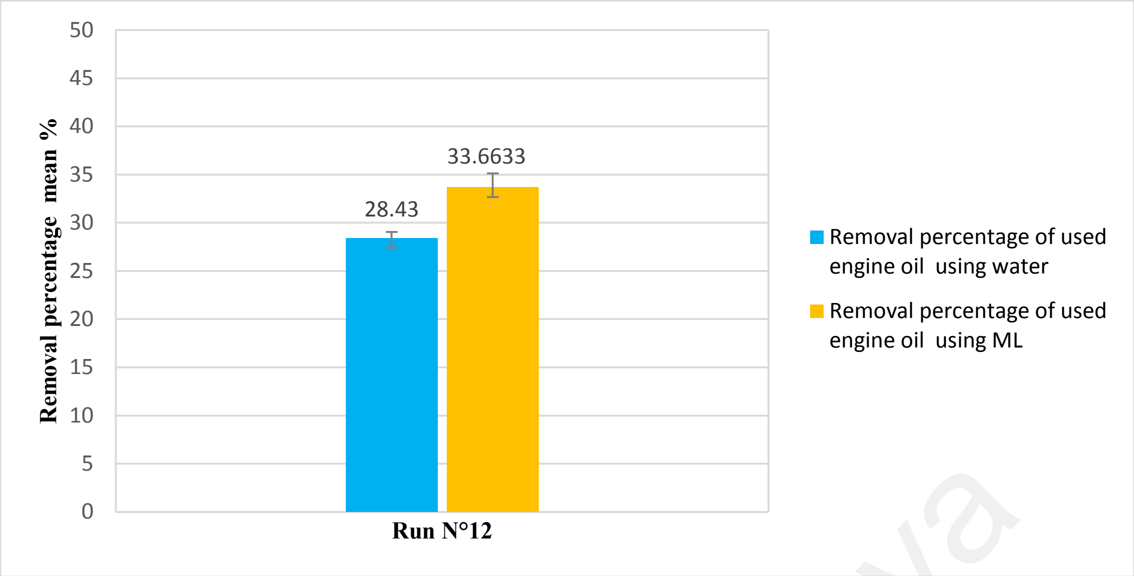


Figure 4.15 Percentage of the removal of used engine oil in Run 12. (Bars indicate standard error (S = 1.45 and S = 0.60) for removal percentage using ML and water, respectively)

4.4.2.4 Fourth group: Run 13, 14, 15 and 16 with temperature fixed at 55°C

A set of runs that were characterized by fixing the temperature at 55°C, while other variables values are shown in Table 3.2. The results showed a significant difference between the removal of used engine oil using control and ML, except for Run 15, where the *p*-value was 0.41. Similar results were obtained using water with 21.09%, 24.42%, 24.64% and 23.12% for Run 13, 14, 15 and 16, respectively (Figure 4.16 to Figure 4.19). However, the results obtained using ML showed some differences, where 40.20% was the highest removal obtained for Run 16 (Figure 4.19), followed by 34.80%, 30.16% and 22.61% in Run 13, 14 and 15, respectively (Figure 4.17, 4.16 and 4.18). The standard error was at acceptable values for all runs.

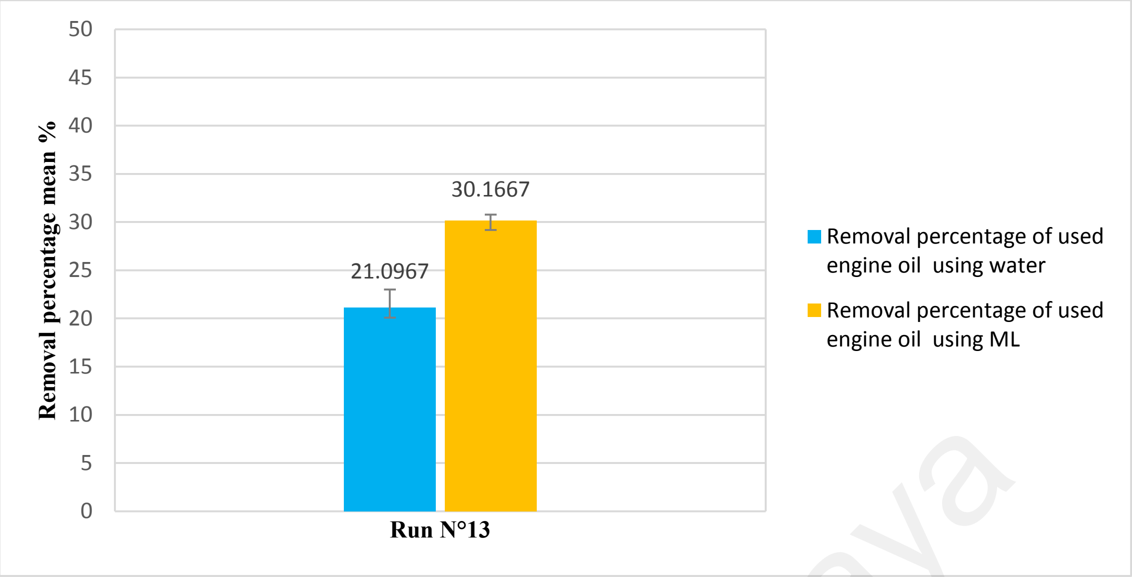


Figure 4.16 Percentage of the removal of used engine oil in Run 13. (Bars indicate standard error (S = 0.60 and S = 1.90) for removal percentage using ML and water, respectively)

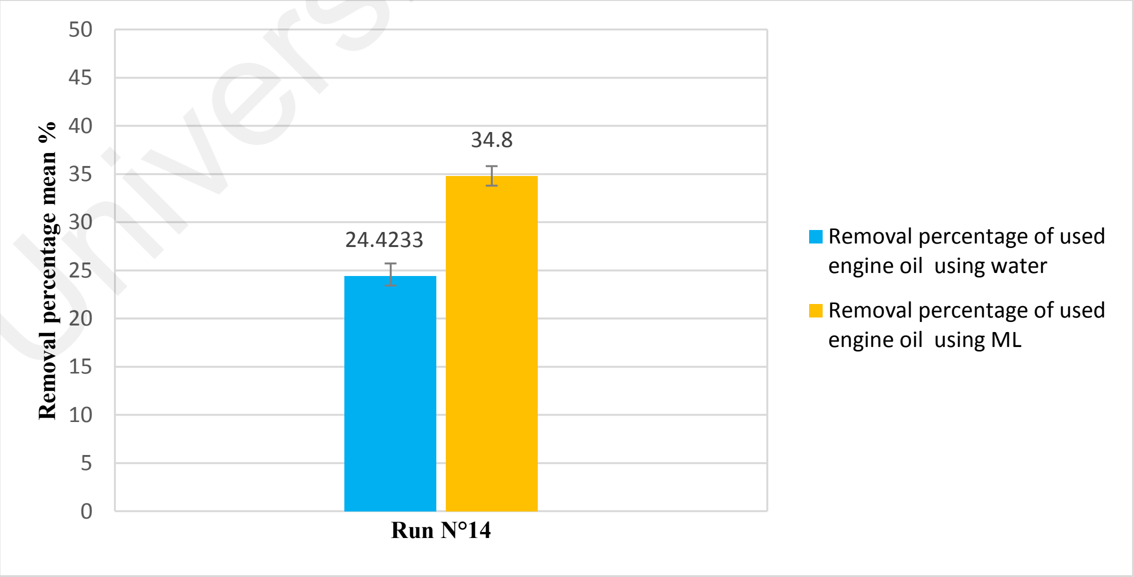


Figure 4.17 Percentage of the removal of used engine oil in Run 14. (Bars indicate standard error (S = 1.00 and S = 1.30) for removal percentage using ML and water, respectively)

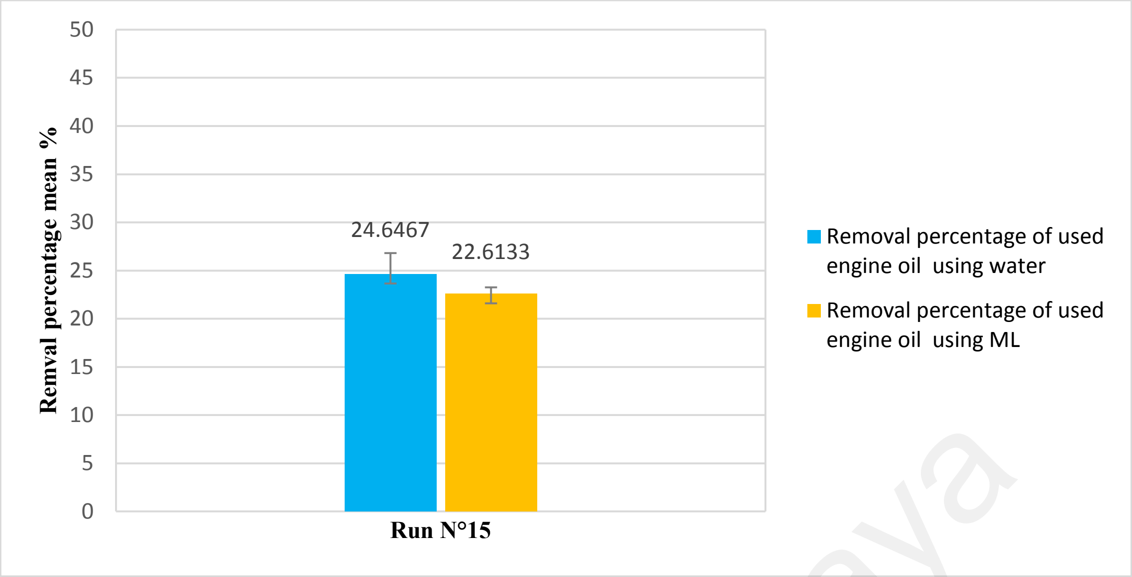


Figure 4.18 Percentage of the removal of used engine oil in Run 15. (Bars indicate standard error ($S = 0.65$ and $S = 2.15$) for removal percentage using ML and water, respectively)

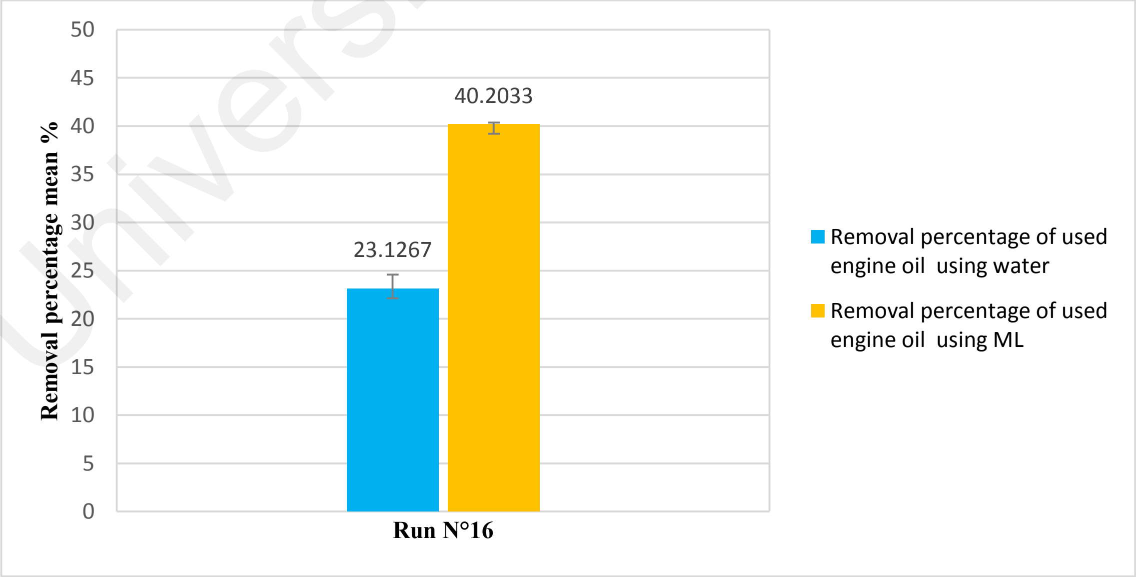


Figure 4.19 Percentage of the removal of used engine oil in Run 16. (Bars indicate standard error ($S = 0.15$ and $S = 1.46$) for removal percentage using ML and water, respectively)

4.4.2.5 Percentage of the removal of used engine oil comparison for 16 Runs

Using SPSS Software and based on *p*-values, it was clear that there is a significant difference between the used engine removal efficiency in soil treated with ML solutions and soil treated with control (*p*-value<0.05), except for Run 3, 12 and 15 indicated *p*-value above 0.05 . Moreover, the calculated total average for the removal of used engine oil was 21.35% and 30.89%, using water and ML, respectively. The *p*-value is less than 0.05, confirming significant difference between water and ML use. The standard error mean were 0.82 and 1.29 for treated soil using ML and water, respectively, indicating that experiments were conducted in optimum conditions (Figure 4.20).

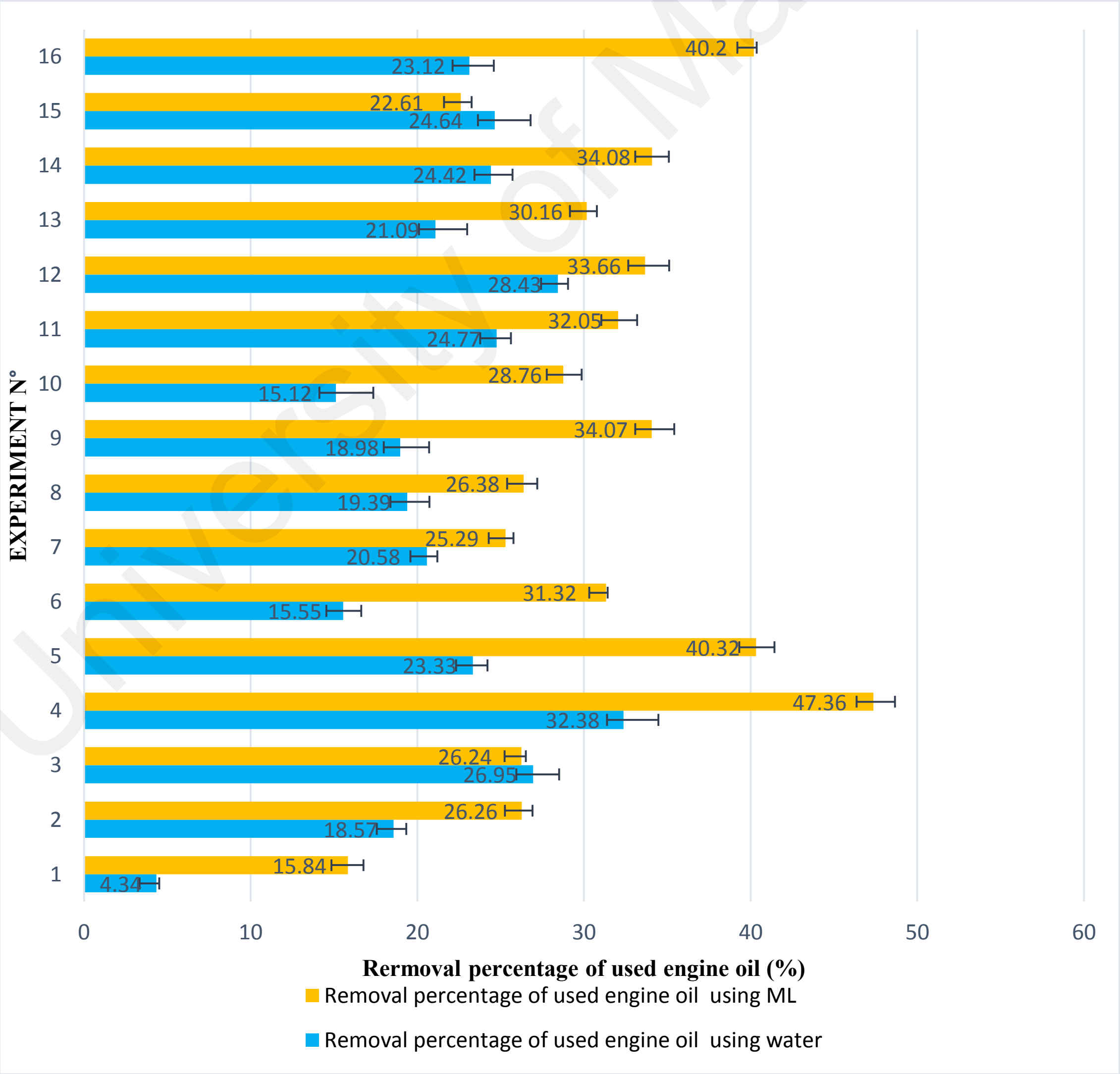


Figure 4.20 The efficiency of used engine oil removal in 16 Runs. (Bars indicate standard error (n= 0.82 and n= 1.29) for ML and water, respectively)

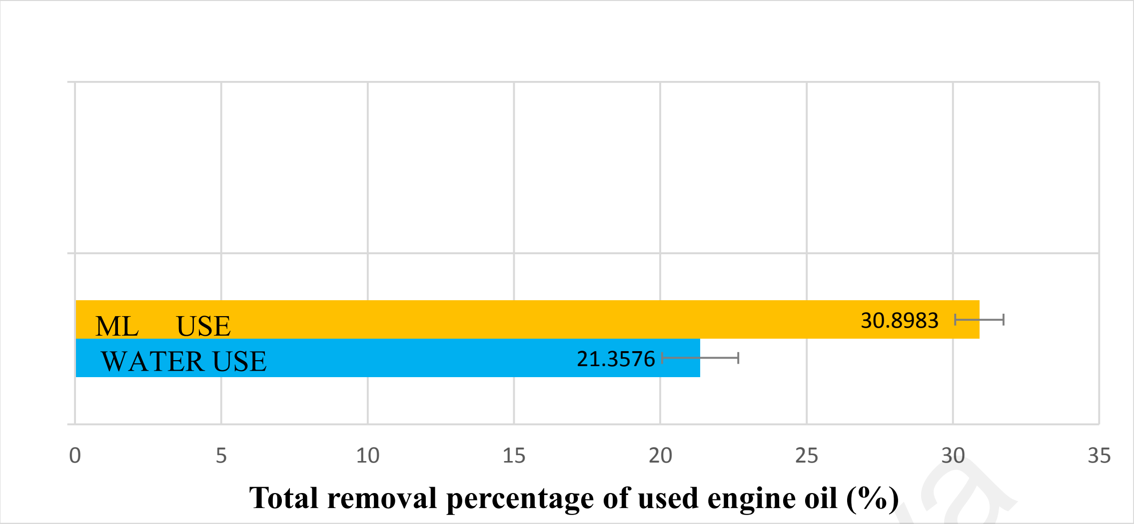


Figure 4.21 Total Percentage of the removal of used engine oil in 16 Runs (Bars indicate standard error (n= 0.82 and n= 1.29) for ML and water, respectively)

4.5 Effect of different variables on removal efficiency

According to Pignatiello (1988), the performance statistic can be chosen as the optimization criterion. The response Table 4.5 shows the performance statistic means calculated using the equation 4. In order to see the effects of the five variables on the removal efficiency of used engine oil. The main effect plots for the performance statistic were established (Figure 4.22 to Figure 4.26).

Table 4.5 Response table of performance statistic means based on the Minitab Software

LEVEL	Washing temperature (A)	Volume of biosurfactant (B)	Concentration biosurfactant (C)	Shaking speed (D)	Washing time (E)
1	27.36	27.85	27.82	27.74	26.68
2	29.36	29.48	28.45	29.06	29.49
3	30.08	29.27	29.61	30.11	30.08
4	30.85	31.47	29.92	29.70	31.45
Δ (Max-Min)	3.49	3.62	2.10	2.37	4.77
Rank	3	2	5	4	1

4.5.1 Effect of temperature on the removal efficiency of used engine oil

Temperature rise from 25°C to 55°C improved the average performance statistic from 27.36 to 30.85, respectively (Figure 4.22). The R^2 value was 0.93 and this meant strong correlations between the increase in temperature and performance statistic values. The efficiency of used engine oil removal increased with temperature, which was similar to the findings by Han *et al*. (2009) and Dai (1995). However, Urum *et al.* (2004) though obtained similar result, recorded constant removal efficiency for all non-weathered soils when it was above 20°C. The weathered soils has better removal efficiency when it was beyond 50°C. Hence, the increased temperature decreases the viscosity of the used engine oil, which improved mobility and this then increases the negative charge potential of the soil silica and reduces the re-adsorption of the used engine oil by electrostatic repulsion, leading to more removal. Similar result was obtained using control (water), but with lower value of performance statistic and correlation value (R^2).

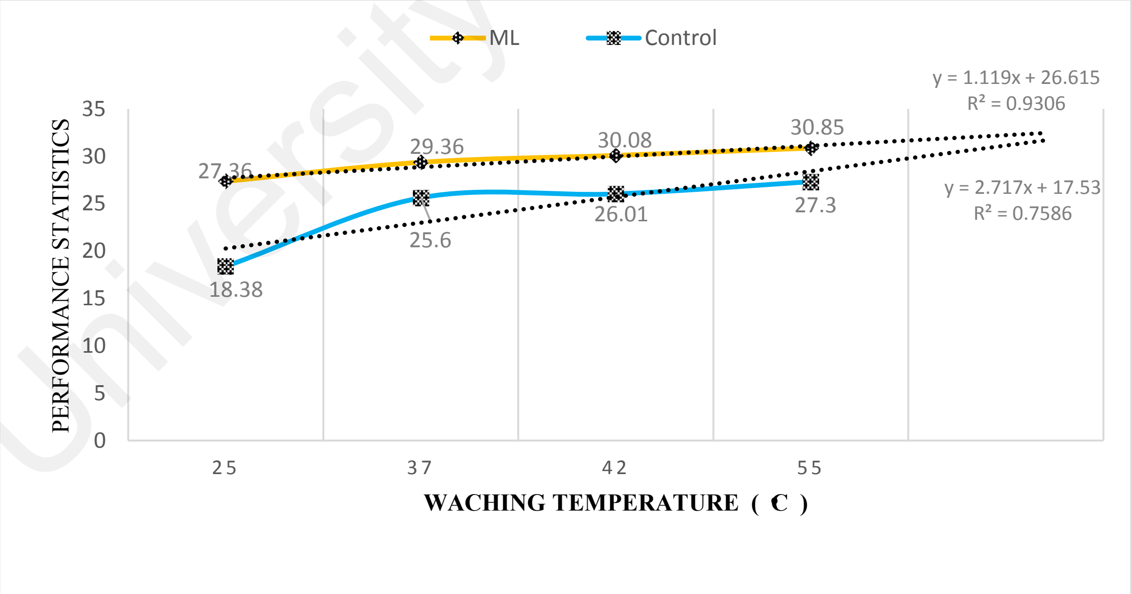


Figure 4.22 Effect of temperature on performance statistic

4.5.2 Effect of ML volume on the removal efficiency of used engine oil

By varying ML volume from 5 to 10 mL, the ML performance statistic increased from 27.85 to 29.48. At 15 mL volume, the performance statistic decreased to 29.27. However, increasing the volume to 20 mL also increased the performance statistic to the maximum level of 31.47 (Figure 4.23). Based on the R^2 value which was 0.85, there was a very strong linear correlation between volume and performance. The decline which occurred at 15 mL volume was probably due to the standard error mean at the point of treatment. Similar results were obtained by Han *et al*. (2009), who pointed out that providing more surfactant in the solution by the increase in volume enhances the interaction between the contaminants and the washing media. Furthermore, distilled water can enhance performance statistic. 18.24 and 24.85 of performance statistic were obtained at 5 mL and 10 mL, respectively. However, beyond 15 mL, the performance was found to be almost constant, indicating the non-influence of water volume in weathered contaminated soils. These results agree with those reported by Deshpande *et al*. (1999).

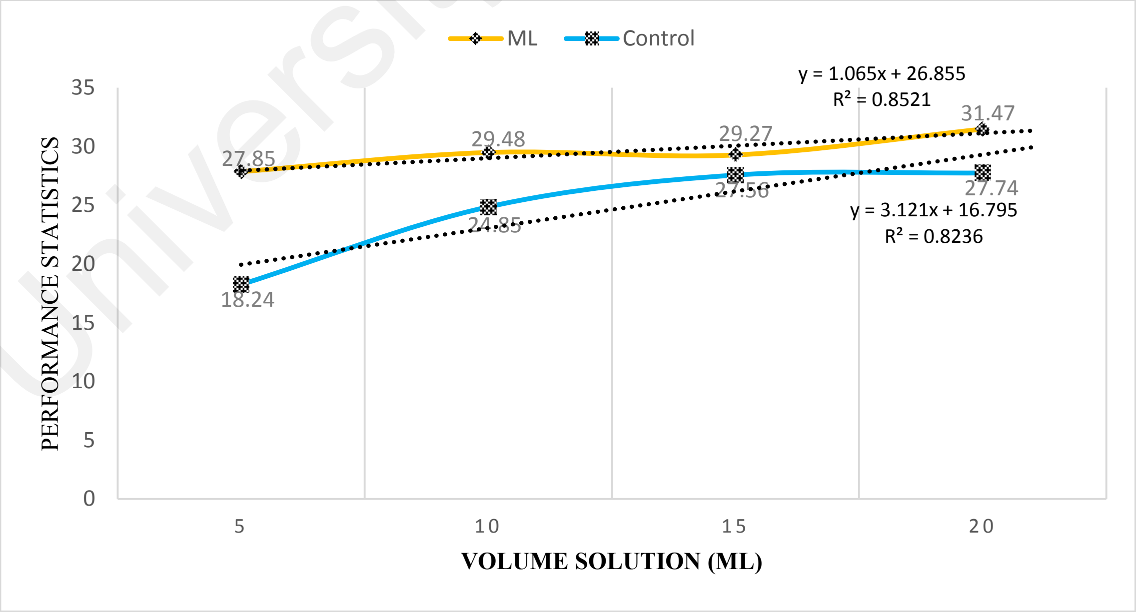


Figure 4.23 Effect of ML volume on performance statistic

4.5.3 Effect of ML concentration on the removal efficiency of used engine oil

It was observed that the increase in washing ML concentration enhanced the used engine oil removal efficiency (Figure 4.24). The minimum removal of used engine oil occurred at the lowest concentration level (0.01% - v/v) and had an average performance statistic value of 27.82. However, the highest of used engine oil occurred when concentration was at the highest level (10% - v/ v solution) and had an average performance statistic value of 29.92. Nevertheless, the removal equilibrium was not attained. The R² value of 0.95 indicated high and strong correlations between washing concentration solution and performance statistic. These results are in agreement with Urum *et al*. (2004) for the rhamnolipid and SDS surfactant which showed maximum efficiency beyond the Critical Micelle Concentration. Similarly, the results are also in accordance with Ang & Abdul, (1991), where the removal of contaminant probably occurred under mobilization, dispersion and solubilisation mechanisms. It can be noted that surfactant used in this research was used without further purification, but the concentrations mentioned in this work were based on the effect of pure biosurfactant as adopted by Han *et al.* (2009).

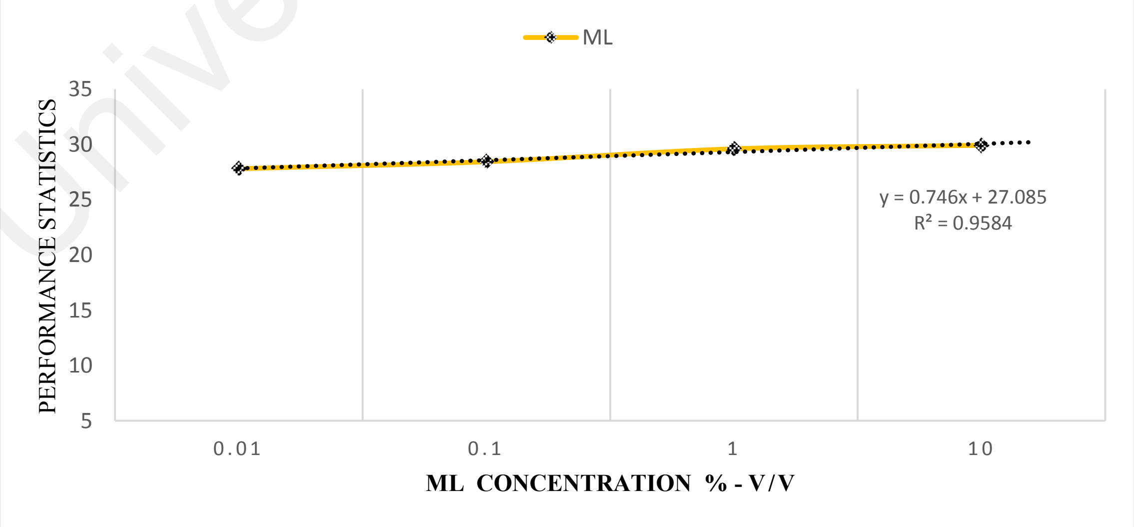


Figure 4.24 Effect of ML concentration on performance statistic

4.5.4 Effect of shaking speed on the removal efficiency of used engine oil

Results of the study indicated that increasing shaking speed from 80 to 160 strokes/min can enhance the performance statistic of the ML (27.74 to 30.11). However, further increase in agitation over 160 (strokes/min) did not improve the removal used engine oil (Figure 4.25). Results showed that the use of water recorded a constant contaminant removal over 120 (strokes/min). Similar results obtained by Urum *et al*. (2004) and Han *et al*. (2009) indicated that the increase in shaking speed can lead to exposure of more surface area of the contaminated soil by the surfactant solution. This action can accelerate the expansion of desorbed contaminant and their interaction with surfactant, which improves mobilization and solubilization mechanisms. Bernardez and Ghoshal (2008) also obtained similar results, where they found that the increase in agitation speed from 130 to 370 rpm decreased the remaining contaminants from 11 mg g^{-1} to 8 mg g^{-1} dry soils. Generally, all research obtained from literatures indicated a constant removal efficiency after a certain limit of speed agitation; therefore, increase in agitation speed did not enhance removal efficiency.

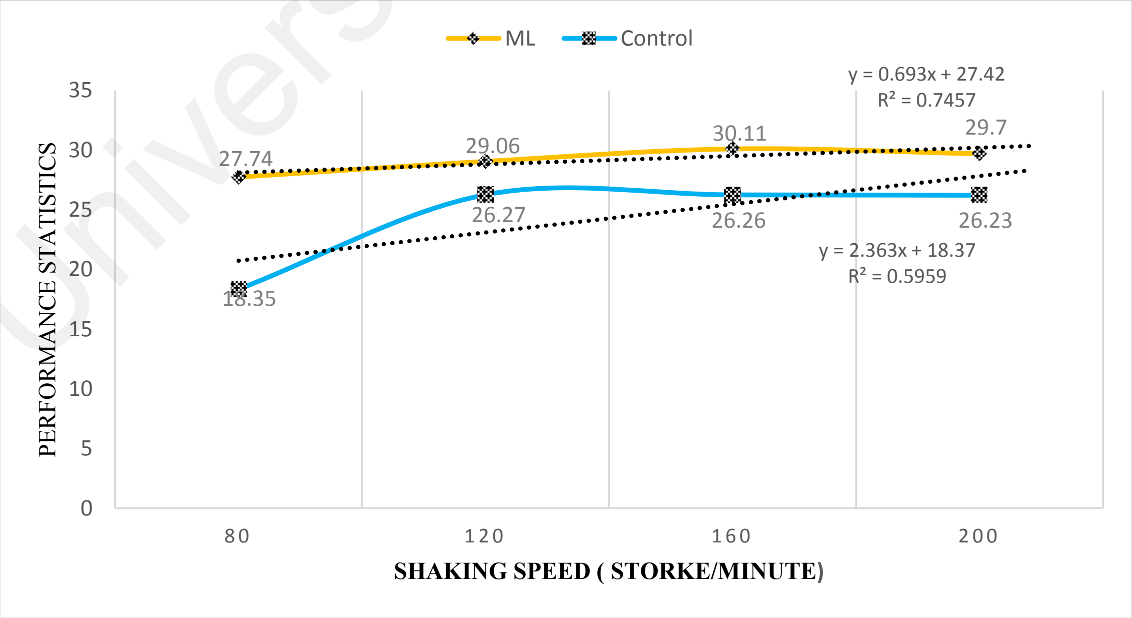


Figure 4.25 Effect of shaking speed on performance statistic

4.5.5 Effect of washing time on the removal efficiency of used engine oil

The washing time showed high similarity effects as ML concentration and washing temperature. A linear relationship was observed with a high value of R^2 (0.91). The performance statistic of ML increased gradually along with the increase in washing time. From 5 min to 20 min, the performance increased from 26.68 to 31.45, respectively (Figure 4.26). However, the removal equilibrium was not attained at the maximum washing time of 20 minutes which indicated that the washing of weathered contaminated soil need considerable time. Control has shown the same trend of performance, but with lower values; 18.11 and 28.16 at 5 and 20 min, respectively. Significant difference was found between the ML washing time and water for removal efficiency of used engine oil. Results are in agreement with the results found by Urum *et al*. (2004) and Han *et al*. (2009). Han *et al*. (2009) observed 85% oil removal results were obtained only in the first 5 min, but did not reach removal equilibrium after 1 hour of washing.

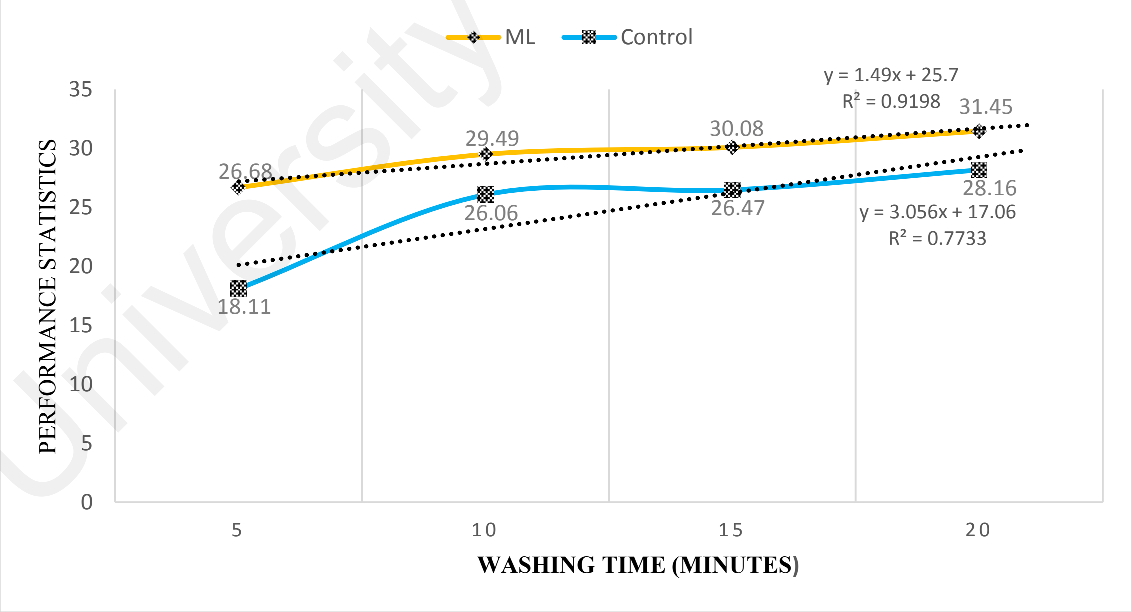


Figure 4.26 Effect of washing time on performance statistic

4.6 Results of experiments in the Optimum conditions

According to Urum *et al.* (2004), the maximum point on the curve for each variable plot was chosen as the optimum point for the treatment. Based on the performance statistic curves, the optimum washing conditions determined are shown in Table 4.6. According to these results, at 55°C, 20 ml volume, 160 strokes/min agitation, 10%-v/v concentration and 20 min washing time, the highest removal efficiency can be achieved (>47.36%). However, the removal equilibrium was not attained at these maximum points, except the shaking speed which indicated that maximum removal efficiency can be reached on the third level (160 strokes/min). According to the range (Δ) between the maximum and minimum value of performance statistic for each parameter, it can be concluded that increasing the variables levels except for the shaking speed enhances contaminant removal efficiency to more significant values. Similar results were obtained by Urum *et al.* (2004) concerning temperature, where 50°C was the optimum point for all surfactants used, as well as, shaking speed at 160 strokes/min for Sodium Dodecyl Sulfate and rhamnolipid surfactant. However, optimum conditions for concentration and volume solution variables were based on properties of each surfactant.

Table 4.6 Optimum conditions for washing soil treatment

Washing temperature (°C)	Washing LM concentration (% - v/v)	Washing ML volume (ml)	Shaking speed (stroke/min)	Washing time (min)
55	10	20	160	20

4.7 Most influential variables for the treatment results

After evaluating equation 4 and following the performance statistic response table, the washing time was found to be the most influential variable for the treatment with Sigma factor of 4.77 (Section 3.6). The result obtained mostly agreed with Han *et* *al*. (2009), and differed from Urum *et al*. (2004). The volume of the ML and washing temperature also has important influence on the treatment (Δ= 3.62 and Δ=3.49 respectively).

4.8 Determination of signal-to-noise ratio effect

In order to observe the effects of noise in this treatment process, each experiment was conducted in triplicates under the same conditions but at different times, and based on the response table for signal-to-noise means (Table 4.7), and the established effect plot for SN (Figure 4.27 to Figure 4.31). Signal-to-noise or SN number for each experiment was calculated using data obtained for table 4.4 and using Equation 5 a 6 (section 3.7).

Table 4.7 Response table for signal-to-noise means based on Minitab Software

LEVEL	Washing temperature (A)	Volume of ML (B)	Concentration ML (C)	Shaking speed (D)	Washing time (E)
1	27.03	24.66	24.94	25.39	23.78
2	24.21	22.69	26.37	26.76	23.42
3	24.39	28.44	27.49	24.54	26.20
4	23.33	28.16	27.15	22.54	25.55
Δ	3.7	5.75	2.55	4.22	2.78
Rank	3	1	5	2	4

It was observed that the volume of ML and shaking speed had the largest effect on the output of the washing process, while concentration and washing time had the smallest effect on the process. From these results, it is evidently necessary to reduce the effects of the noise, which are a smallest errors, on the volume solution, shaking speed and washing temperature variables, by using an advanced incubator shaker, which can allow the flowing of electricity current in a constant manner and changing samples without decreasing temperature at intervals. However, errors come from the volume of solution reading was probably caused by random errors, particularly in solution level reading.

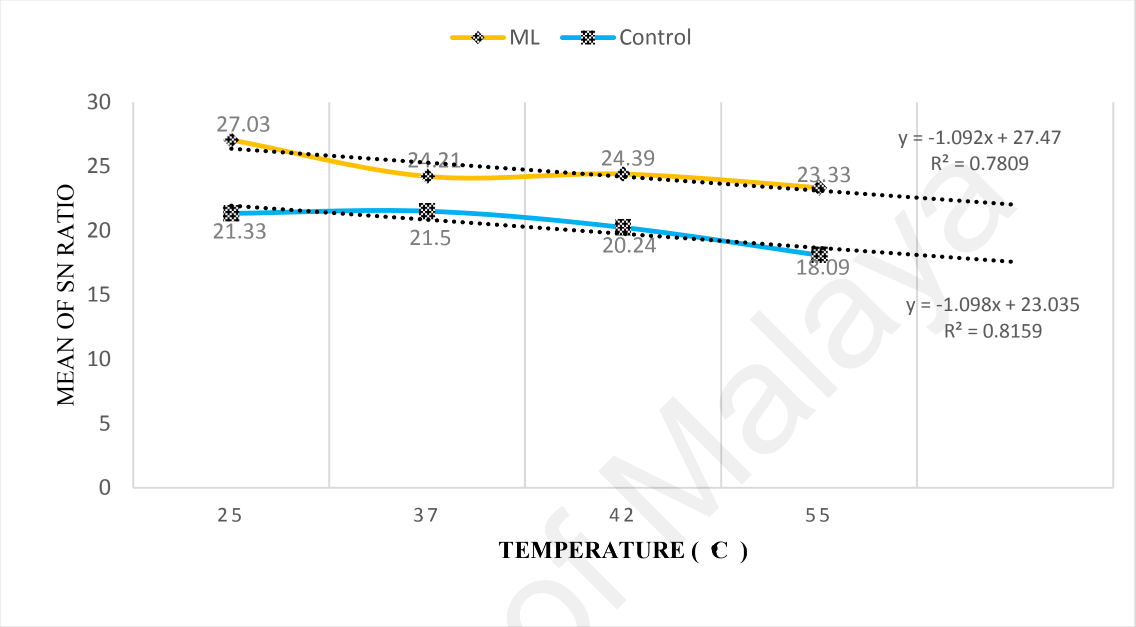


Figure 4.27 Effect Plot for SN Temperature

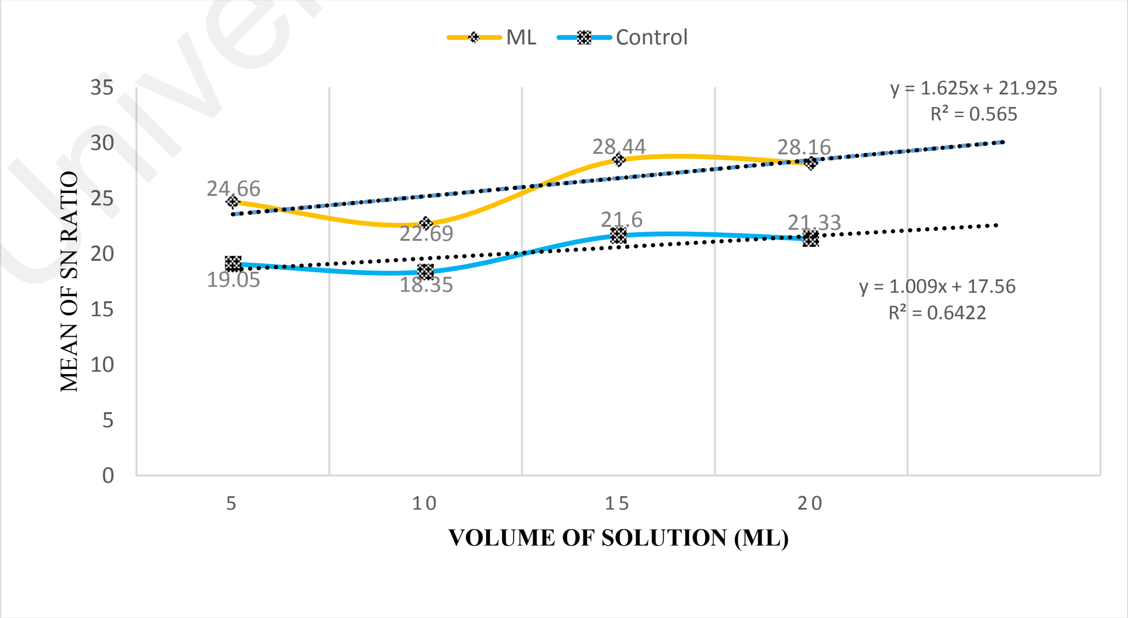


Figure 4.28 Effect Plot for SN volume

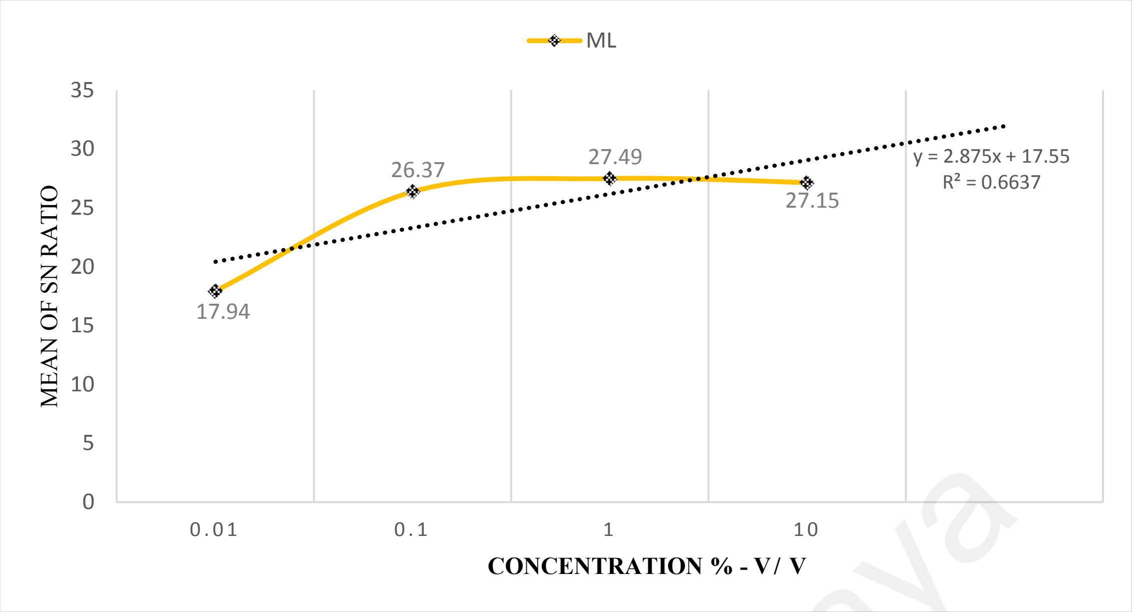


Figure 4.29 Effect Plot for SN concentration

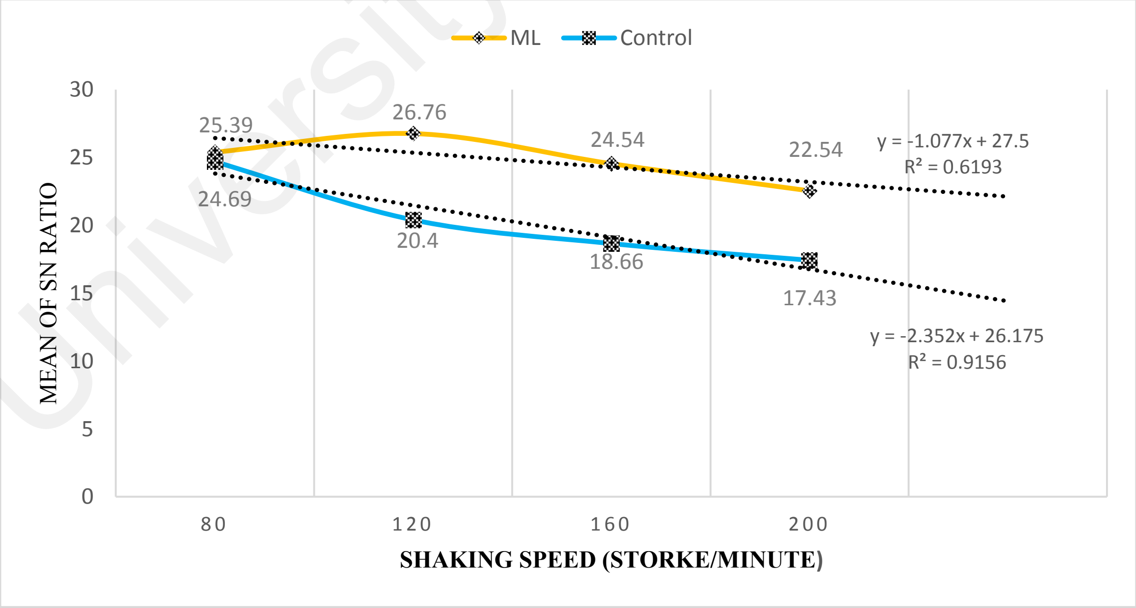


Figure 4.30 Effect Plot for SN shaking speed

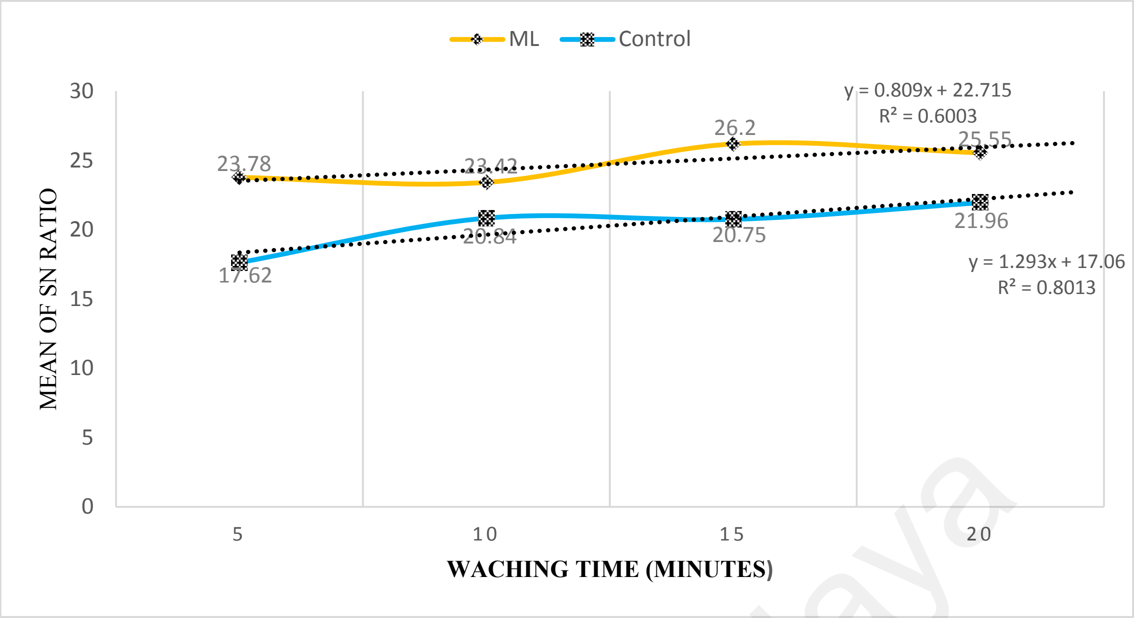


Figure 4.31 Effect Plot for SN time

From Figure 4.27 to Figure 4.31, it was observed that increase in temperature and shaking speed decreased the SN Ratio of the washing treatment. However, increase in volume, concentration and washing time increased the SN Ratio. Thus, water and ML had the same trend of signal-to-noise effect.

4.9 Comparative results with GC-MS data

The analysis of used engine oil for untreated contaminated soil using GC/MS helps to follow the predestination of hydrocarbon elements after the treating process. However, using weathered contaminated soil with unknown chemical compositions and properties of initial contaminants will cause difficulties to determine hydrocarbon predestination and new compounds which can form under the weathering process. The initial and remaining used engine oil was extracted using n-hexane, then analysed and identified based on the mass spectra and retention time (Urum *et al*. 2004; Dadrasnia *et al*., 2013).

Figure 4.32 shows the GC/MS Chromatogram of initial used engine oil extracted from untreated weathered contaminated soil. The chromatogram showed that all hydrocarbons with less than C_{24} (light elements C_{12}- C_{24} were lost and the first compound, which is 1, 2-Benzenedicarboxylic acid ($C_{24}H_{38}O_4$), appeared in the GC/MS chromatogram profile was at 41.470 minutes retention time. This result is in agreement with Garcia and Brebbia (1988) who had indicated that most Aliphatics smaller than C_{16} were reduced to trace levels after heating the oil contaminated soil for 7 days at 60°C.

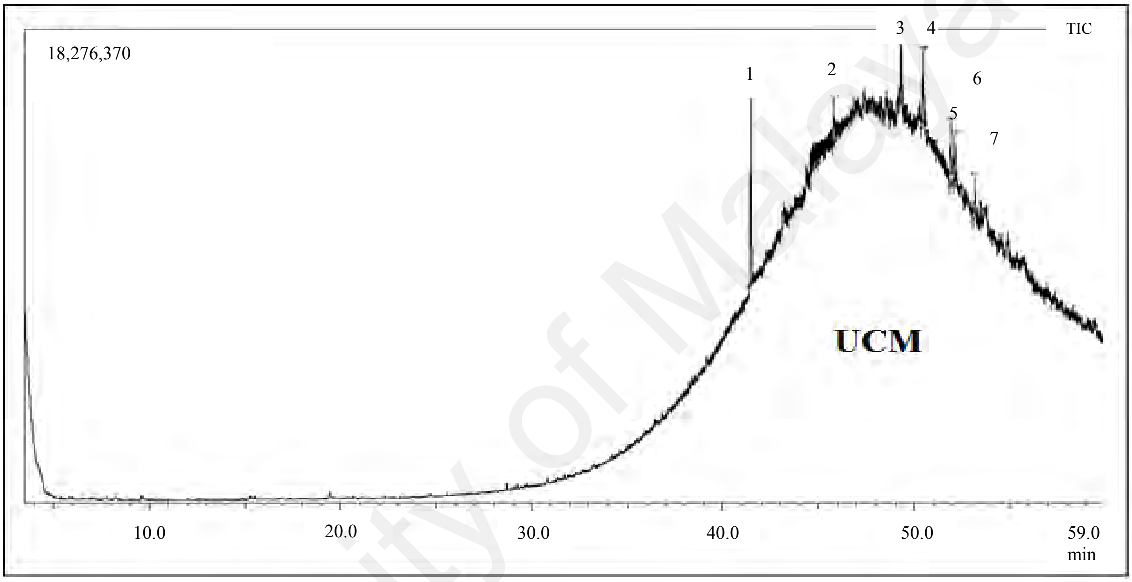


Figure 4.32 GC/MS Chromatogram of initial used engine oil extracted from untreated weathered contaminated Soil

In addition, it was noted that the base of the chromatogram have risen leading to formation of Unresolved Complex Mixture (UCM), which are materials that cannot be detected by the GC/MS; only a few resolved peaks were detected ranged between 1 to 7 (Figure 4.32). Thus, the remaining heavy elements ($C_{24}$to C_{40}) in weathered contaminated soil will tend to bind and adhere in the soil particles and make their removal difficult as compared to non-weathered contaminated soil with less UCM. Similar results were obtained by Urum *et al*. (2004) using weathered soil contaminated with heavy oil.

In order to calculate and estimate the removal percentage of used engine oil after the treatment, the best results that were obtained i.e. Run 4 was chosen to be analysed. A quantitative comparison was made based on the area peaks which have the same retention time in 3 Chromatogram profiles (Figure 4.33 to Figure 4.34)

The GC/MS Chromatogram analysis (Figure 4.33 and Figure 4.34) showed that a significant proportion of linear and cyclic n-alkanes ($C_{12} - C_{20}$: Benzene, Dodecane, Hexadecane, Cyclopropane and Dodecyl acrylate) appear in both contaminated soil washed using ML and distilled water. These were not detected in untreated weathered contaminated soil (Figure 4.32). These results are helpful to interpret the oil removal mechanism. Table 4.8, which created based on GC/MS report results, presented the comparison of area peaks at retention time 41.47, 49.31 and 50.47 minutes for the GC-MS chromatogram for untreated contaminated soil, water treated soil and ML treated soil, respectively. The comparison can provide and explain the process of hydrocarbon degradation and outputs

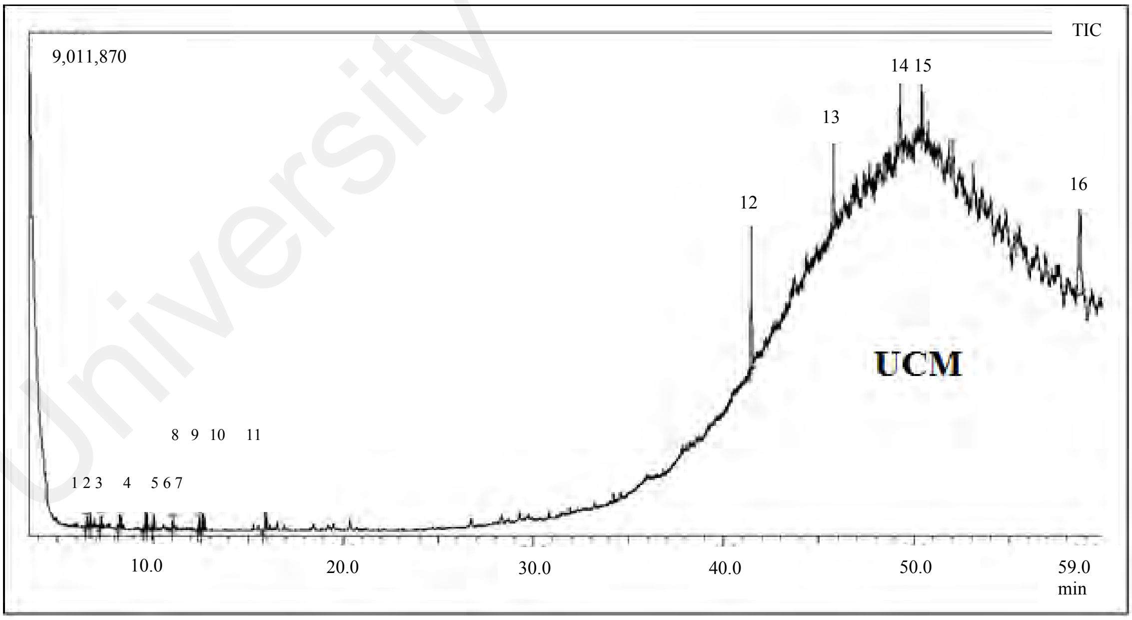


Figure 4.33 GC/MS Chromatogram of remaining used engine oil extracted from contaminated soil treated using ML; 20 mL volume; 10% - v/v; 200 strokes/min shaking speed; 20 min washing time; 25°C washing temperature.

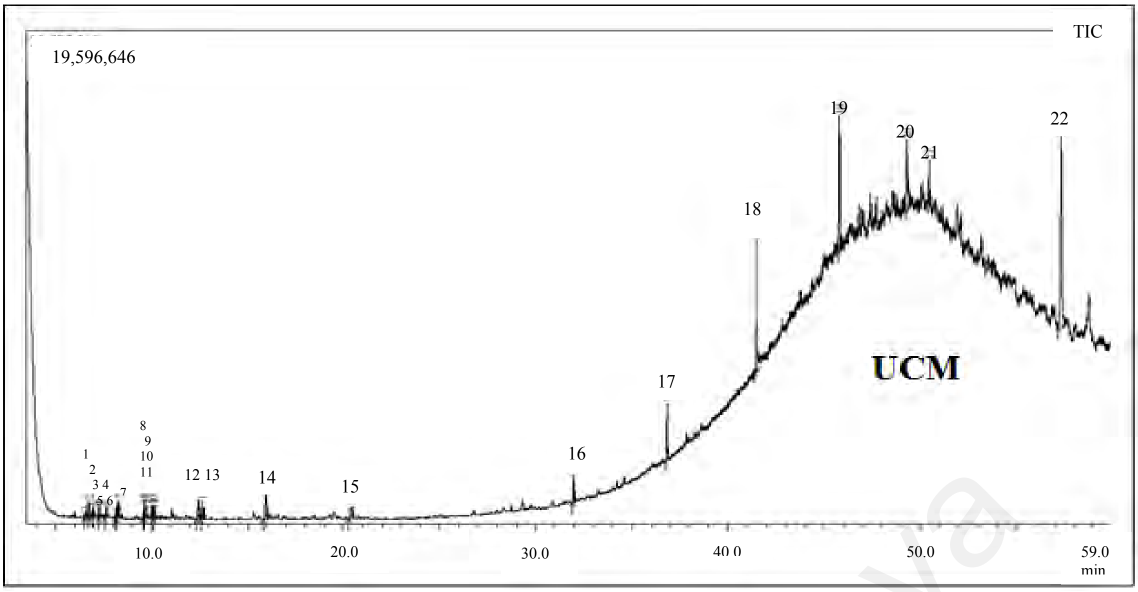


Figure 4.34 GC/MS Chromatogram of remaining used engine oil extracted from contaminated soil treated using distilled water (control); 20 mL distilled water; 200 strokes/min shaking speed; 20 min washing time; 25℃ washing temperature

Table 4.8 Area peaks at retention time 41.47, 49.31 and 50.47 minutes

Samples	Peaks number	Area of peaks	Rentention time (minute)
Untreated contaminated soil	Peak N1	5039747	41.47
	Peak N3	1046755	49.31
	Peak N4	1142693	50.47
contaminated Soil Treated using ML	Peak N12	1545590	41.47
	Peak N14	278618	49.31
	Peak N15	223257	50.47
Contaminated Soil Treated using water	Peak N18	3327912	41.47
	Peak N20	694148	49.31
	Peak N21	676850	50.47

It is clear that reduction in aliphatic and aromatic compounds indicating by reduction in area of peaks of untreated contaminated soil after treatment process (Table 4.8) were the major cause of appearance of new light n-alkane hydrocarbons such as

Benzene, Dodecane, Hexadecane, Cyclopropane and Dodecyl acrylate, probably under degradation and oxidized indicated by peaks 1 to 11 (Figure 4.33) and peaks 1 to 17 (Figure 4.34). It was also clear that the degradation in the area peaks in the contaminated soil treated using ML were higher than those treated using water. However, certain compounds which appeared at area peaks 5, 6 and 7 of the untreated contaminated soil GC-MS chromatogram (Figure 4.32) were converted to acidic compounds; Propionic acid or 2,2-dimethyl at area peak (Figure 4.33) and Hexadecanoic acid or tetradecyl ester at area peak number 22 (Figure 4.34). Similar results were obtained by Han *et al.* (2009) who had found that the proportion of small n-alkanes ($C_{16} - C_{23}$) in the residual crude oil gradually increased. Results in this study also agree with Shallu and Hardik (2014) who had studied biodegradation of Poly Aromatic Hydrocarbons. Anthracene was oxidized by Mycobacterium enzymes in the 1, 2 positions to form cis-1, 2-dihydroxyl and 2-dihydroanthracene then converted it to 1, 2-dihydroxyanthracene and finally, to the ring fission product cis-4-(2hydroxynaphth-3-yl)-2-oxobut-enoic acid; by the same pathway, Catechol was degraded to simple aliphatic compounds.

4.10 Bioremediation technique results

The experiments which were carried out in triplicates show slightly different results. The results were measured at 15, 30, 45 and 60 days in order to determine the effectiveness of the remaining bacteria in the degradation of banded used engine oil based on different time, water and aeration. The results are tabulated in Table 4.9

Table 4.9 Mean of degradation removal percentage at selected time.

Days	0	15	30	45	60
Mean of removal percentage without water and agitation	49.16	55.49	58.16	62.33	64.84
Mean of removal percentage with water and agitation	49.16	57.13	66.74	75.91	83.58

Using SPSS Software, independent-samples T-test analysis was done with all experiments in order to compare results and to confirm the existence or absence of any similarities or significance between contaminated soils treated by adding water and agitation and those without water and agitation at 15, 30, 45 and 60 days. The results for the standard errors and *p*-values are shown in Table 4.10.

Table 4.10 *P*-value and standard error results

Days		0	15	30	45	60
- water - agitation	Standard error	0.2	0.73	0.16	0.44	0.32
+ water + agitation	Standard error	0.2	0.38	0.74	0.51	0.67
(level of marginal significance)	*p*-value	0.00	0.44	0.00	0.00	0.00

4.10.1 Results obtained at 15 days of bioremediation

During the 15 days of bioremediation (Figure 4.35), the results showed no significant difference in degradation percentage of used engine oil between set-up without water and agitation and setup with water and aeration. The results showed a very slight increase in the degradation of used engine oil, only 55.49% and 57.13% for set-up without water and agitation and set-up with water and agitation, respectively. Based on the independent-samples T-test analysis, the *p*-value was found to be 0.11 (> 0.05). This indicates that no significant difference between the results was obtained from the two set-ups. Time probably was the main reason to the difference in removal efficiency because bacteria need more time to grow to make significant difference. Fifteen days of bioremediation increased the degradation percentage only by 6.33% and 7.96% for set-up without water and agitation, and with water and agitation, respectively (Table 4.9). Addition of water and aeration give only 1.64% difference. As each experiment was done in triplicates, the standard error was found to be 0.73 and 0.38 for set-up without water and agitation, and with water and agitation, respectively, indicating the good and accurate conditions of bioremediation (Table 4.10).

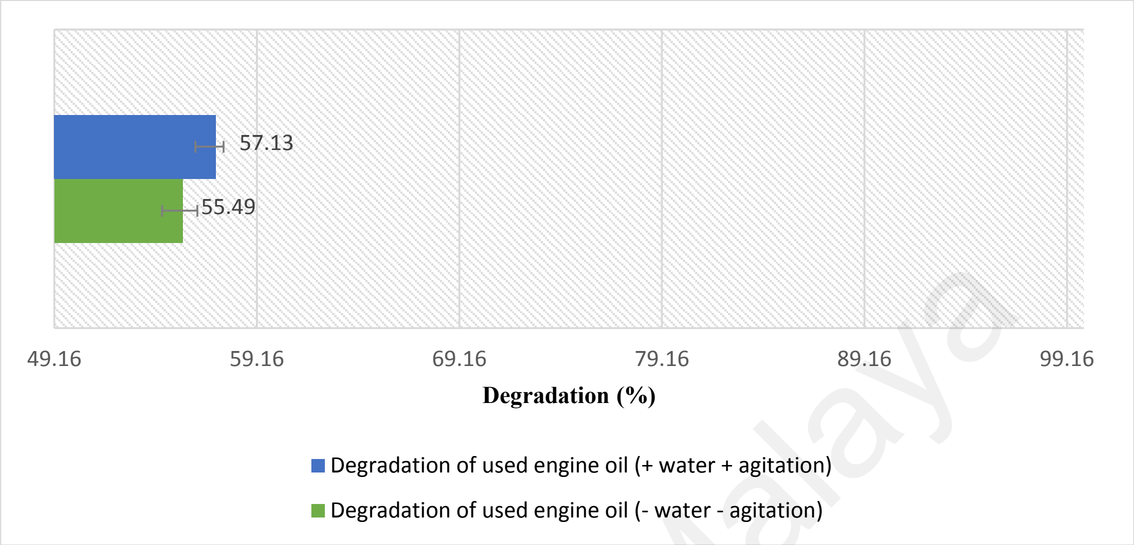


Figure 4.35 Degradation of used engine oil after 15 days treatment. (Bars indicate standard error $S = 0.73$ and 0.38 for set-up without water and agitation, and with water and agitation, respectively)

4.10.2 Results obtained at 30 days of bioremediation

During the 30 days of treatment (Figure 4.36), the results showed a rising continuation of used engine oil degradation percentage as compared to those at 15 days. The degradation percentage of used engine oil in for set-up without water and agitation and with water and agitation were 58.16% and 66.74%, respectively. The Independent-samples T-test analysis using SPSS Software showed a significant difference in the results, where the degradation of used engine oil in set-up without water and agitation increased by 2.67% as compared to the results obtained after 15 days (55.49% to 58.16%). However, for set-up with water and agitation a significant increase in the degradation of used engine oil recorded 9.61%. The 9.61% rise can be explained by the increase in bacteria activity and growth based on the availability of nutrients, water and oxygen. The standard error was found to be 0.16 and 0.74 in set-up without water and agitation, and with water and agitation respectively, indicating a good bioremediation conditions. The *p*-value was at 0.00, proving the significant difference between the results of each set (Table 4.10).

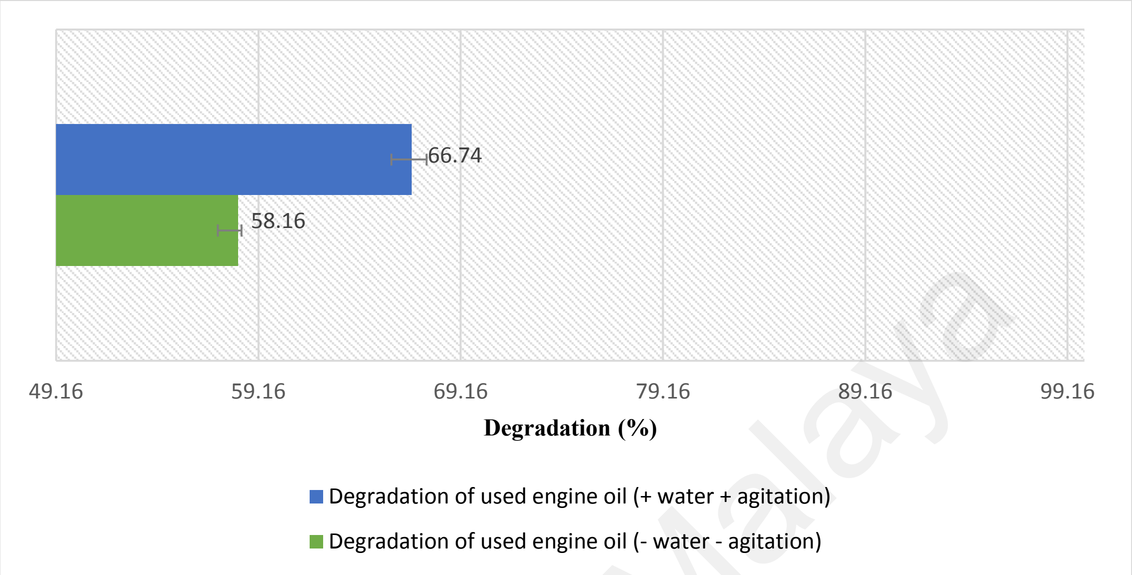


Figure 4.36 Degradation of used engine oil after 30 days treatment. (Bars indicate standard error *S* = 0.16 and 0.74 for set-up without water and agitation, and with water and agitation, respectively)

4.10.3 Results obtained at 45 days of bioremediation

After 45 days of bioremediation (Figure 4.37), the results showed an effective continuation in degradation of the used engine oil in both set-ups. The continuation of bacteria growth and activity lead to higher degradation efficiency. 62.33% of used engine oil was degraded from treated soil without water and aeration, indicating a 4.17% augmentation compared with the results obtained at 30 days. Similarly, for the set-up with water and agitation, added 9.19% excess degradation was recorded as compared to those obtained at 30 days. The standard error was found to be at 0.44 and 0.51 for set-up without water and agitation, and with water and agitation, respectively. The *p*-value was 0.00, indicating a variance between the results in both set-ups (Table 4.10).

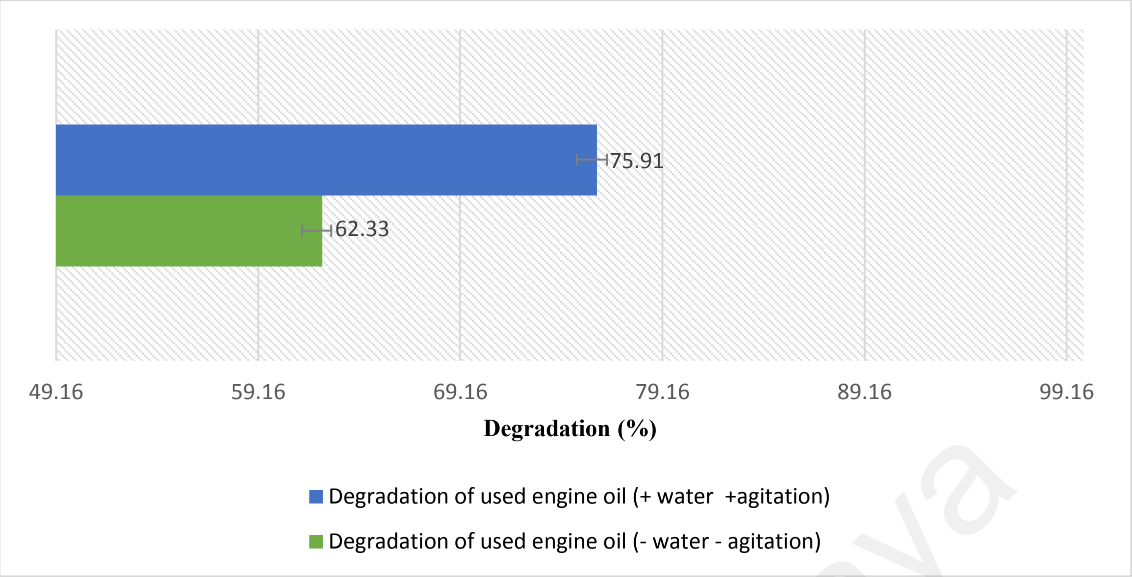


Figure 4.37 Degradation of used engine oil after 45 days treatment. (Bars indicate standard error $S = 0.44$ and 0.51 for set-up without water and agitation, and with water and agitation, respectively)

4.10.4 Results obtained at 60 days of bioremediation

The results obtained from Figure 4.33 indicate an increase in the degradation of used engine oil, but without the same degree of performance as at 30 and 45 days. The removal reached 83.58% in set-up added with water and aeration. Similarly, the degradation of used engine oil reached 64.84% in set-up without water and aeration. Thus, the lack of performance was probably caused by a deficiency in nutrient elements from the contaminated soil treated with addition of water and aeration. Bacteria used up the most of the nutrients at the earlier stage. On the other hand, contaminated soils treated without water and aeration also indicated a drop in performance, probably caused by total deficiency of water content in soils. Similarly, the Independent-samples T-test analysis showed good conditions for bioremediation process. The standard error was found to be only 0.32 and 0.67 in set-up without water and agitation and set-up with water and agitation, respectively (Table 4.10)

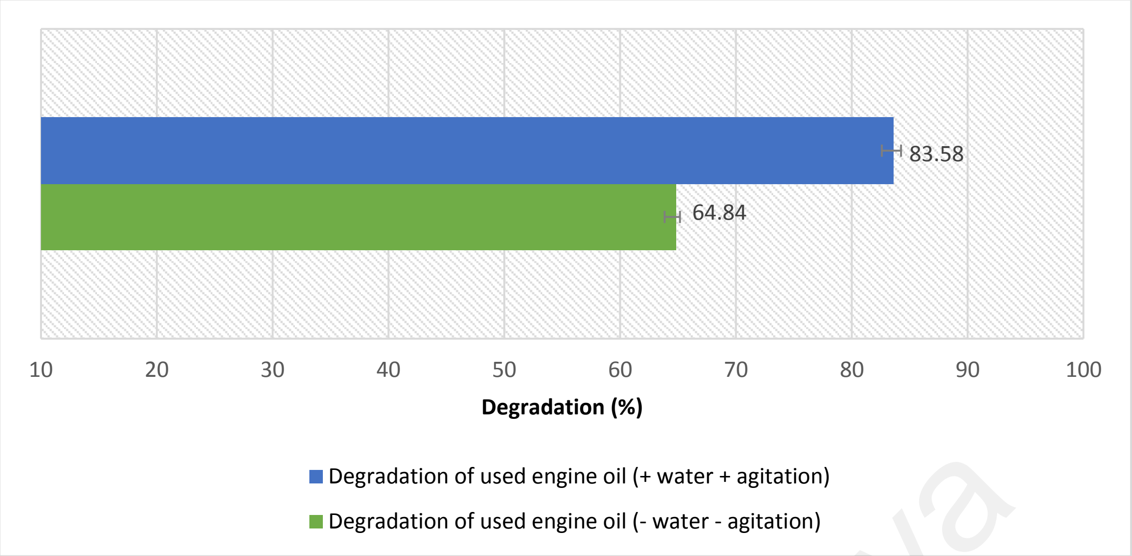


Figure 4.38 Degradation of used engine oil after 60 days treatment. (Bars indicate standard error $S = 0.32$ and 0.67 for set-up without water and agitation, and with water and agitation, respectively)

4.10.5 Comparison of results obtained at 15, 30, 45 and 60 days of bioremediation

From the results shown in Figure 4.39, a strong correlation between the time parameter and the degradation of used engine oil were present in both set-ups, indicated by R^2 of 0.97 for set-up without water and agitation and 0.99 for set-up with water and agitation. However, beginning from 45 to 60 days, a clear decrease in the degradation of the used engine oil was observed despite reaching its maximum level at 60 days of treatment in both set-ups. As previously mentioned, deficiency in carbon, nitrogen and sulphur in the contaminated soil added with water and aeration was probably the main reason for the decreased performance. Bacteria used up the majority of nutrients. However, water content deficiency is probably the reason of the leaking performance lack in soils without water and aeration. Dadrasnia and Salmah (2015) using the bacillus 139I and tea leaf as amendments for soil contaminated with crude oil recorded above 89% of crude oil degradation in period of 2 months. Also, Gestel *et al.* (2003) reported that 85% of diesel oil degraded in soil amended with different composts in period of 12 weeks. In contrast, Chang et al., (2010) found highest degradation of semi and non-volatile petroleum hydrocarbons in soil amended with nutrients only compared with those amended with bacteria.

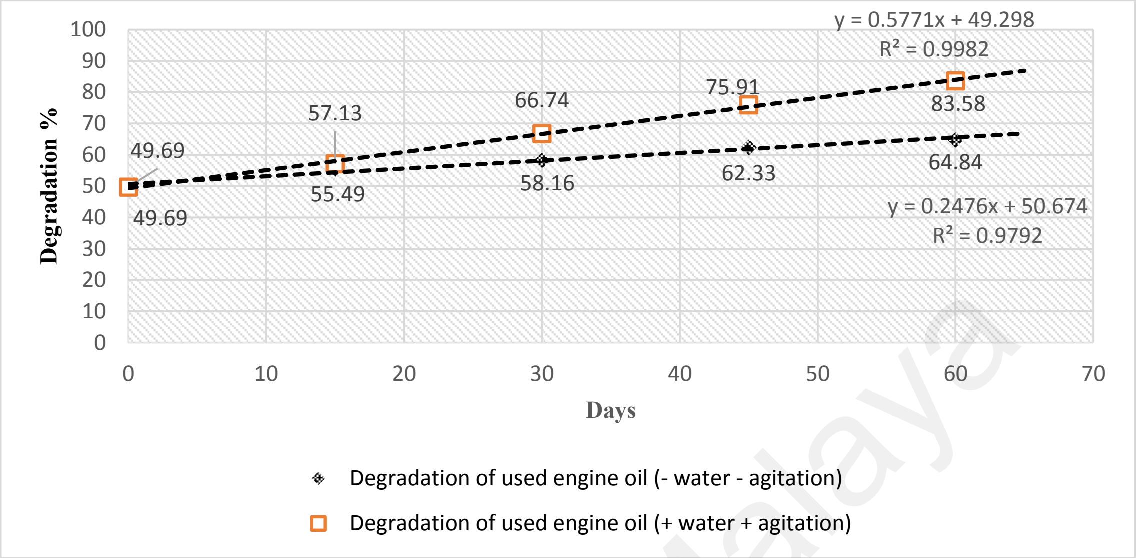


Figure 4.39 Degradation of used engine oil comparison at 15, 30, 45 and 60 days of treatment.

From the linear equation shown in Figure 4.39 and the fact that there is no deficiency in nutrient and water, it is easy to forecast the future results. At 199 days of treatment, degradation of used engine oil can reach 100 % in soil without the addition of water and aeration addition. However, only 88 days is enough to degrade all the remaining used engine oil in soil added with water and aeration.

The washing treatment and bioremediation results showed that the washing technique had removed almost half of the used engine oil concentration, and the existence of bacteria in the washing solution, with nutrients, water and oxygen lead to a removal of up to 83.58% in a period of just two months. The results showed an increase in the degradation of used engine oil with time, which is the most influential variable in the treatment of weathered contaminated soils. However, the deficiency in nutrients affected the degradation performance after 30 days of bioremediation (Table 4.11).

Table 4.11 Rate of used engine oil degradation between intervals

Interval (days)	Rate of excess (%) without water and agitation	Rate of excess (%) with water and agitation
0 - 15	1333	73.7
15 - 30	2317	.314
30 - 45	4347	.347
45 - 60	2324	7317

Set-up with added water and agitation showed a clear decrease in excess rate of 9.61% to 7.67% for the used engine oil degradation after 30 days of remediation, indicating a retrogradation of degradation performance (Figure 4.40). The rate of excess also decreased from 2.67% to 2.51% in set-up without addition of water and agitation. Thus, it is as important that nutrient such as carbon, nitrogen and sulphur, oxygen and water are provided sufficiently in order to guarantee bacterial growth and acceleration in the degradation rate of contaminants.

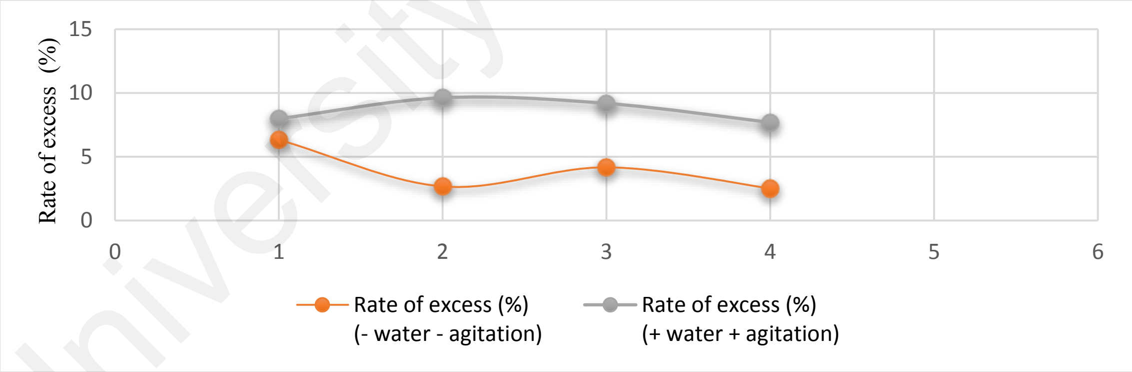


Figure 4.40 Used engine oil excess rate degradation during treatment period.

4.11 Recommendations

According to the results, in order to enhance used engine oil in degradation, it is important to improve certain physical and chemical properties in the contaminated soil, and also modify the conditions of treatment. As a result, the following are some suggestions:

- As removal of used engine oil did not reach equilibrium through the alteration of environmental variables, it is important to increase in the value of all variables, except for shaking speed. Moreover, the temperature needs to be increased beyond 55°C, but below 80°C (prevent evaporation process of washing solution which can affect treatment results). At temperature of 80 °C, washing time of 30 minutes, volume solution of 10 $\mathrm{ml.g^{-1}}$ and agitation speed of 350 rpm; Han *et al*. (2009) obtained 97% of crude oil removal efficiency.

- In order to enhance the contaminant removal efficiency, the suggestion is to combine the used ML with some environmental friendly salts. Under optimum conditions Han *et al*. (2009) obtained 97% of crude oil removal efficiency by adding Soduim salts to alkyl polyglucosides surfactant.
- Based on the results obtained using the washing technique, it is suggested that the combination of technique with other *ex-situ* techniques such as Landfarming can to be applied. This will allow the remaining bacteria in the treated soil to degrade the remaining hydrocarbons using the remaining biosurfactant and soil nutrients as food. Combining these two techniques enhanced degradation up to 83.58% within 60 days.
- The C: N ratio was found to be at 23:1 indicating a deficient in the nitrogen element due to microbial activities. Addition of nitrogen to the soil will enhance activity of microbes leading to more degradation of hydrocarbons during the bioremediation process (Thapa *et al*., 2012; Mesic *et al*., 2012).

CHAPTER FIVE

CONCLUSION

The contaminated soil used in this study has 56% sand with 4.42% carbon, 1.23% hydrogen, 0.19% nitrogen and 1.82% sulfur. Results showed that the soil which pH was pH 6.9, has 40% porosity, 3.39% water content, and 27.64 mg/g of used engine oil in the dry soil.

The application of *Bacillus salmalaya strain* 139I degraded 83% of banded used engine oil from the soil system over a period of two months, using the combination of washing and bioremediation techniques. However, a deficient in nitrogen availability, due to microbial activities, has a clear influence in reducing the efficiency of *Bacillus* 139I.

Taguchi experimental design method, which used for washing technique, was found to be a good and suitable design to determine the most optimum conditions and their influence on the treatment output. The highest used engine oil removal achieved was 47.36% where washing time, of 20 minutes, was found to be the most influential variable in the treatment. As a results, bioremediation of washed contaminated soil was applied based on time variable, and 60 days of treatment able to remove 83% of used engine oil from the soil.

GC/MS analysis revealed that banded heavy elements of used engine oil (C_{24} to C_{40}) were converted to proportions of linear and cyclic n-alkanes (C_{12} to C_{20}) during the removal mechanism

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REFERENCES

Abdul, A. S., Gibson, T. L., & Rai, D. N. (1990). Selection of surfactants for the removal of petroleum products from shallow sandy aquifers. *Ground Water, 28*(6), 920-926.

Abdulsalam, S., Adefila, S., Bugaje, I., & Ibrahim, S. (2013). Bioremediation of soil contaminated with used motor oil in a closed system. *Journal of Bioremediation and Biodegradation,* published November23, 2012**.**

Achuba, F., & Peretiemo-Clarke, B. (2008). Effect of spent engine oil on soil catalase and dehydrogenase activities. *International Agrophysics, 22*(1), 1.

Adedokun, O. M., & Ataga, A. (2007). Effects of Amendments and bioaugmentation of soil polluted with crude oil, automotive gasoline oil and spent engine oil on the growth of *cowpea* (*Vigna unguiculata L. Walp*). *Scientific Research and Essays, 2*(5), 147-149.

Adriano, D. C., Chlopecka, A., & Kaplan, D. I. (1998). Role of soil chemistry in soil remediation and ecosystem conservation. *Soil Chemistry and Ecosystem Health* (*soil chemistry analysis*), 361-386.

Aitani, A. M. (2016). 25th Annual Saudi-Japan Symposium: Catalysts in Petroleum Refining and Petrochemicals, King Fahd University of Petroleum and Minerals, Dhahran, Saudi Arabia, December 7–8, 2015. *Catalysis Surveys from Asia, 20*(1), 59-62.

Ang, C. C., & Abdul, A. S. (1991). Aqueous surfactant washing of residual oil contamination from sandy soil. *Groundwater Monitoring & Remediation, 11*(2), 121-127.

Ayoola, S., & Ajani, E. (2007). Histopathological effects of *cypermethrin* on *juvenile Nile tilapia (Oreochromis niloticus*). *African Journal of Livestock Extension, 5*, 1-13.

Ayoola, S., & Alajabo, O. (2012). Acute Toxicity and Histopathological Effects of Engine Oil on *Sarotherodon melanotheron* (Black Jaw Tilapia). *American-Eurasian Journal of Toxicological Sciences, 4*(10), 48-55.

Balaji, V., Arulazhagan, P., & Ebenezer, P. (2014). Enzymatic bioremediation of polyaromatic hydrocarbons by fungal consortia enriched from petroleum contaminated soil and oil seeds. *Journal of Environmental Biology, 35*(3), 521.

Bernardez, L. A., & Ghoshal, S. (2008). Solubilization kinetics for polycyclic aromatic hydrocarbons transferring from a non-aqueous phase liquid to non-ionic surfactant solutions. *Journal of Colloid and interface Science, 320*(1), 298-306.

Bhattacharya, M., & Biswas, D. (2014). Enhancement of waste engine oil biodegradation by optimization of media using factorial design study. *Indian Journal of Biotechnology, 13*, 293-300.

Box, G. (1988). Signal-to-noise ratios, performance criteria, and transformations. http://dx.doi.org/10.1080/00401706.1988.10488313.pdf (Accessed on 23 March 2012).

Brown, J. N. (2002). Partitioning of chemical contaminants in urban stormwater. A thesis submitted for the degree of doctor of philosophy at University of Otago. Dunedin. New Zealand.

Colwell, R. (2009). Oil refinery processes: a brief overview. Process Engineering Associates, LLC. Excellence in applied chemical engineering. http://www.processengr.com/ppt_presentations/oil_refinery_processes.pdf (Accessed on 2009).

Cooperband, L. (2002). Building soil organic matter with organic amendments. *Center for Integrated Agricultural Systems*. http://www.cias.wisc.edu/wp-content/uploads/2008/07/soilorgmtr.pdf (Accessed on 16 September 2002).

Chang, L. K., Ibrahim, D., & Omar, I. C. (2011). A laboratory scale bioremediation of Tapis crude oil contaminated soil by bioaugmentation of Acinetobacter baumannii T30C. *African Journal of Microbiology Research, 5*(18), 2609-2615.

Chang, W., Dyen, M., Spagnuolo, L., Simon, P., Whyte, L., & Ghoshal, S. (2010). Biodegradation of semi-and non-volatile petroleum hydrocarbons in aged, contaminated soils from a sub-Arctic site: laboratory pilot-scale experiments at site temperatures. *Chemosphere, 80*(3), 319-326.

Dadrasnia, A., & Ismail, S. B. (2015). Bio-enrichment of waste crude oil polluted soil: amended with *Bacillus 139SI* and organic waste. *International Journal of Environmental Science and Development, 6*(4), 241.

Dadrasnia, A., Shahsavari, N., & Emenike, C. (2013). Remediation of contaminated sites. Book edited by Vladimir Kutcherov and Anton Kolesnikov, ISBN 978-953-51-0927-3, Published: January 16, 2013.

Dai, Q., & Chung, K. H. (1995). Bitumen sand interaction in oil sand processing. *Fuel, 74*(12), 1858-1864.

Deshpande, S., Shiau, B., Wade, D., Sabatini, D., & Harwell, J. (1999). Surfactant selection for enhancing ex situ soil washing. *Water Research, 33*(2), 351-360.

Dominguez-Rosado, E., & Pichtel, J. (2003). Chemical characterization of fresh, used and weathered motor oil via GC/MS, NMR and FTIR techniques. Paper presented at the Proceedings of the *Indiana Academy of Science*. 112(2): 109-116.

Garcia-Martinez, R., & Brebbia, C. (1998). Oil and hydrocarbon spills, modelling, analysis and control. 1998; 372 p; Computational Mechanics Publications; Southampton (United Kingdom); 1. *International conference on oil and hydrocarbon spills, modelling, analysis and control*; Southampton (United Kingdom); Jul 1998; ISBN 1 85312 526 1.

Griffiths, R. A. (1995). Soil-washing technology and practice. *Journal of Hazardous Materials, 40*(2), 175-189.

Han, M., Ji, G., & Ni, J. (2009). Washing of field weathered crude oil contaminated soil with an environmentally compatible surfactant, alkyl polyglucoside. *Chemosphere, 76*(5), 579-586.

International Council on Clean Transportation (2011). An introduction to petroleum ref Refining and the production of ultra-low Sulfur gasoline and diesel fuel. Voice: 301-951-9006.

Ijah, U., & Ukpe, L. (1992). Biodegradation of crude oil by *Bacillus strains* 28A and 61B isolated from oil spilled soil. *Waste Management, 12*(1), 55-60.

Ismailov, N. (1983). Effect of Oil pollution on the nitrogen cycle in soil. *Microbiology*. Information Systems division. National Agricultural Library. United States, 52(6): 794-798.

Kamath, R., Rentz, J., Schnoor, J. L., & Alvarez, P. (2004). Phytoremediation of hydrocarbon-contaminated soils: principles and applications. *Studies in surface science and catalysis, 151*, 447-478.

Khan, F. I., Husain, T., & Hejazi, R. (2004). An overview and analysis of site remediation technologies. *Journal of Environmental Management, 71*(2), 95-122.

Koning, M., Hupe, K., & Stegmann, R. (2008). Thermal processes, scrubbing/extraction, bioremediation and disposal. *Biotechnology Set, Second Edition*, 304-317.

Krull, E. S., Skjemstad, J. O., & Baldock, J. A. (2004). Functions of soil organic matter and the effect on soil properties*: Cooperative Research Centre for Greenhouse Accounting*, p. 92-107.

Lopes, P. R. M., & Bidoia, E. D. (2009). Effect of surfactant in automotive lubricant oil biodegradability. *Department of biochemistry and microbiology,* V. 28, n.3, p.225-233.

McLean, E. (1982). Soil pH and lime requirement. Methods of soil analysis. Part 2. Chemical and microbiological properties. *Methods of Soil Analysis 2*, 199-224.

Mesić, M., Birkas, M., Zgorelec, Z., Kisić, I., Jurišić, A., & Šestak, I. (2012). Carbon content and C/N ratio in Pannonian and Mediterranean soils. http://bib.irb.hr/datoteka/570357.4._45-54.pdf

Milala, M., Blessing, D., & Abdulrahman, A. (2015). Effects of spent engine oil on soil physicochemical properties of and microorganism (bacteria). *Asian Journal of Science and Technology, 6*(02), 1032-1035.

Mohammed, M. A. (2004). Treatment techniques of oil-contaminated soil and water aquifers. *International Conference on water resources and arid environment*. http://repository.ksu.edu.sa/jspui/bitstream/123456789/2442/1/Treatment-Techniques-Oil-Contaminated-Soil-Water-Aquifers.pdf.

Moldes, A. B., Paradelo, R., Rubinos, D., Devesa-Rey, R., Cruz, J. M., & Barral, M. T. (2011). Ex situ treatment of hydrocarbon-contaminated soil using biosurfactants from *Lactobacillus pentosus*. *Journal of agricultural and food chemistry, 59*(17).

Murphy, B. W (2014) Soil organic matter and soil function–Review of the literature and underlying. https://www.environment.gov.au/system/files/resources/20051db1-51ba-49e1-9758-66bd2c8c1cc5/files/soil-organic-matter.pdf (Accessed on May 2014).

Nixon, H., & Saphores, J.-D. (2003). used oil policies to protect the environment: An overview of Canadian experiences. *University of California Transportation Center*. https://escholarship.org/uc/item/79c9b9h6#page-3 (Accessed on 9 January 2003).

Nixon, H., & Saphores, J.-D. (2007). Impacts of Motor Vehicle Operation on Water Quality in the United States-Clean-up Costs and Policies. *University of California Transportation Center*. https://escholarship.org/uc/item/4bv8v8gj#page-3 (Accessed on 1 January 2007).

Nwite, J., & Alu, M. (2015). Effect of different levels of spent engine oil on soil properties, grain yield of maize and its heavy metal uptake in Abakaliki, Southeastern Nigeria. *Journal of Soil Science and Environmental Management, 6*(4), 92-99.

Obayori, O. S., Salam, L. B., & Ogunwumi, O. S. (2014). Biodegradation of fresh and used engine oils by *Pseudomonas aeruginosa LP5*. *Journal of Bioremediation & Biodegradation,* 5:213. doi:10.4172/2155-6199.1000213. (Published on 23 January 2014).

Odjegba, V., & Atebe, J. (2007). The effect of used engine oil on carbohydrate, mineral content and nitrate reductase activity of leafy vegetable (*Amaranthus hybridus L.*). *Journal of Applied Sciences and Environmental Management,* 11(2).

Pandey, R., Herbert, A., & Pandey, A. (2014). Soil Decontamination by Soil Washing Technique Using Surfactant. http://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.676.8014&rep=rep1&type.pdf (Accessed on January 2014).

Pavel, L. V., & Gavrilescu, M. (2008). Overview of *ex situ* decontamination techniques for soil cleanup. *Environmental engineering and management journal, 7*(6), 815-834.

Peng, S., Wu, W., & Chen, J. (2011). Removal of PAHs with surfactant-enhanced soil washing: influencing factors and removal effectiveness. *Chemosphere, 82*(8), 1173-1177.

PIGNATIELLO JR, J. J. (1988). An overview of the strategy and tactics of Taguchi. *IIE transactions, 20*(3), 247-254.

Rice, C. W. (2002). Organic matter and nutrient dynamics. *Encyclopedia of soil science, 2*, 1180-1183.

Riser-Roberts, E. (1992). Bioremediation of Petroleum Contaminated Sites. *Boca Raton, Florida*. (Published on 15 July 1992).

Rosen, M. J. (1989). Surfactants and interface phenomena. *Surfactant Research Institute Brooklyn College*. http://mx.nthu.edu.tw/~yucsu/5855/Lec08.pdf.

Rowell, D. L. (2014). Methods & applications: Routledge. Soil science: Methods and Applications. *Department of Soil Science. University of Reading.* http://www.sitehostplus.com/soil-science-methods-applications.pdf.

Samuel, A.-A. (2011). Biodegradation of Used Lubricating Engine Oil Contaminated Water Using Indigenous Hydrocarbon Degrading Microbes in a Fixed Bed Bioreactor System. *Department of Environmental Science, Kwame Nkrumah University of Science and Technology*. http://dspace.knust.edu.gh/bitstream/123456789/5749/1/SAMMYS%20THESES.pdf (Accessed on August 2011).

Sansoni, B., & Iyengar, G. (1980). Elemental analysis of biological materials: current problems and techniques with special reference to trace elements. http://www.iaea.org/inis/collection/nclcollectionstore/_public/11/564/11564331.pdf#page=67. (Published on February 1980).

Santa Anna, L. M., Soriano, A. U., Gomes, A. C., Menezes, E. P., Gutarra, M. L., Freire, D. M., & Pereira, N. (2007). Use of biosurfactant in the removal of oil from contaminated sandy soil. *Journal of Chemical Technology and Biotechnology, 82*(7), 687-691.

Schumacher, B. A. (2002). Methods for the determination of total organic carbon (TOC) in soils and sediments. *Ecological Risk Assessment Support Center, 2002*, 1-23.

Semer, R., & Reddy, K. R. (1996). Evaluation of soil washing process to remove mixed contaminants from a sandy loam. *Journal of Hazardous Materials, 45*(1), 45-57.

Stevenson, F. (1982). Humus chemistry. Genesis, composition, reactions. *Department 0f Agronomy, University of Illinois.* 26-54.

SU, S. L., Singh, D., & Baghini, M. S. (2014). A critical review of soil moisture measurement. *Measurement, 54*, 92-105.

Terzaghi, K., Peck, R. B., & Mesri, G. (1996). Soil mechanics in engineering practice. Third edition. *Technology and Engineering*. 95-6616

Thapa, B., Kc, A. K., & Ghimire, A. (2012). A review on bioremediation of petroleum hydrocarbon contaminants in soil. *Journal of science, engineering and technology, 8*(1), 164-170.

United Nations Industrial Development Organization (2004). A survey of soil remediation technology. *Institute for Capacity Development.* https://institute.unido.org/wp-content/uploads/2014/11/17-survey_of_soil_ remediation_te hnology.pdf

United States Environmental Protection Agency (2012). A Citizen's Guide to Excavation of Contaminated Soil. *Office of Solid Waste and Emergency Respon,* https://cluin.org/download/Citizens/a_citizens_guide_to_excavation_of_contaminated_soil.pdf.

United States Department of Agriculture (2002). Treatment of petroleum contaminated soil in cold, wet, remote regions. *Technology and development program,* https://www.fs.fed.us/td/pubs/pdfpubs/pdf02712801/pdf02712801_300dpi. pdf. 0271-2801.

Urum, K. (2004). Biosurfactant enhanced treatment of petroleum oil contaminated soils. A thesis submitted towards fulfilment of the requirements for the degree of Doctor of Philosophy. Chemical engineering and Physical. Edinburgh.

Urum, K., Pekdemir, T., & Çopur, M. (2004). Surfactants treatment of crude oil contaminated soils. *Journal of Colloid and interface Science, 276*(2), 456-464.

Urum, K., Pekdemir, T., & Gopur, M. (2003). Optimum conditions for washing of crude oil-contaminated soil with biosurfactant solutions. *Process Safety and Environmental Protection, 81*(3), 203-209.

Usman, M. M., Dadrasnia, A., Lim, K. T., & Fahim, A. (2016). Application of biosurfactants in environmental biotechnology; remediation of oil and heavy metal. *Bioengineering*. (Published on 26 July 2016).

Van Gestel, K., Mergaert, J., Swings, J., Coosemans, J., & Ryckeboer, J. (2003). Bioremediation of diesel oil-contaminated soil by composting with biowaste. *Environmental Pollution, 125*(3), 361-368.

Vazquez-Duhalt, R. (1989). Environmental impact of used motor oil. *Science of the Total Environment, 79*(1), 1-23.

Williams, J. (2006). Bioremediation of contaminated soils: A comparison of in situ and ex situ techniques. http://home.engineering.iastate.edu/~tge/ce421-521/jera.pdf.

Xing-hui, X., Li, L., & Jia-lin, X. (2000). Mixed surfactant washing of petroleum contaminants from the soils and sediments in the unsaturated zone. *Journal of Environmental Science, 12*(1), 108-114.

Zhang, L., Somasundaran, P., Ososkov, V., & Chou, C. (2001). Flotation of hydrophobic contaminants from soil. *Colloids and Surfaces A: Physicochemical and Engineering Aspects, 177*(2), 235-246.