Development and evaluation of a remediation strategy for the oil lakes of Kuwait

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“Whilst registered as a candidate for the above degree, I have not been registered for any other research award. The results and conclusions embodied in this thesis are the work of the named candidate and have not been submitted for any other academic award”

Meshari Saad Almutairi

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“Success is just one step beyond the point at which defeat had overtaken you”

Napoleon
Abstract
During the occupation of Kuwait by Iraq in 1990/1991, Iraq’s armed forces destroyed more than 700 oil wells. The resulting oil flowed out and formed a large number of oil lakes. This also led to the contamination of soil, which has been left untreated in the deserts of Kuwait for more than two decades now. The untreated contaminated soil has the tendency to pollute the underground watercourses as well as affecting the ecology and humans health.

Laboratory scale experiments were carried out to assess the efficiency of soil washing according to the removal of the oil residue. A non-ionic biosurfactant, “saponin”, was employed under a range of conditions (different water type, washing time, stirrer speed, surfactant concentrations, temperature and mass/volume ratio). Results identified that the optimum washing parameters in terms of time, stirrer speed, surfactant concentrations, temperature, mass/volume ratio were found to be 20 min, 1,000 rpm, 0.5 wt% (weight percent solutions), 50°C and ratio of 1:3, respectively. The optimum washing parameters were investigated under varying washing modes namely, mechanical stirrer, ultrasonication, combination of ultrasonication and mechanical stirrer and combination of mechanical vibration and stirrer. The removal efficiency of oil residue generally enhanced with the sequential washing, results showed that 90 %, 80 %, 75 % and 60 % of total petroleum hydrocarbons (TPH) was removed after the third wash by using the combination of ultrasonication and mechanical stirrer, mechanical stirrer, ultrasonication, combination of mechanical vibration and stirrer, respectively. The soil washing by using combination of ultrasonication and mechanical stirrer reduced the mean values of TPH from 330,000 to 10,000 mg/kg, which are lowered than the upper limits (TPH <10,000 mg/kg) of Kuwait Environmental Protection...
Agency (KEPA). Samples of contaminated sand were analysed by gravimetric method. This approach of soil washing would be a promising alternative for remediation of Kuwait oil contaminated sand.

It was found that the soil washing process aided the oil residue to be transferred from solid to the aqueous phase. Wastewater obtained from soil washing technique was investigated by using various wastewater treatment (adsorption, anoxic treatment, aeration treatment, combination of anoxic treatment with coagulation/flocculation and combination of aeration treatment with coagulation/flocculation). The combination of anoxic treatment with coagulation/flocculation and combination of aeration with coagulation/flocculation were capable of lowering the mean of COD values from 48,000 mg/l to 14,110 mg/l and 4,784 mg/l, respectively. This investigation suggests that combination of aeration with coagulation/flocculation proved an effective technique to treat the oily wastewater.

This project investigates the potential use of oil-contaminated sand containing TPH <10,000 mg/kg on some engineering properties of hardened concrete by its experimental evaluation of its workability by slump test, compressive strength, hammer test and water absorption test using British Standard (BS) methods. The results of this investigation found that no major change was noted for the slump test conducted either with the contaminated specimens or clean specimens. while sand containing less than 10,000 mg/kg crude oil contaminants was able to reduce the compressive strength of the concrete by about 17.0 %. Enhancement of water absorption on average by the oil contaminated sand was about three times than that of clean sand.
Direct detection of saponins in soapnuts (*Sapindus mukorossi*) using Fourier transform infrared (FTIR) spectroscopy is investigated in this project. Potassium bromide (KBr) powder was mixed with dry extracted powder of soapnuts and compressed to a thin pellet for examination process. The outcome of the FTIR spectra of saponin demonstrated characteristic triterpenoid saponin absorptions of OH, C=O, C-H, and C=C, while the glycoside linkages to the sapogenins were indicated by the absorptions of C-O. The significance of this study is that saponin absorption peaks are directly detectable in crude aqueous and 95.0 d% ethanol extracts of soapnuts powder using FTIR spectroscopy thereby eliminating the need of further expensive and exhaustive purification steps. The extracts of soapnuts were screened for saponins along with controls by phytochemical tests, while advanced spectroscopic techniques like ultrafast liquid chromatography (UFLC) and ultra-performance liquid chromatography quadrupole-time of flight-mass spectrometry (UPLC-QTOF-MS/MS) were also implemented to validate the saponins.
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<table>
<thead>
<tr>
<th>Organisation</th>
<th>Abbreviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kuwait Oil Company</td>
<td>KOC</td>
</tr>
<tr>
<td>Kuwait Institute of Scientific Research</td>
<td>KISR</td>
</tr>
<tr>
<td>United State Environmental Protection Agency</td>
<td>USEPA</td>
</tr>
<tr>
<td>Kuwait Environmental Protection Agency</td>
<td>KEPA</td>
</tr>
<tr>
<td>Kuwait University</td>
<td>KU</td>
</tr>
<tr>
<td>Ministry of Education</td>
<td>MOE</td>
</tr>
<tr>
<td>National Cleaning Company</td>
<td>NCC</td>
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<tr>
<td>United Nation</td>
<td>UN</td>
</tr>
<tr>
<td>United Nations Compensation Commissions</td>
<td>UNCC</td>
</tr>
<tr>
<td>Kuwait National Focal Point</td>
<td>KNFP</td>
</tr>
<tr>
<td>International Agency for Research on Cancer</td>
<td>IARC</td>
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<tr>
<td>World Health Organisation</td>
<td>WHO</td>
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<tr>
<td>Fourier Transform Infra-Red</td>
<td>FTIR</td>
</tr>
<tr>
<td>Scanning Electron Microscope</td>
<td>SEM</td>
</tr>
<tr>
<td>House of Quality</td>
<td>HOQ</td>
</tr>
<tr>
<td>Total Petroleum Hydrocarbons</td>
<td>TPHs</td>
</tr>
<tr>
<td>Total Resolved Hydrocarbons</td>
<td>TRH</td>
</tr>
<tr>
<td>Polycyclic aromatic hydrocarbons</td>
<td>PAHs</td>
</tr>
<tr>
<td>Polychlorinated biphenyl</td>
<td>PCB</td>
</tr>
<tr>
<td>Benzene Toluene Ethylbenzene Xylenes</td>
<td>BTEX</td>
</tr>
<tr>
<td>Diesel Range Organics</td>
<td>DRO</td>
</tr>
<tr>
<td>Biochemical Oxygen Demand</td>
<td>BOD</td>
</tr>
<tr>
<td>Chemical Oxygen Demand</td>
<td>COD</td>
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<tr>
<td>Flocculent Mass</td>
<td>Floc</td>
</tr>
<tr>
<td>Total dissolved solid</td>
<td>TDS</td>
</tr>
<tr>
<td>Primary settled sludge</td>
<td>PSS</td>
</tr>
<tr>
<td>Liquid Plant Fertilizer</td>
<td>LPF</td>
</tr>
<tr>
<td>Ordinary Portland Cement</td>
<td>OPC</td>
</tr>
<tr>
<td>parts per million</td>
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xxx
<table>
<thead>
<tr>
<th>Unit</th>
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<tbody>
<tr>
<td>seconds</td>
<td>s</td>
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<tr>
<td>centimeter</td>
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<tr>
<td>gram</td>
<td>g</td>
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<tr>
<td>kilogram</td>
<td>kg</td>
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<td>litre</td>
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<td>kilometres</td>
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<tr>
<td>molar</td>
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<tr>
<td>milligram</td>
<td>mg</td>
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<tr>
<td>minutes</td>
<td>min</td>
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<tr>
<td>millilitre</td>
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<tr>
<td>millimeter</td>
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Chapter Overview

The outline below provides the different chapters in this study so as to assist a reader in understanding the flow of the investigation procedure that was followed and the main sections that are dealt in each chapter. Including literature review and conclusion, the thesis comprises of six chapters.

1. Chapter 1 provides the background for Kuwait oil lakes. Related literature concerning these issues is reviewed in this chapter, which underpins the theoretical foundations of this research. These topics which will be considered within this chapter consist of history, oil properties, oil fate as well as health and environmental effects of oil pollution and engineering solution to the problems. It also explores a number of techniques employed by other authors throughout the world in the remediation to oil contaminated soil. This chapter reviews primary treatment in greater detail by providing a thorough literature review on previous oily wastewater applications.

2. Chapter 2 discuss the research strategy as well as the hypothesis, aims and objectives. The purpose of this investigation in this chapter is to explore the issues of Kuwait oil lakes. The current chapter deals with any decision concerning a future remediation method requires –as a prerequisite – the basic information, which result from an assessment of the current situation of Kuwait oil lake. A preliminary survey performed periodically will up-date the information and will support and facilitate the management's decision- making process. In this chapter, House of Quality (HOQ) is made to select the suitable method for treating the Kuwait oil contaminated sand.

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3. Chapter 3 describes the methodology and experimental procedures used in this study. It also describes the sampling and the data-collection procedure. This chapter identifies that there is no standard method for the extraction of oil from soil. Therefore, a basic initial test was carried out and several parameters were altered to improve the extraction process. Moreover, this chapter focuses primarily on the methodology for the determination of pH, electrical conductivity, and electron microscopy of the particle surface by using the scanning electron microscope (SEM). Also, this chapter addresses the oily wastewater obtained from washing Kuwait oil sand by measuring the concentration of removed oil, flocculent mass (flocs), surface tension, turbidity, chemical oxygen demand (COD), and biochemical oxygen demand (BOD). In the present chapter, various microbial analysis was conducted such as isolation of bacteria, aerobic growth, preparation method for solid media and liquid media. Further, determination of metal contents, engineering application and extraction of bio-surfactant were addressed in this chapter.

4. Chapter 4 presents the preliminary conceptual design for the remediation of Kuwait oil sand. It also compares the washing efficiency through various methods at different phase, e.g., mechanical stirrer, ultrasonication, combination of ultrasonication and mechanical stirrer and combination of vibration and mechanical stirrer. On the other hand, the present chapter consists of the preliminary design of oily wastewater treatment to enhance the treatment efficiency. It is also an important attempt at showing the advantages of using the combination of aeration system with coagulation/flocculation process during treatment of oily wastewater.
5. Chapter 5 discusses the effected parameters during soil washing process, which include; water type, washing temperature and concentrations of saponin, duration of washing, stirrer speed and ratio of volume to mass. Furthermore, this chapter provides illustrations, discussion and an analysis of the data obtained. This chapter addresses the results from the numerous treatment tests performed on oily wastewater. In addition, chapter 5 measures the BOD of oily wastewater samples by means of the respirometry. The theory behind various methods of disinfection and respirometry measurement are included in thorough details. The analytical measurements for COD values were developed, which recognized that the chloride (Cl\(^-\)) could be act as the main interference when measuring the COD values. This chapter evaluate the potential of using the treated soil on some engineering properties of hardened concrete. Finally, the last section presents the results from direct detection of saponins in soap nuts using fourier transform infrared (FTIR) spectroscopy. Project summarization was evaluated at the end of this chapter.

6. To conclude, Chapter 6 endeavours to provide responses to the research problem and whether to agree with or discard the hypothesis. The conclusion from this research will offer suggestions on probable solutions and conclude the study. Finally, the last sections of this chapter presented future work and recommendations. The pilot study, which is sponsored by KISR to verify the efficiency of the employed method in achieving the targets of the project.
Chapter 1 Literature Review

1.1 Introduction

The State of Kuwait sustained significant and widespread environmental damage resulting from the Iraqi invasion in August 1990. During the 1991 Gulf War, estimates of 798 oil wells were set ablaze, 149 damaged and 45 gushing oil (Petroleum Economist 1992; Petroleum Energy Center (PEC), 1999). Several oil lakes, i.e., oil accumulation in depressions, were formed. Approximately 12 billion gallons of seawater, which contained substantial amount of salt was used to extinguish around 20 to 25 million barrels of ignited crude oil. The gushing oil and fallout from the oil fire plumes spread over the desert surface covering vast areas stretching from Kuwait to the Kingdom of Saudi Arabia. Within the vicinity of the oil wells, oil deposits were formed with various sizes of lakes and films or oil crust spreading across areas same distance away from the wells. As a result, contaminations of soil and groundwater took place due to oil spray and combustion products from oil fires (Al-Awadhi et al., 1996).

The spilled oil which accumulated in low lying areas within Kuwait desert, created more than 300 large oil lakes. These spots comprise a combination of water (28 % average), salt (in excess of 10 %), oil and sand known as “oil lakes” spread over the surface of land. Among these lakes, 45 major lakes were in Burqan and between Maqwa and Ahmadi oil fields. There were another 23 minor lakes (Al-Ajmi et al., 1994; El-Baz et al., 1994; Kwarteng 1998). It is fortunate that the Kuwait Oil Company (KOC) has succeeded in recovering around 21 million barrels from the oil lakes since the end of the Gulf War. The spilled oil was recovered by pumping from the oil lakes to the oil gathering center, then exported as untreated oil (KOC, 2015).
With oil lakes covering approximately 116 km² (Al-Naseem and Al-Duwaisan, 2011), representing 0.65% of the total Kuwait land area was covered by oil lakes, as illustrate in Table (1-1).

Table 1-1: Volume of oil trenches and oil lakes in Kuwait (Source: Al-Naseem and Al-Duwaisan, 2011).

<table>
<thead>
<tr>
<th></th>
<th>Average depth (cm)</th>
<th>Extent (km²)</th>
<th>Volume (x10⁶ m³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry oil lakes</td>
<td>25</td>
<td>98 – 100</td>
<td>25</td>
</tr>
<tr>
<td>Wet oil lakes</td>
<td>64</td>
<td>7</td>
<td>5</td>
</tr>
<tr>
<td>Oil contaminated piles</td>
<td>173</td>
<td>9</td>
<td>15</td>
</tr>
<tr>
<td>Oil trenches and pipeline spills</td>
<td>351</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>116</td>
<td>50</td>
</tr>
</tbody>
</table>

This huge scale of contamination has created environmental issues to the ground, air, coast and ground water. Also, the depth of these formed oil lakes vary from a few centimetres to a few metres, which constitute more than 60 million barrels of crude oil. Altogether, around 660 million barrels of crude oil were released to the environment and as a result, approximately 55 million tons of contaminated soil remained in the lakebeds. The existence of such a phenomenal amount of oil over a substantial land area represents a main environmental concern. No other petroleum catastrophe in history came near to equalling the extent of this event (Al-Ajmi et al., 1994; El-Baz et al., 1994; Kwarteng, 1998). As time goes by, low number of benzene ring structure of polycyclic aromatic hydrocarbons (PAH) has been evaporated on exposure to high temperature. The fine soot particles in the form of oil mist became hard while smaller and shallower oil lakes became dry thus forming tarmats.
The fine soot particles that is formed when burning oil wells which able to change the soil beneath to black. Under the extreme weather conditions, these contaminants continue to disintegrate slowly. The thickness of tarmat varies from a few millimetres to approximately two centimetres. Based on research by Kwarteng (1998), a number of these oil lakes were covered with a veneer of sand and therefore could not be detected from the surface. In addition, at certain places, the oil travelled to new sites owing to the occasional flash floods and heavy showers. There have not been any large scale methods implemented to remediate the affected areas thus far. As such, the environment is exposed to these untreated oil lakes and contaminated surfaces. Subsequently, the government of Kuwait filed claims with the United Nations Compensation Commission (UNCC) seeking compensation to remediate and rehabilitate the areas that sustained environmental damage throughout the State of Kuwait.

In 1991, the UNCC issued to sell Iraqi oil for distributing money as compensation for damages suffered during the invasion. In 2003, Kuwait was awarded 3 billion dollars from the UNCC for environmental rehabilitation (UNCC, 2004). The rehabilitation task will be a combined effort among the KOC, the UNCC and the Kuwait National Focal Point (KNFP). KOC is scheduled to commence bidding for contracts worth hundreds of millions of dollars in the forthcoming months and this is expected to continue thereafter every year. In fact, Kuwait is the first country that claimed for an environmental disaster through the United Nation (UN) for compensation to rehabilitate environmental damage. According to experts, the entire process is expected to take approximately 25 years.
1.2 Previous Studies on Oil Contamination

Contamination caused by petroleum products is an international phenomenon which varies in scale and magnitude from minor, such as leakage at base tanks, to major disasters like damage to a petroleum refining plants (Clark, 1995). The World’s annual production of petroleum products exceeds 2 billion tonnes. The resulting contamination of the environment due to petroleum-based products has created many significant problems (Atlas and Philp, 2005). Our environment is always susceptible to a range of operations within the petroleum business, such as exploration, production and transportation (Amro, 2004), with leaking at underground tanks being the most frequent cause of contamination (Nadim et al., 2000). Severe environmental hazards can also be caused by crude oil contamination, including contamination to ground and groundwater.

According to Li et al. (2008), oil contaminated soils are considered as a major risk to the health of human and environment. The process of weathering causes contaminants to become heavy, more viscous and dense, including increased adhesive strength of crude oil to soil (Urum et al., 2004; Chen et al., 2007). As such, research has shown that (Cho et al., 1997a; Urum et al., 2004; Han et al., 2009), contaminated soils that are exposed to weathering are often harder to remediate than soils that have just been contaminated. It has been suggested by investigators (Friend, 1996; Snead, 2001; Bunjan et al., 2005; Al-Rawas et al., 2005; Zhou et al., 2013; etc) that contaminated sand could be eliminated or remove by using various techniques such as thermal desorption, bioremediation, physical and chemical remediation. They also proposed to employ the oil contaminated sand in construction materials for road base or a topping layer for car parks.
1.3 Properties of Crude Oil

This section endeavours to form a good basis for further understanding of the nature of oil from basic principles.

1.3.1 Oil Types

Generally, crude oil has been created by the action of geologic heat and compression of the remains of animals and plants over millions of years. Discharged oil has specific characteristics that can offer an initial assessment of the possible pathways, exposures and impacts to the mankind, ground water and ecological system. Nonetheless, the characterisation process can be difficult given the wide variations of both crude and refined oils with diverse physical and chemical properties.

According to Bobra and Callaghan (1990), different oil wells from the same zone can produce crude oils with profoundly different characteristics, and even the oil extracted from the same well can have characteristics that differ according to the depth and the year it is produced. Oils can be categorised into different groups in accordance with their expected short-term performance and their potential effects to natural resources. Based on energy information administration (EIA) and safe drinking water foundation (SDWF), these characteristics of various types of crude oil and oil fractions are listed in Table (1-2).
Table 1-2: The properties of each oil group (Source: EIA, 2004; SDWF, 2014).

<table>
<thead>
<tr>
<th>Type of Crude Oil</th>
<th>Density (kg/m³)</th>
<th>Volatility</th>
<th>Remediation Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extra-Heavy</td>
<td>&gt; 1000</td>
<td>No evaporation process</td>
<td>Difficult</td>
</tr>
<tr>
<td>Heavy</td>
<td>920 to 1000</td>
<td>Around one-third will volatile within 1 day</td>
<td>Most effective when conducted rapidly.</td>
</tr>
<tr>
<td>Medium</td>
<td>870 to 920</td>
<td>Moderately volatile</td>
<td>Can be very effective.</td>
</tr>
<tr>
<td>Light</td>
<td>870</td>
<td>Highly volatile</td>
<td>Not required</td>
</tr>
</tbody>
</table>

1.3.2 Chemistry of Petroleum Hydrocarbons

Oils are composed of a complex blend of trace elements and organic compounds. The most general petroleum elements are carbon (83-87 %) and hydrogen (10 - 14 %), with significant minor elements of sulphur (0 - 8 %), nitrogen (0 - 1 %) and oxygen (0.0 - 0.5 %) (Speight, 1999). Understanding of petroleum hydrocarbons and how they behave in the environment is necessary to assess remedial technologies associated with hydrocarbon contamination. As the name implies, petroleum hydrocarbons are generally compounds of hydrogen and carbon atoms.

Total petroleum hydrocarbons (TPH) represent various compounds of hydrocarbon occurring in crude and refined petroleum, including TPH-gasoline range (TPH-g), TPH-diesel range (TPH-d), and TPH-residual range (TPH-r) (Battelle, 2007). Accordingly, to Oklahoma department of environmental quality (ODEQ) (2012) TPH-g, TPH-d and TPH-r are defined as carbon chains between \((C_6-C_{10})\), \((C_{10}-C_{28})\) and \((C_{28}-C_{35})\). These compounds are characterised by chemical composition and structure, which can be classified into four main structural forms:
aliphatic, aromatic, resins and asphaltenes compounds. Association for environmental health and sciences (AEHSa) (1998a) classified that aliphatic compounds are alkanes, alkenes, alkynes and cycloalkanes, and aromatic compounds are monoaromatics and PAHs. The carbon atoms in alkanes are joined by single bonds and referred to as saturated hydrocarbons. While alkenes, alkynes and cycloalkanes are referred to as unsaturated hydrocarbons and characterised by carbon-carbon double bond, carbon-carbon triple bond and single bond carbon ring structure, respectively (Duckworth and Perry, 1986).

Aromatic hydrocarbons are characterised as containing at least one 6-carbon benzene ring. Zapff-Gilje et al. (2001) explain that aromatic compounds are unsaturated like benzene in chemical behaviour. Monoaromatics are characterised by one carbon benzene ring made up of six carbon atoms, while PAH are characterised into two groups based on their molecular structure. These are low molecular weight (LMW) compounds, which have a core structure comprising two to three carbon benzene rings and high molecular weight (HMW) compounds, which have core structure four or more carbon benzene rings. In addition, asphaltenes is defined by Ficken et al. (2002) as high molecular weight of the polar fraction that is present as a micro colloid in the crude oil with varies in molecular weight (from 500 to several thousand). Further, the asphaltenes are identified as a shiny, black and friable solid, while the resins are shiny, dark brown and thick viscous liquid to semi-solid.

In addition to organic compounds, crude oil also contains a wide range of metal contents obtained from different sources. The variations in trace elements are diverse and may consist of elements including vanadium, nickel, iron, aluminium, sodium, calcium and copper (NRC,
A study conducted by Bhattacharyya and Shekdar (2003) showed that, concentrations of the metal contents in oily sludge generated from petroleum refinery processes are 27–80 mg/kg for chromium (Cr), 17–25 mg/kg for nickel (Ni), 32–120 mg/kg for copper (Cu), 0.001–0.120 mg/kg for lead (Pb), and 7–80 mg/kg for zinc (Zn). Additionally, high concentration of heavy metals in oily sludge was reported by Da Rocha et al. (2010) and Roldán-Carrillo et al. (2012). They found that the concentration of the metal contents obtained from refinery was 500 mg/kg for Cu, 480 mg/kg for Cr, 565 mg/kg for Pb, 1299 mg/kg for Zn, 480 mg/kg for Ni, and 60,200 mg/kg for iron (Fe), respectively. The corresponding amounts of these fractions are dependent on multiple factors like the migration, source, age, geological history, and change of crude oil. Recent evidence suggested that microorganisms are the chief agents for the biodegradation of molecules of environmental concern in the terrestrial and aquatic environments, this also includes petroleum hydrocarbons. (Alexander et al., 1982; Swanell and Head, 1994).

1.3.3 Environmental Chemistry of Oil
A few important properties of hydrocarbons can be reviewed in terms of their susceptibility to the natural processes of weathering that petroleum hydrocarbons undertake with time when they are exposed to the environment. Amongst these, volatilization, leaching into water and microbial degradation are the main parameters listed by AEHS (1998b). According to AEHS (1998b), the rate of weathering is subject to the nature of hydrocarbon environment. However, it also depends largely on the physical and chemical properties of the hydrocarbon constituents. Compounds with lower boiling temperature are likely to be highly volatile such as gasoline as well as diesel constituents (AEHS 1998a; Trapp, 2001). As stated by AEHS (1998b), the
aliphatic compounds are generally more volatile than aromatics, and their volatility reduces with increase in molecular weight (AEHS, 1998b). The compounds which are highly soluble are more susceptible to the leaching processes. As the electrons within the aromatic ring structure are already de-localised, aromatic hydrocarbons tend to be fairly polar in comparison with aliphatic compounds. As such, aromatics compounds are likely to have higher water solubility compared to aliphatic hydrocarbons.

Moreover, the solubility reduces with the increase in molecular weight (AEHS 1998b). According to Singer and Finnerty (1984), n-alkanes of intermediate length (C10 –C20) are the preferred substrates and likely to be readily degradable among a number of various petroleum fractions, where shorter chain compounds are more toxic (Klug and Mar- kovetz, 1971). According to Barths (1986), alkanes with longer chains like wax (C20-C40) are hydrophobic solids and therefore not easy to degrade owing to their lower solubility and bioavailability; branched chain alkanes are also degraded at a slower rate compared to the equivalent normal alkanes (Singer and Finnerty, 1984).

Yeast and fungi are considered hydrocarbon-degrading bacteria and they are broadly available in marine, fresh water and soil environments. According to Hanson et al., (1997), both yeast and bacteria seem to be the key degraders in aquatic ecosystems whereas in soil habitats, the key degraders are bacteria and fungi. Also, rate of biodegradation depends upon the environmental conditions such as the presence of oxygen and the presence of microbial community as well as the susceptibility of the hydrocarbons.
In principle, straight-chain alkanes tend to degrade faster than branched alkanes or cycloalkanes (AEHS, 1998a). The Benzene Toluene Ethylbenzene Xylenes (BTEX) compounds are likely to biodegrade easier than PAHs and the ability for PAHs to biodegrade reduces with the increase in the number of benzene rings within the chemical structure (AEHS 1998b; Balba, 1993). The rate of biodegradation for cycloalkanes are fairly inconsistent, however, tend to be much slower compared to alkanes and frequently involve a number of microbial species (Perry, 1981). As mentioned by Atlas, (1981) extremely condensed aromatic and cycloparaffinic structures, tars, bitumen and asphaltic elements have the highest boiling points and demonstrate the highest resistance to biodegradation.

The product of petroleum hydrocarbons in soil known as asphaltene seems to be able to resist microbial degradation (Bossert and Bartha, 1984). According to Jobson et al. (1972), such residual material from the degradation of oil has been suggested to be analogous and might also be considered as, humic material. Owing to its inert properties, insolubility and similarity to humic materials are less likely to be hazardous to the environment.

The final outcome of the weathering processes can be comprehended that the compounds that are more volatile, soluble, and degradable tend to disappear quicker, while the more persistent, recalcitrant compounds will be left behind (AEHS, 1998b). This includes compounds with HMW especially larger PAHs, whose resistance to these processes is offered by the stability imparted by the resonance structures of the benzene rings (AEHS, 1998b; Kanaly, 2000).
1.3.3.1 The effect of weathering on crude oil properties

The combustion of crude oil is a complicated process as crude oil consists of a mixture of hydrocarbons. The physical properties such as viscosity, density and composition are constantly changing as the crude oil is combusted. According to Iwata et al (2001), the combustion properties are understood to differ for different types of oil. Studies by Jokuty (2005) and Pichler & Hense (2012), investigated the combustion properties and vapour pressures of a number of oils aimed at having a good prediction of the fire hazard thereby adapting appropriate fire and safety responses.

Among the main properties of crude oil which relates to flammability is vapour pressure. According to Roberts (2011), the rate of liquid evaporation depends on vapour pressure. For crude oil, its vapour pressure is essential to the industry as the bubbles of gas will start to evolve from the oil as soon as the oil is depressurised into a state lower than its vapor pressure. Subsequently the emission of gas tends to turn into an explosion hazard if the vapour pressure is not controlled stringently. VOCs have high vapour pressure which is sufficient to allow it to evaporate into the atmosphere as a gas at ambient temperatures. Upon exposure to the environment, oil will undergo a natural weathering process subject to the local environment either terrestrial or aquatic. Evaporation is regarded as the main weathering process for land-spills. As mentioned by Gunter (2009), aquatic spills are more complex as the weathering process involves spreading of the oil, evaporation, dispersion, dissolution, sedimentation, oxidation, biodegradation and emulsification. As the oil is emulsified, the flammability of the oil is usually reduced to a non-flammable state, while evaporation on its own can also achieve this in a number of circumstances.
Evaporation is the phase change of the volatile constituents of the oil from liquid to gas. Through the evaporation process, these volatile constituents will blend with the air within the atmosphere and result in the formation of a combustible gas mixture. A number of models have been formulated to estimate the rate of evaporation of multi-component oils in both aquatic and land-based environments established within the past 30 years (Stiver & Mackay, 1984; Okamoto et al., 2010 and Fingas, 2012). While Stiver & Mackay (1984) believed that the rate of evaporation or extent of weathering is usually complex according to the elements, such as temperature, waves and wind, Fingas (2005) reckons that the effect from elements, for instance waves and wind is small.

In most of these models, the oil evaporates at a logarithmic rate against time. As the oil undergoes weathering process, the oil composition changes due to the loss of lighter components. The constantly changing composition of the spilled oil causes further problems in predicting its flammable behaviour. Conventional heavy oils would possibly have as small as 10 % mass loss resulting from evaporation, taking into account a logarithmic rate which only allows a short period for the potential for ignition. This is the opposite of the conventional medium and light crudes which may lose as much as 40 and 75 % mass, respectively (Fingas, 1994). Dilbit crudes usually comprise 20 – 30 % volume diluent (lights) and weathering of these crudes tend to be severe than heavy crude oils (however less than light crude oils).

In accordance with Wu et al (2000) and Torero et al (2003), the most current researches investigating the effect of weathering on flammability have emphasized on the practicality of in-situ burning in the case of off-shore oil spills. The intention of putting an oil slick into flame
in order to minimize damage to the environment is not a new concept (Carrier et al., 1992). The weathering and quick evaporation of the VOS, SVOC and heavy crude oils and dilbit in general indicate that the flammability of the crude spills decrease with time (Fritt-Rasmussen, 2012).

In marine environments evaporation is usually speeded up by the increase in surface area due to the oil dispersion. Should the spills occur on land, the heavier asphaltenic oil has the shortest time frame for in situ burning and becomes non-flammable in not more than 9 hours whereas the light and waxy crudes takes about 18 - 72 hours. As a result, in-situ burning may become more challenging for asphaltenic oils for example, bitumen from oil sands sources, even if, the diluent component introduced to the crudes may possibly prolong the flammability window (Tsapralis & Zhou, 2014).

Owing to these limited flammability windows, there have not been many crude spills that have resulted in fire related cases. However, crude oil spills have the tendency of creating a hazardous environment with the possibility of ignition.

1.3.4 Oil Fates and Weathering
Upon the release of oil into the environment, a broad spectrum of chemical, physical and biological processes occurs during the transformation of the released oil. These processes are collectively known as weathering, which describes the action of altering the composition, performance, exposure and the level of toxicity of the oil (Gitschlag, 1992).
Weathered oil is made up of fairly insoluble chemicals and often combines to form tarmats or tar balls. It is highly likely that birds or other animals can encounter the oil-contaminated soil, with potentially serious effects, as shown in Figure (1-1).

![Figure 1-1: Bird trapped in Kuwait Oil Lakes (Abbas, 1991).](image)

The main processes of weathering are evaporation, biodegradation, adsorption and solubilisation. In general, these processes will take place for all oil discharges in the soil. The volatility and solubility of some hydrocarbons can influence the toxicity and bio transformation of oil residue (Niehaus et al. 1999). As a result, the concentration of carbon will be decreased. As reported by Prince (2003), both the properties of spilled oil and the microorganism interaction may change with time.
As illustrated in Figure (1-2), the composition ratio of heavier and lighter carbons tends to decrease over the course of time due to the biodegradation processes under the conditions like; pH 6.0-8.0, moisture content 50 %, temperature 20°C and adequate content of nitrogen (N): phosphorus (P) with ratio 10:1, respectively.

![Figure 1-2: carbon composition of crude oil with time due to biodegradation process (Source: Prince et al., 2002).](image)

After being in the terrestrial surrounding for a prolonged duration, the weathered chemical residues may resist degradation and desorption, besides they have sufficient time to interact with other soil constituents (Tang et al., 2012). Further, Certini (2005) explained that the covalent bonding among organic compounds within oil residues and humic polymers such as humin, humic acid, and fulvic acid present in soil is capable of forming stable dialkylphthalates, long-chain alkanes, and fatty acids which can resist microbial degradation.
1.4 Source of Crude Oil Pollution

The spill of crude oil means discharging crude petroleum hydrocarbons into the surroundings as a result of human activity. The most common and notable cause of contamination results from manufacturing and refining activities, such as leakages and spillages from oil tankers or accidents whilst transporting the oil. During the processing and production of oil, significant amount of oil sludge or slops are created contributing towards pollution to the environment, whilst utilising the useful storage spaces in the tanks or other storage containers. According to Francis and Stehmeyer (1991), the oil sludge that is usually present in oil processing and production facilities contains various concentrations of waste oil (40–60%), wastewater (30–90%) and mineral particles (5–40%).

1.4.1 Impact of Hydrocarbons

In view of the presence of the high-concentration of toxic substances, inappropriate way of disposing oily sludge may present serious dangers to the existing environment. Upon arrival at the terrestrial environment, oily sludge tends to disrupt the physical and chemical characteristics of the receiving soils causing the morphological change of the soil (Robertson et al., 2007). Rushton et al. (2007) explained that petroleum contaminated soil can be detrimental to the health of people and animals, affecting the liver, lungs, kidney and nervous system, causing cancer, disorder of immune and reproductive system. Based on study conducted by International Agency for Research on Cancer (IARC, 1983), shown that the benzene, one of the components of hydrocarbons, has also been considered to be a human carcinogen. As far as petroleum hydrocarbons compounds (PHCs) are concerned, the PAHs are the main threats as they are considered genotoxic to humans and other ecological receptors.
(Robertson et al., 2007). Within the oil residue, PHCs are able to travel down through the soil profile moving into the groundwater which is interconnected to other water system causing serious adverse consequences for example reduced diversity and population of fish in the marine system (Trofimov and Rozanova, 2003; Wake, 2005). PHCs in oil sludge may also affect the soil enzymes (such as hydrogenase and invertase) activity and present toxic threats to soil microorganisms (Suleimanov et al., 2005). In most risk assessments of potential risk of exposure to a complex mixture of carcinogenic PAH cannot be assessed by its original concentration, the basis chemical is Benzo(a)Pyrene B(a)P equivalent, which was used as a reference to adjust the concentration of the original compound (IARC, 1987).

The soils contaminated with oily sludge may lack in nutrient content, hindering germination of seeds, which results in restricted growth or death of plants (Al-Mutairi et al., 2008). In addition, oil spill can reduce the infiltration of water into a soil, and the nutrient mobility (Kirk et al., 2005) and is capable of travelling through cell membranes with ease into plants (Adam and Duncan, 2002). Consequently, this can hinder the growth of plants within this contaminated soil (Kaimi, 2007). Particularly, constituents with high molecular weight in oil residue and their products could stay close to the soil surface and form a layer around seeds. This could cause reduction in water availability and limited exchange of water/air, and finally lead to a slower rate of germination (Adam and Duncan, 2002; Tang et al., 2012).

Owing to the high viscosity, components of oil residue can be retained in soil pores, adsorbed onto the soil mineral components’ surface, or create a continuous cover on the surface of the oil (Trofimov and Rozanova, 2003). All these factors tend to decrease hydraulic conductivity,
hygroscopic moisture and water retention capability i.e. soils’ wettability (Trofimov and Rozanova, 2003; Suleimanov et al., 2005). A long term (a couple of years) hydrophobicity of agricultural soils contaminated with oily wastes has been reported in western Canada despite the fact that a number of soils contaminated with PHCs take up water in the end and remain wet (Robertson et al., 2007). Therefore, this pollution can also lead to the organic pollution of groundwater, limiting its use and causing environmental concerns, economic loss and affecting the farming productivity of the soil (Wang et al., 2008).

Disposing oil contaminated soil into the environment can cause numerous toxic hazards from the heavy metals and PHCs. The majority of heavy metals have hazards and have cumulative effects. In particular, the combined effects of heavy metals and petroleum hydrocarbons compounds in the soils contaminated with oily sludge may cause more complications.

Specifically, the joined effects of PAHs (i.e. typical elements in oily sludge) and heavy metals were considered as highly toxic in comparison with PAHs on its own (Raab and Feldmann, 2003). This was confirmed by a study conducted by Barbara and Bozena (2003), who concluded that soils contaminated with heavy metals and PAHs possessed a higher inhibitory effect on microorganisms and plant growth compared with soils mixed with either PAHs or heavy metals only. Also, the inhibitory effect was evident during the early phase of the growth of plants and microorganisms. Shen et al. (2005) noted that the effect of interaction between heavy metals (Cd and Zn) with PAHs was heavily dependent upon the contamination time.
Owing to the environmental risk from the oil residues, a number of regulations, for example the United State Environmental Protection Agency (USEPA), have outlined stringent guidelines to deal with handling, storage and disposal of oily sludge (USEPA, 2011). For instance, the regulation has specified that all surfaces impoundments which are used either for treatment or storage of hazardous waste should have two layers of lining or else be taken out of service.

Bhattacharyya and Shekdar (2003) found that the risk of fire hazard and issues with odor remained eminent even when the lagoon for disposing of oil residue were lined with cement and bricks. The oil residue from refinery disposed in landfills or lagoons has been considered as a stationary source of atmospheric pollution such as volatile organic compounds (VOCs) and semi-volatile hydrocarbons (SVOC) (Cheremisinoff and Rosenfeld, 2009). These emissions of air pollutant can cause detrimental risk to workers and neighboring communities (USEPA, 2011).

1.4.2 International Standards
Based on Table (1-3), there is no international standard for maximum permissible TPH and PAH concentration in contaminated soil. Various agencies (including USA, Canada and Republic of Korea) have a common level of 2,000 mg/kg TPH for contamination threshold in industrial area. However, Moen et al. (1986) and the USEPA (1995) reported that the concentration for each individual PAH for uncontaminated soil were 0.1 and 0.01 mg/kg, respectively.
Table 1-3: International maximum permissible for contaminated soil with TPH and PAH.

<table>
<thead>
<tr>
<th>Generic Clean up level</th>
<th>TPH (mg/kg)</th>
<th>PAH (mg/kg)</th>
<th>Details</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Residential</td>
<td>50</td>
<td>-</td>
<td>Use of any level more than the standard residential is considered a risk based remediation</td>
<td>ODEQ. (2012)</td>
</tr>
<tr>
<td>Industrial</td>
<td>500</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>In surface soil</td>
<td>2,000</td>
<td>-</td>
<td>The levels of soil TPH are default values, rather than risk based, and no specific sites are consider</td>
<td>USEPA. (2002)</td>
</tr>
<tr>
<td>In subsurface soil</td>
<td>5,000</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>In any type of soil</td>
<td>-</td>
<td>0.01</td>
<td>Lower limit of application of 0.01 mg/kg (expressed as dry matter) can be ensured for each individual PAH</td>
<td>USEPA. (1995)</td>
</tr>
<tr>
<td>In any type of soil</td>
<td>-</td>
<td>&lt; 0.1</td>
<td>Soils containing 0.1 – 1.0 mg/kg individual PAHs are considered slightly contaminated and soils containing 1 – 10 mg/kg individual PAHs are considered to be significantly contaminated.</td>
<td>Environment Canada. (2008)</td>
</tr>
<tr>
<td>Commercial</td>
<td>800</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Industrial</td>
<td>2,000</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Uncontaminated soil</td>
<td>-</td>
<td>0.1</td>
<td>Dutch B (Indicative value for further investigation) can be ensured for each individual PAH</td>
<td>Moen et al. (1986)</td>
</tr>
<tr>
<td>Uncontaminated soil</td>
<td>10,000</td>
<td>-</td>
<td>Kuwait regulatory cleanup level of soil TPH</td>
<td>Kuwait Environmental Public Authority (KEPA). (2012)</td>
</tr>
</tbody>
</table>
1.5 Engineering Approaches for Soil Remediation

The ‘soil remediation’ terminology relates to the efforts which seek to eradicate or reduce the risks connected to contaminated soil. There are various ways of achieving this objective and the most appropriate option is governed by various factors, such as the type of contaminants, the site conditions and financial constraints. Nathanail and Bardos (2004) describe remediation as the action of removing, degrading or transforming contaminants to safe and stable substances. *Ex-situ* and *in-situ* remediation technologies for remediation of petroleum-contaminated soils can be categorised depending on their treatment mechanism as physical, chemical, thermal adsorption, biological and electrochemical remediation (Riser-Roberts, 1998), *ex-situ* soil washing have been developed lately in an effort to remediate soils contaminated with petroleum (Sui and Ji, 2010).

1.5.1 Bioremediation

The aim of a biological remediation process is the degradation of contaminants to harmless products and end products through the use of microorganisms. Biological treatments have considerable scope for incorporation with other remediation processes and are applicable with contaminated soil (Nathanail and Bardos, 2004). Biological remediation is implemented at low cost compared to the other alternative techniques (Feng and Aldrich, 2000; Zhao *et al.*, 2011), although a wide variation exists in terms of the performance, treatment time and environmental consequences of these techniques. Federal contaminated sites action plan (FCSAP) in Canada reported that bioremediation in 2008-2009 comprised the most significant portion (21%) of the remediation activity, as shown in Figure (1-3). In addition, different criterions for various popular bioremediation processes are listed in Table (1-4).
Table 1-4: Characteristics of various bioremediation (Source: Van Deuren et al., 2002; Lodolo, 2005; Koning et al., 2000; Sinha et al., 2010).

<table>
<thead>
<tr>
<th>Type of remediation</th>
<th>Main target contaminates</th>
<th>Duration time</th>
<th>Cost (£/ton)</th>
<th>Limitations and conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Land farming</td>
<td>VOCs, SVOCs, PAH and pesticide</td>
<td>6-24 months</td>
<td>67 - 75</td>
<td>No specific conditions</td>
</tr>
<tr>
<td>Vermiremediation</td>
<td>BTEX, PAH and heavy metals</td>
<td>6-12 months</td>
<td>100</td>
<td>Uniform</td>
</tr>
<tr>
<td>Biopiles (ex)</td>
<td>VOCs, SVOCs, PAHs and BTEX</td>
<td>&lt; 6 months</td>
<td>19 - 56</td>
<td>Uniform</td>
</tr>
<tr>
<td>Bioventing (in)</td>
<td>VOCs, SVOCs PAHs</td>
<td>12-30 months</td>
<td>11 - 60</td>
<td>Uniform, unsaturated and low permeable</td>
</tr>
<tr>
<td>Windrow</td>
<td>VOCs, SVOCs and PAH</td>
<td>6-12 months or &gt;</td>
<td>186 - 223</td>
<td>No specific conditions</td>
</tr>
<tr>
<td>Phytoremediation</td>
<td>VOCs, SVOCs, heavy metals, PAH</td>
<td>12 months or &gt;</td>
<td>37 - 112</td>
<td>Contaminations in shallow soil</td>
</tr>
</tbody>
</table>

*Prices converted from Euro to British Pound Sterling on 8 Feb 2015.

Brief descriptions of bioremediation techniques employed in the remediation of contaminated soil are described in the following section.
1.5.1.1 Land Farming

Land farming is a process in which the contaminated soil is excavated, mechanically, applied into lined beds and tilled to aerate the waste. For instance, polluted soil is placed in a thin layer no more than 0.4 m thick on the surface of the ground. During the remediation process, aerobic microbial activity aims to degrade, transform, and immobilise the contamination. The rate of microbial degradation can be optimised by controlled the soil conditions such as moisture content, aeration, nutrients and pH (Van Deuren et al., 2002; USEPA, 2004).

1.5.1.2 Vermiremediation

Vermiremediation is slowly becoming a less expensive and more acceptable method of clean up for chemically contaminated sites. This approach involves the use of earthworms to bio-accumulate the contaminants within their bodies and biodegrade or bio-transform them into safe products with the assistance of enzymes. Ali et al. (2006) and Sinha et al. (2010) reported that the earthworms have high resistivity to a number of chemical pollutants present in the soil such as organic pollutants and heavy metals. One of the main features of this economic and environmental importance is that polluted land is not only cleansed but also improved in terms of its physical, chemical and biological quality.

1.5.1.3 Biopiles

Excavated soils are combined with soil amendments and placed on a treatment area. Nutrients and oxygen are inserted to the contaminated soil by air injection system buried under the soil to pass oxygen through the soil either by vacuum or by positive pressure in order to enhance aerobic biodegradation (Slenders et al., 1997; Schulz-Berendt, 2000).
1.5.1.4 Bioventing

Bioventing process injects air into the contaminated land, where the movement of air is designed to maximize biodegradation while minimizing volatilization (Koning et al., 2000; Nathanail and Bardos, 2004). This process is applicable for treating permeable soils because huge volume of air is required to degrade VOC.

1.5.1.5 Windrow

Waste composting and Treatment windrows systems are very similar in their approaches. Contaminated soils are put together with compositing materials (e.g. as wood chips, bark or compost) and placed in windrows to increase aeration and improve the soil structure. Regular turning is carried out to transfer of oxygen into contaminated soils and thus supporting aerobic degradation of organic contaminants (Van Deuren et al., 2002).

1.5.1.6 Phytoremediation

The utilisation of plants in improving degradation as well as reducing the volume, mobility or toxicity of contaminants in soil, underground water or any other polluted media is known as phytoremediation, which is a general term used since 1991 (McCutcheon, 2003). Also, It is offers a high potential in dealing with a range of environmental problems, such as soils contaminated with hydrocarbons or heavy metals and other hazardous chemicals. Many benefits can be offered by employing the phytoremediation method in either minimising risks or saving in costs as compared against traditional excavation and landfilling method. Even though it is not a universally applicable and acceptable solution.
1.5.2 Physical and Chemical Remediation

Physical processes can be used to treat organic and inorganic contaminants and to separate contaminated soil from uncontaminated materials or soil as concentrate through the exploitation of physical differences between the soil and contaminants (e.g. volatility, behaviour in electric field) or between their physical properties (e.g. density, particle size, etc.) and soil properties (Slenders et al., 1997; Nathanail and Bardos, 2004). The different criterions of various physical remediation processes are listed in Table (1-5).

Table 1-5: Characteristics of physical and chemical techniques (Source: Van Deuren et al., 2002; Lodolo, 2005; Koning et al., 2000).

<table>
<thead>
<tr>
<th>Type of remediation</th>
<th>Main target contaminates</th>
<th>Duration time</th>
<th>Cost (£/ton)</th>
<th>Limitations and conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solidification/Stabilisation</td>
<td>Organic compounds, heavy metal.</td>
<td>&lt; 6 months</td>
<td>56 - 158</td>
<td>Uniform</td>
</tr>
<tr>
<td>Electro-remediation</td>
<td>Organic compounds, heavy metal.</td>
<td>6-12 months</td>
<td>48 - 145</td>
<td>Saturated soil</td>
</tr>
<tr>
<td>Soil venting and air sparging</td>
<td>VOCs, SVOCs</td>
<td>12 months or &gt;</td>
<td>15 - 97</td>
<td>Low percent fines and correct moisture content</td>
</tr>
<tr>
<td>Soil washing</td>
<td>Organic compounds, heavy metal.</td>
<td>&lt; 6 months</td>
<td>104 - 297</td>
<td>Uniform</td>
</tr>
</tbody>
</table>

*Prices converted from Euro to British Pound sterling on 8 Feb 2015.

There are several physical and chemical remediation techniques available for soil remediation which are described in the following section.

1.5.2.1 Solidification/Stabilisation

Solidification/ Stabilisation (S/S) process is also termed as in-situ fixation or immobilisation which is the alteration of organic or inorganic compounds to immobilise condition through injection or infiltration of stabilising agents into an area of contaminated soil (Slenders et al.,
Contaminants are physically bound or enclosed within a low-permeability mass (solidification), or chemical reactions are induced between the stabilising agent and contaminants to reduce their mobility (stabilisation). For potential projects, it is important to have good understanding of the hydrological regime and it can apply to moderate to high permeability soils as well as various types of contaminants.

1.5.2.2 Electro-remediation

Electro-remediation is an *in-situ* procedure which entails the use of low intensity direct electrical current across pairs of electrode inserted into the ground on both sides of contaminated area. This triggers electro-osmosis and ion migration. Subject to their charge, contaminates travel toward respective electrodes. The procedure can be improved using surfactant to accelerate the removal of contaminants at the electrodes. This *in-situ* soil processing technology is primarily a separation and removal technique for extracting contaminants from saturated or unsaturated soil, sludge and sediment, furthermore, the technique is only suitable for sites where the soil is saturated with water (Yang *et al.*, 2005).

1.5.2.3 Soil vapour extraction (SVE)

Soil vapour extraction (SVE) is *in-situ* technique used in treatment approaches by injecting gas (usually air or oxygen) into the saturated zone to volatile contaminants and to stimulate biodegradation by augmenting subsurface oxygen concentrations (Bardos *et al.*, 2000). The remediation system of SVE can be applied successfully for VOCs in relatively moderate to high permeability geologic soil.
Among others, SVE is considered as a useful to extract LMW compounds due to high vapour pressure. Nevertheless, this method is not suitable for organic compounds with low volatility, for example PAHs and unable to expel super heavy oil pollutants which contain high concentrations of resins and bituminous materials (Ji and Guo, 2010). Under high pressure of injection it is easy to fracture the soil material which leads to flows of oxygen into the subsurface, thereby starting aerobic contaminant decomposition process (Held and Dorr, 2002).

1.5.2.4 Soil washing

Soil washing uses liquids (usually water, occasionally combined with solvents or surfactant) and mechanical processes to clean soils. Fine soil fractions are often the higher contaminated part of the soil. If the fine soil fraction content exceeds 30-40 % it may not be cost effective to carry out the separation as an additional treatment stage (Wallace and Cork, 1998; Feng and Aldrich, 2000).

1.5.3 Thermal Desorption

Thermal desorption heats the contaminated soil to increase the volatility of pollutants to a gas phase or melt the contaminants. The preparation of this technique is by means of a sintering strand, fluid bed, or rotary kiln plants, which is regarded as rapid clean-up times but with the most expensive remediation method. It is possible for VOC and SVOC to be vaporized, then rise to the unsaturated zone where they are extracted using vacuum followed by treatment. The time it takes depends on three major factors: type and amounts of chemicals present; size and depth of the polluted area; type of soil and situations present (Koning et al., 2000).
1.6 Overview of Soil washing Technologies

Soil washing has been extensively researched for the remediation of soils and sands contaminated with crude oil constituents (Deshpande et al., 1999; Vreysen and Maes, 2005; Santa Anna et al., 2007). Stirring has been utilised to enhance the efficiency of traditional methods using hot water for elution (Feng and Aldrich, 2000). Previous studies (Feng and Aldrich, 2000; Shrestha et al., 2009; Sutkar and Gogate, 2009) have concluded that soil washing techniques aided by ultrasonication has the capability to perform better compared with traditional soil washing methods.

1.6.1 Ultrasonication

Ultrasonication or sonication is one of the most effective, economical, and powerful cleaning methods owing to its ability to penetrate into microscopic holes. In order to bring about effective removal of contamination, "frequency sweeping", (a frequency modulation of the ultrasonication output generated) prevents "standing waves" from being generated and ensures extreme homogeneous energy distribution in the cleaning bath. The frequency results in high-energy acoustic cavitation, which covers the formation, development and implosive collapse of bubbles in a liquid. The acoustic cavitation in the aqueous stage causes the transformation of dissolved species through pyrolysis and radical reactions. Also, acoustic cavitation generates high velocity micro jets, which are high velocity shock waves that encroach the surface, erode the adsorbate and cause vortex micro-streaming in the solid particles’ holes and the interface of solid and liquid (Na et al., 2007).
The occurrence of acoustic cavitation in liquids can encourage sonophysical and sonochemical effects. In the field of environmental engineering, sonochemical effects concern the degradation of organic pollutants utilizing pyrolysis and radical reactions (Mason and Lorimer, 2002). Sonophysical effects on the other hand, relate to mixing and cleaning by means of micro jet, micro-streaming and shock waves. Naturally, these two effects take place at the same time, sonophysical effects can be more vicious in heterogeneous systems which entail solid, turbulent liquid movement, and gradient the velocity of cavitation bubbles in the vicinity (Mason and Lorimer, 2002; Gogate et al., 2011; Chen, 2012). They hypothesized that the liquid medium absorbs acoustic energy from ultrasound waves, and then later on will flow along the spread directions of the sound waves. Therefore, the effectiveness of the contaminant removal by the ultra-sonication system can be further improved through sonophysical effects.

In the case of removal of adsorbed materials from solid particles, it has been evidenced that ultrasonication is found to be an effective technique by splitting the solid and liquid within high concentration solutions as well as reducing the oil/water (o/w) emulsion’s stability (Ye et al., 2008; Li et al., 2013). During the propagation of the ultrasonication waves within the medium of treatment, compressions and rarefactions are generated. The cycle of compression applies a positive pressure towards the medium and pushing together all the molecules. The negative pressure is exerted through the rarefaction cycle by pulling the molecules from one another resulting in the generation of the microbubbles which will then grow. If the growth of these microbubbles is excessive such that it become unstable, they can collapse aggressively creating a sudden wave which results in the generation of extremely high pressure and temperature within just a few milliseconds, directed towards the surface of solid particles (Pilli et al., 2011).
This theory was confirmed by Plesset (1973) and experimented by Coleman et al. (1987) as shown in Figure (1-4). According to Mason & Lorimer (1991) and Suslick (1989), the resultant shock waves produce high localised pressures and temperatures as high as 1000 atm and 5000 K, respectively. Therefore, extreme conditions with highly localised pressures and temperatures led to the development of the field of sonochemistry.

Figure 1-4: Confirmation of bubble collapse (A) Theoretically by Plesset (1973) and (B) Experimentally by Coleman et al. (1987).

In addition to cleaning the solid particles’ surface, ultrasonicationation is also able to infiltrate into various sections of a multiphase system, which are not accessible by other means of washing methods (Swamy and Narayana, 2001). This scenario is termed as ultrasonication leaching whereby solvents, surfactant or leaching reagents are able to enter solids pores’ internal parts with ease and improves the mass transfer of contaminants through solid matrix (Feng and Aldrich, 2000). Among others, the factors that may influence the efficiency of oil removal from oil sludge by means of ultrasonication treatment are salinity, presence of surfactant, solid particle size, intensity, duration of ultrasonication treatment, water content in emulsion, temperature, ultrasonication power, frequency of ultrasonication, and the
concentration of contamination (Feng and Aldrich, 2000; Kim and Wang, 2003; Na et al., 2007). For example, a study carried out by Ji and Guo (2010) involved tests to enhance the removal of severely biodegraded heavy oil (SBHO) from weathered contaminated soil by using ultrasound with hot water at 70.0°C. The results indicated that ultrasonication enhanced the elution of the minerals and elementals of the weathered soil and removed 75.5% of the SBHO fraction after 30 min.

The applicability of soil washing processes by ultrasonication for industrial use are rare and most studies have been conducted in lab-scale systems with ultrasound generators “horn-type” (Feng and Aldrich, 2000: Shrestha et al., 2009), which can only treat a limited quantity of oily sludge effectively. Due to the limited transmittance of the ultrasound, expensive equipment and costly maintenance may also prevent the application of horn type sonication systems at a commercial scale (Sutkar and Gogate, 2009). Conversely, Son et al. (2011) reported that the plate-type ultrasound is a “green” solution for soil remediation in a considerably short period compared to the ultrasonication horn system in large scale processing. Previous research deduced that applications using ultrasonication method could improve the efficiency of surfactants in the elution of hydrocarbon compounds considerably (Kim and Wang, 2003; Mason et al., 2004). Moreover, the effectiveness of contaminant removal by the soil washing technique can be further improved from physical effects in acoustic cavitation by using combination of ultrasonications (plate type) with mechanical stirring (Nam et al., 2009; Son et al., 2011). They concluded that the sonophysical effects plays significant role for mass removal of diesel from soil particles to aqueous phase and allow pollutant to transfer inside the pore, which barely desorbed by using mechanical mixing alone.
1.6.2 Surfactant

The term “surfactant” is originated from the expression “surface active ingredient”. These amphiphilic molecules consist of two main ingredients namely water soluble head group and water insoluble tail group also known as hydrophilic and hydrophobic, respectively. While hydrophilic head helps surfactant molecule dissolve in water phase, hydrophobic tail influences it to assemble at the interfaces in an effort to reduce surface and interfacial tension and therefore improving the mobility and solubility of insoluble organic compounds (hydrophobic) (Mulligan, 2009).

As majority of surface of oil contaminants are naturally hydrophobic, the main purpose of utilising surfactants is to minimize the hydrophobicity of the oil phase to such a level that it is wetted by the water phase and separate itself from the surfaces of the soil. As such, surfactants are employed in order to improve the surface actions of the surfactant/oil/soil systems. Also, chemical surfactants are effective in reducing both the viscosity of oil and the interfacial tensions of water and oil (Al-Sabagh, 2000; Liu et al., 2004). However, there may not be true solubility of petroleum compounds in water and it has been observed that surfactants can also increase pseudo solubility of hydrophobic contaminants (Chu, 2003; Pekdemir et al., 2005). As an example, in the presence of saponins, PAH solubility increases thus making them more available to degrading bacteria (Soeder et al., 1996). The utilisation of surfactant in the removal of organic contamination from solid matrices is considered as an affordable and comparatively speedy procedure and can potentially treat contaminants in larger quantities. The categorisations of surfactants are made according to the type of head group, i.e. cationic, anionic or non-ionic.
Non-ionic surfactants are neutral which result in lower affinity to electrolytes in the solution. With regard to soil washing, if there are metal ions, salt etc. present in the solution, an electrolyte is introduced to the aqueous surfactant solutions for improving the efficiency of oil removal from soil (Zhong et al., 2003). As such, the non-ionic surfactants are able to prolong the contact time in the water bath. According to Mulligan et al. (2001), anionic and non-ionic surfactants are more frequently used in soil remediation as they are less expected to sorb onto surfaces of the soil. Some of the benefits of non-ionic surfactants are that they have a high solubility in cold-water, low microbial toxicity and better wetting action (Kim and Wang, 2003; Zhao et al., 2005). Cuypers et al. (2002) and Conte et al. (2005), they revealed that non-ionic surfactants are able to desorb hydrocarbon compounds from soil.

The environmental impact of surfactant has increased the awareness of using biosurfactant as environmentally friendly cleaning product. Nevertheless, traditional synthetic surfactants were utilised by Hirata et al. (2009), which has caused difficulties in downstream processes due to their toxic characteristics and non-biodegradability even after 8 days. They conducted the biodegradability test under oxygen consumption method according to the organisation for economic co-operation and development (OECD) for testing chemicals at 20°C.

Consequently, owing to lesser toxicity, better biodegradability and stability under the environmental conditions (e.g. temperature and pH) and generally less cost, biosurfactants are more suitable to environmental applications than their synthetic counterpart (Muthusamy et al., 2008). Therefore, it appears that biosurfactant are the way forward.
1.6.2.1 Overview of biosurfactants

There are a number of synthetic surfactants that tend to be toxic to microorganisms, which can reduce the number of degraders within the soil (Sandbacka et al., 2000). One of the methods to deal with toxicity issue is by means of introducing biosurfactants (either produced by surfactant producing microorganisms or natural compounds that perform as surfactants).

According to Hunt et al. (1994) and Oberbremer et al. (1990), biosurfactant-producing microorganisms have been recommended as a substitute to chemically produced surfactants for improving the hydrophobic compounds' availability. They can be categorized into five groups namely (1) Glycolipids, (2) Lipopeptides, (3) Phospholipids, fatty acids, and neutral lipids, (4) Poly-metric bio-surfactant, and (5) Particulate bio-surfactant (Mulligan, 2009). Bio-surfactants generally are anionic or neutral with only a handful which are cationic for instance those that contain amine groups. The structures of these bio-surfactants consist of amphiphilic molecules with a hydrophobic moiety which is fatty acid and hydrophilic moiety (e.g., carbohydrate, carboxylic acid, phosphate, amino acid, cyclic peptide or alcohol) (Mulligan, 2005).

A study was performed by Urum and Pekdemir (2004) in an effort to investigate whether the synthetic surfactants namely (sodium dodecyl sulfate, SDS) and biosurfactant namely (aescin, lecithin, rhamnolipid, saponin and tannin) are applicable for washing oil contaminated soil. The behavior of synthetic surfactant and biosurfactants in water–oil, soil– water and oil–soil systems were measured and their characteristics such as surface tension, interfacial tension, solubilization, sorption to soil, emulsification and foaming were compared. Separation of crude
oil from the contaminated soil by biosurfactants was enhanced at different concentrations, for example SDS and rhamnolipid removed up to 42% while lecithin and oil removed about 80%. With regard to the effectiveness of contaminant removal, their research also demonstrated that saponin at the concentrations of 0.2 to 0.5 mass% at volume/mass ratios of 1:3 have resulted in equal or improved efficiency in comparison with chemical surfactants despite their bulky molecular structures. They suggested that bulky molecular structures of saponin or other biosurfactants might also enhance the efficiency of the oil removal. Generally, biosurfactant assisted oil removal technique have the potential to be a simple, comparatively fast and effective with huge potential in treating large quantity of oil contaminated sand.

A series of tests were conducted by Urum et al. (2004) at laboratory environment for biosurfactant (rhamnolipid) and a synthetic surfactant (sodium dodecyl sulfate, SDS) by means of a rotary shaker in an effort to remove hydrocarbon oil from soils under various washing conditions. Tests were conducted at varying duration (5 to 20 min), surfactant concentration (0.004 to 5 mass%), shaking speed (80 to 200 strokes/min), temperature (5 to 50°C) and volume (5 to 20 cm³). The ratio of soil to surfactant solution used was 5 g: 45 ml. The results showed that the effectiveness of crude oil elimination was significant and within the repeatability range of ±6%. The most effective conditions on crude oil removal from soil were as follows: concentration of surfactant, washing temperature, soil cation exchange capacity and pH. The result indicated that, the weathered contaminated soil samples are often harder to remediate than soils that have just been contaminated soil.
Based upon findings by Urum et al. (2005) in utilising synthetic and biosurfactant for soil washing, this research paper looks into an efficient and reliable technique to remove crude oil from contaminated soil. Their work entails an \textit{ex-situ} method utilizing air sparging assisted stirred tank reactor aided by two surfactants. The outcome of various constraints such as washing time, volume/mass ratio, surfactant concentrations, temperature) were examined by various washing techniques namely, stirring, air sparging and the combination of stirring and air sparging. It was revealed from the results that soil washing with SDS solution was able to remove about 80\% of the crude oil from non-weathered samples. In its conclusion, the researchers confirmed that the oil separation was more effective using solutions prepared from seawater than those prepared using distilled water. Their research also demonstrated that combination technique is more effective in minimizing the quantity of oil in contaminated soil when compared to being applied individually. The focus in this project is therefore to utilise biosurfactants with low toxicity and better biodegradability.

1.6.2.2 Critical Micelle Concentration (CMC)

Critical Micelle Concentration (CMC) is the term used to describe the surfactant concentration at the instance micelles start to form, or when a concentration level of surfactant is at or above a certain level will not cause any effect. This concentration refers to the condition whereby the surfactant starts to demonstrate the minimum surface tension. The measurement of surface tension of the surfactant solutions can be performed by means of a Du Nouy tensiometer with a technique known as platinum ring method. In the soil washing research, surface tension test can be used to decide the optimum surfactant concentration.
The hydrophobic tail of the surfactants will be inclined to gather to form a micelle “an aggregate of surfactant molecules dispersed in a liquid colloid” with a hydrophobic core (Santharam et al., 1997). This finding concurs with the study undertaken by Deshpande et al. (1999) and Urum and Pekdemir (2004), which demonstrated that surfactant solution may have no impact on the oil removal properties at concentration higher than their CMC level. As such, the most effective removal of crude oil was registered at concentration below their CMC value. This suggests that the most effective removal of crude oil happens at concentration below the CMC values and does not exhibit any improvement of oil removal at higher concentration.

1.6.3 Removal of Metals from contaminated soil

According to Doumett et al. (2008), soil contamination by metals and heavy metals is one of the key environmental issues experienced worldwide. The efficiency of the soil washing is mainly dependent upon the capability of the removing solution to dissolve the metal contaminants present within the soils. The bond strength between the metals and the soils usually cause difficulties in the process of cleaning. It is therefore essential that only extractants that are able to dissolve the metals optimally be thoroughly selected for the use of soil washing.

Lately, surfactants have proven to be useful in the remediation of environment by removing pollutant from soils and sediments (Mulligan, 2005; Torres et al., 2012). This can be achieved by adding surfactant into the washing solution in an effort to aid heavy metal desorption.
1.6.4 Civil Engineering Applications

A number of research projects from the literature examine the impact of crude oil products upon geotechnical characteristics of various types of soils with sand in particular. According to Al-Sanad et al. (1995) and Ajagbe et al. (2012), oil contaminated sand can be utilised as concrete additives as they are able to change and improve some properties of hardened or fresh concrete. Numerous studies have endeavored to utilise soil contaminated with oil in the hot mixture of asphalt concrete. Series of negotiations have been conducted in Massachusetts with Massachusetts Department of Environmental Quality and Engineering (DEQE). These negotiations have come to an agreement that contaminated soils are permitted to be used which contains 3% of either oil, gasoline or kerosene with a maximum of 5% contaminated soil to substitute aggregate in order to produce asphalt concrete mix of superior quality (Czarnecki, 1998).

A study by Evgin and Das (1992) focused on the stress strain behaviour of dense and loose sand under saturated condition with water and oil. They revealed that oil contaminated sand reduced its angle of internal friction and improved its volumetric strain. As concluded by Ghaly (2001) in his study, the sand’s angle of internal friction decreases by increasing the percentage level of the oil. A study to investigate the effect of crude oil upon the Kuwaiti sand’s geotechnical properties was also carried out by Al-Sanad et al. (1995). It has been demonstrated in their studies that the introduction of the crude oil has improved the compressibility of the sand. Researchers have begun investigating the compressive strength of concrete mixed with contaminated sand and aggregate. The study by Hamad et al. (2003) emphasized on the consequences of used engine oil on the characteristics of fresh and hardened concrete. The
experimental method includes twenty concrete mixes which were organized in two groups with water/cement ratios of 0.59 for one group and 0.62 for the other. The conclusion from their study indicated that the concrete slump and entrained air volume in fresh concrete were higher due to the used engine oil however, has no major effect on the overall strength of the hardened concrete. Diab (2012) also conducted an investigation on the effect on compressive strength of low and high strength concrete immersed in mineral oil. Concrete cubes were immersed for a period of six months in engine oil and their performance was compared with similar cubes placed in oil free solution, used as benchmark. The experiment involved five varying concrete mixes aimed to assess the harmful effect of mineral oil on concrete strength for both low and high strengths. The author concluded that the oil on the surfaces of concrete has avoided the concrete specimens from attaining higher strength. Also, the presence of mineral oil in the concrete mix improved the concrete’s compressibility however its modulus of elasticity reduced.

Another study by Ajagbe et al. (2012) stressed on the impact on the concrete compressive strength of sandy soil contaminated with crude oil. All specimens used consisted of a concrete mix of 1:1.8:2.7 with a water cement ratio of 0.5. The introduction of crude oil to the specimens was by percentage of the sand’s weight (2.5, 5, 10, 15, 20 and 25 %) in order to contaminate the mix. Altogether, there were 147 samples prepared and experimented comprising 21 control and 126 contaminated specimens. For the samples with 2.5, 5, 10, 15, 20 and 25 % contamination, it was found that their concrete compressive strength dropped by 18, 40, 60, 75, 80 and 90 %, respectively. It has been concluded in researches that the compressive strength of concrete was reduced by increasing the presence of oil contaminated soil.
1.7 Treatment of Industrial Wastewater

Effluent containing hydrocarbons can originate from various industries, oil refining process, oil storage, transportation shipyard wastewater, fuel storage containers, cleaning of ship bilges, and petrochemical production process, while the quantity of wastewater generated is subject to the employed extraction technique and the oil extraction rate (Ahmed et al., 2007; Davis and Cornwell, 2012; Machýin-Ramýírez et al., 2008).

Oily wastewater from such process may cause harm to the human health and ecological when the contamination leach to ground water or get close to wildlife or plant life. Further, oily wastewater produced comprises a various types of organic pollutants and presents extremely high content of salt. Within the oily wastewater, oil and grease present in wastewater may exist in various conditions namely free, dispersed or as “emulsified oil” (petroleum hydrocarbons compounds which stay in stable suspension and emulsified to the wastewater). They can be distinguished mainly from the size. Within a mixture of oil-water, free oil is characterised with its size of droplet exceeding 150 μm, the range of size for dispersed oil is between 20-150 μm while emulsified oil is described with its droplets smaller than 20 μm (Quevedo et al., 2009).

It is a real need for a good oily wastewater treatment to address the present issues in the subject of environmental engineering. Some of the oily wastewater treatment offered presently consist of vacuum evaporation, flocculation, adsorbents, coagulation, centrifugal devices, ultrafiltration and deep bed filtration, flotation, membrane separation technology, combined technologies, advanced oxidation process, etc (Apostol et al., 2011; Peng et al., 2014).
There are advantages and disadvantages in each treatment all subjects to the difficulty of operations, capital and operational expenditures. No technology method which is satisfactory in dealing all conditions or contaminations is presently available. Moreover, the physical processes include filtration, ultrafiltration, centrifugation, reverse osmosis and heating, this pretreatment method was often employed in special cases such as small volume of oily wastewater. The applicability of the secondary treatment such as biological, chemical and physical are able to destabilise colloidal particles by breaking down the oil emulsion. Study conducted by Peng et al. (2014), demonstrated that the pretreatment by biological–physicochemical means was able to breakdown the long chain of complex organic compounds in oil emulsion, and convert into small molecules thereby increasing the surface area.

As a result, the interaction of the micro-organisms with the oily wastewater was improved and the solubility of oil was enhanced as well, which allow the oily substances to be available for biodegradation, consequently the process efficiency improved. Also, Colores et al. (2000) reported that the exclusive property of oil dispersed into water allows the biodegradability of crude oil to be improved because of exposed oil spill surface increased with such surfactant. While the secondary treatment by chemical process could be achieved by neutralizing the negative charge on emulsion oil through using alum or polymer to the solution, furthermore, the pH needs to be modified into alkaline range to encourage the formation of inorganic salt followed by gravity separation (Dick, 1982).
1.7.1 Adsorption Techniques to Treat Wastewater

Adsorption is a process whereby molecules of a dissolved substance gather on and stick to the surface of an adsorbent solid. Strangely, the word ‘adsorption’ was initially introduced by Kayser in 1881 to define the acceptance of gases by carbons (Çeçen and Aktaş, 2011). The process of adsorption takes place when the attractive forces at the surface of sorbent overwhelmed the attractive forces of the liquid. These methods are principally adopted by chemical plants which generate wastewater with high concentration of organic compounds and heavy metals. Adsorption method utilising non-conventional adsorbents, for instance woodchips, peat, wool, silk, and water hyacinth pulp powder are also known to have been employed to remove organic substances and heavy metals from wastewater (Anirudhan and Sreekumari, 2011).

Owing to less energy requirements and the benefit of being environmentally friendly, activated carbons have generated huge interests in the adsorption processes for treatment of wastewater. Study conducted by Srivastava and Tyagi (1995), demonstrated that the rate of adsorption on molecules depends on large surface area adsorbed, whereas a very large surface could be provided by the internal pore structure of the sorbents material. For a considerable period of time, activated carbons have been regarded as the top adsorbent to be utilised in removing organic substances from industrial wastewater, however, large-scale for commercial use are considered as expensive and ineffective (Kadirvel et al., 2003).
1.7.2 Biodegradation

Biodegradation is considered one of the key elements of natural elimination, the change in compounds structure mediated by living things including microorganisms. Extensive research in microbial biodegradation has been undertaken in the last two decades for the purpose of eliminating hydrocarbon compounds present in wastewater contaminated with oil in view of its effectiveness, economical factor, simplicity, significant process and friendliness to the environment (Gomes et al., 2009; Sponza and Gok, 2011).

Generally, the degradation of organic matter by microorganisms depends upon the action of microbial metabolism. Alexander (1999) reported that the variation of bioavailability could be effected by various microbial population. For example, a certain compound might be readily degraded and bioavailable by microbial population “A”; nevertheless, different microbial population “B” with same compound and under the same environment might not degrade or bioavailable to that specific microorganisms group, therefore the compounds are persisted. Due to various biochemical and physical processes, the composition of a material can transform almost instantly as soon as it is discharged into the environment.

Many microorganisms are able to biodegrade a wide range of TPHs (Bystrom and Hirtz, 2002). However, the time required to degrade these substances varies upon their structure and molecular weight. These SVOC having recalcitrant features and low solubility can be present in the environment for quite some time. Further, according to Colwell et al. (1977), it has been broadly acknowledged by the scientific world that there is no microbial species that can entirely degrade any particular oil. From a number of petroleum fractions, highly condensed aromatic,
tars, bitumen and asphaltic materials have the highest boiling points and exhibit the maximum resistance to biodegradation (Bossert and Bartha, 1984).

1.7.2.1 Impact of salinity on biodegradation

Microorganisms are acknowledged to tolerate changes in their external environment by means of osmotic tolerance where variations in external concentration level of salt are tolerated by the microorganisms. In accordance with Stanier et al. (1987), this acceptance is achieved by variations in the strength of intracellular ion subject to the ionic strength of the external medium. There has been contradicting information in the literature on the impacts of salinity towards the efficiency of microorganisms in organic removal during the treatment procedures. Some researchers claim that high salinity of NaCl may have some serious effects to the performance of biological processes of the treatment of wastewater (Kargi and Dincer, 1999; Wang et al., 2005). A number of studies demonstrated that until the salt concentration reaches 3 %, no inhibitory effect was observed on the aerobic activated sludge performance (Hashad et al., 2005). Study conducted by Salvadó et al. (2001), proposed that NaCl of 0.5 – 5 % will lower the performance of the biological process in wastewater treatment plants.

1.7.2.2 Aerobic treatment

In treating domestic sewage, microbial-based techniques have been widely used within the last 100 years. The advancement of technologies has offered excellent opportunity for the destruction of organic contaminants that are easily biodegradable under aerobic environments. As such, techniques comparable with those employed in conventional domestic wastewater treatment have operated without major issues in treating a number of industrial wastewaters
(Zhao et al., 2006). The effectiveness of aerobic biological wastewater treatment method is subject to the presence of adequate dissolved oxygen (DO) as the elimination of organic contaminants can be sped up with increased concentration of DO (Liu et al., 2008). With the presence of oxygen, aerobic degradation is regarded as comparatively straight forward, and affordable for degrading organic contaminants. Suspended microbial biomass, known as activated sludge was recycled to treat wastewater in order to eliminate organic compounds. The activated sludge technique has been considered as the main treatment for treating petrochemical wastewater owing to their commercial value, economic value, reduce the amount of sludge formed and safeguarding the microorganisms (Ma et al., 2009). This is achievable by the microorganisms’ metabolic reaction and the microorganisms’ development is associated with the organic pollutants’ degradation within the wastewater. The metabolic rate of microorganisms within activated sludge system can be influenced by the water salinity caused by plasmolysis action. According to Woolard and Irvine (1995), however, the survival of halophilic microorganisms is possible in high salt concentration. As such, a mixture of biological processes would be necessary to enhance the effectiveness of the treatment (Lu et al., 2009).

1.7.2.3 Anoxic treatment

The organisms in the anoxic (limited oxygen supply) treatment may utilise the nitrate acting as electron acceptor and consequently discharging nitrogen oxides. For an efficient denitrification processes to take place, an easily biodegradable source of carbon compounds under anoxic conditions are degraded to methane, CO₂ and H₂S. It should be noted that the micro-organisms in the anoxic system are able to use sulfate as an electron acceptor as well, and release natural
gas and hydrogen sulfide via the fermentative metabolism and acetogenic bacteria with methanogens or sulfidogenesis. According to Gallert & Winter (2005) methane and CO₂ are considered as the main products in anaerobic or anoxic environments if sulfate is absent, but sulfide and CO₂ are considered as the main products when sulfate is present. The key difference between anaerobic and anoxic techniques is the electron acceptor and the donor. The free oxygen or bound oxygen (NO₂⁻, NO₃⁻) is absent in the anoxic process however, the bound oxygen is present in the form of much diluted dissolved oxygen (which is lower than 0.5 mg/l), whereas the matter electron acceptor is nitrate or sulfate and electron donor used in heterotrophic processes is organic. The reduced sulfur solution like hydrogen sulfide performs as electron donors for anaerobic phototrophic bacteria or offers energy for the growth of the colorless sulfur bacteria (Astrid et al., 1995).

1.7.3 Coagulation-Flocculation Treatments
Combination of secondary treatment such as biological treatment with coagulation-flocculation is regarded as an economical technology and answers various technical issues associated with pollution (Chen et al., 2000). As mentioned by Metcalf and Eddy (2002), coagulation can be regarded as a process usually used to treat wastewater by adding inorganic salts to destabilise colloidal particles so that particles flocculate as a result of the collisions of particles. The suspended solids have a negative charge, so that coagulants are able to allow the colloids to be neutralised by the positively charged coagulant, as a result net charge of the particles are reduced during coagulation. Flocculation is considered as a physical process which allows small particles to agglomerate into a large floc that can be settled down easily by gravity (Csempesz, 2000).
The removal efficiency of stable emulsion of oil in water can be enhanced by using ferric salts, aluminum and sometimes with organic polymers (Edwards, 1995; Al-Ani, 2012). The mechanism of coagulation and flocculation sketched in Figures (1-5).

![Diagram](image)

Figure 1-5: Schematic attraction diagram of colloidal destabilisation, A) Coagulant added; B) Charge neutralization; and C) Colloidal particles destabilize.

However, the turbidity can be increased during the coagulation process by insoluble compounds that generated through the addition of chemical. Therefore, further treatment processes are required such as filtration or sedimentation, which can be conducted in with coagulation to reduce total suspended solid (TSS) and turbidity (Kawumara, 1991). Based on the literature, flocculation used to be conducted in same time with coagulation.

1.7.3.1 Treatment with chemical coagulants

The chemicals commonly used as inorganic salts include Al$^{3+}$ and Fe$^{3+}$, which they have been used as a coagulant to separation and sedimentation of suspended particulate from different types of water (Rizzo et al., 2008; Chen et al., 2009). Aluminum sulfate is acknowledged as a coagulant and frequently utilised as the first choice reagent in treating wastewater due to a
number of benefits such as affordability, effectiveness even with least dosage, low toxicity and easily available (Apostol et al., 2011). There is a strong affinity for aluminum sulfate to form insoluble complexes with several ligands. This is particularly true with polar molecules as well as functional groups containing oxygen for instance hydroxyl or carboxyl groups (O’Melia et al., 1999). Following this, organic hydroxides attain a negative charge and reacts with aluminum cation. Therefore, the destabilisation of colloidal particles occurs easily by charge neutralization between aluminum cation and organic anions. In order to satisfactorily complete the destabilisation of colloidal particles, the dosage requirement of aluminum coagulant is subject to the concentration of colloids.

One of the widely used chemical coagulants in treating wastewaters is ferric chloride (FeCl$_3$). Upon introduction to the wastewater, FeCl$_3$ acts by forming insoluble iron salts with its phosphates at alkaline pH. Commonly, FeCl$_3$ is able to modify the double layer strongly, speed up the aggregation of the supernatant and hydrolyze to produce products dependent on pH of the solution (Feng and Nansheng, 2000). Hydroxo-complex flocs can be made by FeCl$_3$ in solution at pH 6 to 8, which provides large sorptive surface and easy sedimentation.

The determination of the accurate dosage of coagulant is best performed by jar experiments and full-size assessments. Several health and environmental issues could be caused by the use of aluminium coagulants, such as neuropathological, epidemiological and alzheimer’s disease (Polizzi et al., 2002; Banks et al., 2006). Moreover, the resultant excessive iron of the ferric coagulants could cause foaming, corrosion, colour, staining unpleasant metallic taste and odor (Li et al., 2008).
1.7.3.2 Destabilisation of colloidal

The colloidal particles present in emulsified oil usually have a net surface charge which is negative. The colloids size of approximately (10 µm) is such that the attractive forces between the colloidal particles are significantly weaker than the electrical charge resisting forces. The traditional method to destabilise colloids can be shown in three different mechanisms: (a) Charge neutralization (b) Double-layer (C) Sweep flocculation.

In charge neutralization, the coagulant metal of positive charged tends to attract the negatively charge colloids by electrostatic interaction. During the neutralization stage, the flocs start to agglomerate by promoting the contact between particles to enhance the growth of the destabilised floc. However, adding excess charge of Al$^{3+}$ or Fe$^{3+}$ increases the risk of charge reversal, which is effect on neutralization phase, and re-dispersed the colloidal with positive charge particles in water (Wen et al., 2006).

Additionally, the double-layer covering the particle comprises of ions which are attached to the surface, also known as stern layer. After this layer, there is a diffuse layer, where the ions move freely. When the particles get closer to each other within the double layer, they repel each other electrostatically causing them to separate from each other which result in a stable suspension. With the ionic strengthening of the solution, the double layer becomes compressed and the particles get nearer to one another. This resulted in the particles getting attracted to each other, creating flocs and making the suspension unstable (Ritvo et al., 2003). As mentioned in American Water Works Association (AWWA, 1999), electrolytes for example NaCl are acknowledged to assist in double layer compression.
According to Jean and Lee (1999), the opposite charged ions that exist improve the flocculation by means of bridging among the particles and assisting them to form aggregates which ultimately flocculate. Double-layer compression causes destabilisation processes as the concentration of salt get closer to that of the seawater. While, sweep-floc coagulation (alternatively referred to as sweep flocculation or sweep coagulation) was described as the adsorption and bridging enmeshment of both particulate organic and inorganic solids in forming huge and formless flocs (licsko, 2004). Further, the required coagulant dosages depend on the colloid concentration, which is measured in terms of surface area, that means the large surface area needs a lots of coagulant because of the concentration of the colloid would be in higher range. In this case, restabilisation will not occur because much of the coagulant is used by the colloids.

1.7.4 Metals Removal from Wastewater
According to Chipasa (2003), heavy metals toxicity is well documented and at some particular concentrations, they are lethal to higher organisms, microorganisms and plants. As such, their presence in wastewater is a real concern to the environment as well as substantially reduces microbial activity. This resulted in the disturbance to the processes within biological wastewater treatment. Microorganisms’ existence and activities within biological wastewater treatment are essential to the treatment process. Microorganisms are not able to biodegrade heavy metals however tend to accumulate the metals through binding them as cations on the surface of cell membrane within a passive transport process (Beveridge and Doyle, 1989). Cells regardless of whether they are alive or dead are able to bind metal ions.
According to Volesky and Holan (1995), biosorption is a metabolism-independent binding of heavy metals to living cells, non-living biomass, or microbial extracellular polymers. Surface exposure of metal binding biopolymers enhances microorganisms’ metal binding properties based upon biosorption as well as microbial metabolic activities (Beveridge and Doyle, 1989).

In order to stay alive under metal stressed environment, bacteria has developed a few forms of mechanisms in tolerating to accept heavy metal ions. According to Nanda et al. (2011), based upon the dependence on the biosorption mechanisms, cell's metabolism can be categorised as metabolism dependent and non-metabolism dependent.

1.8 Measurement of Oxygen Consumption

1.8.1 Biochemical Oxygen Demand (BOD)
Measurement of biochemical oxygen demand is an empirical method carried out using predetermined laboratory procedures in assessing the relative oxygen requirements of wastewater, polluted water and wastewater. The values of BOD signify the quantity of biodegradable organic material (carbonaceous demand), therefore BOD is defined as biochemical oxygen demand. The determination of BOD could be performed in a conventional way by properly aerating a water sample by placing it in an airtight bottle and incubating the sample in a dark room at a temperature of 20°C for a pre-set duration. While oxygen consumed in the water is then determined upon completion of the incubation period.

1.8.1.1 The mathematical model for BOD
In a BOD test, the rate at which microorganisms utilised organics was considered as a first order reaction, so the equation is: the rate of organic matter utilised by microorganism is
proportional to the amount of organic available, this was described mathematically by a first-order kinetics equation (Davis and Cornwell, 2012). Figure (1-6) shown that the Lo represents the total oxygen equivalent to the total mass of organics at time = 0, the term Lt is the amount remaining at time = t, while k is the reaction constant of carbonaceous deoxygenation. The oxygen equivalent consumed is equaled to the BOD exerted, which can be found from the difference between the value Lo, and Lt. So the equation is:

\[ \text{BOD exerted} = \text{Ultimate BOD} - \text{BOD remaining at that time} \ldots \ldots \ldots \ldots \ldots \text{E. q (1 - 1)} \]
\[ \text{yt} = \text{Lo} - \text{Lt} = \text{Lo} - \text{Lo} e^{-kt} = \text{Lo} (1 - \text{Lo} e^{-kt}) \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \text{E. q (1 - 2)} \]

Where: \( \text{yt} \) = the BOD consumed (mg/l), \( \text{L} \) = the ultimate first stage BOD (mg/l), \( k \) = the rate constant of oxygen consumption (t\(^{-1}\)) to the base e, \( t \) = time in days.

Figure 1.3: BOD and oxygen equivalent relationships
Various methods are commonly used to determine k and UBOD (L0) from the results of BOD
test such as, the log differences method, the least-squares method, the series method, the slope
method, the method of moments, and the graphical method. In 1937, Thomas had developed
the graphical slope method and this method was used for many years for computing and
evaluating the kinetics parameters and the constants of BOD curve (Davis and Cornwell, 2012).
The proposed method by Thomas (1950) had originated from the similarity between two series
mathematical function. Which depends on the equation of BOD rate:

@

1/3

DEF@

=

G
(?HI) G/K

+

? L/K
M(HI) G/K

… … … … … … … … … … … … … … … … … … … … . … . 6. 7 (9 − N)

Plot (t /BODt)1/3 as ordinate against t as abscissa, and linearizes the data into a straight line
with intercept a and slope b. The slope (b) and the intercept (a) of this line are determined by
substituting:

k=6

D
P

and Lo =

G
MPQ D

to k and estimate the UBOD … … … … … … … . … … . 6. 7 (9 − T)

Thomas method is considered as valid, accurate and easy to be implemented (Penn et al., 2004;
Oke and Akindahunsi, 2005).

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1.8.2 Chemical Oxygen Demand (COD)

Chemical oxygen demand (COD) refers to a standard method commonly utilised in measuring indirectly the quantity of organic and inorganic oxidisable components in wastewater. The COD value outlines the oxygen equivalent of the organic content that can be oxidized to carbon dioxide and water by potassium dichromate ($K_2Cr_2O_7$) using silver sulfate ($Ag_2SO_4$) as a catalyst under acidic conditions ($H_2SO_4$). It is also used as an indication of the quantity of organic pollutants that exist within the tested specimen (Lee et al., 1999).

However, there are a few shortcomings in the titration method of determining the level of dichromate. Such as, longer time consumption, low sensitivity, complexity of procedures, use of costly ($Ag_2SO_4$) and toxic chemicals (Cr and Hg), and incomplete oxidation of pollutants (Domini et al., 2006; Wang et al., 2012).

Subsequently, air pollution could be caused by using the titration method (Han et al., 2011). A few alternative techniques based upon colorimetric processes have been used and explored to determine the value of COD. While these techniques demonstrate a few benefits over the old COD determination techniques, they are not precise. The key positive points of the colorimetric COD technique are a wider linear range, cost effectiveness, simplicity, shorter in duration of response and easy automation. Meanwhile, the COD vials comprise of potassium dichromate, regarded as a strong chemical oxidising agents of organic compound added to sulfuric acid (50 %). The standard method employs the ratio for $HgSO_4$: Cl equal to 10:1 for chloride concentration of up to 2000 mg/l in which the excess of chloride can be covered by the formation of $HgCl_2$.
The COD values can be over-estimated by oxidising the chloride ions occurring above 2000 mg/l. This is as a result of the oxidation of the chloride ions which can be expressed by the equation as follows:

\[
\text{Cr}_2\text{O}_7^{2-} + 6\text{Cl}^- + 14\text{H}^+ \rightarrow 3\text{Cl}_2 + 2\text{Cr}^{3+} + 7\text{H}_2\text{O} \quad \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \text{E.q (1 – 5)}
\]

A number of researchers have found that this 10:1 ratio in samples contain chlorides higher than 2000 mg/l place a major role in reducing the error, which allows the COD value to be in moderation (Baumann, 1974; Freire and Sant’anna, 1998). There are a few types of industry wastewaters with chloride concentrations in the excess of 15 g Cl/l, e.g. wastewater from chemical industry (Tuin et al., 2006; Ramos et al., 2007) and from petroleum (Nacheva et al., 2008). Baumann (1974) carried a study with the intention to utilise the open flux method using HgSO₄: Cl ratio of 10:1 for masking the chloride. So as to estimate the accurate value, he proposed to include the sample-specific chloride factors in the calculations.

However, samples containing high chloride concentration demonstrate high standard deviation when oxidised, as such the exact correction factor cannot be used accurately in analyzing the saline wastewater samples. Besides, fairly large quantities of samples are required in the open reflux method, with high quantities of reagents and substantial glassware.
1.8.3 Biodegradability ratio (BOD/COD)

The biodegradability of organic compounds by the microbial activity in aqueous medium evaluated through biochemical oxygen demand (BOD) test. While, the chemical oxygen demand (COD) test is used to measure the oxygen equivalent of the organic matter fractions in wastewater that can be easily oxidized chemically by using potassium dichromate in an acid solution. The COD test considered as a very useful tool since it has the ability to run the test quickly. In the assessment of biodegradability, the ratio of BOD/COD has also been utilised. This ratio provides a gross index of proportion of the organic compounds present that can be degradable aerobically within a specified period such as 5 days for BOD₅ (Mantzavinos, 1996). Therefore, it is essential to determine ration between BOD and COD, so that biodegradability can be estimated by using result of (BOD/COD), once BOD values are known.

The ratio of BOD₅/COD has been widely utilised as benchmarks for improvement in biodegradability, whereby a zero value signifies non-biodegradability with an increase in the ratio indicates improvement in biodegradability (Alvares et al., 2001). Low value of BOD₅/COD ratio of 0.1 or lower typically shows that their resistance to conventional treatment is excellent (Imai et al., 1998; Koch, 2002).

A study was undertaken by Gilbert (1987) on non-biodegradable substituted aromatic compounds in wastewater. He discovered that with the BOD₅/COD ratio of higher than 0.4, the biodegradability of the wastewater was enhanced. It is essential to perform the analysis on the COD and BOD of the wastewater as the results are required to monitor and evaluate the wastewater effectively according to the monitoring agencies and specified industries.
1.8.4 Discharged Regulations For Oily Waste Water

The increasingly stringent regulation to protect the environment requires the proper relevant technology to be applied in treating any wastewater originating from oil manufacturing plants. In developed countries, for instance the United States of America, the ceiling daily allowance for oil and grease effluent was 35 mg/l with the monthly average of lower than 17 mg/l (Cheryan and Rajagopalan, 1998). These regulations were revised for a daily maximum of oil, and grease discharged up to 15 mg/l with the monthly average of 8 mg/l (USEPA, 2012). Table (1-6), shows the allowable levels of the major constituents of wastewater.

Table 1-6: Limitations of the treated waste drainage water discharged into sewerage (Source: KEPA, 2014).

<table>
<thead>
<tr>
<th>Pollutant</th>
<th>Unit</th>
<th>Maximum limit</th>
</tr>
</thead>
<tbody>
<tr>
<td>BOD</td>
<td>mg/l</td>
<td>500</td>
</tr>
<tr>
<td>COD</td>
<td>mg/l</td>
<td>750</td>
</tr>
<tr>
<td>TSS</td>
<td>mg/l</td>
<td>300</td>
</tr>
<tr>
<td>Emulsified Oil &amp; Grease</td>
<td>mg/l</td>
<td>5</td>
</tr>
</tbody>
</table>

1.9 Case Study: Kuwait Oil Lakes

1.9.1 Kuwait Geography

Kuwait is located within the northwestern corner of the Arabian Peninsula between latitudes 28°30'N and 30°00'N and longitudes 46°30'E and 49°0'E. Its total land area amounts to 17,818 square kilometers. The State of Kuwait is 170 km from west to east and 2000 km from south to north. The country’s most prominent geographical feature is Kuwait Bay, a large natural inlet that extends 40 km into mainland. Having almost triangular shape, and bordered by the
Kingdom of Saudi Arabia to the south and south-west, the Republic of Iraq to the north and west, with 290 km of Arabian Gulf (coastline) along its eastern boundary (Al-Sulaimi et al., 1993). Most of Kuwait land is formed of a level desert. Without rivers or mountains, it’s sandy desert soil slopes gently towards the sea where coastal marshes, mud flats and salt depressions within the vicinity of the northern section of Kuwait bay are habitats to a number of bird species. The land elevates to a maximum level of 306 m above sea level to the southwest of the country. The surface sediments are smooth sand layers, which are usually covered with a loose thin layer of coarse sand or fine gravel. With some remote hills, the ground surface is typically flat to slightly rolling desert plain. Based upon research by Omar (2006), belowground soils are dominantly very deep, quartz soils, sandy textured, structure-less, occasionally with CaCO₃ at depth.

The characteristic of Kuwait is a desert-type environment with very minimum rainfall (mean 110 mm/year), which usually takes place between November and April, exceptionally high temperatures during July and August (mean temperature of 37.4°C, maximum mean temperature of 45.0°C and the highest temperature recorded is 51.9°C) (Kuwait Meteorological Department (KMD), 2012), short warm winters, high sunshine hours, low humidity level and mostly dry conditions. Owing to the exceptionally high rates of evaporation and very low moisture content in the soil, the amount of precipitation that infiltrates into the groundwater is very minimal. Currently, the main water supply sources are from non-typical water resources such as brackish water, seawater distillation and reutilising of treated wastewater. The quality of these waters is as saline as 1000-45,000 mg of total dissolved solid (TDS) for each litre (l).
1.9.2 Greater Burgan Oil Field

There are a total of 10 oil fields and 909 oil wells within the State of Kuwait and grouped based upon whether they are located in the northern or southern sections. Bahra, Ratga, Sabriay, and Raudhtain oil fields which contain 143 oil wells are situated in the northern area. As displayed in Figure (1-7), nearly 70% of the Kuwait’s operational oil wells are located in the southern oil fields include Ahmedi, Maqwa and Burgan, which are combined to become the “Greater Burgan” oil field, together with Greater Burgan oil field, Umm Gudair, Manigish and Wafra, which are also located within the southern area.

Figure 1-7: Location of oil fields in Kuwait (Source: KEPA, 2014).
Oil lakes were created in nine major oil fields with varying contamination levels. According to Al-Ghunaim (1997), the total number of oil wells in the Greater Burgan is 636, of which 491 were burning, 33 were gushing and 66 damaged. Within the 10 months of fire, a number of oil fields particularly within this area were nearly flooded with crude oil. Within the oil fields, a number of oil lakes of different sizes were created. Burgan field is considered the most adversely affected by the oil spillage in 1991, as it constitutes 40% of the total contaminated volume. The estimated oil lake areas for the Greater Al-Burqan vary from 14.07 km$^2$ (Al-Ajmi et al., 1994) to 35.45 km$^2$ (Kwarteng, 1998), 24.14 km$^2$ (Kwarteng, 1999) and 24.40 km$^2$ (Omar et al., 2006).

The large variation in area shown is because of the different methods used. Each method has its own accuracy and the inherent characteristics of the oil lakes vary subject to whether they are dry, leaking or covered with sand layer. Furthermore, Saeed et al. (1997) and Din et al. (2008) had observed during work in Kuwait oil field that the oil lakes contain crude oil and partially combusted oil with soot. They found that five categories of oil contaminated soil layers namely soot, tarmat, wet oil lakes and dry oil lakes were identified during the field investigation. The fifth category is clean, where no contamination was detected. In addition, the limited accuracy in estimations for the area and volume is due to the inadequate ground information is inadequate. In performing the estimation for the total volume of contaminated soil, it is necessary to understand the area involved and the average depth of contamination in each lake. According to Al-Sulaimi et al. (1993), Balba et al. (1998), former studies have performed characterization and quantification of certain areas, however, the areas and depths have not been accurately estimated.
Besides, only a few sites have been evaluated to provide good volume estimation for the whole oil field. Also, quite a few oil lakes are present today because of the subdivision of original oil lakes by oil recovery actions and development of infrastructure, as shown in Figure (1-8).

Figure 1-8: Sketch map showing the location of pollution area covering Greater Burgan oil fields (source: Din et al., 2008). All the pictures were taken on January 2012 (A) Oil Lakes, (B) tarmat, (C) Soot and (D) less affected area.
1.9.3 Total Petroleum Hydrocarbons (TPH) Concentration

The soil concentrations of TPH from three different locations of oil lakes in Kuwait one from the northern part and two from the southern part, have been investigated, with variations from 13,000 to 19,000 mg/kg of TPH being found for different soils at these locations (Al-Mutairi et al., 2008). In addition, Din et al. (2008) reported that in Burgan oil field, the average concentrations of TPH for oil lakes nearby is 25,000 mg/kg, whereas that in uncontaminated soil varies from 7 to 20 mg/kg. According to Balba et al. (1998) the ground layer comprised material polluted by oil, which differed from hard crusty soil to sticky tarry sludge containing varied range of TPHs from 133,000 to 694,000 mg/kg, they shown that the oil contents were steadily reduced as the ground gets deeper and the oil concentration became insignificant at a depth of 60 - 80 cm below ground. Furthermore, Cho et al. (1997 a & b) reported that the average concentration of (TPHs) was about 500 mg/kg for the mixture of contaminated soil from different oil lakes at different depth.

1.9.4 Side Effects of Contamination by Oil Lakes

Evaporation, caused by the severe heat, has resulted in the formation of a thick sludge comprising sand, extremely weathered crude oil and oily layer. This caused various ways to clarify the contaminations such as oil lakes, wet oil lakes, dry oil lakes, oil-contaminated piles, oil trenches and related oil spills. The surrounding environment is exposed to the oil lakes with all the contaminants left on the surfaces. These oil lakes coupled with the contaminated soil can potentially change the properties of the soil and likely poses a hazard to the environment if left untreated.
Polluted desert resulted in the widespread death of plant and animal and infiltrate into the soil strata where it reaches the sole fresh water aquifers available in northern Kuwait. As the oil lake poses risk to fresh groundwater source in the north, the sludge remains an obstacle to the development of oil production and the operation of the oil field. This is over and above the potential ecological and human risk related to these contaminated soil (oil lakes) in the north and south of Kuwait (Kwarteng, 1999). Based on a research conducted by Al-Hashem (2011), it was revealed that extended exposure to oil pollution may increase the quantity of contaminants in the environment which will result in detrimental effect to the liver pathology in many types of wild animals. In addition, the effects from the oil lakes were causing harm to the migrating birds as some were discovered with thick oil when they fell onto oily ground. Birds drowned in the oil lakes (El-Baz et al., 1994) Figure (1-9), as they were confused by the reflection.

Figure 1-9: View of birds capsized in Burgan oil lake, at Burgan oil field in the state of Kuwait, 15 Nov 2014.
These oil lakes have also turned to be death traps for dragonflies and ground beetles as well as reptiles. According to Snashall (1991) high levels of volatile PAH poses a risk to human health. Hydrocarbon pollution is extended to the water field in north Kuwait, which is likely to contaminate the aquifers in north Kuwait which are used as strategic reserve for fresh ground water in the emergency situation. Therefore, the oil lakes are considered as vital important pollution and environmental task in Kuwait (Mukhopadhyay et al., 2008). It is essential to monitor the status of the oil lakes and contaminated surfaces since they cause threat to the human health and environment in long run. A number of studies have been undertaken and this inclination will progress into the future as Kuwaiti’s oil lakes have been considered the first of its kind for this type of environment.

Table 1-7: Description of oil lakes in Kuwait desert (Source: Al-Awadhi et al., 2004).

<table>
<thead>
<tr>
<th>Source</th>
<th>Number of oil lakes</th>
<th>Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lakes in north oil field</td>
<td>172</td>
<td>Leaching to fresh groundwater</td>
</tr>
<tr>
<td>Lakes within 1 km</td>
<td>69</td>
<td>Residential and operational areas</td>
</tr>
<tr>
<td>Lakes within 0.5 km</td>
<td>71</td>
<td>Paved road, wells and pipelines</td>
</tr>
<tr>
<td>Lakes in Burgan oil field</td>
<td>123</td>
<td>Operational areas, pipelines and leaching to shallow groundwater</td>
</tr>
<tr>
<td>Lakes in southern oil fields</td>
<td>79</td>
<td>Unclassified</td>
</tr>
</tbody>
</table>

Based on Table (1-7), the oil lakes in Kuwait are considered as a hazard to the human health, wild life and groundwater. Therefore, oil lakes need to be cleaned up when they cause risk to human health and ground water.

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1.9.5 Effect of Natural Factors on Oil Lakes.

1.9.5.1 Temperature

Based on Section 1.9.1, the average summer temperature in Kuwait soars to 45°C. The desert heat has caused most of the volatiles present within the oil to evaporate. While the properties of the remaining components such as carbon residue and asphaltenes are less volatile, denser and highly viscous than the original oil (Al-Sulaimi et al., 1993).

1.9.5.2 Wind and dust storms

A study was undertaken by Lulwa and Yacoub (1998) to investigate the risk of dust from the oil lakes and contaminated soil to human health. They concluded that the southern oil lakes and the northern oil lake caused serious effects like human exposure of B\[a\]P equivalent of 7.0 mg/kg and 22.2 mg/kg, respectively. The wind and dust storms prevalent in the region are likely to cause B[α]P levels higher than the acceptable maximum of 1 mg/kg as defined by the Environment Agency UK (2002). It is likely that wind and dust storms play significant role in threat to human health. Figure (1-10) shows a thin layer of sand blown by the wind that has covered a part of oil lake.
Figure 1-10: Wind-blown sand has covered some parts of the oil lakes in Burgan oil field in the state of Kuwait, 13 Jan 2012.

1.9.5.3 Rainfall

Hydrocarbon pollution extends to the water field in north Kuwait. The aquifers in north Kuwait are valued as a strategic reserve for fresh ground water for emergency situations. Therefore, the oil lakes are considered as a significant pollution and environmental task in Kuwait (Mukhopadhyay et al., 2008). The followings mechanisms can potentially cause pollution to the groundwater (Al-Sulaimi et al., 1993):

I. Oil infiltration directly from the oil lakes
II. Rainwater infiltration
(i). Oil infiltration directly from the oil lakes

Sulaimi et al. (1993) reported that, the crude oil is only capable to infiltrate into the ground up to a maximum depth of 2 to 3 m. Given the ground water table of more than 17 m at the contaminated sites, the probability of crude oil direct infiltration polluting the groundwater is considered remote. This was confirmed by a study conducted by Hamed (2005), who concluded that for the next 100 years, is very unlikely that the hydrocarbons will reach the groundwater.

(ii). Leaching by rainwater infiltration:

Rainfall intensity can play an important role in transporting water soluble pollutants from the surface of the ground to the water table. An effort to measure the rates of infiltration was carried out by Al-Sulaimi et al. (1993) at various sites at north oil field using the double ring infiltrometer method. The results revealed a large disparity in the infiltration rates ranging from 4 to 67 cm/hr. According to Hamed (2005), the rates of infiltration for dry season (April to October) and wet season (November to March) were 10 - 40 and 5 - 30 cm/hr, respectively. At these rates, it will require about 2 to 20 days for the rainwater to percolate up to 20 m below ground to reach the water table.

1.9.6 Studies On Remediation Oil Lake in Kuwait

Studies have been carried out by Kuwait Institute for Scientific Research (KISR) and the Japan Petroleum Energy Center (Al-Awadhi et al., 1998). Three techniques of different basis have been assessed individually at a large extent such as land farming, bioventing and windrow, as shown in Figure (1-11).
It has been revealed that after six months of observation, the priority of degradation was for aliphatic compound, while low molecular weight (LMW) PAHs decreased to 3-ring, such as phenanthrene, anthracene, fluoranthene and pyrene. After one year they discovered that, TPH has decreased to a maximum of 82.5, 74.2 and 64.5 % by landfarming, windrow piles and bioventing, respectively, subsequently, improvements in removal of aromatic compounds of greater ring number after 15 months (Balba et al., 1998).

As reported by Al-Hashash et al. (1998) several alternatives have been adopted such as taking no action, self-cleaning using cyanobacteria, phytoremediation, and various types of bioremediation with fungi that are still in the experimental phase of being developed and have not yet to be used in the contaminated sites. Taking no action may cause severe repercussions. After a period of five years of preliminary treatment, leaving the oil lakes exposed to severe weather conditions will result in the remaining oil to be transformed into a semi-solid mass. It is not an easy task to get rid of this mass.
The other option is using phytoremediation. Preliminary study on moderate to mildly contaminated lands indicated that plants from the composite family “number of smaller flowers clustered together” which were growing in black, and oil contaminated sand always had white clean roots (El-Nemr et al., 1995). The soil surrounding the roots was found to be clean whereas the sand within close proximity was still contaminated. Nevertheless, the concentrations of the salt in the contaminated soil were high due to the large volumes of seawater used to extinguish the oil well fires and the subsequent evaporation of the water.

These high salt levels influenced the selection of an appropriate bioremediation technique. For example, phytoremediation would probably not work well on soils with high salt concentrations unless salt tolerant plants were used (El-Nawawy et al., 1992). Another study was conducted by Cho et al. (1997a) showed that the separation of petroleum hydrocarbon by means of bacteria was enhanced in the liquid medium circulating flasks (see Figure 1-12) at 30°C for a 2-month duration.

It was also revealed that the saturated compound was biodegraded at a much faster speed than other compounds. However, the rate of biodegradation for aromatic portion was extremely time-consuming. As a result, it is likely that the aromatic compound degrading bacteria is less in the polluted soil within Kuwait.
Figure 1-12: Extraction of petroleum hydrocarbon utilising bacteria (Source: Cho et al., 1997a).

Cho et al. (1997b) carried out laboratory scale bioremediation experiments using a mixture of the soils from different oil lakes in a ratio of 1:1:1. Before using the soil samples, they were sieved and mixed thoroughly. Then, amendment materials with soil were incubated in glass columns with a volume of about 2 L glass bottles. The author found that the biodegradation of oil hydrocarbons were accelerated by adding eight kinds of surfactants, two basic nutrients such as (Hyponex and bark manure), four types of materials (baked diatomite, microporous glass, coconut charcoal, an oil-decomposing bacterial mixture), and eight different type of surfactants such as (alkylphenyl ether sulfonate, dioctyl sulfosuccinic acid, alkylbenzenesulfonate, olefin sulfonate, alkylphenyl ether sulfonate ester, alkylsulfonate ester, alkylphenyl ether, and alkyl ether). The bioremediation was improved among tested soil amendment materials and coconut charcoal. Latter, 15 to 33 % of the contaminated oil was degraded through 43 weeks incubation.
1.10 Summary

It is essential for the contaminated soil to be treated to prevent hydrocarbon contamination of the ground water aquifer and reduce the risk to both human health and the ecological system. Methods of remediation or disposal of the contaminated soils typically are landfills, thermal desorption, bioremediation, physical and chemical remediation as well as potential uses as fill materials in the construction industry. Fears have arisen over the adverse effects on health caused by these processes and this has prompted extensive studies that are focused on reducing to an acceptable level, <10,000 mg/kg (KEPA). The literature review process revealed that only limited study has been done in remediation of the Kuwait oil lakes. Also, these studies evaluated the impact of oil lakes on aspects of environmental health and the general ecology.

As discussed in Section 1.9.6, bioremediation is unlikely to be successful given the high concentration of TPH and high concentration of the metal salt in Kuwaiti contaminated soil. Problems associated with the cleanup of oil-contaminated soil in Kuwait oil lakes have demonstrated that there is a need to establish a new remediation technique that are feasible, having less side effects, quick, low cost and environmentally friendly. This project promised to add a new dimension to the remediation field, as it focuses on the improvement brought about by the selected process when it is applied to weathered soils. In the following chapter the chosen technique will be described and its merits evaluated.
2 Chapter 2 Research Approach

2.1 Rationale

The scenario in Kuwait is exceptional as the contamination is primarily caused by crude oil which has been weathered in an open environment for 25 years. In time, this has caused most of the volatile substances present within the crude oil to evaporate into the atmosphere leaving behind a residue of heavy compounds. Moreover the contaminants in different situations present a “cocktail” of various types of pollutants. It follows, therefore, that different types of remediation are required to deal with these many variants but, thus far, different remedial methods whilst being both lengthy in time and high in cost, have yielded only limited success. The higher levels and wider ranges of TPH have shown interesting patterns in the Kuwait oil lakes for future research. Critically, these contaminated sites have not yet been restored so that the continued threat remains to both the environment and human health.

2.2 Aims and objectives

• The main aim of this research is to assess if soil washing can remediate oil contaminated Kuwaiti sandy soil.

2.2.1 Objectives

For carrying out this study, the following objectives were set:

• Undertake review of relevant literature, specifically on crude oil contamination in Kuwait.

• Appraise the nature of the contamination in the Kuwait oil lakes.

• Review and assess the appropriate soil remediation technologies based on lab studies and suggest a suitable method for the treatment of Kuwaiti oil lake.
• Develop a laboratory scale soil washing system.
• Investigate the effect of oil contaminated sand on the engineering properties of hardened concrete.

2.3 Research Strategy

A two-stage research strategy was devised to address the complex treatment of contaminated soil. During the first stage, the contaminations levels present in the soil sample will be investigated. At the end of stage one, a method of remediation will be selected and then evaluated by means of a lab-scale test. Owing to the lack of accurate and important basic data for the oil lakes it was necessary to conduct a field investigation; this was carried out by visual inspection, comprehensive photographic documentation and sampling together with chemical and physical analysis. The area that was the worst affected by the oil spillage, in 1991, was the Greater Al-Burqan oilfield in the Kuwaiti desert and it was from here that the soil samples that were to be used for testing were taken. The information from this investigation was crucial as it allowed the selection of remediation options which had not, hitherto, been possible. On completion of all essential preliminary procedures, including the initial characterisation of the soil, it was possible to commence the evaluation of the selected process under laboratory conditions.

The House of Quality analysis system (HOQ), is a function deployment tool that can be utilised to collate conditions required and develop the specifications for various soil remediation techniques as well as communicate these specifications to select the optimum technique for remediation on a case by case basis.
The process of selecting the most suitable remediation technique needed a decision-making tool to be created and this utilised a set of United Nations (UNCC, 2004) requirements as its basis for selection of soil washing process selected for this project. This research comprises of two main phases, namely phase 1 and phase 2:

P1) Improve the efficiency of the soil washing treatment in the reclamation of Kuwaiti oil lake.
P2) Treatment of the “oily wastewater” residues produced by soil washing.

2.3.1 Research for Phase 1
The principal goal of this research is to conduct research into the use of soil washing technique for the removal of oil residue from sand. During the early stages of experimentation, tap water and distilled water were used and evaluated as the washing agents. However, the supply of fresh water is limited in the state of Kuwait. Therefore, the most practical and economical was to use seawater. It has been suggested in the literature that biosurfactants play a significant role in enhancing the soil washing process. This hypothesis led to the selection of saponin as the biosurfactant, using a decision-making table. A series of experiments were designed to assess the number of variables. This includes washing temperature (25°C, 50°C, 70°C), various concentrations between 0.001 and 2 wt% of water volume, duration of washing (10 min, 20 min, 30 min), stirrer speed (500 rpm, 1000 rpm, 1500 rpm) and ratio of mass to water volume (1:3, 1:6). The critical micelle concentration (CMC) is considered as an important characteristic for the concentration of surfactants. Surface tension of oily wastewater is determined with a tensiometer by using the Du Nouy ring method to obtain an accurate determination of the CMC value. Subsequently, the washing efficiency was investigated through various methods at different phases, e.g., mechanical stirrer, ultrasonication, a combination of ultrasonication with
mechanical stirrer and a combination of mechanical vibration and stirrer. The outcome of this research is expected to provide a better understanding of the removal of the oil residue under various parameters as well as providing useful information with regard to utilising saponins in soil washing techniques in remediating organically contaminated soils. However, the behavior of oil residues and sand surfaces on particle size distribution during the treatment process are still issues to be resolved. Consequently, a scanning electron microscope (SEM) test was used to observe and increase understanding of the changes in the granular surface structure of oil contaminated sand both before and after washing. When soil washing process completed, several contaminations were obtained in the washed water, including high values of COD and BOD, presence of metal contents, and oil residue which able to cause significant human and environmental problems. Therefore, the wastewater resulted from soil washing process can be considered as a source of contamination which required to be treated and this necessitated the implementation of phase 2.

2.3.2 Research for Phase 2
The oil residues present in washed water obtained from the washing of Kuwaiti oil contaminated can be classed as pollutants. International requirements and legislation such as KEPA covering the conservation of the environment are stringent. They place increasing demands on the official bodies responsible for wastewater treatment and are the reason for the creation of phase 2. This phase provides a conceptual and preliminary design of an oily wastewater treatment. The work consists of three sections; the first of these is an adsorption test which was carried out sequentially to find the optimum reduction of COD and segregation of flocs. Several technologies such as centrifuge, activated carbon and woodchips have been
carried out. The second phase was devised to find the optimum coagulant doses of alum and ferric chloride and, then to select the more efficacious of the two. The third stage was devised to combine the bio-treatment such as anoxic treatment or aeration treatment, with the coagulant selected from the second phase. This kind of work is vital for successfully meeting the treatment objective, with a system being chosen that offers cost effectiveness, feasibility, short time and low cost in treating wastewater contaminated with oil residue.

2.4 Lab Scale System

The increasing influence of legislation governing health and safety and environmental issues has been felt not only in industry but also in universities. Small scale lab experiments are safer, presenting a lower risk of contact with hazardous chemicals and they are more environmentally friendly as well as producing less waste. The organic chemistry laboratory is more complicated than its general chemistry counterpart requiring many kinds of equipment. For this reason it is desirable to have a set of small scale apparatus to conduct experiments safely and conveniently. To perfect a large scale model it is necessary first to conduct lab-scale study to measure the optimal conditions and select the best method required to achieve the desired results within the timeframe. One of the main advantages of the lab scale is that it provides indications of feasibility, whether similar rates of efficiency may be achieved in pilot scale. Therefore, lab-scale was required to be considered. The importance of empirical testing on a lab-scale is that it allowed a greater number of replications of the experiment to be studied in a range of different conditions. The ensuing discussion examines the theories that underpin soil remediation and treatment for wastewater and will be followed by a comparative evaluation in the subsequent chapters.

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Site investigation was formulated to recognise, assess and then suggest most suitable technique to remediate the oily contaminated soil. The greater Al-Burgan oil field, in the desert southeast Kuwait was selected for this study. Extreme care was taken in selecting appropriate section of the site as areas in proximity to the oil fields have been reported to contain unexploded ordnance. The soil samples from the Burgan oil field were collected at the edge of the lake No. 105, in September 2011 (Figure 2-1). A hand shovel was used to remove about 3 cm of oily sludge, after which oil contaminated soil was collected to a depth of approximately 30 cm, below the surface level of oily-sludge. Subsequently, the samples were placed into plastic containers and transported to the laboratory for further analyses. This project has been focused on the contaminated layer below the oil sludge layer, meanwhile the sludge is taken by the KOC. This showing a major source for future research, but outside the scope of this project.

Figure 2-1: The collection process for contaminated soil.
The main parameters measured were the depth of contamination, concentration of total petroleum hydrocarbon (TPH, gravimetric method), colour of the soil (munsell colour chart) and the particle size of the soil (wet sieving). The bulk of the contaminated soil to be dealt with has oily soil characteristics, and in some areas the oily soil is under liquid oil that would also need to be ameliorated. Any method for remediation of the tar mat and soot would need to take into account that they occur over an extensive area and form a thin layer on the soil surface.

2.5.1 Field Survey
These oil lakes have been sitting in the elevated temperature (51.9°C) for many decades now. It can be seen from Figure (2-2) that the field data, these layers of contaminated oil can be segregated based on their colour and properties consistency.

Figure 2-2: The layers in the oil lake at Burgan oil field in the state of Kuwait, 13 Jan 2012, depth measured in (cm).
In the same figure (2-2), the colour of liquid oil is black and non-coherent, oily soil is dark brown to black with a moderate to slightly hard consistency whilst tar mat is also black in colour with a hard consistency. These oil lakes are as deep as 70 cm below ground and as a result, the oil has infiltrated the soil to various depths subject to the condition and characteristics of the soil below the surface.

A thick oily sludge deposit has covered the affected areas with a thicker layer, and oil has seeped through aided by gravity and percolation (Al-Ajmi et al., 1994; Kwarteng, 1998). Beside this, the quality of oil has deteriorated which is caused by prolonged exposure to the extreme weather. The volatile hydrocarbons within the oil structure have been lost and the oil has endured changes in its chemical and physical properties (Al-Hashash et al., 1998; Mukhopadhyay et al., 1998; Balba et al., 1998). Omar et al. (2006) suggested that contaminated soils in general have three dimensions and form layers, as they get deeper into the ground. The nature of layers and the manner they are arranged provide indication of the category of contamination, the three contamination categories described below are exhibited in Figures (2-3; 2-4 and 2-5):

A) Oil Lake – comprises liquid oil of 1 to 70 cm thick which is above 18 to 100 cm (or higher) of oily soil

B) Dry Oil Lake – comprises tar of 1 to 3 cm thick which is above 1 to 179 cm (or higher) of oily soil, and

C) Tarmat and Soot – comprises 0.2 to 1 cm layer oily layer in the upper soil above ‘clean’ sand.
Figure 2-3: Wet oil lake contains oil residual over the soil that has a black colour, odor and consistency of liquid.

Figure 2-4: Dry oil lake category contains a tar layer over the soil that has a black colour and consistency of semi liquid.

Figure 2-5: Tar mat layer over the clean sand that has a hard consistency.
Various types of soil layers contaminated with oil cannot be differentiated using only the analytical results. As such, field investigation is key in classifying these contaminations for example, tarmat is very consistent while liquid oil has weak consistency, as they possess varying characteristics and requiring different methods to remEDIATE.

2.6 Determination of Remediation Options

In carrying out decision making process, a number of techniques could be utilised, for example, pair wise comparison chart, decision matrix, force field analysis, cost benefit analysis, and HOQ (Russel and Taylor, 2006). Out of these available methods, the HOQ was selected in the present research work. HOQ is a simple decision mechanism which can be employed at various phases of product manufacturing and advertising. Moreover, HOQ provides assistance to engineers in focusing on specified needs and decide on the best sequence in the case that the process goes ahead. Upon outlining and assigning weight to each specification, personnel responsible for decision making ought to define weighted symbols among the processes that constitute the interlink between the proposed processes and specifications, and non-weighted symbols between the processes themselves. Towards the end, the accumulated score for each process is determined by calculating the sum of the product of the specified weight by the equivalent symbol weight (John and Clausing, 1988; Russel and Taylor, 2006). Based on a research conducted by Ejbarah et al. (2011), reported that the HOQ was used to investigate and assess the best remediation technique for the Kuwaiti oil lake such as soil flushing, in situ steam injection, ex situ land farming and in situ land farming. Various types of multi criteria analysis such as pairwise comparison chart, objective tree and weighted objective tree were developed to evaluate the use of HOQ technique against the set requirements. The total weighted score of
each method was calculated to assess the final decision. They suggested that the best two techniques to match the stated objectives are the *ex situ* land farming and *in situ* steam injection which they can be either perform solely or in series. In this research eight more techniques were evaluated using assigned weights obtained by (Ejbarah *et al.*, 2011), which is explained in detail in the following sections.

2.6.1 Establishing House of Quality
For the purpose of selecting the most appropriate treatment method, the chemical and physical properties of the lake as well as, unexploded ordnance, weathered soil and local conditions were taken into consideration. Relationships were established between pre-set objectives outlined by UNCC and KNFP and various methods of soil remediation. The descriptions of the UN requirements for appropriate soil remediation strategy are:

a) capable in remediating severely contaminated soil

b) able to operate under severe weather conditions (extreme temperature)

c) simple to operate and assemble/dismantle parts

d) generates least side effect on soil, groundwater, air, employees and neighbouring environment

e) does not need longer time duration

f) in compliance with the requirements of Environmental Protection Agency (EPA)

g) good previous success rate in Kuwait or comparable conditions

h) no direct use of thermal treatment

In an effort to assign weights to these criteria, Ejbarah *et al.* (2011) carried out a series of discussions with consultants, environmental engineers and scientists and undertook extensive
literature review. Based upon the outcomes of their study, weights were assigned to each objective creating the weights of objectives (see Table (2-1)). The outcome of this study is utilised these assigned weights to distinguish, appraise various solutions and determine the importance of the objectives listed.

Table 2-1: Weights of objectives of engineering characteristics (Ejbarah et al., 2011).

<table>
<thead>
<tr>
<th>Engineering characterisation</th>
<th>Assigned weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>1) Reduction in major contaminants</td>
<td>0.36</td>
</tr>
<tr>
<td>2) Can be used in Kuwait’s climatic conditions</td>
<td>0.18</td>
</tr>
<tr>
<td>3) Not causing health problems to the worker</td>
<td>0.12</td>
</tr>
<tr>
<td>4) Simple to operate and assembly</td>
<td>0.07</td>
</tr>
<tr>
<td>5) Generates least residuals</td>
<td>0.03</td>
</tr>
<tr>
<td>6) Creates least equipment contaminants</td>
<td>0.03</td>
</tr>
<tr>
<td>7) The least pollution to air</td>
<td>0.027</td>
</tr>
<tr>
<td>8) Only small area needed</td>
<td>0.02</td>
</tr>
<tr>
<td>9) The least pollution to ground water</td>
<td>0.018</td>
</tr>
<tr>
<td>10) Not causing noise pollution</td>
<td>0.015</td>
</tr>
<tr>
<td>11) Requires shorter duration</td>
<td>0.01</td>
</tr>
<tr>
<td>12) In compliance with the requirements of EPA</td>
<td>0.06</td>
</tr>
<tr>
<td>13) Previous experience in Kuwait or similar surroundings.</td>
<td>0.06</td>
</tr>
<tr>
<td>Total score</td>
<td>1.26</td>
</tr>
</tbody>
</table>

2.6.2 Investigating alternative methods
A number of remediation strategies have been designed in an attempt to reduce the effects of petroleum pollution on the environment. Due to the large differences in the physical, chemical and biological characteristics of the contaminants, as well as the large number of soil remediation methods available, selection of an appropriate and economical technology for the remediation of particular contaminants can be difficult. For this study ten soil remediation
techniques: land farming, windrow, phytoremediation, vermiremediation, bioventing, soil washing, biopiles, electro-remediation, solidification/stabilisation and thermal were assessed using HOQ matrix system. These approaches have been selected in accordance with previous attempts to remediate hydrocarbon-contaminated soil.

2.6.3 Relationship Symbols and Weights
A range of remediation alternatives is presented in (Section 1.5), to highlight on why soil washing is the preferred treatment for Kuwait oil contaminated sand. This chapter explains the decision making process behind the selection. In choosing the most appropriate method, a list of each remediation option was reviewed. The relationship between each soil remediation method and the characteristics are evaluated in Table (2-2), the main aim of this section is to select the best remediation method among the alternatives. The definitions of the symbols used with their corresponding values are listed below:

1. Strong positive relationship (●) with a weight of (9)
2. Positive relationship (○) with a weight of (3)
3. Moderate relationship (▽) with a weight of (1)

The definition of the interrelationship symbols are also set to investigate the potential of applying the selected remediation method in sandy soil. The followings are the symbols used:

1. The technique work more suitable (▲) without problems.
2. The technique work suitable (◇) with a few concerns.
3. The technique less suitable (▼) to work.

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Table 2-2: Interrelationship matrix among several remediation techniques for Kuwaiti oil contaminated sand.

<table>
<thead>
<tr>
<th>Row</th>
<th>Weight Chart</th>
<th>Relative Weight</th>
<th>Customer Importance</th>
<th>Maximum Relationship</th>
<th>UN Requirements (Explicit and Implicit)</th>
<th>Remediation Options</th>
<th>Column 1</th>
<th>Column 2</th>
<th>Column 3</th>
<th>Column 4</th>
<th>Column 5</th>
<th>Column 6</th>
<th>Column 7</th>
<th>Column 8</th>
<th>Column 9</th>
<th>Column 10</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>1%</td>
<td>1</td>
<td>9</td>
<td>Short treatment time</td>
<td>Land farming</td>
<td>▼</td>
<td>o</td>
<td>▼</td>
<td>▼</td>
<td>o</td>
<td>o</td>
<td>•</td>
<td>o</td>
<td>•</td>
<td>•</td>
</tr>
<tr>
<td>2</td>
<td>7</td>
<td>7%</td>
<td>7</td>
<td>9</td>
<td>Easy to operate</td>
<td>Windrow</td>
<td>o</td>
<td>o</td>
<td>o</td>
<td>•</td>
<td>o</td>
<td>o</td>
<td>▼</td>
<td>▼</td>
<td>▼</td>
<td>▼</td>
</tr>
<tr>
<td>3</td>
<td>2</td>
<td>2%</td>
<td>2</td>
<td>9</td>
<td>Requires small area</td>
<td>Biopiles</td>
<td>o</td>
<td>o</td>
<td>o</td>
<td>o</td>
<td>o</td>
<td>•</td>
<td>o</td>
<td>•</td>
<td>▼</td>
<td>▼</td>
</tr>
<tr>
<td>4</td>
<td>6</td>
<td>6%</td>
<td>6</td>
<td>9</td>
<td>Experience in Kuwait</td>
<td>Phytoremediation</td>
<td>•</td>
<td>•</td>
<td>•</td>
<td>▼</td>
<td>▼</td>
<td>▼</td>
<td>•</td>
<td>▼</td>
<td>▼</td>
<td>▼</td>
</tr>
<tr>
<td>5</td>
<td>18</td>
<td>18%</td>
<td>18</td>
<td>9</td>
<td>Applicable for Kuwait’s weather</td>
<td>Vermiremediation</td>
<td>o</td>
<td>▼</td>
<td>▼</td>
<td>▼</td>
<td>▼</td>
<td>▼</td>
<td>▼</td>
<td>▼</td>
<td>▼</td>
<td>▼</td>
</tr>
<tr>
<td>6</td>
<td>36</td>
<td>36%</td>
<td>36</td>
<td>9</td>
<td>Reduces high contaminants</td>
<td>Soil washing</td>
<td>o</td>
<td>o</td>
<td>o</td>
<td>o</td>
<td>▼</td>
<td>•</td>
<td>o</td>
<td>▼</td>
<td>▼</td>
<td>▼</td>
</tr>
<tr>
<td>7</td>
<td>3</td>
<td>3%</td>
<td>3</td>
<td>9</td>
<td>Minimum residuals</td>
<td>Electro-remediation</td>
<td>•</td>
<td>•</td>
<td>•</td>
<td>•</td>
<td>▼</td>
<td>o</td>
<td>•</td>
<td>▼</td>
<td>▼</td>
<td>▼</td>
</tr>
<tr>
<td>8</td>
<td>2</td>
<td>2%</td>
<td>1.8</td>
<td>9</td>
<td>Minimum ground water pollution</td>
<td>Thermal treatment</td>
<td>▼</td>
<td>▼</td>
<td>▼</td>
<td>▼</td>
<td>▼</td>
<td>▼</td>
<td>▼</td>
<td>▼</td>
<td>▼</td>
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</tr>
<tr>
<td>9</td>
<td>3</td>
<td>3%</td>
<td>2.7</td>
<td>9</td>
<td>Minimum air pollution</td>
<td>Solidification/stabilization</td>
<td>▼</td>
<td>▼</td>
<td>▼</td>
<td>▼</td>
<td>▼</td>
<td>o</td>
<td>▼</td>
<td>▼</td>
<td>▼</td>
<td>o</td>
</tr>
<tr>
<td>10</td>
<td>2</td>
<td>2%</td>
<td>1.5</td>
<td>3</td>
<td>Not cause noise pollution</td>
<td></td>
<td>o</td>
<td>o</td>
<td>o</td>
<td>o</td>
<td>o</td>
<td>o</td>
<td>▼</td>
<td>▼</td>
<td>▼</td>
<td>o</td>
</tr>
<tr>
<td>11</td>
<td>6</td>
<td>6%</td>
<td>6</td>
<td>3</td>
<td>UN and EPA recommended</td>
<td></td>
<td>o</td>
<td>o</td>
<td>o</td>
<td>o</td>
<td>o</td>
<td>▼</td>
<td>▼</td>
<td>▼</td>
<td>▼</td>
<td>o</td>
</tr>
<tr>
<td>12</td>
<td>12</td>
<td>12%</td>
<td>12</td>
<td>9</td>
<td>Not cause human health effect</td>
<td></td>
<td>o</td>
<td>o</td>
<td>o</td>
<td>o</td>
<td>▼</td>
<td>▼</td>
<td>o</td>
<td>▼</td>
<td>o</td>
<td>o</td>
</tr>
<tr>
<td>13</td>
<td>3</td>
<td>3%</td>
<td>3</td>
<td>9</td>
<td>Minimum equipment contaminants</td>
<td></td>
<td>•</td>
<td>•</td>
<td>o</td>
<td>•</td>
<td>▼</td>
<td>▼</td>
<td>o</td>
<td>▼</td>
<td>o</td>
<td>o</td>
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</table>

Customer Competitive Assessment

<table>
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<th>Direction of Improvement</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
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<td>•</td>
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</tr>
</tbody>
</table>

Relative Weight:
- 8% for Column 1
- 8% for Column 2
- 7% for Column 3
- 8% for Column 4
- 8% for Column 5
- 12% for Column 6
- 18% for Column 7
- 9% for Column 8
- 14% for Column 9
- 9% for Column 10

Technical Importance Rating:
- 321 for Column 1
- 323 for Column 2
- 307 for Column 3
- 331 for Column 4
- 326.4 for Column 5
- 516.6 for Column 6
- 768 for Column 7
- 370 for Column 8
- 690.4 for Column 9
- 400.8 for Column 10

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2.6.4 Evaluating Alternatives

Several methods are now available in remediating hazardous contaminated soils, as shown in Section 1.5. Quite a number of these techniques are either expensive or not able to remove the contamination in entirely. According to Li et al. (2008), soils contaminated with crude oil represent a significant risk to the environment and human health. As a result of the weathering process, higher binding strength of crude oil to soil is achieved (Section 1.3.3). As such, it is usual that weathered contaminated soils are more difficult to remediate in comparison with soils which are recently contaminated. Crude oils are of low water solubility, high adsorption onto soil matrix and present very low rate mass transfer rate for biodegradation (Banat et al., 2000). In order to choose the most appropriate method, an exhaustive list of each remediation option was reviewed. As exhibited in Table 2-2, a matrix was formulated to show the inter-relationship, while the weights and alternatives were ascertained and assessed against the requirements. Hence, weighted alternatives have been formulated. The process began by converting the set symbols to their corresponding values. Each value was later multiplied by the weight of its characteristic.

This process was repeatedly carried out for all the characteristics and the total sum was derived at the final stage. As seen in Table (2-2), the relative weight for bio-piles is 7 %, there is a similar weight score recorded among land farming, windrow, phytoremediation and vermicremediation, the outcome shows that the score of relative weight was 8 %. Likewise, the result also exhibited that the score of relative weight for electro-remediation, and solidification/stabilisation as 9 %, while the score of relative weight for bioventing, thermal adsorption and soil washing were 12, 14 and 18 %, respectively. The major change in the soil
materials takes place during the thermal treatment stage. Usually, high-temperature procedures damage the organic compounds and clay minerals, hydroxides are being transformed into oxides and main minerals are changed to fines. For thermally treated soils which are transformed to slurry, their values of pH are quite high (pH 11). In addition, compared with other thermal and bio-remediation technologies, soil-washing method has some major benefits. From the results, the soil washing appears to be the best method in view of its total score while thermal cannot be employed based on UN requirements. A large number of remediation methods have been designed in an attempt to reduce the effects of petroleum pollution on the environment. Due to the large differences in the physical, chemical and biological characteristics of the contaminants, selection of an appropriate and economical technology for the remediation of particular contaminants can be difficult.

For this study nine remediation technologies were considered to assess their suitability to treat soil contaminated with hydrocarbons. These technologies were land farming, windrow, phytoremediation, vermiremediation, bioventing, soil washing, biopiles, electro-remediation and solidification/stabilisation. Based on the evidence collected, each soil remediation technique was categorised (see Table 2-3) either as less suitable (*), suitable (**), or more suitable (***) respectively, for the treatment of sandy soil contaminated with hydrocarbon. Different criteria were considered to select appropriate methods such, soil constraints, implement in surface soil, if any further treatment, compounds removal, time-scale for clean-up and cost. Bioremediation is a degradative or transformative approach aimed to stimulate microorganisms to grow and live on contaminants as its source of food and energy by introducing a conducive environment for the microorganisms. Nonetheless, it is time
consuming and hard to confirm whether the contaminants have been entirely eliminated. In the biological remediation of HMW which are less biodegradable could be left behind. While greater concentration of hydrocarbons can become toxic or act as an inhibitor to the microorganisms, the presence of exceptionally low concentration may prove insufficient for the microbial activities sustenance. PAHs with higher molecular structure are known to be carcinogens. In general, bioremediation is not suitable to be used as treatment of inorganic contaminants. Soil contaminated with oil is particularly challenging to be treated by bioremediation due to the fact that excess oil produces droplets or films on soil particles, which becomes a major obstacle against microbial degradation (Urum et al., 2004).

Each of the remedial methods were also assessed against their capability to treat sandy soil. In employing physical treatments, the physical properties of the contaminants or the contaminated medium are utilized to destroy, segregate or accommodate the contamination. One of the physical treatments is soil washing and is regarded as commonly effective and affordable method and requires shorter completion time (when compared against biological treatment). This technique seems to be one of the best techniques in handling broader spectrum of organic contaminants especially petroleum hydrocarbons. It has been acknowledged that soil washing is capable to treat coarse material better than soils with higher silt contents (Mulligan et al., 2001). This is due to the fact that coarse materials are highly porous for the liquid which allows it to bind lower amount of contaminants due to their smaller particle surface areas per unit volume (Wang & Keller, 2008). Therefore, in order to substantially reduce the amount of the most contaminated soils, it is recommended to separate the fine soil (clay and silt) from the coarse soil (sand and gravel) before or during the processes (Anderson et al., 1999).
The employment of surfactants has been proven to be a viable method in the extraction of more hydrophobic compounds from soils for instance, organic contaminants (Mulligan et al., 2001). With the presence of electrolytes, the CMC of anionic surfactants is significantly reduced resulting in the increase of aggregation number and size of micelles as asserted by Bai et al. (1998). In the design of a soil washing technique, it is vital that the interaction between surfactant and salt and their resultant effect on surfactant performance is thoroughly understood. This will be considerably central as non-ionic surfactants are not affected by the presence of electrolyte in the solution. This is exceptionally interesting in the field application of soil washing as the Kuwaiti oil contaminated soil is likely to contain electrolytes, for example Ca\(^{2+}\), Mg\(^{2+}\), Na\(^+\) and K\(^+\) and is perhaps from the seawater employed in the firefighting activities.

The results obtained in this study showed that the soil washing appeared to be the most appropriate option for the treatment of sandy soil contaminated with oil. Some of the benefits are their cost effectiveness, scalability and its exceptional ability to remove oil from contaminated soil in short time. It has less profound side effects, while removal of contaminants can be controlled by enhancing washing parameters. Based on Table (2-3), the selected techniques require about one year to achieve the remediation target and is the cheapest of the available approaches, with the maximum treatment cost of £40 per tonne of soil. This method can be employed on its own, or performed in series. By using ex-situ techniques for soil washing, the coarser material can be used for construction work such as sub grade fill or backfill, landfill capping or in the production of concrete etc.
Table 2-3: Compilation of characteristic of various soil remediation techniques from previous studies (* Less Suitable, ** Suitable, *** More Suitable).

<table>
<thead>
<tr>
<th>Remedial Option</th>
<th>Land-farming</th>
<th>Windrow</th>
<th>Phyto-remediation</th>
<th>Vermi-remediation</th>
<th>Bioventing</th>
<th>Soil Washing</th>
<th>Bio-piles</th>
<th>Electro-remediation (in)</th>
<th>Solidification/stabilisation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Further treatment required</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>***</td>
</tr>
<tr>
<td>Air emission</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>***</td>
<td>*</td>
<td>***</td>
<td>*</td>
<td>*</td>
<td>***</td>
</tr>
<tr>
<td>Leaching wastewater</td>
<td>*</td>
<td>***</td>
<td>*</td>
<td>***</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>***</td>
<td>***</td>
</tr>
<tr>
<td>Compound removal</td>
<td>TPH &gt;C30 are less degradable, PAH e.g. 2-4 rings</td>
<td>TPH &gt;C30 are less degradable, VOCs, SVOCs, heavy metals and PAH e.g. 2-4 rings</td>
<td>TPH &gt;C30 are less degradable, VOCs, SVOCs, heavy metals and PAH e.g. 2-4 rings</td>
<td>TPH &gt;C30 are less degradable, VOCs, SVOCs, heavy metals and PAH e.g. 2-4 rings</td>
<td>TPH &gt;C30 are less degradable, VOCs, SVOCs, heavy metals and PAH e.g. 2-4 rings</td>
<td>Separate the organic compound. Soluble inorganic and metals salts.</td>
<td>TPH &gt;C30 are less degradable, VOCs, SVOCs, heavy metals and PAH e.g. 2-4 rings</td>
<td>Heavy metals, inorganic and soluble organic.</td>
<td>Immobilise contaminant, such as: heavy metals, organic and inorganic compound</td>
</tr>
<tr>
<td>(1) Koning et al. (2000); (2) Lodolo (2005); (3) Mike et al. (2006); (4) Nathaniel &amp; Bardos (2004); (5) USEPA (1991); (6) Sinha et al. (2010)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
2.6.4.1 Selection of surfactant

Surfactants have been employed considerably in soil washing, in order to enhance the efficacy of oil removal for the rehabilitation of hydrophobic contamination. A number of parameters ought to be taken into consideration in the selection of surfactants to be used for oil removal, such as effectiveness, cost and toxicity, ability to recycle, degradation products, regulatory acceptance, and biodegradability. Economic factors need to be evaluated during selection of the appropriate surfactant; Mulligan et al. (2001) suggested that the cost of the product is often considered as the primary selection criteria for surfactant. Besides, one particular consideration is that the high cost to produce bio-surfactants may affect the viability of their industrial application (Banat et al., 2010). A comparison of different surfactant and biosurfactant with their properties and product cost is given in Table (2-4).

Physicochemical behaviour of surfactant plays a significant role in solubilisation of hydrocarbons. Hence, the charge-type of surfactant required to be considered while classified to selecting the appropriate surfactant. Biodegradation is a key selection criterion as it is essential to reduce the environmental impact. The selection of the most suitable surfactant or bio-surfactant depends upon its ability to biodegrade in wash waters with minimal impact on its surroundings. Other properties that must be considered include availability, good wetting and emulsification characteristics.
Table 2-4: Comparison of biosurfactant and synthetic surfactant.

<table>
<thead>
<tr>
<th>Type of Surfactants</th>
<th>Classification</th>
<th>Biodegradable</th>
<th>Toxicity</th>
<th>Available for large quantity</th>
<th>Cost (£) (100g)</th>
<th>References</th>
<th>Remark</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aescin</td>
<td>Non-ionic</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>3445 (*)</td>
<td>[3]</td>
<td>Expensive</td>
</tr>
<tr>
<td>Sodium dodecyl-sulphate</td>
<td>Anionic</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>60 (**)</td>
<td>[4]</td>
<td>Non-degradable</td>
</tr>
<tr>
<td>Alkyl-polyglucoside</td>
<td>Anionic</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>16 (**)</td>
<td>[7]</td>
<td>Non-degradable</td>
</tr>
<tr>
<td>Tween80</td>
<td>Non-ionic</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>7.40 (**)</td>
<td>[4,6]</td>
<td>Non-degradable</td>
</tr>
<tr>
<td>Tannin</td>
<td>Anionic</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>-</td>
<td>[3]</td>
<td>Slow-biodegrade</td>
</tr>
<tr>
<td>Synperonic</td>
<td>Non-ionic</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>15 (*)</td>
<td>[5,6]</td>
<td>Non-degradable</td>
</tr>
<tr>
<td>Rhamnolipid 90%</td>
<td>Anionic</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>2,370,000 (*)</td>
<td>[2,3]</td>
<td>Expensive</td>
</tr>
<tr>
<td>Triton X-100</td>
<td>Non-ionic</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>5.12 (*)</td>
<td>[1,4,6]</td>
<td>Non-degradable</td>
</tr>
<tr>
<td>Saponin 98%</td>
<td>Non-ionic</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>122 (**)</td>
<td>[2,3,6]</td>
<td>Selected, available in less purity 60%.</td>
</tr>
<tr>
<td>Tyloxapol</td>
<td>Non-ionic</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>221 (*)</td>
<td>[1]</td>
<td>Non-degradable</td>
</tr>
<tr>
<td>Lecithin</td>
<td>Anionic</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>364 (**)</td>
<td>[3,6]</td>
<td>Expensive</td>
</tr>
<tr>
<td>Brij 35</td>
<td>Non-ionic</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>4.5 (*)</td>
<td>[1]</td>
<td>Non-degradable</td>
</tr>
</tbody>
</table>

Boonchan et al. (1998); (2) Mulligan (2009); (3) Urum et al. (2003); (4) Zhou et al. (2008); (5) Paterson et al. (1998); (6) Zhou et al. (2011); (7) Urum & Pekdemir (2004). *Sigma-Aldrich (8 February 2015); **Fisher scientific (8 February 2015)
2.6.4.2 Overview of Saponin

Research shows using biosurfactants have advantages of removing crude oil from polluted soils. Based on the Table (2-4), saponin has been selected for this project. Saponins are glucosides with non-ionic biosurfactants properties and foaming characteristics. They are extensively distributed in the territory of plants (Roy et al., 1997; Tewari et al., 2011), for e.g., vegetables, fruit pericarp, soybeans, peas, beans and herbs, whereas saponin which are commercially produced and obtained largely from *Sapindus mukorrossi* soapnuts, *Yucca schidigera* and *Quillja saporina*.

The name ‘Saponin’ is originated from the word ‘sapo’ in Latin language which means soap, owing to the foam generating characteristic in aqueous solution by stirring action. The solutions are amphiphilic glycosides in which the polar components include sugars (pentoses, hexoses or uronic acids) which are connected covalently to a non-polar group. Saponin’s structure is in the form of lipid soluble polycyclic aglycones connected to a single or multiple sugar chains and water-soluble sugar residues (Hong et al., 2002). The aglycone element also known as sapogenin can be either steroid ($C_{27}$) or triterpene ($C_{30}$). According to Shiau et al. (2009) and Sharma et al. (2013) saponin consist of several functional groups such as, OH, COOH, carboxylate, acetate group and esteric band. In general, the glycone elements of saponins are oligosaccharides. It is possible for oligosaccharides to be connected to the sapogenin by means of either ether or ester bond at one or two glycosylation sites resulting in the monodesmosidic or bidesmosidic saponins, correspondingly. According to Natori et al. (1981), glycone linkage to three sites (tridesmosidic) in a sapogenin is uncommon.
For this study, steroidal experiment consisting of 27 carbon (C\textsubscript{27}) spirostane skeleton, with a six-ring structure (Figure 2-6) having a 414.62 Da molecular weight with its molecular formula of C\textsubscript{27}H\textsubscript{42}O\textsubscript{3}, was utilised.

![Saponin Molecule](image)

Figure 2-6: General chemical structure of saponin (Source: Fisher Scientific, UK).

Saponins solubilisation of hydrocarbons tends to be affected by a number of environment conditions such as pH and ionic strength (Mitra & Dungan, 1997). Ionisable function groups exist in the saponin molecules, with some glucoronic acid and saponin solutions groups present with some charges. Therefore, the ionic strength may have some influence towards the CMC values for the saponin solution followed by the solubilisation of the oil residue. For instance, the effectiveness of saponin solubilisation for oil demonstrated a substantial difference from anionic rhamnolipid biosurfactants and non-ionic surfactants synthetic (Zhou et al., 2011). In addition, the authors concluded that the saponin was effective in improving PAHs’ solubilisation such as phenanthrene compared to anionic rhamnolipid or synthetic non-ionic surfactants. Further, saponin can also be utilised to eliminate organic pollutants present in contaminated soils.
Zhou et al. (2013) found that the potential of improving the washing efficiency for phenanthrene contaminated soil utilizing saponin was investigated and compared with those of Tween 80, a synthetic non-ionic surfactant. From the result, it was revealed that the highest percentage of organic matter removal from the contaminated soil using saponin was approximately 87% whereas Tween 80 resulted in 91%. This is attributed to Tween 80’s capability of sorption onto soil which is much superior. It was concluded by the authors that saponin demonstrated an encouraging performance in soil washing. As such, it should be able to offer good prospect in soil washing technology in an effort to remediate soils contaminated by organic compounds. Moreover, addition of low levels of electrolyte to non ionic surfactant solution did not show any effect on improvement of solubilisation of organic compounds. As concluded by Paria (2008), a minor improvement of solubilisation occurred at high concentrations of electrolyte as a result of the “salting out” effect of the non-ionic surfactants. According to Oakenful and Sidhu (1989) saponins form micelle-like aggregates when dissolved in water when it approaches the CMC value. The molecules remain separated at a level lower than this concentration. Oleszek and Hamed (2010) highlighted that the physical properties vary abruptly by increasing the concentration levels surpassing the CMC at which the micelles begin to form.

Another characteristic of saponins is their hemolytic property, which is the ability of saponin to breakdown red blood cells by releasing the internal components and hemoglobin into the surrounding fluid. Different saponins have very different hemolytic activities, and there is a wide variation among different animal species in the susceptibility of their erythrocytes to undergo hemolysis by saponins. Extensive investigations were carried out by Cheeke (1989),
to correlate the capacity of saponin with hemolytic activity, the author found that the chemical structure of hydrophobic glycone and other polar group has a high hemolytic activity. Therefore, the hemolytic activity of saponins cannot be ascribed to their detergent-like properties but must be related to an ability to interact with some constituents of the cell membrane. There have been only very limited studies carried out using saponin to remove hydrocarbons from soil. In this current work, oil residue from Kuwait was used as a soil specimen. In an effort to improve the washing process, bio-surfactant “Saponin” was chosen.

2.7 Statistical Analysis

All statistical analysis was examined using Statistical Package for the Social Sciences (SPSS) for Windows software (Version 21.0). All experiments were repeated at least three times in order to confirm the replicates data. The statistical data such as the removed oil concentrations carried out under a range of parameters and various washing methods are expressed as the mean (M) and standard deviation (±SD). In an effort to identify the homogeneity of various techniques against the different parameters adopted in the study, the distinction between two or more groups were examined for significance by means of one-way analysis of variance (ANOVA) with Post hoc Scheffe method. Moreover, the paired t-test was carried out to examine the significant difference of the means between two different groups. The differences amongst groups were regarded as significant at $p$ value of 0.05. The normality of the results was measured using a Shapiro-Wilk’s test ($p > 0.05$) with visual check of their box plots and histograms. Furthermore, descriptive statistics with Skewness and Kurtosis tests were used as reference values to determine normality, while the values for these parameters should be zero ($z$) or close to zero (-1.96 – 1.96).
3 Chapter 3 Development of Analytical Method

3.1 Introduction

This chapter describes the methodology and experiments conducted in the project along with the sampling and data-collection procedures. After specimen collection, the soil was stored in the oil/hazardous waste disposal bag to avoid contaminants from volatilising. All experiments were carried out in the University of Portsmouth, Kuwait University and KISR.

3.2 Sample Preparation

3.2.1 Preparation of Oil contaminated Sand
The sample of oil contaminated sand was obtained from Kuwaiti oil lake, as explained in Section 2.5. In order to enhance the efficiency of washing system, the process requires the soil to be homogeneous before the washing. 1 kg of sample was screened manually through 13.5 mm sieve for 2 min, to segregate materials finer than gravel coarse aggregate and to separate out the larger clumps of congealed oil residue.

3.2.2 Preparation of Artificial Seawater
A standard volumetric flask of 1000 ml was used for preparing artificial seawater. 45 g of tropic marine sea salt were weighed to mimic Kuwait seawater, and then transferred into the volumetric flask, the tropic marine sea salt was supplied by C.D, Aquatics (UK). Subsequently, the weighed tropic marine sea salt was added to 1000 ml of distilled water. When the preparation process complete, the solution retained in a refrigerator with a temperature of 4°C.
3.2.3 Preparation of Saponin

Saponins selected in this study were a large assembly of glycosides with foaming characteristics. The purified (98 %) saponin was purchased from Fisher Scientific Ltd, Loughborough (UK). It was used without further purification as supplied. 5 g of saponin was added with 1 l of artificial seawater to obtain required concentrations of saponin 0.5 wt%. The solution of artificial seawater and saponin were mixed for 10 min and stirred at a constant speed of 200 rpm to dissolves the saponin.

3.2.4 Preparation of Glucose Solutions

The preparation of glucose with distilled water was carried out using 0.25 g of the glucose with 250 ml of distilled water in a 500 ml glass beaker, while the head of the flask was sealed. A glucose stock solution was then made with 1 g/l concentration. The flask was placed into a magnetic stirrer. The solution was mechanically stirred at a constant speed of 200 rpm for 10 min to provide a uniform distribution of the glucose.

3.3 Characteristics Measurement of Oil Contaminated Sand

There are several methods to determine the total oil content such as total organic carbon (TOC), total carbon (TC), total petroleum hydrocarbon (TPH) etc. TOC is the measure of organic carbon present in oil, grease and a lot more of organic compounds. TOC estimates any possible effects on the human health. However, total carbon is the sum of three carbon forms namely elemental, organic and inorganic carbons. The term total organic carbon is different to total carbon, which refers to carbon in organic compounds only.
Due to the lack of information regarding the concentration of petroleum hydrocarbons in the Kuwait oil sand, this study was designed to measure the total petroleum hydrocarbon concentration (TPH), due to its ability to determine a broad family of several hundreds of chemical compounds present in crude oil. The most important reason for selection of TPH over other methods is that the core contaminants to be analysed are petroleum based which would delineate the level of oil pollution while the other methods are less relevant in this case. Another reason for the choice of TPH is that it gives a direct indication of the degree of pollution resulting from oil which is required by regulatory agencies. Based on environmental sciences division UK (ESD, 1993) TPH is commonly used gross parameter for quantifying environmental contamination originated by PHC products such as fuels, oils, lubricants, waxes and others. Traditional wet chemistry methods for determining TPH level is based on extracting the contaminant from the soil sample. The TPH level in the extracted solution is then determined by several methods such as gravimetric or Gas Chromatography-Mass Spectrometry (GC-MS) measurement calibrated by an EPA calibration standard.

3.3.1 Analysis of Oil residue Using Gas Chromatography-Mass Spectrometry (GC-MS)

Any extraction procedure used in analytical chemistry is primarily intended to detach the analyte from its matrix quantitatively, speedily and utilising as little solvent as possible (Saim et al., 1997). A procedure known as accelerated solvent extraction (ASE) has been employed for the quantitative extraction of a number of semi-volatiles comprising Polycyclic Aromatic Hydrocarbons (PAHs), phenols, polychlorinated biphenyls (PCBs) as well as total petroleum hydrocarbons (Li et al., 2003). An instrumental method known as Gas Chromatography Mass Spectrometry (GC-MS) consisting of a gas chromatograph (GC) coupled to a mass
spectrometer (MS) in an effort to separate, identify and quantify complex mixture of chemicals. This proves to be the most appropriate method to analyse hundreds of compounds with comparatively low molecular weight. The compound itself has to be thermally stable and satisfactorily volatile so that it can be analysed using GC-MS technique.

3.3.1.1 Extraction process

Contaminated soil (1g) was mixed with Hydromatrix concentrate (Varian, UK) at a ratio of 1:1 w/w. The mix was then ground vigorously to such an extent that it appeared dry and powdery. The sample was then added to 22 ml stainless steel extraction vial (Dionex, UK) partitioned with disposable cellulose filters (Dionex, 52 UK) and acid washed sand (VWR, UK). Accelerated solvent extractor (ASE) 200 (Dionex, UK) was utilised to perform the extraction process by using Dionex method 324.

The ASE had the following Extraction conditions:

- solvent for TPH 1:1 hexane: acetone (Fisher Scientific, UK)
- solvent for PAH 1:1 acetone: dichloromethane (Fisher Scientific, UK)
- heat= 5 min
- static= 5 min
- flush= 60 % volume
- purge= 60 sec
- pressure= 1500 psi
- oven temperature= 200°C
- purge= turn on nitrogen gas first (12 bar)
To facilitate the extraction process, the ASE cell is left in an ASE carousel. Whilst extraction takes place, hexane/acetone, 1:1 was introduced into the extraction cell up to 50 ml. Upon extraction, the extract was flushed out of the cell into a collection vial with the help of nitrogen, to be analysed. GC-MS was utilised to perform the analysis on the extract. Then, 1.5 ml of the specimen solution was pipetted into the GC auto sampler vial (Technopath, UK) and capped by crimp caps.

3.3.1.2 Measuring the levels of oil residue by GC-MS

The GC-MS has been widely used in recent environmental research due to its effectiveness with regards to many major classes of organic samples. Analyses of oil residue in terms of TPH were performed on a Varian 43GC and a Varian 21-MS IT with an auto-sampler CP-8400 (Figure 3-1) in order to examine and assess traces of petroleum hydrocarbons in the soil sample. A DB-5 MS capillary column (15 m x 0.32 mm i.d. x 0.1 µm film) was used for the GC–MS. A GC-MS works by mixing the specimen solution with helium and air in the column. The GC-MS had the following conditions: injector temperature, 300 °C; oven temperature program, 50 °C for 2 min – 300°C for 20 min at 5 °C/min. 1 µl sample of the sample was introduced into the GC inlet by injection in which it vapourised and swept onto a chromatography column by helium which is used as the carrier with a constant flow rate of 1.0 ml/min at 300°C and then carried on to the MS console. Electron impact mode was used for ionisation of the sample. The MS was scanned from m/z 35–550, with 3 min solvent delay and a 150-threshold count.
3.3.1.3 Result and Discussion

Preliminary study was conducted for the Kuwait oil sand, the initial results from the GC-MS analyses indicated that the concentrations of TPH varied from 183,432 to 212,993 mg/kg. No traces of PAH compounds were found in the sample by this analysis. The GC profile for TPH showed that after 15 minutes the TPH level started to increase, indicating that the sample contains long chain hydrocarbon compounds (Figure 3-2).
Before carrying out GC analysis, chemical modification of the sample is essential to eliminate interfering compounds that may affect the quality of the data obtained. Hence, a high content of asphaltenes was removed to get a correct reading for aliphatic and, aromatic compounds and to protect the GC. However, the main portion of the diesel oil is not characterized as most of the constituents cannot be resolved. In the chromatogram, these constituents look like a ‘hump’, which is termed unresolved complex mixture’ (UCM), containing branched, cyclicalkanes and polar transformation products (Marchal et al., 2003).

The resolved hydrocarbons are named ‘total resolved hydrocarbons (TRH). The TRH and UCM combine to become TPH. In the GC-MS, the TRH are shown as peaks, which are non-degraded hydrocarbons, as shown in Figure (3-3).
However, this method faced some challenges during the measurement, one of the main difficulties caused by GC column which was blocked after each run. This necessitated the development of another alternative method for the analysis of TPH from soil samples, as given in the next section.

3.3.2 Analysis of Oil Residue Using Gravimetric Method

Currently, there is no standard method for determination of TPH in contaminated soil with petroleum hydrocarbon products (USEPA, 1999b). A number of researchers (Saeed et al., 1997; Parra-Barraza et al., 2003; Li et al., 2012) have developed and utilised various methods for this purpose. Most of the techniques for separating oil from the sand in the laboratory involves ASE, soxhlet extraction or ultrasonication.
Ultrasonication with aid of an appropriate solvent has been selected to extract oil from the sand, since it is easily available with high extraction efficiency and economical. The amount of oil residue can then be calculated by gravimetric technique.

3.3.2.1 Method development for gravimetric analysis

In order to determine the oil in the oil-contaminated soil, a basic initial test was carried out by altering several parameters to improve the extraction process which are:

I. Different sample weight
II. Type of solvent
III. Different extraction time.

3.3.2.2 Gravimetric method for analysis of oil residue

Gravimetric method is based on two phases, the first phase aimed to extract the oil residue from sand while the second phase aimed to determine the percentage of total petroleum hydrocarbons recovered from contaminated sand. The oil was extracted from the sand based on the following procedure. One gram of contaminated sample was placed into a screw-capped bottle with 5 ml of acetone: hexane solvent (1:1 v/v) and washed for 5 minutes in ultrasonication bath. The solution of crude oil and mixture of acetone: hexane was pipetted into a clean and dry weighed round-bottomed glass flask. The ultrasonication cycle was then repeated for four times using fresh solvent in each cycle. The isolated sand was left to air dry for 2 hours and then noted the dry weight. The collection apparatus, conical flask, distillation head with condenser side arm and water bath were prepared and fixed to determine the percentage of total recoverable petroleum hydrocarbons. The water bath temperature was
maintained at 52°C, to accelerate the evaporation of the acetone: hexane mix. Finally, when the acetone: hexane mix was recovered, the round glass flask was dried, then the total weight of crude oil in the soil sample was re-weighed to calculate the concentration of isolated oil residue

\[
\text{mg Oil/sample g} = \frac{(W_b - W_a) \times 1000}{\text{g sample}} \quad \text{E. q(3 - 1)}
\]

Where, the weight of the crude oil sediment plus the round flask is \(W_b\), while the weight of the round flask is \(W_a\). While, the percentage removal of oil residue is calculated by

\[
\text{Oil residue\%} = \frac{W_d - W_f}{W_d} \times 100 = \text{E. q(3 - 2)}
\]

Where, the initial concentration of the weathered oil in the soil (g) before washing is \(W_d\), while the concentration of oil residue in the soil (g) after washing is \(W_f\).

3.3.2.3 Different sample weight

Using the basic method described in (Section 3.3.2.2) starting with the most polluted sample, the 5 g and 1 g weights of the oil-contaminated samples were selected depending on contamination level.

I) Result

The 1g of contaminated soil was cleaned in three cycles whilst the 5g of contaminated soil required six cycles. After the washing process is completed, the weight of the oil-contaminated soil sample after extraction and the weight of the crude oil were calculated using the equation in (Section 3.3.2.2), data is presented in Table (3-1).
Table 3-1: The weight and concentration of oil residue in contaminated sand (n=3).

<table>
<thead>
<tr>
<th>Sample Size (g)</th>
<th>Weight of Sample (g)</th>
<th>Weight of Crude Oil Sediment (g)</th>
<th>Concentration of Crude Oil (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample 5 g</td>
<td>5.20</td>
<td>1.80</td>
<td>346,000</td>
</tr>
<tr>
<td>Sample 1 g</td>
<td>1.00</td>
<td>0.33</td>
<td>330,000</td>
</tr>
</tbody>
</table>

II) Discussion

One-gram sample was cleaned in 30 min with 15 ml of solvent, in comparison with the 5 g sample, which needs 60 min with 150 ml of solvent to complete cleaning. However, the interaction between the extraction solvent and oil contaminants was unlikely to be changed by increasing the weight of the soil sample. Therefore, 1 g was recommended in this study.

3.3.2.4 Type of solvent

This technique involved determining the suitable solvents for the extraction of crude oil from sand in a short period with high extraction efficiency and a less harmful product. There is a wide range of solvents which can be used, such as hexane, dichloromethane (DCM) and chloroform. The selected solvents for this experiment are DCM, DCM: hexane (1:1 mix) and acetone: hexane (1:1 mix), these solvents were found to be the most effective solvent compared to other solvents in isolation of organic compounds from soil (Li et al., 2012; Osman and Saim, 2013). The selected solvents were tested using the same procedures carried out in three trials by applying the basic method described in Section 3.3.2.2.
I) Results

The ultrasonication process cleaned 1 g of contaminated soil, in three washes all with different solvents trialed. After the completion of the solvent recovery process, the weight of sand for all three samples and recovered oil were calculated, as exhibited in Table (3-2).

Table 3-2: Analysis of the oil contaminated samples and crude oil concentrations.

<table>
<thead>
<tr>
<th>Sample + DCM</th>
<th>Weight of the sample (g)</th>
<th>Weight of the crude oil sediment (g)</th>
<th>Concentration of oil residue (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Trial 1</td>
<td>1.21</td>
<td>0.33</td>
</tr>
<tr>
<td></td>
<td>Trial 2</td>
<td>1.10</td>
<td>0.27</td>
</tr>
<tr>
<td></td>
<td>Trial 3</td>
<td>1.20</td>
<td>0.31</td>
</tr>
<tr>
<td>Sample + DCM: Hexane (1:1mix)</td>
<td>Trial 1</td>
<td>1.09</td>
<td>0.35</td>
</tr>
<tr>
<td></td>
<td>Trial 2</td>
<td>1.22</td>
<td>0.39</td>
</tr>
<tr>
<td></td>
<td>Trial 3</td>
<td>1.23</td>
<td>0.37</td>
</tr>
<tr>
<td>Sample + Acetone: Hexane (1:1mix)</td>
<td>Trial 1</td>
<td>1.00</td>
<td>0.34</td>
</tr>
<tr>
<td></td>
<td>Trial 2</td>
<td>1.08</td>
<td>0.37</td>
</tr>
<tr>
<td></td>
<td>Trial 3</td>
<td>1.23</td>
<td>0.42</td>
</tr>
</tbody>
</table>
II) Discussion

Based on Table (3-2), the highest crude oil concentration on average was 341,000 mg/kg for the solvent combination of acetone: hexane (1:1 v/v). Chloroform is not recommended in this study since it affects the central nervous system, such that people who are exposed to chloroform with a certain amount can become unconscious. The mixture of acetone: hexane has a high extraction efficiency due to its dependency on the solvent mixture polarity for eliminating oil contaminants.

Moreover, different authors have used them in the extraction of petroleum products from soil (Somasundaran et al., 1997; Zhang et al., 2001). This experiment has shown that the best solvent system of choice for the extraction of oil from sand was mixture of acetone: hexane (1:1 v/v).

3.3.2.5 Different ultrasonication cycles

The main aim of trying a different ultrasonication cycle is to investigate whether a reduction of cycle improved the extraction process. The previous experiments (Section 3.3.2.4) required 5 ml of acetone: hexane (1:1 v/v) for 10 minutes per cycle for three cycles and these results were then compared with an alternative experiment, which used 5 ml of acetone: hexane (1:1 v/v) solvent mixture to clean 1g of soil sample for four cycles with a shorter cycle time of 5 minutes for each cycle.
I) Result

This test demonstrates that the four cycles were required if the sample washed for 5 minutes per cycle, therefore a total volume of 20 ml of solvent was required to complete the extraction process. However, samples washed within 10 minutes per cycle required three cycles, therefore, 30 ml of solvent was required for the whole process and the outcomes are presented in Table (3-3).

Table 3-3: The concentration of oil residue after 5 min and 10 min (n=3).

<table>
<thead>
<tr>
<th></th>
<th>Concentration of crude oil (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Trial 1</td>
</tr>
<tr>
<td>5 min</td>
<td>332,000</td>
</tr>
<tr>
<td>10 min</td>
<td>304,000</td>
</tr>
</tbody>
</table>

II) Discussion

Based on Table (3-3), there are no significant differences in concentration of oil residue was observed irrespective of the washing cycle time. The outcome showed that the mean values for 5 and 10 minutes were 344,000 and 333,000, respectively (Post-hoc Scheffe test, $P < 0.05$). The previous experiment (Section 3.3.2.2) required three cycles, 15 ml of solvent and 10 minutes per cycle to wash two samples in one hour. The alternative experiment used more solvent with less time, so that three samples could be washed in one hour. Therefore, this outcome suggested the choice of 5 minute cycle over the 10 minute cycle.
Validation of gravimetric method

One-gram of clean sand was mixed with 5 ml of fresh crude oil. The crude oil was obtained from (KOC). The mixture of sand and crude oil were washed in 20 minutes with 20 ml of solvents in four wash cycles (Section 3.3.2.2). After the completion of the solvent recovery process, the weight of sand and recovered oil were calculated, as exhibited in Table (3-4).

Table 3-4: Analysis of the oil contaminated samples and crude oil concentrations.

<table>
<thead>
<tr>
<th></th>
<th>Trial (1)</th>
<th>Trial (2)</th>
<th>Trial (3)</th>
<th>Trial (4)</th>
<th>Trial (5)</th>
<th>Trial (6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight of sand (g)</td>
<td>10.0</td>
<td>10.07</td>
<td>10.09</td>
<td>10.03</td>
<td>10.02</td>
<td>10.03</td>
</tr>
<tr>
<td>Volume of fresh crude oil (ml)</td>
<td>5.00</td>
<td>5.00</td>
<td>5.00</td>
<td>5.00</td>
<td>5.00</td>
<td>5.00</td>
</tr>
<tr>
<td>Weight of vial (g)</td>
<td>13.51</td>
<td>13.53</td>
<td>13.44</td>
<td>13.20</td>
<td>13.45</td>
<td>13.30</td>
</tr>
<tr>
<td>Weight of vial + sand + crude oil (g)</td>
<td>28.51</td>
<td>28.60</td>
<td>28.53</td>
<td>28.23</td>
<td>28.47</td>
<td>28.33</td>
</tr>
<tr>
<td>Weight of round bottom flask (g)</td>
<td>110.20</td>
<td>105.40</td>
<td>110.98</td>
<td>111.13</td>
<td>110.94</td>
<td>108.30</td>
</tr>
<tr>
<td>Weight of crude oil + round bottom flask after separation (g)</td>
<td>115.00</td>
<td>110.30</td>
<td>115.77</td>
<td>116.00</td>
<td>115.88</td>
<td>113.17</td>
</tr>
<tr>
<td>Volume of crude oil after separation (ml)</td>
<td>4.80</td>
<td>4.90</td>
<td>4.79</td>
<td>4.87</td>
<td>4.94</td>
<td>4.87</td>
</tr>
<tr>
<td>Weight of vial &amp; sand after separation (g)</td>
<td>23.31</td>
<td>23.57</td>
<td>23.49</td>
<td>23.22</td>
<td>22.85</td>
<td>23.10</td>
</tr>
<tr>
<td>Weight of sand after separation (g)</td>
<td>9.80</td>
<td>10.04</td>
<td>10.05</td>
<td>10.02</td>
<td>9.40</td>
<td>9.80</td>
</tr>
<tr>
<td>Percentage of the recovered oil (%)</td>
<td>96.00</td>
<td>98.00</td>
<td>95.80</td>
<td>97.40</td>
<td>98.80</td>
<td>97.40</td>
</tr>
</tbody>
</table>
The outcome of Table (3-4) indicated that the previous experiment (Section 3.3.2.2) was able to recover about 97.20% of the crude oil. This study suggested that the selection process in recovery of the crude oil is influenced by four principal characteristics: (1) minimise the cost of the extraction solvent by reducing the quantity of solvent (2) the solvent must dissolve the crude oil in the shortest time (3) high extraction efficiency and (4) select the solvent that has the least side effect. In this study, the temperature of the extraction was not examined since the volatility of the selected solvents was already high.

3.3.3 Potential Hydrogen Ions (pH) in Soil Sample
The pH of oil contaminated sand was measured based on BS ISO (10390: 2005). 30 g of oil contaminated sand sample was dried for 24 h in a drying cabinet at 40°C (Lec, UK), re-weighed and placed into beaker containing 150 ml of distilled water and mixed for 10 min by mechanical stirrer (CP Cole-parmer). The mixture was then left to settle for one hour at room temperature. The samples were then read using pH meter (model, Jenway 3305, UK) after being calibrated for pH 7.

3.3.4 Electrical Conductivity.
The oil-contaminated soil was mixed with distilled water to measure the electric conductivity (in units of µS/cm) with a ratio of 1:5 weight to volume. The conductivity of the oil-contaminated soil was measured by using a Jenway 4010 conductivity (BS EN 13038: 2011).
3.3.5 Surface Tension

Surface tension of the oily wastewater at various concentration of saponin were determined using a Du Nouy tensiometer (Type: K20. KRÜSS, Korea) using a platinum ring method, as presented in Figure (3-4). Each experiment was conducted by using 50 ml of the sample in a 150 ml beaker. During the process, the distance between the liquid surface and immersed ring was kept approximately 5 mm to insure the ring is just below the liquid surface. The pre-programmed software in the tensiometer measures the surface tension of the surfactant solutions.

Figure 3-4: Du Nouy tensiometer.
3.3.6 Oil agglomeration
The combination of ultrasonication with a mechanical stirrer was used to mix 100 g of the oil contaminated soil with 150 ml of saponin solution in a 250 ml beaker. The washing parameters used in this study were as follows: washing temperature of 50°C, 0.5 wt% of saponin, stirring speed of 1000 rpm and washing time of 20 min (Washing method is given in next Chapter 4, Section 4.2.3). Repeated the experiments three more times and separated the oil aggregates from washed sand using forceps. The agglomerates hence formed were dried for 24 h at 20°C and then measured the average size of agglomerates in millimetres using a ruler.

3.3.7 Particle size distribution
Mechanical sieve analysis was carried out by using sieve shaker (Retsch Sieve Shakers AS 200). The analysis was used to determine how the grain size of contaminated particles influenced the oil removal. The process was carried out by using three sieves with pores sizes of 0.60mm, 2.38 mm, 5.66 mm and a pan. The procedure was performed by using tap water from a nozzle spray placed on the top sieve. 250 g of contaminated sample was placed on the largest sieve size on top. Rinsing process was continued for approximately 5 min, until the sieving solutions become clear.

3.3.8 SEM Preparation and Equipment
SEM (JOEL JSM-6301D field emission) was used to scan the particle surface using a thin and strong electron beam. For an imaging function, the detection of electrons was carried out backscattered so as to allow the examination of the sample surface regardless of its thickness. 1 g of the particles was dried for 48 hours at room temperature and the samples were mounted.
on a gold coater (Edwards Sputter coat SI50B) for 1 min by using both electrical field and Argon gas. Upon completion of gold coating, the scanning of the samples was performed by the computer enhancement method.

3.4 Characteristics Measurement of Oily Wastewater

3.4.1 Analysis of Hydrocarbons in Oily Wastewater

The washing process of oil contaminated sand generates wastewater containing surfactants, hydrocarbons and metals. It is necessary to determine the concentration of total petroleum hydrocarbons in the waste water as well. The oil was extracted from the waste water based on the following procedure. 5 ml of oily wastewater was placed into a screw-capped bottle with 15 ml of a mixture of acetone: hexane solvent (1:1, v/v) and washed for 5 minutes in an ultrasonication bath so that the ratio of sample to the extraction solvent remains 1:3. The solution of crude oil and mixture of Acetone: Hexane was pipetted into a clean and dry weighed round-bottomed flask. The ultrasonication cycle was then repeated for three times which results in the formation of two immiscible layers, the top layer being mixture of organic solvents and crude oil and the bottom layer being clear water. This layer of water was pipetted out and noted the weight. The collection apparatus, conical glass flask, distillation head with condenser side arm and water bath were prepared and fixed to measure the total recoverable of petroleum hydrocarbons. The water bath temperature was maintained at 52°C, to accelerate the evaporation of the acetone: hexane mix. When the acetone: hexane mix was recovered, the round glass flask was dried, then the total weight of oil residue was re-weighed to calculate the concentration of isolated oil residue.
\[ \text{mg Oil/sample ml} = \frac{(W_b - W_a) \times 5000}{\text{ml sample}} \] 

Where, the weight of the oil residue sediment plus the round flask is \( W_b \), while the weight of the round flask is \( W_a \).

3.4.1.1 Different ratios

Different ratios such as (1:3, v/v) and (1:5, v/v) of oily wastewater sample to the mixture of extracting solvent were tested by applying the basic method described in Section 3.4.1.

III) Result

The amount of oil residue in terms of TPH was calculated by using two different ratios of oily wastewater: solvent (1:3, v/v) after three cycles and the ratio of (1:5, v/v) after two cycles of the washing process. The weight of the oily wastewater sample after extraction and the weight of oil residue was calculated using the equation in section 3.4.1, data is presented in Table (3-5).

<table>
<thead>
<tr>
<th>Trial</th>
<th>Concentration of crude oil (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Trial 1</td>
</tr>
<tr>
<td>Ratio (1:3)</td>
<td>62,000</td>
</tr>
<tr>
<td>Ratio (1:5)</td>
<td>63,000</td>
</tr>
</tbody>
</table>

The difference in ratio of oily wastewater: the extracting solvent does not reflect much difference in the TPH value.
IV) Discussion

The oily wastewater sample with ratio of (1:3) was cleaned in 15 min with 45 ml of solvent, in comparison with the ratio of (1:5), which needs 10 min with 50 ml of solvent to complete cleaning. However, the interaction between the oil contaminants and extraction solvent was unlikely to be changed by increasing the ratio of sample to the mixture of solvent from (1:3) to (1:5). Therefore, ratio of sample to the mixture of solvent (1:3) was recommended in this study.

3.4.1.2 Checked the extraction method by recovery process

10 ml of distilled water was mixed with 5 ml of fresh crude oil (Source: KOC). The mixture of distilled water and crude oil were washed in 15 minutes with 45 ml of solvents in three washes (Section 3.4.1). After the completion of the solvent recovery process, the weight of distilled water and recovered oil were calculated, as exhibited in Table (3-6).

Table 3-6: Analysis of the oil contaminated samples and crude oil concentrations.

<table>
<thead>
<tr>
<th></th>
<th>Trial (1)</th>
<th>Trial (2)</th>
<th>Trial (3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight of water (ml)</td>
<td>10.0</td>
<td>10.00</td>
<td>10.00</td>
</tr>
<tr>
<td>Weight of fresh crude oil (ml)</td>
<td>5.00</td>
<td>5.00</td>
<td>5.00</td>
</tr>
<tr>
<td>Weight of vial (g)</td>
<td>17.60</td>
<td>16.55</td>
<td>15.40</td>
</tr>
<tr>
<td>Weight of vial &amp; water &amp; crude oil (g)</td>
<td>32.60</td>
<td>31.55</td>
<td>30.40</td>
</tr>
<tr>
<td>Weight of round flask (g)</td>
<td>110.20</td>
<td>105.40</td>
<td>110.98</td>
</tr>
<tr>
<td>Weight of crude oil &amp; round flask after separation (g)</td>
<td>114.70</td>
<td>109.70</td>
<td>115.28</td>
</tr>
<tr>
<td>Weight of crude oil after separation (ml)</td>
<td>4.80</td>
<td>4.50</td>
<td>4.60</td>
</tr>
<tr>
<td>Weight of vial &amp; water after (g)</td>
<td>27.80</td>
<td>26.65</td>
<td>25.60</td>
</tr>
<tr>
<td>Weight of sand after (g)</td>
<td>10.20</td>
<td>10.10</td>
<td>10.20</td>
</tr>
<tr>
<td>Percentage of the recovered oil (%)</td>
<td>96.00</td>
<td>90.00</td>
<td>92.00</td>
</tr>
</tbody>
</table>
The outcome of Table (3-6) indicated that the previous experiment (Section 3.4.1.1) was able to recover about 93% of the crude oil. This study is limited only to hexane solvent, as such the results found that the weight for distilled water was slightly increased which may be due to the polarity of the water and hexane. This property may be leads to hydrogen bonding and strong interactions between water molecules and hexane.

3.4.2 Measuring the Amount of Flocculent Mass (Flocs)
A 50 ml of oily wastewater was filtered through Whatman 1.2 μm GF/C filter paper (Fisher Scientific, UK), with vacuum suction. The filtered paper was dried in an oven at 45°C for 24 h, and then cooled at room temperature. The total weight of filter papers with the remaining residual was re-weighed to 4 decimal places. The weight of dry flocs (g) was calculated based on the following formula:

\[
\text{Flocs (g)} = \text{final filter paper weight (g)} - \text{initial filter paper weight (g)} = \ldots \text{E. q (3 – 4)}
\]

3.4.3 Turbidity
The water’s light transmitting properties is known and measured as turbidity. It is one of the essential assessments to inform the quality of wastewater which concerns colloidal and residual suspended solid. Kuwait regulation for the maximum limits of pollutants industrial wastewater permissible to be discharged into the sea, the treated water turbidity must be lower than or equal to 50 NTU. In this work, the nephelometer was adjusted at 0 using 40 (Turbid meter, model DRT 100B. Partech, UK) as shown in Figure (3-5). Then 100 ml of the treated water sample was transferred into the nephelometer tube. During measurement, the sample was ensured that it did not contain any air bubbles. The pre-programmed software in the nephelometer measured the turbidity of the sample.
3.4.4 Potential Hydrogen Ions (pH) in Water Sample
The pH of oily wastewater was measured based on BS EN ISO (10523: 2012). 100 ml of oily wastewater sample was collected in 150 ml beaker to submerge the tip of the probe. The probe was rinsed with oily wastewater sample before the measurement for conditioning. The samples were then read using pH meter (model, Jenway 3305, UK) after being calibrated at pH 7.

3.4.5 Measuring Biochemical Oxygen Demand (BOD) by OxiTop
It has become feasible to measure the BOD throughout a long period of time due to the respirometric BOD system, for example OxiTop. The utilization of the OxiTop system has made the measurement of BOD possible as the measured values are kept automatically within the system. During the incubation, microorganisms use up oxygen and release carbon dioxide and these gases remain dissolved. The values as measured can be documented throughout long
periods of time even after a few days to a few weeks. In reality, this would aid to assess the treatment system’s efficiency as well as the strength of the wastewater. In comparison to the other BOD measurement methods, the microbial biodegradation in the OxiTop bottle could be able to represent the degradation process in natural conditions. Furthermore, the oxygen needed for the operation is supplied by the graduated measuring flask, which consist of dissolved oxygen and the amounts of oxygen above the solution. Moreover, a good exchange of gases between gas phases and aqueous phase were maintained through using constant stirrer stirring. As such, the sample ought to be diluted prior to incubation in an effort to bring the demand and supply of the oxygen into the correct balance < 4000 mg/l, which considered as a maximum permissible concentration of BOD values for a determination by OxiTop. Therefore, the BOD value was required to be estimated before analysis.

3.4.5.1 Respiration measurements

The standardised biodegradability test such as organisation for economic co-operation and development (OECD, 2008) and international organisation for standardisation (ISO, 1999) were used to ensure reproducible and comparable results can be achieved by using OxiTop control system. All samples were collected in 250 ml reagent amber bottles and kept in an incubator to prevent exposure to light for a period of 28 days at 20°C ±1°C. So as to ensure maximum mixing of the samples, magnetic bar stirrers were included in the bottle. Rubber sleeve was used to seal the bottle top to ensure it was leak proof. All bottles were fitted with wet seals covered with caps which acted as vapour seal over the top of the bottle seals in order to guarantee that no evaporation takes place.
As an additional measure, sodium hydroxide (NaOH) pellets, 2 pellets for each measurement, were utilised to absorb carbon dioxide. The nitrification bacteria were inhibited in the present experiment by adding one drop of N-Allylthiourea (C4H8N2S) ATU to the measurement solution. Once the samples are secured tightly and water sealed, and then they were incubated. The components of the OxiTop measuring system are shown in Figure (3-6). For a total stabilisation, the sample may need a very long incubation period which was impractical.

![Figure 3-6: Respirometric measuring system (A) Stirring platform and incubator cabinet (B) control system, measuring heads and graduated measuring flask (C) NaOH pellets, ATU, rubber sleeve and magnetic stirrer bars.](image)

3.4.5.2 Dilution requirements

The rates of the reaction to pollutant concentrations differ between microorganisms, these impacts can be minimised or even eradicated by proper dilution of the samples. The prepared representative dilutions are dependent totally upon the anticipated value of the BOD. Samples having high values of BOD have to be diluted extensively to ensure the adequacy of the oxygen in the used bottles. Therefore, distilled water was used to dilute the sample with ratio of 1:10.
3.4.5.3 Preparation of stock solution

Two stock solutions were prepared during this study using 250 ml volumetric flasks were used. The first stock was prepared using saponin and distilled water at a ratio of 1:10 (A), and second stock was prepared using oily wastewater and distilled water at a ratio of 1:10 (B) (see Figure 3-7). All samples were manually shaken. In addition, primary settled sewage (PSS) taken from the Petersfield Sewage Works (Southern Water) and liquid plant fertilizer (LPF) were utilised as the amendment substance during the BOD test. A set of 4 sub-stocks were prepared using 50 ml volumetric flasks by adding 2.5 ml of amendment substance into the volumetric flasks and making up to 50 ml using stock solutions A and B. Exactly 22.7ml of the respective substock was transferred using a graduated pipet into the BOD bottles.

![Figure 3-7: The preparation of the stock solutions for (A) Saponin solution at 1:10; (B) Oily wastewater at 1:10.](image-url)
3.4.6 Conventional Seeding Material (CSM)

In the case of unavailability of obtaining seed sources, seeds can be developed and adapted in a laboratory. This can be performed by isolating the bacteria from Kuwait oil sand to extract the initial population of microbial. This approach is considered as one of the appropriate method for biodegradation enhancement (Dua et al., 2002). In order to evaluate whether the microbial population is sufficient, an experiment was undertaken to assess how the seed in the BOD test sample performs. The seed adaptation was monitored by using Oxitop to test biodegradability of oily wastewater.

3.4.6.1 Microbial seed for BOD test

The main purpose of seeding the samples is to make certain that the activity and survival of microorganisms is sufficient. A number of samples contain components which are usually not degradable by the microorganisms present in wastewater. In order to achieve the required microbiology for oil degradation, the wastewater samples were seeded with the bacteria. It is generally accepted that inoculating microorganisms into an established environment is not a straightforward task (Kastner et al., 1998). In the current research, the microbial populations were allowed to be grown in nutrient broth to make the seeding adaptation easily and more accurate. The culture was obtained from an agar plate of bacterial by using sterilize inoculating loop. The isolated colonies of bacteria were allowed to grow under standard conditions of nutrient broth (OXOID, UK). The colony was spread in the conical flask of liquid media. Then, incubate conical flasks at 37°C for 48 hr. The liquid media was required to transfer the same amount of bacteria in Oxitop bottles. For formulation of BOD-CSM, microorganisms were isolated from oil contaminated soil as explained in next section.
3.4.6.2 Preparation of solid media

Standard conical flask of 250 ml was used for preparation of solid media. The flask was filled with 100 ml distilled water and 2.8 g of nutrient agar (OXOID, UK). The conical flask was placed on the hotplate stirrer (CB302), to boil the solution to dissolve completely. Then, the mixture was sterilized using Autoclave (TOMY SX-500) at 121°C for 15 min. The solution was then cooled at room temperature and pipetted carefully into the Petri dishes to avoid bubbles to the extent possible. Subsequently, allowed to solidify at room temperature for 20 min and 10 ml of nutrient agar was dropped in to petri dish.

3.4.6.3 Preparations of liquid media

A conical flask of 300 ml was used for preparation process of the liquid media. The media was prepared by using 200 ml of distilled water with 2.6 g of nutrient broth to provide the required characteristics. The solution was mixed in orbital shaking action for 10 min to distribute the nutrient broth into the conical flask. Then, the mixture was sterilized using an Autoclave (TOMY SX-500) at 121°C for 15 min. The solution was cooled at room temperature for 30 min.

3.4.6.4 Isolating of bacteria

Generally, bacteria grouped into two kingdoms, aerobic and anaerobic, based on their need for oxygen to generate energy for growth. Therefore, the isolated bacterial was considered as facultative aerobes. Experiment was conducted by mixing 10 g of the Kuwaiti oil contaminated sand with 50 ml of isotonic solution 0.9 % NaCl in a 250 ml glass beaker, while the head of the flask was covered with sealer, as shown in Figure (3-8).
The flask was placed into a magnetic stirrer (CB302) at the room temperature. The mixture of oil contaminated sand with isotonic solution was mechanically agitated for 24 hours and stirred at a constant speed of 50 rpm to provide a uniform distribution of the isotonic solution during process. The isotonic solution was used in this experiment to keep the external concentration of solutes equal to cell’s internal environment. The filtration method was used for isolating the microbial consortium (BS 6068-4.12:1998). Isotonic solution was concentrated by filtration through 0.45 µm of charged nylon membrane (SIGMA, UK), and then a funnel filter assembly was used to separate the precipitate. Subsequently, the filtered sample was used as inoculant for isolation of bacteria. 1 ml of filtered sample was dropped into solid media plate. Then, the plates were incubated at 37°C for 24 hr.
After this period, two different colonies were identified in the agar plate, as presented in Figure (3-9). Each culturing experiment was repeated ten times to confirm the colony obtained.

Figure 3-9: The mixture of two different colonies were isolated, A) *Bacillus subtilis* and B) *Bacillus mycoides*.

The DNA of Bacteria’s were identified by Dr. Esmaeil al saleh from KU-College of Science - Dept. of Biological Sciences, (Appendix, B). The sequences were assigned to representatives that have been identified from the main eubacterial lineages in accordance with the highest score and 16S sequence similarity of 97 % and 99 % for *Bacillus subtilis* and *Bacillus mycoides*, respectively.
3.4.7 Measuring Chemical Oxygen Demand (COD) by Colorimetric Method

COD was measured to estimate the oxidizable organic matter in the water sample. 100 ml of sample were homogenised for 5 min in overhead stirrer (CP Cole-Parmer). The oily wastewater was diluted (distilled water to oily wastewater, 100:1) in a 100 ml conical flask, and manually shaked for 5 min. The cap from a COD vial provided in the kit was removed and 2 ml of diluted sample was micro-pipetted into the vial. The lids were sealed tightly and the vial mixed gently several times to mix the contents. The vial was placed in the preheated digester block and allowed to react with COD vial (0-1500 ppm) containing 86 % sulphuric acid and potassium dichromate. The vials were digested in a COD reactor hot block (DRB200, Hach, UK) as shown in Figure (3-10), and heated for two hours at 150°C. The block was turned off and allowed to cool for 15 min. The digested samples and reagent blanks were measured in a pre-programmed colorimeter (DR890, Hach, UK) photometer. The results are expressed as the number of milligrams of oxygen consumed per litre of sample (mg/litre COD). Subsequently, the colorimeter reading was multiplied by the ratio factor.

Figure 3-10: Hach COD Reactor.
3.4.7.1 Dilution of samples

To obtain the appropriate depletion of COD in the incubated samples, the optional dilution of samples was prepared in 25 ml of conical flask by using (25:1) ratio of distilled water to sample, and shaken manually for 5 minutes. While the oily wastewater was prepared in 100 ml of conical flask by using (100:1) ratio of distilled water to oily wastewater, and kept shaken for 5 minutes as well.

3.4.8 Development of COD Analytical Method

For further experimental study in this subject is required to study the impacts of the salt concentrations exceeding 2000 mg/l. Therefore, titrimetrically by open-reflux digestion methods, and colorimetrically (photometrically) with USEPA (1983) Method 410.4, Hach Method 8000 were utilised in analyzing saline water, which contain in the excess of 50 mg/l COD, and below 2000 mg/l of chloride concentration.

Generally, Hg-free vials can avoid the risks of the toxic mercury. It is also reduces the disposal cost of test vials in comparison with test vials containing mercury. Unlike COD results have been obtained by using Hg-free vials during the COD measurement. In order to detect the interference generated, relationship of COD data using Hg vials compared with Hg-free vials needs to be established for this particular waste. This interference were prevented by adding excess mercuric sulfate to a maximum concentration of 5000 mg/l, provided that the mercuric sulfate to chloride ratio of 10:1 is kept. The accuracy of the results obtained were confirmed in comparative experiments to ensure the validation of results.
This way would support the results, further the results obtained using titration method were considered as reference to confirm the data. The measurements of COD were carried out on (COD; 96 samples), the number of corresponding samples analysed at various stages were tested. Further, there are various range of COD (Hach) testing vials such as ultra-low range, low range, high range and high range plus. Therefore, the percent of chlorides ion in the tested samples need to be determined first to select the accurate range of COD testing.

3.4.8.1 Effects of chlorides on COD

Bounded seas are known to have very high salinity. For instance, the salinity of the Arabian Gulf Sea close to the Kuwait coastline and Saudi Arabia may register between 38 to 50 g/kg (El-Dessouky and Ettouney, 2002; Al-Kandari, 2013). Generally, the seawater is very rich in chlorides, which in excess of half of the percent by weight of dissolved ions, as illustrated in Table (3-7).

The major interference in the conventional dichromate method to determine the COD is from the chloride ions (Zhang et al., 2009; Qu et al., 2011; Zhang et al., 2011), which could be act as the main interference when measuring the COD values. Therefore, the effect of chloride ions on the COD values was investigated in this project. The COD vials consist of potassium dichromate which considered as a strong chemical oxidation of organic compound mixed with sulfuric acid (50%).
### Table 3-7: Main dissolved ions in Kuwait seawater (mg/l) (Source: WC&P, 2005)

<table>
<thead>
<tr>
<th>Dissolved ions at Kuwait Bay (mg/l)</th>
<th>% By weight of dissolved ions</th>
<th>% By weight of seawater</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Chloride</strong> (Cl(^{-}))</td>
<td>23,000</td>
<td>51.110</td>
</tr>
<tr>
<td><strong>Sodium</strong> (Na(^{+}))</td>
<td>15,850</td>
<td>35.220</td>
</tr>
<tr>
<td><strong>Sulfate</strong> (SO(_4^{2-}))</td>
<td>3,200</td>
<td>7.110</td>
</tr>
<tr>
<td><strong>Magnesium</strong> (Mg(^{2+}))</td>
<td>1,765</td>
<td>3.920</td>
</tr>
<tr>
<td><strong>Calcium</strong> (Ca(^{2+}))</td>
<td>500</td>
<td>1.110</td>
</tr>
<tr>
<td><strong>Potassium</strong> (K(^{+}))</td>
<td>460</td>
<td>1.020</td>
</tr>
<tr>
<td><strong>Bicarbonate</strong> (HCO(_3^{-}))</td>
<td>142</td>
<td>0.320</td>
</tr>
<tr>
<td><strong>Strontium</strong> (Sr(^{2+}))</td>
<td>-</td>
<td>0.000</td>
</tr>
<tr>
<td><strong>Bromide</strong> (Br(^{-}))</td>
<td>80</td>
<td>0.180</td>
</tr>
<tr>
<td><strong>Borate</strong> (BO(_3^{-}))</td>
<td>-</td>
<td>0.000</td>
</tr>
<tr>
<td><strong>Fluoride</strong> (F(^{-}))</td>
<td>-</td>
<td>0.000</td>
</tr>
<tr>
<td><strong>Silicate</strong> (SiO(_3^{2-}))</td>
<td>1.5</td>
<td>0.003</td>
</tr>
<tr>
<td><strong>Iodide</strong> (I(^{-}))</td>
<td>-</td>
<td>0.000</td>
</tr>
<tr>
<td><strong>Others</strong></td>
<td>-</td>
<td>0.000</td>
</tr>
<tr>
<td><strong>TDS</strong></td>
<td>44,998</td>
<td>99.993</td>
</tr>
</tbody>
</table>

Several analytical methods have been suggested to address Cl\(^{-}\) interference (Tzeng and Chen, 1993). Hach Water Analysis Handbook (Boyle, 1997) has been followed in this research to specify the required range of Cl\(^{-}\) concentration, see Table (3-8).
Table 3-8: Concentrations of chloride for accurate COD testing recommended by Hach.

<table>
<thead>
<tr>
<th>Vial Type Used (mg/l)</th>
<th>Maximum Cl in sample (mg/l)</th>
<th>Suggested Cl in diluted sample (mg/l)</th>
<th>Maximum Cl in sample with 0.5 HgSO₄ (mg/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ultra low range 0.7 - 40</td>
<td>2,000</td>
<td>1,000</td>
<td>N/A</td>
</tr>
<tr>
<td>Low range 3 - 150</td>
<td>2,000</td>
<td>1,000</td>
<td>8,000</td>
</tr>
<tr>
<td>High range 20 - 1500</td>
<td>2,000</td>
<td>1,000</td>
<td>4,000</td>
</tr>
<tr>
<td>High range plus 200 - 15,000</td>
<td>20,000</td>
<td>10,000</td>
<td>40,000</td>
</tr>
</tbody>
</table>

3.4.8.2 The interference of chloride ion

In order to guarantee that the chloride interference will not affect the results of COD on the Hach Equipment, the effects of concentration need to be thoroughly investigated. The Cl⁻ concentration in mass of marine salt was determined by using two different methods, explained in the following sections. Both these methods are according to the values displayed in Table (3-8).

3.4.8.3 Weight of chlorides in artificial seawater

The percent of chlorides in seawater by weight is 2.3 % (Table 3-7). This means that 1 gram of seawater contains 0.023 grams of chlorides. The mass of seawater can therefore be calculated knowing the density of 1.0250 g/cm³ for artificial seawater at a temperature of 16°C and a salinity of 45 g/l. The following method (1) was performed to determine the concentration of chloride in the artificial seawater.

1g seawater × 2.3 (mass Cl⁻) % = 0.023 grams Cl⁻
1 ml seawater = (1.025 g/10⁻³ l) × (1 × 10⁻³ l) = 1.025 grams
1.025 g of seawater therefore contains 0.0235 grams Cl⁻
When the mass of marine salt (45,000 mg) is added to the 1-litre beaker, the total chloride concentration (Cl\(^-\)) = 1,035 mg. So, the mass of chlorides mass (g) in dissolved ions = 23,570 (mg/l).

The mass of chlorides in one litre of artificial seawater by various concentration of marine salt was therefore calculated following the method described above to check that the maximum concentration of chlorides had not been reached in the Hach COD vials, as presented in Table (3-9).

Table 3-9: Concentrations of chloride in artificial seawater by method (1).

<table>
<thead>
<tr>
<th>Marine salt Added by volume %</th>
<th>Mass of marine salt (g)</th>
<th>Chlorides mass (g)</th>
<th>Chlorides (g/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>5</td>
<td>0.11</td>
<td>2.53</td>
</tr>
<tr>
<td>1.0</td>
<td>10</td>
<td>0.23</td>
<td>5.30</td>
</tr>
<tr>
<td>2.0</td>
<td>20</td>
<td>0.46</td>
<td>10.53</td>
</tr>
<tr>
<td>4.0</td>
<td>40</td>
<td>0.92</td>
<td>21.05</td>
</tr>
<tr>
<td>4.5</td>
<td>45</td>
<td>1.03</td>
<td>23.57</td>
</tr>
</tbody>
</table>

The results show therefore that the addition of 4.5 % of marine salt to the 1-litre beaker contributes 23,570 mg/l of chlorides to the solution. This is significantly higher than 2000 mg/l, which is the maximum range of Cl\(^-\) concentration. The dilution was therefore considered.
3.4.8.4  Sodium chloride in artificial seawater

To roughly estimate the amount of chlorides in seawater, NaCl can be used as an indicator. This is the basis for method 2 described in details below to calculate the concentration of chlorides added to the beaker with the addition of various seawater volumes. The example shown below is for a 10 ml seawater addition to the 1-litre beaker:

Similar salinity of Kuwait seawater 45 ppth (parts per thousand) = 45 g NaCl / litre of seawater:

\[ \text{NaCl} \rightarrow \text{Na}^+ + \text{Cl}^- \]

Atomic weight of sodium Na\(^+\) is 23 g/mol and the atomic weight of Cl\(^-\) is 35 g/mol.

\[ \text{NaCl} \rightarrow \text{Na}^+ + \text{Cl}^- \]

\[
\begin{align*}
1 \text{ mole NaCl} & \rightarrow 1 \text{ mole Cl}^- \\
(35 + 23) \text{ g/mol NaCl} & \rightarrow 35 \text{ g/mol Cl}^- \\
45-\text{g/litre NaCl} & \rightarrow X \text{ g/litre Cl}^- 
\end{align*}
\]

\[ X = 27.156 \text{ g Cl/litre} = 27,156 \text{ mg Cl/l} \]

The values of chloride concentrations added to the artificial seawater in jar tests for various concentrations of marine salts were therefore calculated and summarized in Table (3-10).

Table 3-10: Concentrations of chloride in artificial seawater by method (2).

<table>
<thead>
<tr>
<th>Marine salt added by (volume %)</th>
<th>Artificial seawater added by volume (ml)</th>
<th>Chlorides (g/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>5</td>
<td>3.00</td>
</tr>
<tr>
<td>1.0</td>
<td>10</td>
<td>6.00</td>
</tr>
<tr>
<td>2.0</td>
<td>20</td>
<td>12.10</td>
</tr>
<tr>
<td>4.0</td>
<td>40</td>
<td>24.14</td>
</tr>
<tr>
<td>4.5</td>
<td>45</td>
<td>27.16</td>
</tr>
</tbody>
</table>
Both methods show that the addition of 4.5 % of marine salts to one-litre beakers is able to interfere with the COD readings. Therefore, the dilution needs to be performed during the COD measurement. It seems that method 1 presents the total ionic concentration in seawater, not focus the analysis on specific ions, like Na or Cl, therefore method 2 is considered reliable than method 1. This point suggests to select method 2. Based on Section 3.4.8.2, the concentrations of chloride (20-1500 mg/l) is recommended for accurate COD reading, which are limited by 1000 mg/l of chloride concentration for diluted sample.

3.4.8.5 Colorimetric analysis

During this study, two different vials were used to check the COD values, such as mercury vials and mercury free vials, the procedure of colorimetric method is explained previously in Section 3.4.7.

3.4.8.6 Titration analysis

The conventional procedure using dichromate was employed as a reference to detect the values of the COD of a number of samples. The chemicals used were H₂SO₄ (98 %), C₆H₁₂O₆, K₂Cr₂O₇, Ag₂SO₄, HgSO₄ and Fe(NH₄)₂(SO₄)₂.6H₂O. Results obtained from the tests were compared against the values obtained from the colorimetric analysis. Specific details of the test procedure are as follows:
I. Reagents

The preparation for the digestion solution was arranged by adding 12.258 g K$_2$Cr$_2$O$_7$, which was dried beforehand for 2 h at 103°C in oven and diluted to 1000 ml. The preparation for the sulfuric acid reagent (H$_2$SO$_4$) was performed in accordance with the standard methods, which approved by American Public Health Association (APHA, 1999) for the open colorimetric technique. This reagent was prepared by adding 5.5 g silver sulfate Ag$_2$SO$_4$ to 0.50 l of sulfuric acid, the silver sulfate was dissolved in approximately 30 min by stirring continuously. The ferrous ammonium sulfate (FAS) was prepared by dissolving 98 g of Fe(NH$_4$)$_2$(SO$_4$)$_2$.6H$_2$O in distilled water followed by introducing 20 ml of H$_2$SO$_4$ which is then diluted to a volume of 1 litre. Adjustment: Using distilled water, dilute 25 ml of regular dichromate solution to approximately 250 ml in volume. Introduce 20 ml of concentrated sulfuric acid to the solution, allow it to cool and subsequently carry out titration using FAS titrant, three drops of ferroin indicator.

\[
\text{Normality} = \frac{[(\text{ml K}_2\text{Cr}_2\text{O}_7)(0.25)]}{\text{ml Fe(NH}_4)_2\cdot(\text{SO}_4)_2}} \ldots \ldots \ldots \ldots \text{E.q (3 - 5)}
\]

Sulfuric acid (sp. gr. 1.84) (concentrated H$_2$SO$_4$), silver sulfate (powdered Ag$_2$SO$_4$), mercuric sulfate (powdered HgSO$_4$), and ferroin indicator solution used in the experiment were purchased from Fisher Scientific, UK.
II. Procedure

In order to calculate the COD of the samples by using titration method, which contains chloride higher than 2000 mg/l, the procedures described below should be followed (Burns and Marshall, 1965). One gram of glucose was dissolved in a litre of distilled water and 50ml of which was pipetted into a 500 ml refluxing flask. Add 1 g of HgSO$_4$, a few glass beads, very gently introduce 5 ml of sulfuric acid reagent and mix the solution to dissolve the HgSO$_4$. During mixing, cool the solution simultaneously to prevent potential volatile materials losses. Add 25 ml 0.25N K$_2$Cr$_2$O$_7$ solution and mix thoroughly. Attach flask to condenser and allow the cooling water to flow. 70 ml of the sulfuric acid reagent was poured in the condenser through open end. While adding the sulfuric acid reagent, whirling and mixing should carry on. The mixture for reflux should be mixed thoroughly before applying heat to prevent any potential local heating of flask bottom or blowout of flask contents. In order to avoid ingress of foreign material into the refluxing mixture, the open end of the condenser should be covered with a small beaker and continue refluxing for 2 hours. Allow the condenser to cool and rinse using distilled water. Disconnect the reflux condenser and add distilled water to dilute the mixture to approximately double its original volume. The mixture was allowed to be cool in a room temperature and excess of K$_2$Cr$_2$O$_7$ was titrated with FAS, utilising 0.10 to 0.15 ml (2 to 3 drops) ferroin indicator. While the quantity of the ferroin indicator is immaterial, the same volume should be used in all titrations. The last point of the titration should be taken as the first sharp colour change from blue-green to reddish brown that remain consistent for at least 1 minute. Likewise, reflux and titrate a blank container with the reagents and a volume of distilled water which equals the sample value.
Where $A$ is FAS (ml) used for blank; $B$ is FAS (ml) of used for sample, the normality of FAS is $N$ and milliequivalent weight of oxygen is 8. In the case of oily wastewater, the quantity of $\text{HgSO}_4$ to be added (before concentration) should be calculated based on the 10:1 ratio of $\text{HgSO}_4$:$\text{Cl}$, utilising the quantity of $\text{Cl}$ available in the sample. Similar procedure was conducted for a blank reagent.

3.4.9 Measurement results

A series of experiments were designed to assess the interference among different samples. This includes actual water samples, which was collected from seawater (Portsmouth sea front), different concentration of artificial seawater (45.00, 22.50 and 11.25 g/l), glucose 1 g/l and saponin 0.5 wt%. This measurement has been carried out to verify and confirm the correlation between Hg vial and Hg-free vials.

3.4.9.1 COD for Artificial Seawater.

It has been highlighted that chloride ions consider as the most significant interference for the determination of COD by dichromate method. Table (3-11) shows the COD of artificial seawater prepared using 2.250 g (4.500 %), 1.125 g (2.250 %) and 0.560 g (1.125 %). These samples were prepared to investigate the interference by oxidizing different concentration of chloride ions. Dilution was performed appropriately in accordance with the range specified by Hach Handbook (Boyle, 1997), as explained in Section 3.4.8.2.
Table 3-11: COD of artificial seawater with different ratios by using Hg and Hg-free vials (n=3).

<table>
<thead>
<tr>
<th>Marine salt in (50 ml)</th>
<th>Concentration of chloride ion mg Cl/L</th>
<th>Type of vial</th>
<th>Ratio ml:ml</th>
<th>Mass of chloride ion (mg)</th>
<th>Mean (mg/l)</th>
<th>Std. Deviation (mg/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>COD of artificial seawater 4.500 %</td>
<td>27,156</td>
<td>Hg</td>
<td>1:25</td>
<td>1.086</td>
<td>1,042</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1:50</td>
<td>0.543</td>
<td>1,217</td>
<td>29</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Hg-free</td>
<td>1:25</td>
<td>1.086</td>
<td>2,550</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1:50</td>
<td>0.543</td>
<td>2,907</td>
<td>45</td>
</tr>
<tr>
<td>COD of artificial seawater 2.250 %</td>
<td>13,577</td>
<td>Hg</td>
<td>1:25</td>
<td>0.543</td>
<td>658</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1:50</td>
<td>0.272</td>
<td>776</td>
<td>29</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Hg-free</td>
<td>1:25</td>
<td>0.543</td>
<td>2,199</td>
<td>44</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1:50</td>
<td>0.272</td>
<td>2,533</td>
<td>101</td>
</tr>
<tr>
<td>COD of artificial seawater 1.125 %</td>
<td>6,788</td>
<td>Hg</td>
<td>1:25</td>
<td>0.272</td>
<td>216</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1:50</td>
<td>0.136</td>
<td>283</td>
<td>28</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Hg-free</td>
<td>1:25</td>
<td>0.272</td>
<td>1,725</td>
<td>66</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1:50</td>
<td>0.136</td>
<td>2,150</td>
<td>132</td>
</tr>
</tbody>
</table>

In this table (3-11), shows the comparison between Hg and Hg-free COD vials. The outcome of this study shows that the COD values decreased dramatically by decreasing the amount of marine salt. It can be seen that there are major differences in COD values, when measured with Hg and Hg-free COD vials (Post-hoc Scheffe test, \( P < 0.05 \)). To examine the statistical difference of the colorimetric method, three concentrations of salinities were tested, 27,156 mg Cl/L, 13,577 mg Cl/L and 6,788 mg Cl/L.
The mean difference between Hg and Hg-free COD vials at ratio (1:25) with mercury vials were 1,508, 1,541 and 1,509 mg/L, repetitively. While at ratio (1:50) the difference values were increased to 1,690, 1,757 and 1,857 mg/L, repetitively. Table (3-11) indicated that the mean of COD using Hg vials were reduced about (50 %) by reducing the mass of marine salt from (2.250, 1,125 and 0.560 g), further, Hg-free vials were reduced about (25 %).

The interference generated by oxidising various mass of chloride ions was investigated in this section. Based on Table (3-11) the mass of chloride ion was 0.543 mg Cl\(^{-}\) in both 2.25 % of marine salt with ratio 1:25 and 4.5 % of marine salt with ratio 1:50. With regard to dilution ratio, the mean of COD using mercury vials were 658 and 1,217 mg/l, respectively. This point shows that the COD values was increased about double by adding twice of marine salt.

The COD chemistry reviewed here applies to colorimetric methods, 18 samples were used for the correlation experiments. Figures (3-11), shows a significant linear correlation \(r^2 = 0.939\); Post-hoc Scheffe test, \(p <0.05\) between Hg and Hg-free COD vials for various concentration of artificial seawater such as 1.125, 2.250 and 4.500 % of marine salt at ratio 1:25.
Figure 3-11: Relationship between COD (mg/l) for various concentrations of artificial seawater (A) 4.500 %; (B) 2.250 % and (C) 1.125 %. Where, Hg Vial = - 1546 + 0.947 Hg-free, $r^2 = 0.939$, n= 6.

Based on Figure (3-11), the outcome indicated that the COD values obtained by using mercury COD test vials for artificial seawater 1.125 %, artificial seawater 2.250 % and artificially seawater 4.500 % were approximately 8.0, 3.3 and 2.5 times less, respectively, than those obtained using the mercury free vial. This suggests that the chlorides cause the most serious problem because of their normally high concentration in wastewater samples. This interference was eliminated by the addition of mercuric sulphate to the sample, prior to addition of other chemicals.
3.4.9.2 COD for seawater

Seawater obtained from Portsmouth seafront was used as the reference method to detect the COD values by using mercury and mercury free vials, as illustrated in Figure (3-12).

![Figure 3-12: Mean of COD for Portsmouth seawater measured by using mercury and mercury free COD vials at ratio (1:25) and (1:50) (error bars are indicative of the standard deviation of the mean (n = 3)).](image)

As can be seen from Figure (3-12), COD values using mercury vials at ratios (1:25) and (1:50) were 36 and 44 %, respectively, less than the generated result using mercury free vials. Moreover, the mean of COD values by using mercury vials at ratios (1:25) and (1:50) were 937 mg/l and 1,283 mg/l, respectively (Paired sample test, \( p < 0.05 \)). While, the mean of COD by mercury free vials at ratios (1:25) and (1:50) were 2608 mg/l and 2930 mg/l, respectively (Paired sample test, \( p < 0.05 \)).
Comparison of this study with the previous Section (3.4.9.1), the COD values at ratios (1:25) were reduced about 36 and 40% by using mercury vials for Portsmouth seawater (38,000 ppm) and artificial seawater (45,000 ppm). This highlights the fact that a lower ratio (1:25) and mercury vials are essential for the analysis of high COD at high salinity.

3.4.9.3 COD for glucose

The theoretical COD value of glucose can be determined using stoichiometric considerations. The theoretical COD of a compound with a structural formula C\textsubscript{x}H\textsubscript{y}O\textsubscript{z} can be calculated using the equations described below.

\[
\text{C}_6\text{H}_{12}\text{O}_6 + 6\text{O}_2 \rightarrow 6\text{CO}_2 + 6\text{H}_2\text{O}
\]

\[
\frac{\text{g of } \text{O}_2}{\text{g of glucose}} = \frac{6 \text{ mol}}{\text{1 mol}} \left(\frac{32 \text{ g}}{\text{mol}}\right) = \frac{1.067 \text{ g of } \text{O}_2}{\text{1 g of glucose}} = \frac{1.067 \frac{\text{mg}}{\text{l}} \text{ of } \text{O}_2}{\text{1 g of glucose}}
\]

The COD of 1 g/l of glucose

\[
\text{COD} = 1,000 \left(\frac{\text{mg}}{\text{l}} \text{ glucose}\right) \times \frac{1.067 \frac{\text{mg}}{\text{l}} \text{ of } \text{O}_2}{\frac{\text{mg}}{\text{l}} \text{ of glucose}} = 1,067 \text{ mg/l } \text{O}_2
\]

Glucose solution of 1 g/l was used to optimize the analysis parameters.

The COD values detected using the colorimetric procedures were correlated with those determined by the titration method. While, the titration dichromate method was used as the reference method to detect the COD values of the glucose, as shown in Figure (3-13).
This section examines the normality test of COD values obtained by using mercury vial, mercury free vials and titration method on glucose sample. The normality test was examined based on Shapiro & Wilk (1965) and Razali & Wah (2011). A Shapiro-Wilk’s ($p > 0.05$) and exam scores were approximately normal distributed for all conducted method. Another test was carried out to confirm the normality and to show whether samples data are fit or differ from a normal distribution. As such, a Skewness & kurtosis shows that the data is normally distributed. The outcomes were between (-1.96-1.96), for the mercury vial, mercury free vials and titration method.
method were 1.44 & 0.81, 1.55 & 0.94 and 0.66 & 0.24, respectively. It has been found that the mean of COD values for glucose sample using Hg vial, Hg-free vial and titration method were 1,060 mg/l, 1,054 mg/l, and 1,065 mg/l, respectively, see Figure (3-13). The outcome indicated that there was insignificant effect with the mercury free as compared to the mercury vial or titration method on the COD values for glucose sample. Another experiments were carried out on artificial seawater (4.5%) with glucose at ratios (1:25) using the colorimetric procedures. The glucose was used as the reference to detect the COD values, as shown in Figure (3-14).

Figure 3-14: Mean of COD values for A) artificial seawater; B) artificial seawater with glucose by using conventional mercury and mercury free COD test vials at ratio (1:25) (error bars are indicative of the standard deviation of the mean (n = 3)).
Based on Figure (3-14), the mean values of COD readings for Hg and Hg-free vials for artificial seawater at ratio 1:25 were 1,042 and 2,550 mg/l, respectively. The average of COD values for artificial seawater with glucose using Hg and Hg-free vials were increased about 1,171 and 992 mg/l, respectively, than artificial seawater samples, see Figure (3-14). These results suggest that increasing of COD values were reflected to the COD values of glucose.

3.4.9.4 COD of saponin.

The outcome demonstrated that the average of COD values for saponin (0.5 wt%) at ratio (1:25) by using mercury and mercury free, as shown in Figure (3-15).

![Figure 3-15: Mean of COD values for A) saponin (0.5 wt%); B) artificial seawater with saponin (0.5 wt%) by using mercury and mercury free COD test vials at ratio (1:25) (error bars are indicative of the standard deviation of the mean (n = 3)).](image-url)
Figure (3-15), illustrated that the COD values for saponin with distilled water using mercury and mercury free were 5,450 mg/l, and 5,430 mg/l, respectively (Paired sample test, \( p > 0.05 \)). As expected, there were no–significant different for saponin values when the solution is chloride-ion free.

Another investigation was carried out to compare between saponin (0.5 wt%) with distilled water and saponin (0.5 wt%) with artificial seawater (4.5%) using mercury and mercury free vials, see Figure (3-15). The average of COD values by using mercury and mercury free vials were increased about 1,058 mg/l and 2,550 mg/l, respectively, than saponin (0.5 wt%) with distilled water. The outcome of this study confirmed that the mean of COD values obtained in previous Section 3.4.9.3 for artificial seawater (4.5 %).

### 3.5 Heavy Metal Determination

#### 3.5.1 Introduction

During this project, a number of toxic metals have been found that their concentration levels are greater than the allowable discharge levels in oil contaminated sand and in oily wastewater. Therefore, it is a requirement to remove these heavy metals from these contaminated sand and wastewaters by using suitable treatment methods. National Cleaning Company (NCC), Kuwait, carried out the test for the concentration of metals and heavy metals in the oil contaminated sand, treated sand, oily wastewater sample, aerated water sample and treated water sample based on (USEPA, 1996) method number (SW-846, 6010B).
Seven heavy metals (Zn, Co, Cr, Mn, Pb, Fe and Ni), two metals (Ba and Al) and one non-metal (B) in the samples were determined by using inductively coupled plasma-optical emission spectrometry (ICP-OES) after digestion with a microwave digester (MARS. CEM, USA) following the manual instructions with the addition of nitric acid, hydrofluoric acid and hydrogen peroxide for sample digestion.

3.5.2 Method

A 0.40 g of tested sample with addition of 15 ml of mixture (nitric acid: hydrochloric acid: hydrogen peroxide) (9:1:1) were added into the reaction vessel. Prior to sealing, the vessel containing mixture was allowed to react for about 1 min. Vessels were placed in the rotor of the microwave to start the digestion process. The vessel were heated up to 180°C for 5.5 min and keep at 180°C for 9.5 min. After the digestion process completed, the samples were cooled down for 2 h, and then the digestates were filtered in a clean bottle prior to analysis. The filtered sample was diluted to a final volume of 50 ml using deionized water. The vessels were opened and vented in a fume hood and then introduced into the argon plasma by means of a glass nebulizer and glass cyclonic spray chamber. The concentration for the metal in solution was calculated based on wet weight:

$$R^*(mg/Kg) = \frac{(R * 50)}{sample \ weight} \ E. q(3 - 7)$$

Where, R is reading from Instrument (mg/l), 50 is the dilution factor ml, sample weight in gram and R* is final result. The concentrations of the metal contents in the water were measured without digestion process. 4 ml of water sample was transfer to ICP-OES Spectroscopy.
3.6 Engineering properties of treated sand

This research examines the consequence of treated sand (TPH<10,000 mg/kg) upon engineering properties of concrete such as compressive strength, slump test, water absorption test, and rebound hammer. In the slump test, six specimens were tested. For the compressive strength test, eighteen specimens were tested for 7, 14 and 28 days upon curing with another six replicates tested for absorption. For the rebound hammer eighteen specimens were used.

3.6.1 Soil properties
3.6.1.1 Permeability test

Constant head test BS 1377-5: 1990 (method 5). The permeability of a soil is a measure of its capacity to allow the flow of water through the pore spaces between solid particles. The degree of permeability is determined by applying a hydraulic pressure gradient in a sample of saturated soil and measuring the consequent rate of flow. The coefficient of permeability is expressed as a velocity.

3.6.1.2 Wet Sieve analysis

Mechanical sieve analysis was carried out by using sieve shaker (Retsch Sieve Shakers AS 200). A sieve analysis was used to determine the distribution of grain size of contaminated particles, the process was carried out by using six sieves with pores sizes of 3.35 – 3.00, 2.00, 1.60, 1.00, 0.15, 0.063 and pan. The procedure was performed by using tap water from a nozzle spray placed on the top sieve. 1 kg of contaminated sample was placed on the largest sieve size on top. Rinsing process was continued for approximately 5 min, until the sieving solutions become clear.
3.6.2 Materials and Method

The constituents that make up concrete blocks are cement, water, coarse aggregate and fine aggregate. The quantity of water is essential for the mix to allow proper chemical reaction of the cement as well as desired workability of the concrete. In this study, the designed mix was made of cement: fine aggregate: coarse aggregate at ratio of 1:1.5:4.5, respectively. Building Research Establishment (BRE) form was adapted in the concrete design (refer Appendix, B). Various materials were used to produce the concrete mix for this test (Figure 3-16), these materials are Ordinary Portland Cement (OPC), Kuwaiti washed oil sand, sand, coarse aggregate of 20 mm in size and water. Furthermore, control samples were used to confirm the test results.

![Figure 3-16: Raw materials for casting, A) Coarse aggregate, B) Fine aggregate, C) Clean Sand, and D) Treated sand.](image)

I. Cement

In this study, the cement used is Ordinary Portland Cement in accordance with BS EN 197-1:2011. In the presence of water, it has the attributes to perform as a binding agent. For all the mixes, OPC provided by Kuwait Cement Company was utilised.

II. Water

In order to facilitate concreting, clean potable water was used. Water assists in the cement hydration, which causes the concrete to set and harden (BS EN 1881: 2011).
III. Fine aggregate

The washed sand was collected after the washing process was completed. British Standard BS 1097-6:2013 was used to achieve complete dryness, as well as to ensure accurate water/cement ratio, the sample was dried in an oven at 110°C ±5°C for 48 h, and then cooled at room temperature for 2 h and noted the weight.

IV. Aggregates

The maximum size of coarse aggregate used is 20 mm is based on BRE design (see Appendix, B). Similarly, this aggregate was air dried to achieve saturated surface dry condition aimed to have accurate water/cement ratio.

V. Casting

Using a water/cement ratio of 0.48 (Table B-1, in Appendix, C), the concrete mix designed was produced based upon the guidelines of the BRE Method of Concrete Design (1997). In order to cast the concrete cubes, British Standard BS 1881-125:2013 was adopted. The design mix was prepared for a concrete compressive strength of 30 N/mm² at 28 days. The aim of this work is to investigate the effect of Kuwaiti oily sand upon the concrete compressive strength. In this study, eighteen 100 x 100 x 100 mm concrete cubes were made for compressive strength test and rebound hammer test, while six 150 x 150 x 150 mm were made for water absorption test, as presented in Figure (3-17). The mould was filled with concrete in three layers and manually compacted by means of 16 mm diameter metal rod, rammed at 25 strokes for each layer.
In the preparation of the cubes, a steel plate was placed at the top and bottom of the mould to create a flat uniformly distributed load on the surface of the block. After 24 hours from casting, the mold was dismantled and placed in a water tank for curing BS EN 12390-2 (2009).

3.6.3 Measurement of Workability of Treated Soil

Workability is the term used to describe a number of concrete characteristics related to the easiness to place concrete for instance cohesiveness, mobility, compatibility and finish ability. Example, concrete with low slump value is more appropriate compared with a high slump in a road construction. The appraisal of the Kuwait oil sand concrete’s workability is performed by:

3.6.3.1 Slump test

This test was performed on Kuwait oil sand with (TPH <10,000 mg/kg) samples in the concrete laboratory of the Civil Engineering Department, KU, all in compliance with the method described in BS EN 12350-2, 2009. As shown in Figure (3-18), the slump test was used to measure the concrete consistency.
3.6.3.2 Compressive strength and rebound hammer test

The aim is to investigate the effect of Kuwait oil sand upon the concrete compressive strength. For this experiment, eighteen 100 x 100 x 100 mm concrete cubes were made for compressive strength and rebound hammer test. The cube compressive strength can be computed by dividing the load at failure by the block’s total area. Based upon BS EN 12390-3: 2009, the observation on the strength shall be carried out on three concrete cubes after 7, 14 and 28 days upon curing, all in compliance with the method for rebound hammer test describes in BS EN 12504-2:2012.

3.6.4 Measurement of Durability of Treated Sand

A concrete is considered durable if it provides an acceptable performance within its intended life in a given environment; this covers provision of steel reinforcement protection against corrosion for both reinforced and pre-stressed concrete. There have been a number of durability issues concerning the constituent materials and others caused by the aggressive environment.
3.6.4.1 Water absorption test

The experiment was performed on six contaminated samples of 150 mm\(^3\) concrete cubes. The experiment based upon guidelines of BS 1881 Part 122 (2011), was conducted at the Civil Engineering Department, KU, Concrete and Material Laboratory.

3.7 Method Development for Extraction of Saponin

Any process design aims at finding the most cost-effective raw materials during the pilot and commercial scale. Surfactant being one of the most important raw material used in this research, it was worth acquiring it by extraction from natural source rather than purchasing the purest form which is very expensive. Therefore, this study focussed to reduce the surfactant cost by extracting the saponin (surfactant) from the soapnuts. The overall cost of the process can be reduced by choosing an alternative surfactant called saponin which has the same impact on removal of oil residue from sand.

It has been explained in Section 2.6.4.1 that the saponin obtained from (Fisher Scientific, UK) cost around £113 for 100 g. While, extracted saponin (less purity) cost around £1 for 1 kg. Hence it is necessary to characterise the extracted saponin based on its properties. The easiest way to characterize saponin is by using fourier transform infra-red spectroscopy (FTIR) where the characteristic absorption peaks are directly detectable in crude aqueous and 95 % ethanol extracts of soapnuts powder. While, the qualitative analyses were carried out to determine non-numerical variables about its chemical structure. During this study the qualitative analysis of extracted saponin was conducted using modern spectroscopic methods like ultra-fast liquid chromatography (UFLC) and Ultra performance liquid chromatography quadrupole-time of
flight-mass spectrometry (UFLC-QTOF-MS/MS), thereby eliminating the need of further expensive and exhaustive purification steps.

3.7.1 Materials
Dry fruits of *Sapindus mukorossi* (Lot# HAUS57/30185-1) commonly known as soapnuts were purchased from ISKA GmbH, Germany. Laboratory reagent grade standard saponin (from *Quillaja saponaria*) was purchased from Fisher Scientific, UK. Grade acetonitrile and milli-Q water were obtained from Sigma. Absolute ethanol was purchased from Aldrich, Tryptone soya agar (CM0131 from OXOID, UK). Defibrinated blood from sheep was used for the preparation of blood agar plate obtained from saudi prepared media laboratory (LTD).

3.7.2 Sample Preparation and Extraction of Saponins
The dark brown coloured soapnuts were sorted and outer pericarps were separated from the seeds manually. The soapnuts (500 g) were placed in a vacuum oven (Isotemp Vacuum Oven, Model 281A, Fisher Scientific, UK) at 60°C for 48 h and were ground into a fine powder by using a dry grinder (Stardust), and stored in airtight plastic containers. Dry soapnuts (*Sapindus mukorossi*) powder (50 g) was used for extraction with 95% ethanol at a ratio of 1:10 with 500 ml of extractant in erlenmeyer flasks covered with aluminum foil to reduce evaporation. The suspension was agitated on a hotplate with magnetic stirrer (Thermo Nuova) for 3 hours at 45°C and centrifuged using Sorvall Lynx model 4,000, at 12,000 rpm for 30 min at 25°C. The supernatant obtained was carefully decanted into freeze dryer bottles (Labconco) and placed on a tray layered with aluminum foil. The pellet was collected and added to 500 ml of 95% ethanol to extract and centrifuge at same conditions as described previously. The extracts were
frozen at -80°C for 16-24 h and loaded onto a freeze dryer (Labconco Freezone 18 Freeze dry/Shell Freeze system). The freeze dried sample was then stored in airtight bottles at room temperature. The phytochemical tests on the extracts were carried out according to standard methods as described by Tewari et al. (2011) and Sotheeswaran (1988).

3.7.3 Phytochemical Tests for Saponins
The phytochemical tests on the extracts were carried out according to standard methods as described by Tewari et al (2011) and Sotheeswaran (1988). The extracts of soapnuts were screened for saponins along with controls by phytochemical tests like foam test and hemolysis test.

3.7.3.1 Foam test
Froth forming capacity of saponin was demonstrated by foam test. The samples containing saponin when shaken with water, reduce the surface tension of water and produce a honeycomb froth stable for more than ten minutes. 200 mg of the extracts, standard and water were thoroughly shaken with 2 ml of water and left undisturbed for ten minutes. The persistence of foam produced was observed after 10 min. Samples were screened for saponins by making use of this characteristic property of saponins.

3.7.3.2 Haemolysis Test
All saponins can hemolyze blood, that is, they break down the red blood cells (Fong et al., 1977). This property is used in the screening test for saponin by application of the saponin containing sample on an agar base containing blood.
I. Preparation of Blood Agar Plates

Standard conical flask of 250 ml was used for preparation of solid media. The flask was filled with 100 ml distilled water and 4 g of Tryptone Soya agar powder. The conical flask was placed on the hotplate stirrer (CB302), to boil the solution to dissolve completely. Then, the mixture was sterilized using Autoclave (TOMY SX-500) at 121°C for 15 minutes. The solution was cooled to 45°C, and then 7% of the sheep blood was pipetted carefully into the Petri dishes to avoid bubbles to the extent possible. Subsequently, allowed to solidify at room temperature for 20 minutes and 10 ml of nutrient agar was drooped in to petri dish.

II. Application of Samples

Four wells 4mm deep were cut on the solidified agar equidistant from each other using a sterile well cutter. 120ul each of the aqueous extract, 95% ethanolic extract, standard Quillaja saponin as positive control and sterile water as negative control were carefully dispensed into the wells and kept covered for 24 hours at ambient temperature. Agar Petri Dish was observed for zone of clearance, also the distance from the farthest point of hemolysis to the edge of the well was measured and recorded.

3.7.4 The Qualitative Analysis of Saponin

The qualitative analysis of saponins were also done with the help of modern spectroscopic methods like UFLC and UPLC-QTOF-MS/MS to support this study.
3.7.4.1 Fourier Transform Infra-Red Spectroscopy (FTIR).

Dry powder of the extract obtained was mixed with KBr salt, using a mortar and pestle, and compressed into a thin pellet. Infrared spectra were recorded as KBr pellets on a Jasco Vacuum FT/IR 6300 between 4000–500 cm$^{-1}$. Infrared absorptions were recorded for the direct detection of triterpenoid saponins as described in Kareru et al. (2008).

3.7.4.2 Ultra-Fast Liquid Chromatography (UFLC)

The separation of saponins were achieved using a Shimadzu UFLC system equipped with PDA detector on a Shimpack ODS C$_{18}$ column (50 x 3mm id, 2.2µm). The mobile phase consisted of water (A) and acetonitrile (B). Gradient elution was performed as follows: 5-100% B in 8min, at a flow rate of 2 ml/min by monitoring the eluent at 206 nm. The injection volume and column temperature was 40ul and 25ºC respectively.

3.7.4.3 Ultra Performance Liquid Chromatography Quadrupole-Time of Flight-Mass Spectrometry (UPLC-QTOF-MS/MS)

All mass spectrum of the soapnuts extracts and standard Quillaja saponin were conducted on a Xevo G2-S QTOF LC-MS micromass spectrometer from Waters. An Acquity UPLC class I system from Waters was operated at room temperature with a BEH C$_{18}$ column (50 x 2.1mm id, 1.7 µm). The mobile phase consisted of water (A) and acetonitrile (B) was used at a flow rate of 0.6 ml/min under gradient program 40–60% B in 30 min. The mass spectral identification of saponins was established by monitoring the ESI spectrum in positive mode.
4 Chapter 4 Method Development for Remediation of Kuwaiti Oil Lakes

4.1 Introduction

In order to achieve high washing efficiency with reduced volume of solution and less environmental impact, the use of a saponin solution under the optimum parameters appear to be the best option. This study aims to select the optimum parameters to remove oil residues from Kuwaiti soil. Further, it provides a better understanding of the interaction between optimum parameters and various washing technique, in order to recognize how hydrocarbons behave. Untreated oily wastewater contains toxic compounds that might be mutagenic or carcinogenic. Therefore, the immediate removal of hydrocarbon compounds from oily wastewater is considered as essential issue to protect both the human health and environment. Different experiments were driven in this chapter to remediate, eliminate or reduce the impact of high toxicity level.

4.2 Development of Soil Washing Method

Experiments were conducted in laboratory conditions to determine the optimum washing parameters, and then investigate the selected parameters under different washing technique. The summary of the experimental parameters are listed in Table (4-1). A number of soil washing trials were performed, this included mechanical stirrer, ultrasonication, combined processes of ultrasonication with mechanical stirrer and combined processes of vibrating machine with mechanical stirrer to determine the best soil washing mechanism. Each washing experiment was repeated six times to confirm the data obtained. At the end of each washing, the residue was left to settle down for one hour and then the supernatant liquid was decanted into amber jar bottles.
<table>
<thead>
<tr>
<th>Parameters number</th>
<th>Water types</th>
<th>Washing times (min)</th>
<th>Stirrer speeds (rpm)</th>
<th>Washing temperature (°C)</th>
<th>Washing ratios (mass: volume)</th>
<th>Surfactant concentration (wt%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1) Parameters (Water types)</td>
<td>1) Distilled water 2) Tap water 3) Artificial seawater</td>
<td>20</td>
<td>1000</td>
<td>70</td>
<td>1:6</td>
<td>0.001</td>
</tr>
<tr>
<td>(2) Parameters (times)</td>
<td>Artificial seawater</td>
<td>1) 10 2) 20 3) 30</td>
<td>1000</td>
<td>70</td>
<td>1:6</td>
<td>1</td>
</tr>
<tr>
<td>(3) Parameters (speeds)</td>
<td>Artificial seawater</td>
<td>20</td>
<td>1) 500 2) 1000 3) 1500</td>
<td>70</td>
<td>1:6</td>
<td>1</td>
</tr>
<tr>
<td>(4) Parameters (temperature)</td>
<td>Artificial seawater</td>
<td>20</td>
<td>1000</td>
<td>1) 20 2) 50 3) 70</td>
<td>1:6</td>
<td>1</td>
</tr>
<tr>
<td>(5) Parameters (ratios)</td>
<td>Artificial seawater</td>
<td>20</td>
<td>1000</td>
<td>50</td>
<td>1) 1:3 2) 1:6</td>
<td>1</td>
</tr>
<tr>
<td>(6) Parameters (concentration)</td>
<td>Artificial seawater</td>
<td>20</td>
<td>1000</td>
<td>50</td>
<td>1:3</td>
<td>1) 0.001 2) 0.01 3) 0.5 4) 1 5) 2</td>
</tr>
<tr>
<td>(7) Optimum Parameters</td>
<td>Artificial seawater</td>
<td>20</td>
<td>1000</td>
<td>50</td>
<td>1:3</td>
<td>0.5</td>
</tr>
</tbody>
</table>
4.2.1 Method 1, Mechanical Stirrer:
The soil washing test was carried out mixing 50 g of the oil contaminated soil and 150 ml of saponin in a 250 ml glass beaker. The beaker was placed in a water bath and mixed with a mechanical stirrer (CP Cole-Parmer) under the optimum parameters (Section 4.2); see Figure (4-1).

Figure 4-1: Method 1, soil washing by mechanical stirrer and water bath.

4.2.2 Method 2, Ultrasonication:
For the second trial US Portable ultrasonication bath was utilised. The ultrasonication bath had a cylindrical steel container with an effective volume of 1 l, equipped with an ultrasound generator and a temperature control device. Test was conducted by washing Kuwaiti oil contaminated soil with saponin solution in a 250 ml plastic beaker.
The beaker was placed into an ultrasonication bath (power about 60 W and ultrasonication frequency of 45 kHz), while the washing experiment were conducted under the selected parameters, as mentioned in Section 4.2.

![Figure 4-2: Method 2, soil washing by Ultrasonication.](image)

4.2.3 Method 3, Combination of Mechanical Stirrer and Ultrasonication:

In this trial, a combination of ultrasonication and mechanical stirrer (CP Cole-Parmer) was used as shown in Figure (4-3). Test was carried out by sonified and mixed the Kuwaiti oil contaminated soil with saponin solution under the optimum parameters (Section 4.2).
4.2.4 Method 4, Combination of Mechanical Vibration and Stirrer:
In this trial mechanically vibration Octagon 200 and mechanical stirrer (CP Cole-parmer) were combined at the same time, as shown in Figure (4-4). Washing experiment was conducted by shaking and mixing the Kuwaiti oil contaminated soil with saponin solution in a 200 ml stainless steel flask. The flask was placed into a mechanical vibration (power about 120 W and frequency of 50 Hz) under the same optimum parameters (Section 4.2).
4.3 Materials Required for Oily Wastewater Treatment

4.3.1 Oily Wastewater Sample
Specimens of oily wastewater were taken from washing of Kuwaiti oil sand, as explained previously in Section 4.2. All samples were placed in 25 l amber jar bottles and retained in a refrigerator with a temperature of 4°C.

4.3.2 Seeds Inoculation
Most wastewater samples obtained from the municipal treatment plants have adequate minerals, trace elements and nutrients to degrade the carbon compounds at optimum level. Therefore, these samples can normally be used straight away as the measurement solution. During the bio-treatment “anoxic and aeration”, prime settled sludge (PSS) was utilised as the amendment substance.
4.3.3 Coagulation
Ferric chloride (FeCl₃·6H₂O) and aluminum sulfate hydrate (Al₂(SO₄)₃·16H₂O) were used as coagulants in the coagulation/flocculation process to treat oily wastewater. The stock solutions of the ferric chloride and ‘alum’ were employed at a concentration of 1.000 M (54,000 mg/l) by Fe and 0.238 M (12,848 mg/l) by Al, respectively. The distilled water was used for preparation of stock solutions. Coagulants were supplied by Fisher Scientific, UK.

4.3.3.1 Preparation of coagulants
Two standard conical flasks of 100 ml were used separately for preparation process of the ferric chloride (FeCl₃·6H₂O) and aluminium sulfate hydrate (Al₂(SO₄)₃·16H₂O). The coagulants were prepared by using 50 ml of distilled water with 7.5 g of aluminium sulfate and 50 ml of distilled water with 13.5 g of the ferric chloride. The solution was on a hot plate magnetic stirrer (Fisher Scientific, UK) for 10 minutes at 45°C to allow the coagulants to be distributed and dissolved easily into the conical flask.

4.4 Treatment of Oily Wastewater
There are various technologies such as vacuum evaporation, flocculation, adsorbents, coagulation, centrifugal, deep bed filtration and flotation, membrane separation technology, combined technologies, advanced oxidation process, etc that can be used to treat oily wastewater. Trials were conducted by using four different methods, i.e. adsorption (Appendix, A), coagulation/flocculation, anoxic treatment, combination of anoxic with coagulation/flocculation, aeration, and combination of aeration with coagulation/flocculation. The advantages and disadvantages of individual treatment options and combine treatment are discussed to enhanced treatment of oily wastewater. The results of scientific experiments were determined by measuring COD and floc weight for a fixed period of time.
4.4.1 Coagulation and Flocculation Treatment

Coagulation and flocculation tests were performed in a magnetic stirrer (CB302). The performance of the ferric chloride and ‘alum’ was compared by adding various concentration of coagulant to 50 ml of oily wastewater. The stock solution of coagulants such as ferric chloride and ‘alum’ were prepared previously, as explained in Section 4.3.3.1. Then, the stock solution was diluted to determine the required coagulant dosage. The coagulation tests were carried out using seven glass beakers (50 ml), oily wastewater was completed to the graduated mark of 50 ml beaker by addition amount of coagulant dosage required. After the stock of coagulants dosage were prepared, then introduced to the oily wastewater samples followed by the coagulation tests with rapid stirrer at 700 rpm for 1 min to ensure that the wastewater and coagulant are well mixed. The speed of the mixing was then reduced to 50 rpm for 10 min and allowed to settle in 24 h. Upon settling, 1 ml of supernatant interface was extracted at predetermined distance of 20 mm beneath air-liquid interface with a syringe to measure COD, as explained in Section 3.4.7. Subsequently, the precipitate was separated by using a funnel filter assembled with a weighed filter paper, (Whatman1.2 µm membrane filters WCM). The weight of floc was determined, as explained Section 3.4.2.

4.4.2 Combination of Bio-Treatment and Coagulation/Flocculation Method

Oily wastewater seeded with microorganism was subjected aerobic and anoxic condition. Therefore, the coagulants dosage of ferric chloride and ‘alum’ is required to be empirically investigated, to select the appropriate coagulant. The industrial scale of aeration or anoxic treatment need has to be scaled down in order to simulate the conditions in laboratory scale. Therefore, the simulation methods were carried out by maintaining the primary function of the industrial scale. According to Mueller et al. (2002) and Krause et al. (2003) the main function of aeration treatment is to supply oxygen for microbial growth and biodegradation process.
However, switching the supplied air off provides the anoxic condition (Chai and Lie, 2008). In the present work (USEPA, 1989) has been applied to standardise the aeration systems. Also, the cultures were incubated under anoxic conditions at 37°C to a simulation anoxic treatment (USEPA, 2007).

4.4.2.1 Combination of anoxic and coagulation/flocculation treatment

The incubation of anoxic treatment process was carried out using six reagent amber bottles with volume of 50 ml. Oily wastewater was completed to the graduated mark of 50 ml bottle by addition of 5 ml of PSS. The amber bottles were covered with aluminum foil to reduce evaporation. Then, they were incubated in heratherm incubators (Thermo Scientific) at 37°C, as shown in Figure (4-5). 50 ml of seeded sample was conducted as explained in Section 4.4.1 after 3 and 5 days of incubation.

Figure 4-5: Lab scale study for oily wastewater by anoxic digestion.
4.4.2.2 Combination of aerobic and coagulation/flocculation treatment

The aeration treatment for oily wastewater was conducted in a 2000 ml of a measuring cylinder. The working cylinder was filled with oily wastewater (450 ml) with addition of PSS (50 ml) at 20°C, as shown in Figure (4-6). After 3 and 5 days of aeration process, 50 ml of aerated sample was employed as explained in Section 4.4.1. Three replicate were carried out each time to verify the replicates data. During this process, air was continuously supplied into the reactors by using compressor (model N810FT.18, KNF Neuberger U.K. Ltd) to provide aeration, acclimatisation and immobilisation for microorganisms. The aeration was measured during this process by using air velocity meter (model AVM501, Prosser scientific instruments, Ltd). It was controlled to 5.5 m/s (model VFB-80D-BV, Dwyer instruments international), to avoid the froth from bubbling out of the cylinder.

Figure 4-6: Lab scale study for oily wastewater and saponin solution by aerobic process.
5 Chapter 5 Result and Discussion

5.1 Operating Parameters of Washing Treatment

5.1.1 Introduction
The soil washing was conducted to remove or reduce, hydrocarbon compounds from Kuwait oil sand to an acceptable level (TPH <10,000 mg/kg). By using artificial seawater combined with the bio-surfactant (saponin) under the optimum washing parameters, the oil residues were transferred from the sand to the water. This produced large volumes of oily wastewater that required further treatment. There are two main sections in this chapter. The first section reports the results obtained from the washing sand. The results obtained from treatment of oily wastewater are discussed in the second section. In following sections, the results are separated into four topics, namely measurement of BOD, measurement of COD, engineering properties of treated sand and direct detection of saponins in soapnuts.

5.1.2 Soil Chemistry
Chemical properties were investigated by determining TPH, EC, pH and metal contents of Kuwaiti oil contaminated sand. The outcome demonstrated that the average value of TPH was 367,000 mg/kg (SD = 4,700, n=3). The present preliminary study shows a high average value of electrical conductivity (EC) (2,455 µS/cm) (SD = 440, n=3), the existence of ions could be caused by using seawater to extinguish fires from oil well. Moreover, the average of pH (7.9) for the contaminated sample was found slight higher than the permissible level (7.5) (KEPA, 2014). As illustrated in the Table (5-1), the results indicate the presence of some elements such as Ba, Cr, Fe, Ni, Pb, Cd and Ag.
Table 5-1: The concentration of contamination in Kuwait oil contaminated sand (n=3).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Average concentration of metals (mg/kg)</th>
<th>KEPA limit (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Barium (Ba)</td>
<td>0.78</td>
<td>10</td>
</tr>
<tr>
<td>Chromium (Cr)</td>
<td>0.52</td>
<td>5</td>
</tr>
<tr>
<td>Iron (Fe)</td>
<td>8.10</td>
<td>5</td>
</tr>
<tr>
<td>Nickel (Ni)</td>
<td>0.43</td>
<td>10</td>
</tr>
<tr>
<td>Lead (Pb)</td>
<td>0.70</td>
<td>5</td>
</tr>
<tr>
<td>Cadmium (Cd)</td>
<td>1.10</td>
<td>1</td>
</tr>
</tbody>
</table>

5.1.3 Selection of Washing Parameters

5.1.3.1 Water types

This study was focused on investigations of removed oil using distilled water, tap water and artificial seawater. Each washing experiment was repeated six times to confirm the data obtained. The results of the soil washing were determined under parameters 1 (Section 4.2) with method 1 (Section 4.2.1). The influence of ionic strength on the solubilisation of seawater for oil removal was investigated with changing water type. The electrical conductivity of distilled water, tap water and artificial seawater were 0, 642 and 60,800 µs/cm, respectively. As presented in Figure (5-1), all different types of soil washing method showed removal of oil residue.
This study demonstrated that the percentage of oil removal by distilled water, tap water and artificial seawater were significantly different at 28, 31 and 35 %, respectively (A one-way F (2, 15) = 76.1, Post-hoc Scheffe tests, $p = 0.00$). The Post-hoc Scheffe tests revealed statistically significant differences between oil removal by artificial seawater ($M = 116,974; SD = 3,774$) and tap water ($M = 104,963; SD = 3,154$), ($p = 0.04$). Also, there was significant different between oil removed by artificial seawater and distilled water ($M = 92,720; SD = 3,256$) ($p = 0.00$) as well. There was significant difference between distilled water and tap water ($p = 0.02$).

The results for the experiments on distilled water, tap water and artificial seawater, revealed that the artificial seawater was effective in removing oil from contaminated soil and has potential capability in enhancing oil solubilisation.
5.1.3.2 Washing times

The experiment to remove the oil was carried out at various times such as 10, 20 and 30 min. The washing process was conducted using parameters 2 (Section 4.2) with method 1 (Section 4.2.1). The percentage of oil removal significantly improved as the time increased from 10 min to 30 min as demonstrated in Figure (5-2), (A one-way ANOVA F (2, 15) = 41.6, Post-hoc Scheffe tests, p = 0.00).

![Graph showing mean oil residue after different washing times](image)

Figure 5-2: Mean of oil residue after different washing times. Note: The data are expressed as mean ± SD (n=6).

Post-hoc Scheffe tests revealed statistically significant differences between oil removal at 10 min (M = 168,314; SD = 10,475) and oil removed at 20 min (M = 223,840; SD = 8,963) (p = 0.00).
This improvement can be associated with the increased length of time of contact between the saponin and the soil sample. Therefore, the washing time was believed to correspond to the wetting of the soil with the saponin solutions. Urum et al. (2004) reported that the wettability of contaminated soil and contact angle between the soil and oil increase with washing time. However, there was no significant difference between 20 min and 30 min (M = 226,450; SD = 8,856) (Post-hoc Scheffe tests, \( p = 0.777 \)).

The outcome of this study shown that the percentage of oil removal was increased about 1 % after 20 min of soil washing time, this slight removal of oil residue could be attributed to the nature of weathered contaminated soil. It can be concluded, therefore, washing time of longer than 20 min will not have much effect on oil removal efficiency and therefore uneconomical.

5.1.3.3 Stirrer speeds

These studies were focused on investigations of removed oil at various stirrer speeds (500, 1000 and 1500 rpm). The results of the soil washing were determined by using parameters 3 (Section 4.2) with method 1 (Section 4.2.1). Figure (5-3), demonstrates that the mean of oil removal significantly increased as stirrer speed increased (ANOVA F (2, 15) = 78.6, Post-hoc Scheffe test, \( p = 0.00 \)).
The result of Post-hoc Scheffe test shown that the mean of oil removal significantly improved by increasing the stirrer speed from 500 rpm (M = 154,231; SD = 6,267) to 1000 rpm (M = 223,840; SD = 8,963) ($p = 0.00$) also, from 500 to 1500 rpm (M =227,135; SD = 7,454) ($p = 0.00$) the oil removal was significantly increased as well. Nevertheless, there were no significant differences between 1000 and 1500 rpm ($p = 0.869$). Furthermore, the outcome of this study demonstrated that the percentage of oil removed from soil at 500, 1000 and 1500 rpm were about 54, 67 and 68 %, respectively. It seems to be uneconomical to employ stirrer speed of more than 1000 rpm.

Figure 5-3: Mean of oil residue with various stirrer speeds of mixed solution. Note: The data are expressed as mean ± SD (n=6).
5.1.3.4 Washing temperature

These studies were focused on investigations of removed oil at various temperatures (20, 50 and 70°C). The results of the soil washing were determined by using parameters 4 (Section 4.2) with method 1 (Section 4.2.1), as shown in Figure (5-4).

![Bar graph showing mean oil residue with different washing temperatures.](image)

**Figure 5-4**: Mean of oil residue with different washing temperature. Note: The data are expressed as mean ± SD (n=6).

As presented in Figures (5-4), the mean of removal of oil significant improves by increasing the washing temperature (ANOVA F (2, 15) = 50.5, Post-hoc Scheffe test, \( p = 0.00 \)). At temperatures of 50°C and 70°C, the removal efficiency significantly increased by about 15% (Post-hoc Scheffe test, \( p = 0.03 \)), which could have possibly caused by the foam which was produced consistently during washing, as exhibited in Figure (5-5). The formation of foam enhances the removal of oil from the soil. Rather than the oil remains in the bulk surfactant phase in the form of liquid-liquid dispersion, the oil is transported by the foam resulting in the
formation of a gas-liquid phase as described in the study undertaken by Urum et al. (2004). This could minimize the amount of oil which may disperse on the bulk surfactant phase to form emulsions.

Figure 5-5: Washing oil contaminated soil at 70°C (1), 50°C (2) and 20°C (3).

During this study, the froth forming capacity generated at different temperature conditions were evaluated by foam test. It can be seen from the Figure (5-5) that the formation of foam for the flask (1) > flask (2) > flask (3), because the temperature plays a role in the foaming capacity of saponin. The outcome of this study demonstrated that the percentage of oil removed from soil at 20°C, 50°C and 70°C were about 43, 51 and 67 %, respectively. A lower percentage of removal was however noted at 20°C, this signifies that at this temperature, the weathered oil exhibits adsorptive behaviour with soil causing it to remain glued to the soil. However, it can be concluded that washing at 50°C will make a difference in terms of energy used and therefore, it is uneconomical to wash at 70°C. The froth test showed that the temperature improved foam stabilising properties between the air phase and water interface of the foam. Further, the formation of foam may also favour the removal of oil residue from a mechanical aspect that its easier to remove by simple design when the foam rises to the surface of the system.
5.1.3.5 Washing ratios

This study was conducted to determine the amount of oil removed from 50 g of Kuwait oil sand by altering the ratio of (mass: volume) of washing solutions from 1:6 to 1:3. The results of the soil washing were determined by using parameters 5 (Section 4.2) with method 1 (Section 4.2.1). According to Chu and Chan (2003), soil washing was carried out to clean contaminated soil with hydrophobic organic compounds. They found that the soil washing with a biosurfactant was able to achieve an effective optimum washing condition using a soil to solution ratio of 1:6 (mass/ volume). However, this study shows that decreasing the soil/solution ratio to 1:3 (mass/ volume) enhanced the percentage of oil removal about 17%, see Figure (5-6). It seems that the concentration of saponin was increased with decreasing the volume of solution.

![Figure 5-6: Mean of oil residue at various ratio (Mass/Volume). Note: The data are expressed as mean ± SD (n=6).](image)
The outcome shown that the mean of oil removal significantly increased by altering the ratio of soil/volume of the washing solution from 1:6 (M = 190,696; SD = 6,821) to 1:3 (M = 228,670; SD = 11,060) (Paired sample t-test, t = -7.8, df = 5, p = 0.01). Also, the volume of the wastewater produced after the washing processes complete was reduced to 50%.

As the weight of the oil contaminated soil was maintained, it can be seen that the improvement of oil removal was dependent on availability of surfactant micelles to stabilise the oil contaminants in the solution, also further surfactant monomers were being available to allow oil contaminants to be mobilised.

This study demonstrated that the rates of removal of oil residues from soil with ratios of soil/volume 1:6 and 1:3, were 51 and 67%, respectively. These experiments indicate clearly that the greatest rate of oil removal from samples was achieved with a ratio of 1:3 (mass/volume).

5.1.3.6 Surfactant concentrations

Soil washing was performed in an effort to determine the relation between surfactant concentration and washing efficiency. The washing process was conducted using parameters 6 (Section 4.2) with method 1 (Section 4.2.1) at various concentration 0.001, 0.1, 0.5, 1 and 2 wt% (see Figure (5-7)). It can be seen from the same figure that the decaying curves of TPH concentration with various concentration of saponin showed logarithmic curve trend. Therefore, adjustment was made to the data to demonstrate logarithmic curve thus the following equation:

\[
\text{Oil removal} \left(\frac{\text{mg}}{\text{kg}}\right) = 8,9491 \times \ln(\text{saponin (wt%)}) + 106,717 =\ldots\ldots\ldots\ldots\ldots E. q (5 - 1)
\]
This range of solution concentration understood to be able to effectively remove oil contamination from practical point of view. The result of proves that the percentage of oil removal from Kuwait oil residue specimens improved steadily by increasing the concentration from 0.001, 0.1, 0.5, 1 and 2 wt% were about 35, 44, 64, 67 and 69 %, respectively. As indicated in Figure (5-7), there were non-significant differences among the concentrations of 0.5 wt% (M = 217,173; SD = 11,597), 1 wt% (M = 228,670; SD = 11,060) and 2 wt% (M = 243,392, SD = 10,990), (ANOVA F (4, 24) = 201.77, Post-hoc Scheffe test, p = 0.828) in term of the oil removal from soil samples. Conversely, at concentration of 0.1 wt% (M = 147,170; SD = 12,427), the oil removal had significantly decreased (p = 0.00). Furthermore, at concentration of 0.001 wt% (M = 116,974; SD = 3,774), the oil removal had significantly decreased (Post-hoc Scheffe test, p = 0.004) as well.
These experiments provide a clear indication that there was no significant difference between 0.5 and 1 wt% however, it can be concluded that concentration of saponin at 0.5 wt% will make a difference in terms of money cost spent and therefore uneconomical to wash at 1 wt%.

5.1.3.7 Surface tension

The surface tension was measured during this study to select and evaluate the optimum concentration of saponin required to achieve high efficiency removal of the oil residue. The surface tension of the oily wastewater supernatants was measured at 20 °C as explained in Section (3.3.5) by a Du Nouy tensiometer. The CMC value was determined by plotting surfactant tension versus log of surfactant concentration. CMC was found at the intersection point on the slope where surface tension shows linear decline and baseline of minimal surface tension.

Based on the literature, the most effective removal of crude oil was registered at concentration below their CMC values, and does not exhibit any improvement in removal of the oil residue at higher concentration. In order to measure the surface tension, washing process was conducted using parameters 7 (Section 4.2) with method 1 (Section 4.2.1.) at various concentrations such as 0.001, 0.1, 0.5, 1, 2 and 3 wt%, as shown in Figure (5-8). This experiment was repeated three times for each concentration to check the reproducibility.
The outcome of Figure (5-8) illustrates that the surface tension test of 0.001, 0.1, 0.5, 1, 2 and 3 wt% for various concentrations of saponin solution were 53.2, 41.1, 37.2, 36.0, 35.0 and 35.5 mN/m, respectively. Furthermore, surface tension between air and oily wastewater was measured as 77.2 mN/m. Based upon findings, as the surface tension between oil residue and saponin solution is reduced, the attraction force holding the oil residue and sand is reduced as well. It seems that the reduction of surface tension demonstrates the ability of saponin to remove oil residue from sand.

The proportion of micelles present at the surface of a liquid depends on their concentration. As such, surfactants occupy the surface of the liquid at low concentrations, this study found that only slight change was detected in surface tension at low concentrations of saponin, however Figure (5-8) identified that additional saponin decreases surface tension. This suggests that the removal of crude oil was caused by mobilization that took place due to the drop in the surface
tension. As such, the solution of saponin is introduced, the surface tension decreases until the value of is achieved at concentration of 0.5 wt% and remains constant with slight change after this point. CMC was found to be 0.5 wt%, therefore this concentration was chosen to carry out further experiments during this research.

5.1.3.8 Effect of ionic strength

Ionic strength was calculated to investigate its influence on the removal of oil residue by using artificial seawater and distilled water in the presence of saponin (0.5 wt%). The washing process was conducted using parameters 7 (Section 4.2) with method 1 (Section 4.2.1.). The results of this study presented in Figure (5-9).

![Figure 5-9: Mean of oil residue with different water types. Note: The data are expressed as mean ± SD (n = 6).]
Paired sample t-test was conducted to examine whether there was a noticeable difference between artificial seawater and distilled water in relation to their oil removal efficiency. The test revealed that the mean of oil removal significantly improved by using artificial seawater (M = 217,173; SD = 11,597) than by using distilled water (M = 185,252; SD = 13,241) (t = -3.631, df = 5, p < 0.015). As a result, the efficiency of oil removal was enhanced about 15% by using artificial seawater prepared with 0.5 wt % saponin than distilled water with 0.5 wt% saponin.

Comparing this Figure (5-9) against Figure (5-1), the efficiency of the removal of oil residue using artificial seawater without saponin and artificial seawater with saponin were 35.0 and 64.0 %, respectively. High concentrations of electrolytes in sea water were calculated in Section (3.4.8.4). This experiment calculated the concentrations of Na+ and Cl− in artificial seawater were 17,844 mg Na+/l and 27mg Cl−/l, respectively. This point suggests that the removal of oil residue can be enhanced by the addition of electrolytes to oily wastewater which eases the electrostatic repulsion of the saponin. Moreover, this is due to the electrostatic repulsion of the surfactant ions which may result in oriented aggregation.

The outcome is in agreement with Zhang et al. (1996) that the electronic repulsion of the surfactant ions reduced in the presence of salt, as a result of increased counter ions concentration thereby reducing the CMC of the ionic surfactants and improving their aggregation number. Another study carried out by Bai et al. (1998) debated that as soon as electrolytes such as Na+ is introduced to a bio-surfactant solution, the Na+ attracts the negative charge of the surfactant head group, which will then reduce the electrostatic repulsion between the surfactant head group. As such, the effective head groups will reduce the CMC causing a rise in both number of the micelles as well as their overall internal volume.
5.1.3.9 The Efficiency of mechanical stirrer, ultrasonication and combined technique

In order to determine the effective washing method, several techniques were carried out such as method 1 (Section 4.2.1) method 2 (Section 4.2.2) and method 3 (Section 4.2.3) with parameters 7 (Section 4.2), as shown in Figure (5-10).

![Figure 5-10: Mean of oil residue with various methods at different phases. Note: (A) Mechanical stirrer (B) Ultrasonication, and (C) Ultrasonication and mechanical stirrer. The data are expressed as mean ± SD (n=6) (**) No-significant.](image)

It can be seen from Figure (5-10) that the most effective method for removal of oil from Kuwaiti contaminated sand was as follows: (1) combined techniques of ultrasonication and mechanical stirrer; (2) ultrasonication (3) mechanical stirrer (ANOVA F (8, 45) = 77, Post-hoc Scheffe test, \( p = 0.00 \)). Also, in the same figure, the mean of oil removal was significantly increased by increasing the number of washes. The percentage of oil removal by mechanical stirrer and ultrasonication after the third wash were 75 and 80 %, respectively.
However, a combination technique was able to remove about 90% of the oil residue after the third wash. The combined technique speeded up the chemical and physical reaction time. Therefore, combining the ultrasonication (bath) with the mechanical stirrer is more effective than the individual method. In order to improve the removal efficiency of the combined technique of ultrasonication and mechanical stirrer technique to 96%, it requires the oil-contaminated sand to be washed four times as shown in Figure (5-11). It was observed that washing of 5 times or more is not advisable as the amount of oil removed by the fifth and sixth washing was merely 1 and 2%, respectively. As such, the fourth wash is required only when more than 5% of the crude oil remains in the soil, while the (TPH) in the soil was found less than the acceptable level which is 10,000 mg/kg (<1%) (KEPA, 2012).

Figure 5-11: The percentage removal of oil residue after several washing phases. The data are expressed as mean ± SD (n=6).
5.1.3.10 Washing with Combined Technique of Mechanical Vibration and Stirrer

In order to enhance the effective washing method, other techniques were carried out using parameters 7 (Section 4.2) with method 4 (Section 4.2.4) as illustrated in Table (5-2).

Table 5-2: The percentage removal of oil residue with combination of mechanical vibration and stirrer methods (n=6).

<table>
<thead>
<tr>
<th>Phase</th>
<th>Concentration of oil residue before (mg/kg)</th>
<th>Concentration of oil residue after (mg/kg)</th>
<th>Concentration of removed oil (mg/kg)</th>
<th>Standard deviation (mg/kg)</th>
<th>Removed oil (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st phase</td>
<td>337,000</td>
<td>187,000</td>
<td>150,000</td>
<td>18,000</td>
<td>44.5</td>
</tr>
<tr>
<td>2nd phase</td>
<td>187,000</td>
<td>160,000</td>
<td>27,000</td>
<td>3,000</td>
<td>51.5</td>
</tr>
<tr>
<td>3rd phase</td>
<td>160,000</td>
<td>138,000</td>
<td>22,000</td>
<td>3,000</td>
<td>59.0</td>
</tr>
<tr>
<td>4th phase</td>
<td>138,000</td>
<td>122,000</td>
<td>16,000</td>
<td>2,000</td>
<td>64.0</td>
</tr>
<tr>
<td>5th phase</td>
<td>122,000</td>
<td>115,000</td>
<td>7,000</td>
<td>1,000</td>
<td>66.0</td>
</tr>
<tr>
<td>6th phase</td>
<td>115,000</td>
<td>110,000</td>
<td>5,000</td>
<td>1,000</td>
<td>68.5</td>
</tr>
</tbody>
</table>

Based on previous Table (5-2), the percentage of oil removal increased as washing number increased. The outcome presented that the highest removal efficiency was achieved after sixth wash 67%. The percentage of oil removal by combination of mechanical stirrer and mechanical vibration after the third, fourth and fifth washes were 59, 64 and 66 % (ANOVA $F(5, 30) = 107$, Post-hoc Scheffe test, $p = 0.782$), respectively. Nevertheless, the efficiency of a combination technique was limited, since temperature of artificial seawater was difficult to be control. As such, the water temperature at the end of each washing reached 25°C. That means the temperature plays significant role in reducing the viscosity of oil residue. Therefore, the reactor (shown previously in Figure 4-4) needs to be improved if its need to be used. In order to improve the removal efficiency of the combined technique it requires the oil-contaminated sand to be washed further by combined technique.
5.1.3.11 Initial concentration and particle size distribution

Oil contaminated sand was subjected to sieve analysis (Section 3.3.7). Each fraction of the contaminated soil which belong to the different particle size distribution were washed using parameters 7 (Section 4.2) with method 1 (Section 4.2.1.).

As exhibited in Figure (5-12), higher efficiency of removal was apparent with higher initial oil residue concentration. As anticipated, larger size of oil contaminated sand particles demonstrated better removal efficiency. These phenomena may be attributed to the interaction of the oil residue and the solid surfaces of grains. The bonding force between the oil residue and the sand surface outweighs the bond at the oil residue phases. The existing surface area and capillary force are higher with smaller sized particles and lower concentration of oil residue correspondingly.

The interaction between oil residue and the surface of sand particle gets weaker with thicker oil residue layer at higher concentration. As a result, the oil residue can be removed and desorbed easily at a higher concentration, while low initial concentration with reduced size of sand particles exhibited low removal efficiencies. As such, the removal of oil residue from weathered soil specimens was improved for soils with large fractions.
Figure 5-12: Effect of particle size on the percentage of oil removal (n=3).

The mean percentage of oil removal from different particles size (0.60, 2.38 and 5.66 mm) were about 53 % (SD = 2.5), 65 % (SD = 2.1) and 78 % (SD = 4.4) (Post-hoc Scheffe test, \(p < 0.05\), respectively. Soils with large oil residue fractions possess minimal initial content of oil, as such it may be quicker for them to remove the oil residue under the selected parameters in comparison with samples with finer soils. As their pores contain larger surface area with lesser oil retention, it can be estimated that the angle of contact between the soil and oil would increase, as the contaminated soil is wetted. This point suggested that the saponin solution plays a significant role by breaking through the interfaces between soil surface and oil residue.
5.1.3.12 Sand Grain Cover

Images obtained by SEM were used to investigate the interaction between oil and sand particular and also to assess the removal of agglomeration by using various soil washing w. This analysis was conducted to soil particles before using soil remediation techniques, Figure (5-13), and after using ultrasonication, mechanical stirrer and combined of mechanical stirrer and ultrasonication, Figure (5-14).

Figure 5-13: SEM images showing the surfaces of contaminated soil particles before washing at 90× magnification.
Figure 5-14: SEM images showing the surfaces of washed soil particles prepared after third washed at 25× magnification (left-side images) and at 55 to 90× magnification (right-side images) (A) mechanical agitation (B), ultrasonic (C) and combined technique (D).

As shown, after the third wash, it is apparent that the efficiency of removal is higher in the combined soil washing, while the soil particles were adequately broken up in the water and the effectiveness of the procedure was evident after the third wash. Nevertheless, insignificant difference was noted in the SEM images between the individual ultrasonication and mechanical washing, however the dispersion of soil particles in the water was not as good during the procedure which resulted in inefficient removal.
Ultrasonication treatment aimed to use the reflection of sound by radiation pressure to disturb particulate structure of oil residue, so the consistency of water and oil residue were enhanced by physical impact of sound waves during the process. This point leads to propose that the mechanical stirring enhances the ultrasonication exposure to the soil particles by agitating the soil matrix to aid the dispersion of oil and soil.

This improves contaminant desorption from the particle surface and effectively enhances the ultrasonication removal efficiency by generating internal air bubbles that can reach the surface of the sand. This point leads to high frequency of ultrasonic waves (45 kHz) produced cavitation by alternating high and low pressure waves, these bubbles grown in low pressure phase from micro-bubbles until they formed and compressed at high pressure phase. Consequently, the bubbles collapse to split the oil from fine particles with contaminants from larger parent constituents, as such homogenisation was occurred by breakup of agglomerates of oil residue from sand as shown in Figure (5-14).

Further, the damage to the surface of soil particles may be due to the sonophysical effects which can improve the effectiveness of contaminant removal from the sand. It has been noticed that the water temperature was maintained at 50°C during the washing process. According to Sandilya and Kannan (2010) cavitation was able to keep the water temperature during the process by releasing tremendous energy.
5.1.3.13 Oil agglomerations

This research aimed to investigate the influence of the sequential washing on the size of agglomeration. Washing process was carried out using parameters 7 (Section 4.2) with method 3 (Section 4.2.3). Some of the isolated oil residue was formed as oil agglomerates after the washing process. These agglomerates were found at the surface of the washed sand after decanting the supernatant of the first wash. This study demonstrated that the size of oil agglomerate was significantly increased by increasing number of washing cycles, Figure (5-15). When the oil contaminated soil was washed for a number of times, the size of oil agglomerates could be increased by increasing interparticle contact between oil dispersion and saponin solution.

![Figure 5-15: Measurement image for oil agglomeration after the first wash (A) second wash (B) third wash (C) and fourth wash (D) (n=5).](image)

The outcome demonstrated that the measurements of oil agglomeration after the first wash generated smaller units (M = 9.50; SD = 1.70) than those generated after the second wash (M = 28.60; SD = 4.15), paired test, \( p < 0.05 \). Further, paired comparison analysis revealed that the oil agglomeration after the third (M = 47.20; SD = 5.26) were significantly smaller than those obtained after the fourth wash (M = 110.80; SD = 12.67), Paired test, \( p < 0.05 \). This study suggest that the the extent of agglomeration depends on the amount of oil residue in the contaminated sand. High amount of oil residue leads to the formation of strong agglomerates, which are easy to recover. The oil agglomerations can be separated from the non-agglomeration particles by using high stirring speed to disperse oil residue, saponin solution and oil contaminated sand to provide sufficient number of collisions among them.
5.1.3.14 Removal of heavy metals

In order to determine the effective washing method, parameters 7 (Section 4.2) with method 3 (Section 4.2.3) were carried out to determine the heavy metal removal. During the current research work, heavy metal were determined as explained in Section (3.5). Analysis of trace heavy metals in the washed wastewater sample is shown in Table 5-3.

Table 5-3: The average concentration of metals residue during the washing of Kuwait oil sand (n=3).

<table>
<thead>
<tr>
<th>Metal contents</th>
<th>Before wash (mg/kg)</th>
<th>1st wash (mg/kg)</th>
<th>2nd wash (mg/kg)</th>
<th>3rd wash (mg/kg)</th>
<th>4th wash (mg/kg)</th>
<th>KEPA limit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Barium (Ba)</td>
<td>9.78</td>
<td>0.28</td>
<td>0.26</td>
<td>0.025</td>
<td>0.15</td>
<td>10</td>
</tr>
<tr>
<td>Chromium (Cr)</td>
<td>19.52</td>
<td>0.06</td>
<td>0.05</td>
<td>0.04</td>
<td>0.04</td>
<td>5</td>
</tr>
<tr>
<td>Iron (Fe)</td>
<td>26.10</td>
<td>5.20</td>
<td>5.10</td>
<td>4.35</td>
<td>4.30</td>
<td>5</td>
</tr>
<tr>
<td>Nickel (Ni)</td>
<td>1.53</td>
<td>0.08</td>
<td>0.07</td>
<td>0.07</td>
<td>0.04</td>
<td>10</td>
</tr>
<tr>
<td>Lead (Pb)</td>
<td>1.10</td>
<td>0.06</td>
<td>0.05</td>
<td>0.05</td>
<td>0.03</td>
<td>5</td>
</tr>
<tr>
<td>Cadmium (Cd)</td>
<td>1.10</td>
<td>0.05</td>
<td>0.03</td>
<td>0.03</td>
<td>0.02</td>
<td>1</td>
</tr>
</tbody>
</table>

The outcome of this investigation indicates that most of the detected metals were reduced to insignificant level after the second wash. However, iron seems to be one of the most strongly bound to the soil particles of the seven metals in this study. The result shown that the Fe was slightly higher than the acceptable level (Fe= 5.10 > 5.00) after the second wash. The remaining elements of the metals were considered to be strongly bound within the soil matrix. This research shows that the complexation of metals residue with saponin solution may affect the metal removal. However, the removal of metals from the contaminated soil can be influenced by another chemistry factors such as pH, EC, ion exchange capacity and adsorption (Sposito,
1989; Evans, 1989). Studies conducted by Mulligan et al. (1999) and Hong et al. (2002) revealed that heavy metal for instance, Cu, Pb and Zn were retained because of some complexation with carboxyl within saponin, therefore a stronger bond between the metals and saponin must be the key reason for metal desorption from the soil sample. This indicates that desorption mechanism by saponin is not only due to exchange of ion or micelle function. On the whole, heavy metals co-exist in contaminated soils and complexation of heavy metal with saponin micelles may disturb the solubilisation and elimination of hydrocarbon matters at low saponin concentration. However, by increasing the saponin concentration level, the quantity of micelles correspondingly increased with further collisions taking place with the micelles causing a weaker collaboration between the metal and the micelles. Nevertheless as there appeared to be some attraction between metals and the organic compound as reported by Mulligan et al. (1999), there is a probability that the micelles’ structure is not a seamless sphere but has bent surfactant monomers. As such, this kind of structure could facilitate metal ion entrapment which may inhibit the metal from re-adsorbing onto the soil surface leading to the solubilisation of heavy metals at high saponin concentration (Zhanga et al., 2010).

5.1.4 Discussion
The selected parameters play a significant role in enhancing the washing efficiency. The soil washing parameters were investigated to determine the optimum parameters by using mechanical stirrer only. Then, selecting the effective washing technique among mechanical stirrer, ultrasonication, combination of ultrasonication and mechanical stirrer and combination of mechanical vibration and stirrer. It was found that the critical parameters on the Kuwait oil sand were as follows: using artificial seawater; 2) washing time (20 min); 3) stirrer speed (1,000 rpm); 4) temperature (50°C) and 5) soil/solution ratio (1:3).
5.1.4.1 Optimum parameters

Several key parameters were discussed in this research to select the optimum parameters. The solubilization capabilities of surfactant for petroleum hydrocarbon compounds were tested by Rosen (1989) and Paria (2008). They reported that the order of solubilising capabilities for organic surfactants with similar hydrophobic chain length are; 1) nonionics; 2) cationics; and 3) anionics. Other environmental factors such as ionic concentration, pH, water temperature etc, could be also effect on the solubilisation of the surfactant for organic compound.

A recent study carried out by Zhou et al. (2011) showed that the concentration of saponin can increase or decrease by changing ionic strength of the solution, which allow the petroleum hydrocarbon compounds to be distributed into the micelle of saponin. They concluded that increasing the electrical conductivity could increase separation of oil residue into saponin micelle. Moreover, the results of this research shows that the removal efficiency of oil residue from oil contaminated sand using saponin with seawater was about 15% more than that of saponin with distilled water. This research suggests that the solubilisation effectiveness of saponin for removal of oil residue increased with the increasing electrical conductivity (ionic strength).

The washing time and stirrer speed play a vital role in improving the abrasion between the particles of contaminated soil and the washing strength of the solution. It was found by Bernardez and Ghoshal (2008) that increasing the stirrer speed accelerates the diffusion of the desorbed contaminants and their reaction with the surfactant micelles within the solution, which accelerated the rate of solubilisation. The results of soil washing tests revealed that the elimination of oil residue by increasing the length of washing time or the stirrer speed was
merely 1%. Similarity, increasing the length of washing time or the stirrer speed has no noticeable improvement in the removal of hydrocarbon compounds.

Amongst all the tested parameters, temperature exhibits a direct relationship with the oil physical properties namely density and viscosity. According to Urum et al. (2004) a rise in temperature would reduce the crude oil viscosity resulting in improved oil mobility. Taking into account the broad range of temperatures tested and the oil viscosity, it can be concluded that viscosity plays an important part in the removal of oil from contaminated soil.

Reducing the oil viscosity will activate the oil, forming a good environment for its isolation from the soil. Research shows using surfactants with hot seawater has the advantages of removing crude oil from polluted soils (Urum et al., 2004; Paria, 2008; Han et al., 2009). The outcome shows linear increase pattern against the mean of oil removal. The result from the tests showed that the percentage of hydrocarbon compounds removal at 20, 50 and 70°C were in the excess of 43, 54 and 65%, respectively.

The present work found that the efficiency of oil removal increased as water temperature increased. It seems that the attraction force between the oil and the soil was reduced at high water temperature. In addition, the mobility of oil residue at various water temperature may be due to the foam produced during the washing process. However, the lower efficiency of oil removal was obtained at 20°C. As indicated in Section 1.9.2, the Kuwaiti oil contaminated sand has been weathered in an open environment over 25 years, these results suggest that the oil residue exhibits an adhesive strength with Kuwaiti sand. Therefore, oil residue remains strongly attached with sand particles.
The washing performance is also reliant upon the ratio of soil: surfactant solution. Saponin was used by Pekdemir et al. (1999) and Urum and Pekdemir (2004) to eliminate oil from soil at various ratios (w/v). They concluded that a ratio of 1:3 was able to achieve the most efficient removal process. From the present project work also, the highest efficiency of oil removed was achieved at volume/mass ratios of 1:3. The outcome illustrates that about 64 % of the oil residue was successfully removed from contaminated soil using saponin solution at ratio 1:3. The ratio of soil to saponin solution demonstrates results equally as encouraging as those of the concentration. In comparison a much higher elimination performance of between 68 – 64 % was obtained for 2.0 wt% at ratio 1:6 and 0.5 wt% at ratio 1:3, respectively.

The sample from the Kuwait oil residue is highly hydrophobic. As such, the saponin solution can lower the hydrophobicity of the oil phase to the point where it is wetted, then allow the oil to disengage itself from the soil surfaces. Based on Figures 5-7 and 5-8, the optimal concentration level for saponin (0.5 wt%) is governed by the severity of the oil-contamination in the sand. In order to achieve a high removal rate of metals and oil residue from soil samples, it is necessary to maintain the optimum washing parameters. The outcome shows linear increase against the mean oil removal. The results show that the percentage of oil removal at 0.001, 0.1, 0.5, 1 and 2 wt% were about 35, 44, 64, 67 and 69 %, respectively. This has been caused by the ability of saponin to solubilise efficiently hydrocarbon compounds.

The CMC of surfactant has been defining as a useful tool used to select the concentration of surfactant. It was concluded that the mechanism of solubilisation described in a study by Deshpande et al. (1999) and Mulligan et al. (2001) was the main cause of the removal of crude oil from the contaminated soil specimen. They indicated that the crude oil removal may be caused by the decrease in the surface tensions of the surfactant solutions at or below the CMC.
The result shows that the CMC value of saponin solution was achieved at 0.5 wt%. It can be suggested that this concentration (0.5 wt%) was able to improve mobility of oil residue, further, resulted in the better separation from oil owing to the reduced capillary force, which holds the sand and oil residue together. These results are vital since the costs of remediation process will be increased with increasing in surfactant consumption, soil/solution ratio, temperature, washing time and stirrer speed. It can be concluded, therefore, that the selected parameters improved the treatment efficiency. The oil residue will be made active by enhancing the oil washing parameters, creating a good condition for it to be separated from the soil.

5.1.4.2 Combined technique

As shown previously (Section 1.9.6) different type of remediation deals with different range of contaminates, so that limited successes have been found in remediation of Kuwaiti contaminated sand. It can be suggested that a highly efficient oil removal could be achieved in the combined processes compared to the individual techniques. Therefore, the combination system of ultrasonication with mechanical mixing is considered a promising technology for soil washing process in industrial use, which agrees to the findings of Son et al. (2011). This due to the fact that the mechanical stirring induces macro-scale effects, which allow the desorption rate of the contaminants on the soil particle surface effectively and easily (Feng and Aldrich, 2000). The soil washing by combination of mechanical stirrer and ultrasonication is an essential process in removing oil from contaminated soil. This is in accordance with the findings of Reddi and Wu (1995), who concluded that the ultrasonication wave is capable of increasing the mobility of non-aqueous phase liquid (NAPL) hydrocarbon from soils surface as well as the soil’s porosity causing the viscosity to reduce. The mechanical stirring technique involves mixing at macro-scale which resulted in contaminants desorption merely from the soil particle surfaces (Feng and Aldrich, 2000). As such, this study shows that the mechanical stirrer
was able to keep the sandy particles in circulation during the washing process, and prevent the particles settling. While, ultrasonic waves produced cavitation bubbles by alternating high and low pressure waves. These bubbles collapse to split the oil from fine particles with contaminants from larger parent constituents to water as oily colloidal suspension. Moreover, this vibrant action takes place within the external shell surface of the soil matrix only. The mechanical mixing is necessary to agitated the soil and spread the behavior of the ultrasonication waves to other particles within the soil matrix.

The present work suggests that mechanical stirring can enhance the exposure and dispersion of soil particles to the ultrasonication waves by producing a macro-scale stirrer of the soil matrix during the mixing process, as a result the efficiency of the combined process can be improved, moreover, sequential washing is required in the present work to enhance the desorption of contaminants from the pores.

As explained in (Section 5.1.3.13) SEM image was taken in this study to represent the surface of sand particles before and after the washing technique. The contaminant elimination using ultrasonication technique was effective only within the comparatively outer zone of the soil matrix as the penetration into the soil matrix is difficult in view of the large agglomeration of oil on soil particles as presented in SEM image. However, the contaminant elimination using the combination of ultrasonication and mechanical stirrer produce better results, as the contaminant removal was better from the soil surface as well as from within the pores. The images show that the sand particles without treatment presented mainly as aggregates, and the soil surface was composed of a variety of oil agglomeration. Following treatment with mechanical stirrer, ultrasonication and combined technique, the washed soils were composed of dispersed large particles. Furthermore, the image shown that most of the soil particles were
in the form of “bare patches” after the washing treatment, while the oil agglomeration “lump” was reduced by the combination method more than ultrasonication or mechanical stirrer. Based on the SEM image (Figure 5-14), it seemed that removal of oil residue by using combination processes could be accomplished by different ways such as, direct desorption of contaminants from the surface of the soil particles and the breakage of soil particles with contaminants from larger oil agglomeration. Moreover, the combined technique of mechanical vibration with stirrer was able to improve the percentage of oil removal from 45 to 61 % after fourth wash, while the oil removal efficiency was slightly enhanced after the fifth and sixth wash. This work suggests that the water temperature was difficult to maintain constant at 50°C during the washing process. The current research supposes that the mechanism of sieve shakers allowed the particles to move in a vertical motion with lateral vibration.

As a result of vigorous up and down motion, the contaminated particles closely interacted with the saponin to remove the oil residue. This point leads to suggest that the free rotations allowed the particles to orient differently when they are move up or down and thus, could eventually increase the solubilisation of the oil residue during the washing process. The washing reactor needed to be redesigned to control the water temperature during the washing process.

Figure (5-16), exhibits the concentrations of TPH utilising combined methods of ultrasonication and mechanical stirrer after the first up to the fourth washes. The washing method was continued until the TPH concentration reaches 10,000 mg/kg or lower based on the recommendation by KEPA. It is also observed from the same figure that the decaying curves of TPH concentration after various stage showed an exponential curve trend. Therefore, adjustment was made to the data to demonstrate an exponential curve thus the following equation:
Oil removal \(\left(\frac{\text{mg}}{\text{kg}}\right) = 67,442.5 \times \exp^{-0.807x}\) (Washing Stage), \(\ldots \ldots \ldots \ldots \ldots \ldots \ldots \). \textbf{E. q (5 - 2)}

Figure 5-16: The relationship between the mean of oil removal and the efficiencies after various washing stages \((R^2 = 0.96; n=5)\)

It has been found that higher removable percentage will result in higher TPH quantity removed, this sort of expression may be used as a guide, assist in predicting the number of washing required to determine the desired TPH concentration during the process of washing. This indicates that the soil washing for more than four washes will result in negligible removal percentage with wastage in surfactant and even more wastewater. Furthermore, more washing lechate would be produced requiring a minor treatment procedure (Feng and Aldrich, 2000; Son et al., 2011). As for the oily wastewater, it is necessary for the post treatments to be performed. It is significant to note that the concentration of saponin has influenced the entire washing process.
Sequential washing method were taken into consideration to the potential of minimize the concentration of saponin in the washing phase after the second, third and fourth washes. The outcome demonstrates that the percentage of oil removal within concentration of 0.25 wt% after second, third and fourth washes were 73, 79 and 86 %, respectively. Moreover, the percentage of oil removal within concentration of 0.50 wt% after second, third and fourth washes were 78, 90 and 96 %, respectively.

The outcome of the present work identified that the removal efficiency of oil residue was significantly reduced by decreasing the concentration of saponin solution from 0.50 to 0.25 wt%. Therefore, reducing the concentration of saponin to 0.25 wt% required further washings, which generates more quantities of wastewater to be treated. This point suggests that the concentration of 0.50 wt% is effective in the operation time. Also, the efficiency of removal improves by increasing the number of washing phases. During this study, the combination of ultrasonication with mechanical stirrer under the selected parameters was able to remove metal contents such as (Ba, Cr, Ni, Pb and Cd) to insignificant level after the second wash. However, the Fe was slightly higher than acceptable level (Fe = 5.10 > 5.00).

5.2 Operating Technique of Wastewater Treatment

5.2.1 Introduction
The present work deals with the treatment of oily wastewater produced from washing of Kuwait oil sand, untreated oily wastewater contains toxic compounds that might be mutagenic or carcinogenic as TPH and heavy metals such as zinc, aluminum, cobalt, chromium, barium, manganese, lead, iron, boron and nickel. Various oily wastewater treatment methods were assessed within this study to address the best treatment method.
5.2.1.1 Wastewater quality analysis

Oily wastewaters were collected from washing of Kuwaiti oil residue at various stages of the process (as explained in Section 4.2). These experiments were carried out by determining the preliminary parameters of the oily wastewater such as, pH, TPH, COD and turbidity, the results are shown in Table (5-4).

Table 5-4: Water characteristic analysis (n=3).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Units</th>
<th>Mean ± SD</th>
<th>KEPA limit</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>-----</td>
<td>8.27 ± 0.2</td>
<td>6.5 – 8.5</td>
</tr>
<tr>
<td>TPH</td>
<td>mg/l</td>
<td>67,500 ± 5,500</td>
<td>5.0</td>
</tr>
<tr>
<td>COD</td>
<td>mg/l</td>
<td>48,215 ± 2,720</td>
<td>100.0 – 350.0</td>
</tr>
<tr>
<td>Turbidity</td>
<td>NTU</td>
<td>176.0 ± 12.5</td>
<td>5.0</td>
</tr>
</tbody>
</table>

The mean values of TPH and COD were 67,500 and 48,215 mg/l, respectively, which indicates high organic compounds concentration. Further, low water clarity indicated high turbidity.

5.2.2 Selection of an Appropriate Wastewater Treatment Technology

5.2.2.1 Effect of coagulant dose

Coagulation/flocculation experiments were carried out using various inorganic types, such as ferric chloride and alum (see Section 4.4.1). The selection will be based upon the values of COD, for the solution upon completion of the flocculation process. The outcome of the experiment is shown in Figures (5-17) and (5-18).
Figure 5-17: Mean of COD by various doses of Alum. Note: the data are expressed as mean ± SD (n=3).

Figure 5-18: Mean of COD by various doses of ferric chloride. Note: the data are expressed as mean ± SD (n = 3).
It can be seen from Figure (5-17) that the values of COD significantly decreased from 44,633 to 27,266 mg/l (ANOVA F (6, 14) = 350, Post-hoc Scheffe test, $p = 0.00$) by increasing the coagulant dosage from 0.32 to 1.53 (mg-Al/l). Also, Figure (5-18) shown that the COD values were significantly reduced from 47,300 to 29,133 mg/l (ANOVA F (6, 14) = 231, Post-hoc Scheffe test, $p = 0.00$) by reducing the coagulant dosage from 2.65 to 0.27 (mg-Fe/l). The result of the present work identified that the COD values were reduced by increasing dosages of Al, nevertheless the COD values were reduced by decreasing dosages of Fe. The percentage of aluminium in aluminium sulfate is about 8 % (weight (w)/weight (w)), while the percentage of iron in ferric chloride is about 35 % (w/w), therefore, addition of positive ion of Al is required. Further, the selection was based on the values of floc weight obtained after flocculation/coagulation process was completed. The outcome of the experiment is shown in Figures (5-19) and (5-20).

![Figure 5-19: Mean of flocs by various mass of Alum. Note: the data are expressed as mean ± SD (n = 3).]
The present research work identified that the mean values of flocs were significantly increased from 0.22 to 0.43 g by increasing the coagulant dosage from 0.32 to 1.53 (mg-Al/l) (ANOVA F (6, 14) = 54, Post-hoc Scheffe test, p = 0.00). However, the mean values of flocs were significantly increased from 0.25 g to 0.41 g by decreasing the coagulant dosage from 2.65 to 0.27 (mg-Fe/l), (ANOVA F (6, 14) = 290, Post-hoc Scheffe test, p = 0.00). Furthermore, the outcome shows the generated results from overdosing charge of Fe and Al, for instance increasing the Al dose to 1.81 (mg-Al/l) or decreasing Fe dose to 0.162 (mg-Fe/l) resulted in further increase of COD and decrease of floc weight. In addition, Figures (5-21) and (5-22), reveals a reverse impact on the water clarity by altering the dosages of ferric chloride or alum could be identified. Therefore, design doses have a limitation of overdosing, as such a higher charge of Fe or Al is able to increase possibility of re-stabilisation by causing charge reversal.
Additionally, Figure (5-21) show that further issues could be generated by using ferric chloride, the outcome of the experiment demonstrated that turbidity was effected by transferring the ion colour to water, as a result the generated water required further treatment.

![Figure 5-21: Preliminary study on selecting the best dose of Fe.](image)

It can be evidently shown that the reduction of COD and weight of floc dramatically increased by increasing the dosage of coagulants up to reaching the optimal coagulant dosages, moreover, these significant reductions may be due to the charge neutralization, increasing the presence of cationic ions of Fe or Al can enhance the attraction of the negative charge colloids from emulsified oils through electrostatic interaction. Therefore, the colloids and floc particles were started to agglomerate during the neutralization phase until they become a heavy enough to settle down by gravity.

![Figure 5-22: Preliminary study on selecting the best dose of Al.](image)
Moreover, paired sample t-test was conducted to examine whether there was a significant difference between cationic ions of Fe and Al in relation to their COD reduction, as shown in Figure (5-23).

![Chemical Oxygen Demand (COD) Graph](image)

Figure 5-23: Mean of COD reduction by using the selected dose of Fe and Al. Note: The data are expressed as mean ± SD (n =3).

It is clearly shown that the mean of COD significantly reduced by using Al (M = 27,266; SD = 152) than Fe (M = 29,133; SD = 165) (t = 21, df = 2, Paired sample test, p = 0.002). As a result, the efficiency of COD reduction was significantly enhanced about 6.5 % with alum.

Further, the values of floc weight obtained after flocculation/coagulation process was measured. The outcome of the experiment is shown in Figures (5-24).
Figure 5-24: Mean of flocs obtained by using the selected dose of Fe and Al. Note: The data are expressed as mean ± SD (n = 3).

It can be seen from Figure (5-24) that the agglomerations of floc weights were significant higher by using Al (M = 0.44; SD = 0.010) more than Fe (M = 41; SD = 0.006) (Paired sample test, t = 4, df = 2, p = 0.0057). Also, it was noted that the weight of flocs changes in tandem with the tendencies shown in the COD, moreover, this suggests that the optimal coagulant dosage contributes mainly to achieve the desired results. As such, the conclusion is that the most optimum coagulant was achieved with aluminum sulfate at concentration of 1.53 (mg Al/l). At present, alum is the favored choice as coagulant, over ferric chloride due to the ferric ion colour that is transferred to water, while the use of ferric chloride could be limit the improvement of the coagulation-flocculation process by minimizing the flocs produced and decreasing COD value. This study demonstrated that the coagulant type and optimal coagulant dose will not only reduces treatment costs, but also improve the water characteristics.
5.2.2.2 Anoxic treatment

The combination of anoxic treatment followed by coagulation/flocculation was investigated in this section, as explained in Section 4.4.2.1. Alum was used as a coagulant after three and five days of anoxic process. Figure (5-25), indicate that the mean of COD for oily wastewater for oily wastewater during anoxic process.

![Figure 5-25: Mean of COD reduction using anaerobic digestion process after 3 and 5 days. Whereas, A) oily wastewater; B) oily wastewater with PSS; C) Anoxic treatment; D) combination of anoxic treatment with coagulation/flocculation. The data are expressed as mean ± SD (n = 3).](image)

The result demonstrated that the mean of COD for oily wastewater with PSS was significant reduced from 48,240 mg/l (SD = 378) to 25,720 mg/l (SD = 497) after three day and to 22,034 mg/l (SD = 749) after five day (One way ANOVA F (5, 12) = 3,174, Post-hoc Scheffe test, \( p = 0.00 \)). However, the COD values after anoxic treatment was still high as exhibited in the same Figure (5-25), therefore, further treatment is required to remove the recalcitrant organic compounds to acceptable level. It has been found in previous (Section 5.2.2.1) that the Alum was able to reduce the mean of COD values about 39 %, these results encourage to consider combined use of coagulation/flocculation.
The present work recognized that the COD values for incubated samples was significantly decreased by using coagulation and flocculation process to 17,700 mg/l (SD= 483) after three day and to 14,110 mg/l (SD = 384) after five day (Paired sample test, t = 13.6, df = 2, p = 0.00). This study identified that the combination technique of conventional anoxic treatment with coagulation/flocculation processes enhanced the reduction of COD values about 64 % after three days and 70 % after 5 days. The outcome of this study proposed that the contact time (treatment duration) between the microbes and oily wastewater exhibits a direct relationship on the removal efficiency of oil residue. The combination treatment of anoxic treatmnet followed by coagulation/flocculation does not require electrical energy, therefore, this treatment is considered as an environmentally-friendly treatment method. However, the result shown in Figure (5-26) indicated that there is slight increase in aggregation of floc weight after five days of incubation period.

![Image](image_url)

Figure 5-26: Mean of flocs obtained after 3 and 5 days during the anoxic process. Note: The data are expressed as mean ± SD (n = 3).
The outcome shown that the average floc weight significant changes to 0.50 (SD= 0.016) after three day and to 0.61 g after five day (SD = 0.015) (Paired sample test, $t = -15.24$, df = 2, $p = 0.00$), respectively. It seems that floc weigh increase with longer incubation times. This oily wastewater sample contain high COD in the average of 48,050 mg/l and a large amount of colorants that provide dark brown colour, which considered as a slightly basic (pH 7.5–8.0). Even after being treated using anoxic procedures, the colour of the oily wastewater become a darker brown with high dour, as shown in Figure (5-27).

![Figure 5-27: Fresh oily wastewater samples (A), and after 5 days of anoxic process (B).](image)

Perhaps a chemical reaction was occurred which change the physical priorities of oily wastewater (see Figure 5-27). Recent studies have reported that colour of the blackening of wastewater can be increase with an anaerobic process due to the formation of ferrous sulphide (Poddar and Sahu, 2014; Tikariha and Sahu, 2014). Under the anaerobic conditions, hydrogen sulphide can be produced and combined with divalent metal, such Fe which often black or brown colour.
5.2.2.3 Aerobic Treatment

Alum was used as a coagulant after three and five days of aeration process, as explained in Section 4.4.2.2. The outcome of present experiment is shown in Figures (5-28) and (5-29).

Figure 5-28: Mean of COD reduction using aerobic digestion process after 3 and 5 days. Whereas, A) oily wastewater; B) oily wastewater with PSS; C) aeration treatment; D) combination of aeration treatment with coagulation/flocculation. The data are expressed as mean ± SD (n = 3).

As shown in Figure (5-28), the result demonstrated that the mean of COD for oily wastewater with (PSS) was significant reduced from 48,240 mg/l (SD = 378) to 16,466 mg/l (SD= 406) after three days and to 10,516 mg/l (SD= 697) after five days (ANOVA F (5, 12) = 7,778, Post-hoc Scheffe test, $p = 0.00$). Additionally, this investigation identified that the COD values for aerated samples was significantly decreased by using coagulation and flocculation process to 7,992 (SD = 205) after three days and to 4,784 mg/l (SD = 270) after five days (Paired sample test, $t = 28.3$, df = 4, $p = 0.00$). The present work indicated that the combination of aeration followed by coagulation/flocculation process was able to reduce the COD value about 83 and 90 % after 3 and 5 days, respectively. In addition, Figure (5-29) indicated that there is slight increase in aggregation of flocs weight after three and five days of aeration process.
Figure 5-29: Mean of flocs obtained after various mass of Fe. Note: the data are expressed as mean ± SD (n = 5).

The results shown that there was significant change in aggregation of floc weight to 0.55 (SD = 0.015) after three days aeration and to 0.69 g (SD = 0.013) after five days aeration (Paired sample test, t = 54.7, df = 2, p = 0.00), Figure (5-29). Basically, the physical property of oily wastewater was changed after employing the aeration system such as the colour as shown in Figure (5-30).
It is thought that this change of colouration of the oily wastewater is due to the loss of colloid particles stability by the flocculants thus emulsifies oil within the wastewater. A study carried out by Zhou et al. (2008) stated that the destabilisation of colloidal particles by using high aluminium dosage, an adequate level of oversaturation prompts fast precipitation of a large amount of aluminium hydroxide by the formation of “sweep floc”.

As explained in (Section 2.6.4.2) the hydrophilic part of saponin which is polar due to the several functional groups such as, OH, COOH, and carboxylate group of sugar, acetate group and esteric band. Since these compounds carry a negative charge, the removal of oil emulsion may be due to the complexation of Al with hydroxyl ion (OH\(^-\)). Moreover, these significant enhancements in COD values and flocs weight perhaps due to the flocculants enmeshment (sweep-floc coagulation) produced from overdosing of the flocculants, which tends to give fairly thick layer of the flocculant around the suspended solids, and therefore accelerates the setting of floc as explained in Section 1.7.3.2. Regardless of the high dose of coagulant, leading to the assumption of a sweep floc coagulation mechanism, the dependence to the observed stoichiometric or quasi-stoichiometric relation inclines to suggest that the charge adsorption-neutralization mechanism is performed by high charge of Al.
5.2.2.4 Comparing aeration and anoxic treatment

This investigation was evaluated by measuring the reduction of COD values, as shown in Figure (5-31).

![Figure 5-31: Mean of COD reduction; A) aeration treatment; An) anoxic treatment. B) coagulation/flocculation. The data are expressed as mean ± SD (n = 3).](image)

Based on Figure (5-31), there is significant enhancement in the percentage of COD reduction by the combination of aeration system more than combination of anoxic system, where it increased to 64 and 83 % after 3 days and then increased dramatically after 5 days to 70 and 90 % (ANOVA F (7, 16) = 443, Post-hoc Scheffe test, p = 0.00), respectively. Further, this study was carried out to measure the aggregation of flocs weight, as shown in Figure (5-32).
Figure 5-32: Mean of flocs obtained by different digestion process. A) Anoxic treatment; B) Aeration treatment. The data are expressed as mean ± SD (n = 3)

In Figure (5-32), the results of the average floc weight for aerobic and anoxic digestion were measured, where it was 0.55 and 0.50 g after 3 days and then increased significantly after 5 days to 0.69 and 0.61 g (ANOVA F (3, 8) = 155, Post-hoc Scheffe test, p = 0.00), respectively. It can be seen that the removal quantity of COD and floc were often higher in the aerobic treatment than anoxic treatment. This observation leads to the suggestion that the charge adsorption – neutralization mechanism was achieved with high efficiency by introducing aerobic digestion. Consequently, the reduction in the COD values upon aeration system is probably due to the breakage of stable colloid particles thus presented as emulsifies oil within the wastewater. Therefore, the aeration system for 5 days was recommended in this study.
5.2.2.5 Saponin removal

Aeration process (Section 4.4.2.2) was carried out to measure the removal of saponin during the aeration treatment. Two volumetric flasks were used during this research, first flask was filled with distillate water and saponin (0.5 wt%). While the second flask was filled with artificial seawater and saponin (0.5 wt%), as shown in Figure (5-33). The experiment aimed to compare the initial COD values with the COD values obtained at the end of day five in order to find the decrease in saponin due to biodegradation in terms of COD reduction. The outcome of the experiment shown in Figure (5-34).

Figure 5-33: Lab scale study for saponin removal (0.5 wt%) by aeration system.
This work identified that the COD reductions for saponin prepared with distilled water and PSS were decreased significantly from 6,488 (SD= 222) to 2,018 mg/l (SD= 75), (Paired test, t= 62, df =4, p < 0.05). Therefore, around 69% of saponin seems to be removed during the aeration system. However, the study identified that the mean of COD for saponin prepared with artificial seawater and PSS were significantly reduced from 8,753 (SD= 173) to 4,415 mg/kg (SD= 148) (Paired test, t= 37, df =4, p < 0.05).

The mean of COD for artificial seawater is 2,550 mg/l (SD = 55), while the mean of COD for saponin once the COD value of artificial seawater subtracted becomes 6,203, 1,856 mg/l before and after aeration system, respectively. For this reason, it could be concluded that the average of the COD reduction for saponin in artificial seawater was about 68%, as shown in Figure (5-35).
Figure 5-35: Mean of saponin removal by subtracting the mean of COD values of artificial seawater from saponin. The data are expressed as mean ± SD (n = 3)

Based on Figure (5-35), it seems that there is no significant difference between final COD values of saponin prepared with artificial seawater and saponin prepared with distilled water (Paired test, t= -2.1, df =4, p = 0.106). This experiment is as rule of thumb for measuring the percentage removal of saponin during the aeration process. The outcome of aeration system demonstrated that the result considers as normal distribution (Skewness and kurtosis, z value = ±1.96). While, the percentage removal of saponin removal calculated by:

\[
\text{Saponin removal} = \frac{(\text{COD Final} - 2,550)}{6,203} \times 100 = \ldots \ldots \ldots \ldots \ldots \ldots \ldots E. \ q(5 - 3)
\]

While, COD Final = COD of oily wastewater after five days of aeration, COD for artificial seawater = 2,550 mg/l, and COD for saponin= 6,203 mg/l.
5.2.3 Removal of Metals

Numerous techniques have been considered to remove or reduce the metal contents from water. As explained in Section 1.7.4, the encounter between microbes and metal contents within the environment takes place in a few forms, in somehow they are likely interact with each other. In this investigation, the combination of aeration system and coagulation/flocculation was carried out to measure the removal of metal contents from oily wastewater, aerated sample and treated sample, as illustrated in Table (5-5).

Table 5-5: The average concentration of metals during the water treatment process (n=3).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Oily wastewater (mg/l)</th>
<th>Aerated sample (mg/l)</th>
<th>Treated sample (mg/l)</th>
<th>KEPA limit (mg/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zinc (Zn)</td>
<td>2.302</td>
<td>1.684</td>
<td>0.752</td>
<td>2.0</td>
</tr>
<tr>
<td>Aluminium (Al)</td>
<td>7.260</td>
<td>1.008</td>
<td>9.600</td>
<td>5.0</td>
</tr>
<tr>
<td>Cobalt (Co)</td>
<td>0.520</td>
<td>0.510</td>
<td>0.440</td>
<td>0.2</td>
</tr>
<tr>
<td>Chromium (Cr)</td>
<td>18.610</td>
<td>9.390</td>
<td>0.611</td>
<td>1.0</td>
</tr>
<tr>
<td>Barium (Ba)</td>
<td>8.880</td>
<td>0.388</td>
<td>0.181</td>
<td>2.0</td>
</tr>
<tr>
<td>Manganese (Mn)</td>
<td>0.588</td>
<td>0.300</td>
<td>ND</td>
<td>0.2</td>
</tr>
<tr>
<td>Lead (Pb)</td>
<td>0.920</td>
<td>0.090</td>
<td>ND</td>
<td>0.5</td>
</tr>
<tr>
<td>Iron (Fe)</td>
<td>52.240</td>
<td>6.308</td>
<td>6.197</td>
<td>5.0</td>
</tr>
<tr>
<td>Boron (B)</td>
<td>24.720</td>
<td>9.520</td>
<td>7.742</td>
<td>2.0</td>
</tr>
<tr>
<td>Nickel (Ni)</td>
<td>1.149</td>
<td>0.900</td>
<td>0.020</td>
<td>0.2</td>
</tr>
</tbody>
</table>

The outcome of present work recognized that the mean value of cobalt was almost same in oily wastewater (0.52 mg/l) and aerated sample (0.51 mg/l) while it was slight less in treated sample (0.44 mg/l). Iron content was also highest in oily wastewater with mean of 72.24 mg/l while it becomes 6.31 and 6.20 mg/l in aeration process and combination system of aeration and coagulation/flocculation, respectively.
Nickel content become lowest in treated sample with mean of 0.02 mg/l while it was 1.15 mg/l in oily wastewater sample. Furthermore, aerated sample showed much lesser mean of nickel content (0.90 mg/l). Chromium was observed with higher mean in oily wastewater sample with a value of 19.61 mg/l while it becomes 9.40 and 0.61 mg/l by using aeration process and combination system of aeration and coagulation/flocculation, respectively.

One of the interesting features of the present investigation was the absence of manganese and lead in aerated and treated samples. While in metal, the average reduction of Ni and Ba in the treated sample was recorded as 98 %. The average reduction in non-metal concentration such as B after combination process was 69 %. During this project, the alum has been used for the coagulation process, as expected aluminum was observed to be present in these treated samples with a maximum mean value of 9.60 mg/l.

A study carried out by Xia and Yan (2010) reported that the surfactants are able to increase the bioavailability of metals, which allow the biosorption to be more accessible through enhancing uptake mechanisms of microorganisms. Generally, the cell walls of microbial biomass, mainly composed of proteins, polysaccharides and lipids have abundant metal binding groups such as amino groups, carboxyl, sulphate and phosphate. This type of biosorption, i.e., non-metabolism dependent is relatively rapid and can be reversible. In the case of precipitation, the metal uptake may take place both in the solution and on the cell surface of microorganisms (Ercole et al., 1994). In most cases, these metalloids and metals are present in nature in the form of oxyanions, cations or both in aqueous solution, and mostly in salts or oxides in crystalline (mineral) form or as amorphous precipitates in insoluble form. The phenomenon of bio-sorption is vital in microbial removal of heavy metals.
The concentration of cobalt in the oily wastewater was the lowest and its removal from the wastewater was the lowest, whereas those of barium and nickel were the highest removal. Hence, the removal of heavy metals from wastewater is influenced by their initial contents. According to Sheng-lian et al. (2006), there are many factors that can influence the removal efficiency of heavy metal during the biological processes, of which pH being regarded as the most critical factor for biosorption performance of toxic metals with microbial communities. As shown previously (Table 5-4) the oily wastewater sample considers a slightly basic, which may effect on the removal efficiency of heavy content from wastewater.

Other external elements such as concentration of the metal, metal species, solubility, contact time (treatment duration), temperature, presence of other cations and activated sludge dosage concentration of organic materials in wastewater can also contribute to the heavy metal toxicity (Gikas, 2008). Subsequently, this also affects the heavy metals removal, despite the fact that many studies have demonstrated that heavy metals can be removed in the biological wastewater treatment processes (Volesky and Holan, 1995; Chipasa, 2003; Nanda et al., 2011).

Consequently, the removal level of metal contents form oily wastewater may be difficult to estimate. Based upon the previous explanations, it is apparent that the treatment of wastewater by aeration process seems as an intricate process which relies heavily upon various biological and physicochemical conditions in addition to process operating design and conditions.
5.2.4 Discussion

The immediate removal of hydrocarbon compounds from oily wastewater is considered as an essential issue to protect the receptor. Different experiments were conducted in this study to remediate, eliminate or reduce the impact of high toxicity level. Based on the quality analysis for the oily wastewater sample, it could be concluded that high concentration of TPH, COD and turbidity were found with average values of 67,500 mg/l, 48,215 mg/l and 176 (NTU), respectively. Their unfavourable effects towards health have caused fear to the receptor and have initiated extensive studies focussing on how to find the most effective method to treat the oily wastewater so it can be reuse or disposed of safely. Several technologies were carried out in the present research, such as centrifuging, activated carbon and woodchips (See appendix A). Result of COD values for woodchips, centrifuging and PAC were found to be between 37,400-41,600 mg/l, 37,400-41,600 mg/l and 24,600-28,200 mg/l, respectively, which were very high and not within Kuwait EPA conditions (1000 mg/l), which is the maximum limits of COD in industrial wastewater discharged into sewerage.

Based upon the above observation and discussion in Section 5.2.2.1, the coagulation performance for coagulants was assessed by solely comparing with Al₂(SO₄)₃ and FeCl₃ by determining their capabilities to reduce COD and increase the agglomeration of floc from oily wastewater. It is clearly shown that the mean of COD significantly reduced by using Al (M = 27, 266 mg/l) than Fe (M = 29,133 mg/l), also the study demonstrated that the agglomerations of floc weights were significant increased by using Al (M = 0.44 g) more than Fe (M = 41 g), therefore alum has been selected. As explained in Section 1.7 ensuring proper wastewater treatment is as important for protecting community health and environment. It has been found that the mean of COD values were reduced about 39, 70 and 78% by using coagulation/flocculation with alum, anoxic and aeration process, respectively.
These results encourage to investigate the potential of combine the aerobic or anoxic digestion with coagulation/flocculation. The obtained result demonstrated that COD removal rate for oily wastewater was reduced about 90% using five days of aeration with coagulation and flocculation. Based on the quality analysis of the wastewater, it could be concluded that treatment of wastewater containing high concentration of organic compounds using only a conventional biological process could be considered as a difficult task. In order to success in the treatment of oily wastewater by using aeration system technology is to manage the contact time, this project recommended 5 days. This is to ensure that the biodegradation is able to progress within a realistic time, as a result the level of wastewater toxicity can potentially be minimized. The turbidity of supernatant for oily wastewater was measured before and after the combination of coagulation/flocculation with aeration system, as presented in Figure (5-36).

![Figure 5-36: Mean of turbidity for oily wastewater, aerated sample and treated sample. The data are expressed as mean ± SD (n = 5).](image-url)
The results reveal a moderately enhancement of the clarity of water after five days of aeration, where the average of turbidity was reduced from 159.4 NTU (SD ± 1.2) to 119.4 NTU (SD ± 2.3) after 5 days of the aeration process. The current study demonstrated that turbidity was reduced to 8.2 NTU (SD ± 0.15) after coagulation/flocculation, however the generated water remains difference of colour. This study found that the combination of coagulation/flocculation with anoxic or aeration system were able to be operated at high level of toxics. The aeration process is acknowledged in the present work, due to the high COD removal and floc weight.

5.2.4.1 Destabilization

It is well known that the varying ionic strength is able to effect on the efficiency of coagulation process and then flocs weight. Saponins are categorized as a non-ionic biosurfactant, but contains acidic and ionizable group of glucuronic acid as part of its head group (hydrophilic fraction) (Mitra and Dungan, 1997). Further, some charged species would exist in saponin solutions due to the ionic strength of artificial seawater. So, it seems that the ionic strength of saponin solution may have a potential effect on the destabilisation of colloidal particles during the coagulation/flocculation process.

Based on the literature (Section 1.7.3.2) sodium chloride (NaCl) aids in double layer compression. The present work suggests that the ions with opposite charge play a significant role in improving the flocculation process by bridging the particles and allow the flocs to be formed. The applicability of the secondary treatment with aeration may be able to destabilise colloidal particles by breaking down the long chain of complex organic compounds in oil emulsion, and convert into small molecules by increasing the surface area “colloid concentration”. As a result, the contact time of the micro-organisms in the oily wastewater was increased from 3 to 5 days, which allow the saponin to be available for biodegradation. Soeder
et al. (1996) confirmed that the solubility of hydrocarbon compounds improves with the presence of saponins which makes them available for degradation by bacteria. In addition, Alexander (1999) reported that the surfactants play a significant role in increasing contaminant solubility or adhesion to the surface, which makes the contaminant more accessible to degradation by microorganisms. As such, the solution of saponin and artificial seawater was aerated for 5 days (as explained in Section 5.2.2.5), the outcome shown that the average value of COD was reduced about 70%. This point suggested that the reduction in the COD reading of the oily wastewater is perhaps due to the biodegradation of the saponin, allowing the stability of oil emulsion to be broken down and released easily and it can be seen in the following section that owing to its toxicity effects, oil residue in the wastewater was not biodegradable by microbes. This may be due to the double layer compression and sweep flocculation theory as discussed in Section 1.7.3.2. With the increase in sodium ion concentration in the solution, the double layer becomes thinner and aids in getting the same charge particles nearer. As such, the floc formation becomes quicker and better which improves the settleability of the colloid matter, this causes a speedy decrease in turbidity as schematically shown in Figure (5-37).

![Breakage of colloid particles](image)

Figure 5-37: The emulsion of discrete particle is broken up by saponin biodegrading.
The two means whereby high coagulant dosage can improve the rate of coagulation, they are (a) increasing metal hydroxide precipitate concentration and hence the rate of aggregation, and (b) enmeshing particulates to enlarge the size of aggregates using sweep-floc coagulation (Duan and Gregory, 2003). In this research, adding a high dosage of coagulant precipitated a heavy, sticky and high quantity of flocs, while the settling time was accelerated as well. The outcome of this study demonstrated that the settling time for colloidal particles by adapting the oily wastewater sample under aeration system for 5 days required 15 min, while the sample without aeration required 24 h to settle down. This scenario suggested that the sweep-floc coagulation whereby the colloidal particles are removed from the suspension by means of enmeshment into the aluminum hydroxide Al(OH)$_3$ precipitate, as shown in Figure (5-38).

Figure 5-38: Increasing the concentration of broken colloids allow “sweep-floc” occur.

The process resulted in the creation of a suspension with smaller size and high quantity of flocs. This will therefore eliminate higher quantity of organic particles in view of the increased surface area offered for adsorption. In contrast, low doses of coagulant boosts larger and fewer quantity flocs owing to the faster rate of growth compared to the rate of nucleation. This causes the reduced surface area for the adsorption of organic compounds. This study suggested that the destabilisation improvement of colloidal particles occurred due to the linkage of inter-
particle interactions. This effect results in the generation of large amount of precipitation that permits the colloidal particles to be enmeshed while settling. In such cases, it is not straightforward to define the main mechanism for destabilisation. The oily sludge left in the reactor needs to be evaluated for future potential use. In the present work, it was disposed of properly as hazardous waste.

5.3 Determination of Biodegradability

The biodegradability test was carried out to determine BOD of oily wastewater and saponin solution by using OxiTop method. Aerobic biodegradability was investigated by measuring the amount of oxygen used by bacteria to decompose organic compounds. The samples were seeded by means of introducing micro-organism population. As mentioned in (Section 3.4.6), the bacteria employed in the current research were extracted from the Kuwaiti oil contaminated soil such as *Bacillus mycoides* and *Bacillus subtilis*. Except *Rhodococcus* were brought from KISR. Which they have been widely acknowledged as effective cultures in degrading petroleum organic compounds (Yudono *et al.*, 2009; Ali *et al.*, 2011). Another inoculums were obtained from a wastewater treatment plant PSS.

5.3.1 Analytical Development of BOD

Generally, the bacteria in aerobic process use oxygen to degrade organic compounds, where the amounts of oxygen used and amount of metabolize organic compounds are in direct proportionality. Initially, 28 days was selected as the incubation time since that is the longest travel time of oily wastewater, and all tested samples were examined by using OxiTop as explained in Section 3.4.5. Details of these samples will be given in section discussion. The BOD$_{28}$, initial COD value (COD$_i$) and BOD$_{28}$/COD ratio values are listed in Tables (5-6).
Table 5-6: BOD$_{28}$, COD$_i$, ratio of BOD$_{28}$:COD$_i$, Ultimate BOD and reaction rate k values for oily wastewater and saponin solution by Rhodococcus, Mycoidesis and Bacillus subtilis.

<table>
<thead>
<tr>
<th>Type of solution</th>
<th>Rhodococcus (R) ml</th>
<th>Mycoidesis &amp; Bacillus subtilis (M) ml</th>
<th>Distilled water (D) ml</th>
<th>COD$_i$ mg/l</th>
<th>BOD$_{28}$ mg/l</th>
<th>BOD$_{28}$/COD$_i$</th>
<th>Ultimate BOD mg/l</th>
<th>k</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saponin solution</td>
<td>1.25</td>
<td>-</td>
<td>1.25</td>
<td>2,990</td>
<td>1,007</td>
<td>0.300</td>
<td>1,119</td>
<td>0.24</td>
</tr>
<tr>
<td></td>
<td>1.25</td>
<td>-</td>
<td>1.25</td>
<td>3,050</td>
<td>971</td>
<td>0.300</td>
<td>1,054</td>
<td>0.25</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>1.25</td>
<td>1.25</td>
<td>3,510</td>
<td>1,112</td>
<td>0.300</td>
<td>1,969</td>
<td>0.26</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>1.25</td>
<td>1.25</td>
<td>3,320</td>
<td>1,113</td>
<td>0.300</td>
<td>1,907</td>
<td>0.30</td>
</tr>
<tr>
<td></td>
<td>1.25</td>
<td>1.25</td>
<td>-</td>
<td>5,010</td>
<td>1,715</td>
<td>0.300</td>
<td>3,136</td>
<td>0.34</td>
</tr>
<tr>
<td></td>
<td>1.25</td>
<td>1.25</td>
<td>-</td>
<td>4,690</td>
<td>1,591</td>
<td>0.300</td>
<td>2,864</td>
<td>0.35</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>-</td>
<td>2.50</td>
<td>1,925</td>
<td>85</td>
<td>0.040</td>
<td>141</td>
<td>0.88</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>-</td>
<td>2.50</td>
<td>2,010</td>
<td>57</td>
<td>0.030</td>
<td>122</td>
<td>1.20</td>
</tr>
<tr>
<td>Oily wastewater</td>
<td>1.25</td>
<td>-</td>
<td>1.25</td>
<td>3,990</td>
<td>961</td>
<td>0.200</td>
<td>1,253</td>
<td>0.29</td>
</tr>
<tr>
<td></td>
<td>1.25</td>
<td>-</td>
<td>1.25</td>
<td>4,260</td>
<td>849</td>
<td>0.200</td>
<td>1,057</td>
<td>0.31</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>1.25</td>
<td>1.25</td>
<td>4,180</td>
<td>1,298</td>
<td>0.300</td>
<td>2,419</td>
<td>0.24</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>1.25</td>
<td>1.25</td>
<td>3,795</td>
<td>1,305</td>
<td>0.300</td>
<td>2,229</td>
<td>0.23</td>
</tr>
<tr>
<td></td>
<td>1.25</td>
<td>1.25</td>
<td>-</td>
<td>5,330</td>
<td>1,778</td>
<td>0.300</td>
<td>3,351</td>
<td>0.35</td>
</tr>
<tr>
<td></td>
<td>1.25</td>
<td>1.25</td>
<td>-</td>
<td>5,900</td>
<td>1,807</td>
<td>0.300</td>
<td>3,867</td>
<td>0.36</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>-</td>
<td>2.50</td>
<td>2,900</td>
<td>85</td>
<td>0.030</td>
<td>141</td>
<td>0.88</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>-</td>
<td>2.50</td>
<td>3,010</td>
<td>28</td>
<td>0.001</td>
<td>56</td>
<td>1.30</td>
</tr>
</tbody>
</table>

As illustrated in Table (5-6), the mean of BOD$_{28}$ values for the saponin solution using distilled water D and mixture of Rhodococcus, Bacillus mycoidesis and Bacillus subtilis RM were 71 mg/l to 1,653 mg/l, respectively. While the mean of BOD$_{28}$ values for the oily wastewater using D and RM were 56 mg/l to 1,792 mg/l, respectively. The mean of COD$_i$ values for the saponin solution using D and RM were 1,968 mg/l and 4,850 mg/l, respectively.
While the mean values of CODi for oily wastewater using D and RM were 2,955 mg/l and 3,988 mg/l, respectively. Moreover, the higher values of BOD$_{28}$ were recorded when mixture of bacteria seed was added together with the saponin solution or oily wastewaters. The BOD$_{28}$ values for oily wastewater and saponin solution proposed that the bacteria may not be able to biodegrade the oily wastewater.

Different BOD values were obtained from oily wastewater and saponin solution due to the concentration of organic compounds in the tested samples. These samples contain toxic materials which may be decreased BOD values, therefore these samples were diluted to ratio of (1:10) to reduce the toxic substance or minimize their effects. However, the outcome of this work recognized that the UBOD and $k$ are very similar for different samples and regardless of reactivity status (See Table (5-6)).

The maximum of removal rate $k$ is 0.35 day for saponin solution, which almost similar to the value of 0.36 day for oily wastewater. The outcome shows that BOD removal rate $k$ is in agreement with those found by Orhon et al. (2000) for carbonaceous deoxygenation in most industrial waste waters with mean $k$ 0.32 – 0.43 day at 20°C. Also, maximum values of ultimate BOD for saponin solution and oily wastewater by using RM seed were recorded in the range of 2,864 to 3,136 mg/l and in the range 3,351 to 3,867 mg/l respectively. In the same Table (5-6) the minimum UBOD for sponin solution and oily wastewater were obtained by not using microbial seed.

Referring to Figure (5-39), there is slight difference for the BOD samples tested with varying seed. Generally, the rate of the oxygen consumption decreases as BOD decreases. This study suggested that the hydrocarbon compounds were reduced the activity of microbial.
Figure 5-39: Response curve of oily wastewater and saponin solution as measured by the Oxitop. (S1) sample 1; (S2) sample 2; (A) *Rhodococcus* “R”; (B) *Bacillus mycoides* and *Bacillus subtilis* “M”; (C) *Rhodococcus, Bacillus mycoides* and *Bacillus subtilis* “RM” and (D) distilled water “D”.

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The biological oxidation of organic compounds was nearly completed after twenty days with oily wastewater or saponin solution, the microbial seeds need that long to oxidize the carbon compounds may be due to the high toxicity in the tested samples. As show in curve A Figure (5-39), the BOD values were start from 220 mg/l and end up at the final value of 850-950 mg/l for saponin solution with *Rhodococcus* (R) and oily wastewater with R, respectively. Comparing curve B and A, in the same Figure (5-39), curve B is actually curve A, since all the samples used were contained as much organic material. It seems that the sample of saponin solution and sample of oily wastewater contain equal amount of organic material (BOD) as the rates can differ quite significantly.

Although, curve B in Figure (5-39) shown that biodegradation process was a slightly improved by the mixture of *Bacillus mycoides* and *Bacillus subtilis* M. Curve A and curve B in Figure (5-39), identified that the BOD values of the oily wastewater or saponin solution were increased over time. The concentrations of degradable organics and removal rate $k$ enhanced when mixture of R and M was adding together with the saponin solution or oily wastewaters, as shown in curve C Figure (5-39). It has been seen in Figure (5-39), the BOD values of the saponin solution or oily wastewaters were slightly increased after 18 days.

Refer to curve D in Figure (5-39), initial decline of microbial growth curves was occurred after 20 days of incubation period. During this phase the growth rate begins to slow, therefore this phase was identified as death phase. The reductions value of the BOD curves may be attributed to the following reasons (a) sample did not incubate with sufficient bacteria; (b) bottle leaks from caps; (c) high toxic sample; (d) amendment substance was not used in this test. Actually, not all of samples have the same BOD values. This relates back to the biodegradation activity of the microbial population and the concentration levels of hydrocarbons present in the sample.
Again, different microbial seed were conducted for the BOD test, the samples of oily wastewater and saponin solution were seeded with PSS, LPF and mixture of PSS and LPF. The BOD\textsubscript{28}, COD\textsubscript{i}, ratio of BOD\textsubscript{28}/COD\textsubscript{i}, ultimate BOD and reaction removal rate \( k \) values are listed in (Table 5-7).

<table>
<thead>
<tr>
<th>Type of solution</th>
<th>LPF ml</th>
<th>PSS ml</th>
<th>Distilled water (D) ml</th>
<th>COD\textsubscript{i} mg/l</th>
<th>BOD\textsubscript{28} mg/l</th>
<th>BOD\textsubscript{28}/COD\textsubscript{i}</th>
<th>Ultimate BOD mg/l</th>
<th>( k )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saponin solution</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.250</td>
<td>-</td>
<td>1.250</td>
<td>2,340</td>
<td>452</td>
<td>0.200</td>
<td>520</td>
<td>0.035</td>
</tr>
<tr>
<td></td>
<td>1.250</td>
<td>-</td>
<td>1.250</td>
<td>2,260</td>
<td>423</td>
<td>0.200</td>
<td>494</td>
<td>0.075</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>1.250</td>
<td>1.250</td>
<td>2,105</td>
<td>310</td>
<td>0.100</td>
<td>320</td>
<td>0.200</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>1.250</td>
<td>1.250</td>
<td>2,085</td>
<td>565</td>
<td>0.300</td>
<td>733</td>
<td>0.270</td>
</tr>
<tr>
<td></td>
<td>1.250</td>
<td>1.250</td>
<td>-</td>
<td>2,630</td>
<td>#</td>
<td>#</td>
<td>#</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.250</td>
<td>1.250</td>
<td>-</td>
<td>2,620</td>
<td>#</td>
<td>#</td>
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<td>-</td>
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<td>3,215</td>
<td>254</td>
<td>0.080</td>
<td>277</td>
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<td>254</td>
<td>0.080</td>
<td>279</td>
<td>0.120</td>
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</tbody>
</table>

# Missing sample
Table (5-7) shown that the mean values of $BOD_{28}$ for the saponin solution using D and PSS were 339 mg/l and 438 mg/l, respectively. While the mean values of $BOD_{28}$ for the oily wastewater using D and mixture of LPF and PSS were 254 and 480 mg/l by, respectively. Further, CODi of the saponin solution using D and mixture of LPF and PSS were 1,663 mg/l and 2,625 mg/l by, respectively. Furthermore, the COD of oily wastewater using D and mixture of LPF and PSS were 3,125 and 3,478 mg/l, respectively.

Additionally, the higher values of $BOD_{28}$ were registered when adding the mixture of LPF and PSS together with the saponin solution or oily wastewaters. The $BOD_{28}$ values for oily wastewater and saponin solution indicated that PSS and LPF were also not contributing to the biodegradation of the oil residue in oily wastewater.

The maximum of removal rate is 0.27 day for saponin solution, which higher than the maximum value of 0.19 day for oily wastewater. The outcome proposed that the easily degradable organic compounds can be completely removed than the less degradable organics during wastewater treatment. Therefore, in the oily wastewater samples, relative proportion of the less biodegradable organic compounds were higher, giving lower BOD rate constant than the saponin solution. Also, maximum values of ultimate BOD for saponin solution and oily wastewater were 733 and 728 mg/l, respectively. Moreover, the minimum UBOD were obtained with no microbial seed. This study demonstrated that the level of toxicity in the oily wastewater was high enough to destroy microbial activity. Based on curves A, B and C in Figure (5-40), the rates of $BOD_{28}$ test through PSS or LPF reactions were slightly similar. Referring to curve D, expectedly, there is difference for the BOD samples tested with varying seed.
Figure 5-40: Response curve of oily wastewater and saponin solution as measured by the Oxitop. (S1) sample 1; (S2) sample 2; (A) LPF “N”; (B) PSS “B”; (C) mixture of LPF & PSS, and (D) Distilled water “D”
Based on Curves A and B in Figure (5-40), the rates of BOD$_{28}$ test through PSS or LPF reactions were slightly similar. Since the maximum generated values of BOD$_{28}$ for oily wastewater with LPF and saponin solution with LPF were 480 and 452 mg/l, respectively. Also, the maximum generated values of BOD$_{28}$ for oily wastewater with PSS and saponin solution with PSS were 254 and 565 mg/l, respectively. However, the BOD values were increased when adding the mixture of PSS and LPF together with the saponin solution or oily wastewaters, as shown in curve (C) Figure (5-40).

The results of this study suggested that the BOD$_{28}$ values of the saponin solution or oily wastewaters were slightly increased after 20 days. This point indicated that the degradability process was only occurred on the saponin, which is well known as biodegradable surfactant. The microbial seed may not able to consume enough oxygen to degrade the oily wastewater, since the concentration of toxicity in the oily wastewater was high, which could be capable of destroying the microorganisms.

Bioassay for oily wastewater without seeding exhibited the highest reduction in terms of BOD. During the period of incubation, time lags in the BOD degradation curves for oily wastewater were noticed as presented in curve D Figure (5-40), while the maximum values of the BOD curves for oily wastewater and saponin solution without seeding were 254 and 339 mg/l, respectively. The present results suggest that the BOD test may be needed an appropriate bacteria during the incubation period. Therefore, bacteria considered as vital to enhance biodegradation rate.
There seems to be a significant reduction in $k$ that effect on the overall BOD magnitude as shown in Tables (5-7). The mean removal rate $k$ for oily wastewater without seeding and for oily wastewater with mixture of PSS and LPF were 0.15 and 0.16, respectively. The reduction rate of $k$ differs from one sample to the other, resulted from slow biodegradation rate of the organic compounds or due to the transfer of oxygen at the air-water interface (Zainudin et al., 2010). The experiment suggested that the bacteria were unable to (or could barely) adapt to environment conditions of the oily wastewater. The outcome of $\text{BOD}_{28}$ seems to be resulted from biodegrading of saponin.

5.3.2 Biodegradability Comparison of BOD with COD

Based on Table 5-6 and Table 5-7, the ratios of BOD to CODi for saponin solution and oil wastewater by (PSS) and (LPF) were between 0.070 to 0.300, while the ratios of $\text{BOD}_{28}$ to CODi for saponin solution and oil wastewater by *Rhododcoccus, Mycoides* and *Bacillus subtilis* were between 0.001 to 0.300, which indicate that the biodegradation process is extremely slow (Gilbert, 1987). As suggested by Gilbert (1987), a diluent with a ratio of 0.4 could be regarded as non-biodegradable compounds. The dissolved organic compounds can be completely oxidised when compared to less particulate organics compounds. According to Wang et al. (2011) reported that presence of particulate matter such as oil residue may cause depletion of dissolved oxygen, which has an adverse effect on the biodegradation rate. In this work, the total COD measurement is presumably the sum of COD of dissolved and particulate organic and inorganic compounds in the system. Therefore, BOD to COD ratios were very low. Furthermore, the concentration of toxicity in the sample were significantly high which could slow the biodegradation rate. It may prove beneficial to undertake longer BOD experiments to monitor the long term biodegradability. The outcome of the experiment demonstrated that the long term biodegradability was maintained, however with slower rates and with a slightly enhancement was observed in ratio of $\text{BOD}_{28}$/CODi.
5.3.3 Discussion
Oily wastewater samples often contain toxic materials and need special conditions when running BOD tests. Therefore, the sample was required to be diluted to minimize the toxic substance and their impacts. The main factor influencing the rate of hydrocarbon biodegradation in solid or liquid phase is the presence of microorganisms (Ramos et al., 1991). If microbial populations are present, then optimal growth rates and biodegradation rate of petroleum hydrocarbon can be controlled by providing the optimum conditions such as enough nutrients and oxygen, temperature 15-20°C in marine environments and a pH value of 6-9 (Das and Chandran, 2011).

The biodegradation of oily wastewater and saponin solution were recorded indirectly by measuring oxygen consumption rate of microbial seed. The concentration of saponin for oily wastewater and saponin solution was 0.5 wt %. The mean values of BOD\(_{28}\) for the saponin solution and oily wastewater using RM were 1653 and 1792 mg/l, respectively. In addition, the maximum generated values of BOD\(_{28}\) for saponin solution and oily wastewater using LPF were 452 and 480 mg/l, respectively. Generally, it has not been successful to view any major differences in the rates of oil degradation among the microorganism. The outcome shows that the ultimate BOD values were slightly increased after 28 days for most of the seeded samples. These results suggest that the variance in the rates of biodegradation between various microorganisms could not be much higher if the entire experiment duration is longer than 28 days. According to Leahy and Colwell (1990) adaptation of microbial communities considered vital role to increase the effective of the hydrocarbon degradation rates, particularly when utilising hydrocarbon degrading bacteria. As terrestrial petroleum hydrocarbon degrading bacteria typically use various substrates, the majority is extremely specialised, utilising hydrocarbons as the only source of carbon (Yakimov et al., 2007).
Crude oil comprises a significant range of petroleum hydrocarbons with various chemical properties. One of the typical characteristics of bacteria is its ability to metabolise only certain hydrocarbons such as VOC, SVOC and LMW. According to Al-Saleh et al. (2009) a host of different bacteria is required to degrade a wide range of hydrocarbon compounds. In addition, for the degrading communities of petroleum hydrocarbon, it has been found that the surfactants make the oil more accessible to the degraders (Head et al., 2006). For a number of samples, the BOD of oily wastewater samples were found to less than the BOD of saponin solution with seed. This is probably due to the presence of saponin which is well-known to increase BOD as it is readily biodegradable.

It was noted that the highest ratio of $\text{BOD}_{28}/\text{CODi}$ for oily wastewater samples was 0.36, while the highest ratio of $\text{BOD}_{28}/\text{CODi}$ for saponin solution was 0.35. This provides an indication that the constituents in the samples wastewater were non-biodegradable, which could effect on the microbial degradation rate. Further, these recorded levels of $\text{BOD}_{28}$ and $\text{CODi}$ signal potential pollution problems and may lead to harmful bodies.

Based on Section 1.7.2.1, salt concentration above 3 % lowered the performance of aerobic activated sludge in wastewater treatment plants however, the present work found that the population of microbial seed can tolerate salinity levels 45,000 mg/l (4.5 %), which is the concentration of salinity for artificial Kuwait seawater. This study matched to those found by Salvadó et al. (2001), where it can be noted that even though NaCl concentration of 0.5 – 5 % would lower the performance of the biological process in wastewater treatment plants, the microbes could tolerate the salinity. It appears that the microbial cells used in the experiments were capable of keeping an osmotic balance with their micro environment, thus preventing high salt concentrations from impeding their important metabolic processes.
In view of the lessening of microbial activity during the oily wastewater treatment, it has been found that the high salt concentrations (NaCl) is the main reason for decrease of the COD removal rate, affect biodegradation rate of organic compounds such as VOC, SVOC and LMW (Pendashteh et al., 2012). It can be suggested that the low rate of biodegradation of the hydrocarbons in the oily wastewater could be due to the high concentration of petroleum hydrocarbon compounds and high percentage of salt. Given the distinctive current scenarios in the oily wastewater and the dissimilar bacteria in charge of petroleum hydrocarbon degradation, different approaches and techniques ought to be employed for the development of biological oil spill response in the oily wastewater. The microorganism used for seeding played a significant role in reduction of the reaction rates $k$ of the oily wastewater samples and the ultimate BOD. This case study has clearly indicated that aerobic bio-treatment alone is not a suitable method for treating the oily wastewaters. Based on literature search, it is also noted that studies on rate constant of BOD consumption is very limited and hence this experiment adds novelty to this research.

5.4 Measurement of Chemical Oxygen Demand

COD test is indirect measurement used to determine the amount of organic load in waste water. The colorimetric (Section 3.4.8.5) and titration methods (Section 3.4.8.6) were used to measure the COD of organic contamination in saline solution. There were some challenges in this research in attempting to carry out COD measurements for salt concentrations above 2000 mg/l due to the interference of chloride above this level. The results obtained using method Hg-free vials have been compared with the results obtained using Hg-vials and titration method. Comparative data analyses of the COD results were carried out to detect the interference obtained by chloride ion. The relation between Hg and Hg-free vials were tested on different types of water samples such as oily wastewater and treated water.
5.4.1 COD for Oily Wastewater and Treated Sample.

COD vials, which are available commercially containing mercury and mercury free were employed in assessing the COD values oily wastewater samples. Results obtained from the tests were compared against the values obtained from the titration method, as shown in Figure (5-41).

![Figure 5-41: Mean of COD values for oily wastewater by using A) mercury vials and B) mercury free (error bars are indicative of the standard deviation of the average (n = 3)).](image)

As can be seen from Figure (5-41), the mean of COD values for oily wastewater using mercury vials and titration method were 35,500 and 33,759 mg/l, respectively (Paired sample test, \( p > 0.05 \)). However, this study indicated that the mean of COD values was increased to 48,107 and 47,673 mg/l, by using mercury free vials and titration with mercury free, respectively (Paired sample test, \( p > 0.05 \)). COD vials containing mercury and mercury free were employed in assessing the COD values for treated water samples as well. Results obtained from the tests
were compared against the values obtained from the titration method, as shown in Figure (5-42).

![Figure 5-42: Mean of COD values for treated water sample by using A) mercury vials and B) mercury free (error bars are indicative of the standard deviation of the average (n = 3)).](image)

As can be seen from Figure (5-42), the mean of COD values for treated sample by using mercury free vials and titration method mercury free were 4,283 mg/l and 3,922 mg/l, respectively (Paired sample test, \( p > 0.05 \)). This study indicated that the means of COD values were reduced to 1,725 mg/l and 1,430 mg/l, by using mercury vials and titration method with mercury, respectively (Paired sample test, \( p < 0.05 \)). Comparison of the results obtained using COD vials and titration method that contain mercury for treated water samples were 60 and 63 \%\), respectively, less than the generated result with no mercury. The existing methods have substantial challenges, mainly in high organic concentrations, high salinity and high dilution. This highlights the fact that a high ratio 1:100 and mercury vials are essential for the analysis of high COD at high organic concentrations with high salinity.
Further experiments were carried out as shown in Figures (5-43), the outcome of this study established a linear correlation between mercury and mercury free vials for different sample such as oily wastewater with ratio of 1:100, oily wastewater ratio of 1:50 and treated water.

As shown in Figure (5-43) the plot of test samples suggest that there is a strong linearity with high correlation factor between Hg vials and Hg-Free vials. Probably due to the oily wastewater at ratio 1:100 having double strength of organic and saline solution than oily wastewater at ratio 1:50. For these reasons, the COD values obtained by using Hg vials for oily wastewater, at ratio (1:100) and (1:50) were approximately 1.4 times more than those obtained with Hg-Free vials. Further, treated samples have the same concentration of chloride ion in the oily wastewater at ratio (1:100) but are free of organic petroleum contamination. The COD values for treated sample obtained by using Hg vials were approximately 2.5 times more than those obtained with Hg-Free vials.

Figure 5-43: Relationship between COD (mg/l) for (A) Oily wastewater with ratio of 1:100; (B) Oily wastewater with ratio of 1:50 and (C) Treated water. Where, Mercury Vial = - 1546 + 0.76 mercury free, r² = 1, n= 6. 

As shown in Figure (5-43) the plot of test samples suggest that there is a strong linearity with high correlation factor between Hg vials and Hg-Free vials. Probably due to the oily wastewater at ratio 1:100 having double strength of organic and saline solution than oily wastewater at ratio 1:50. For these reasons, the COD values obtained by using Hg vials for oily wastewater, at ratio (1:100) and (1:50) were approximately 1.4 times more than those obtained with Hg-Free vials. Further, treated samples have the same concentration of chloride ion in the oily wastewater at ratio (1:100) but are free of organic petroleum contamination. The COD values for treated sample obtained by using Hg vials were approximately 2.5 times more than those obtained with Hg-Free vials.
5.4.2 Discussion

Oily wastewater is produced which contain high percentage of organics with a comparatively high salinity. The samples were diluted enough to bring the COD results in range of 20 to 1500 mg/L, as explained in Section 3.4.8.2. Measured values of diluted samples were adjusted based on the dilution factor however, major errors can be found as a result of high dilution. According to Han et al. (2011), the low oxidation capabilities of the chemicals involved, due to the sort of organic pollutants in the wastewater leads to issues of poor reproducibility and accuracy. In reality, a COD test assesses the content of carbon based materials through measurement of quantity of oxygen that will react with the sample. The transaction of oxygen is where the name is taken from, hence chemical oxygen demand. The present research work have utilised the standard open reflux and the colorimetric method for analysis of samples containing high salinity (45 g NaCl/l) and high COD value up to 48,107 mg COD/l for oily wastewater sample using mercury free vials. Comparison of standard opened reflux with mercury and colorimetric COD vials that contain mercury shows that our COD results are more reliable. Further, these results suggest that the colorimetric method with mercury vials can be reliable for salinities of 45 g NaCl/l.

In general, employing colorimetric method is more appealing to most researchers than using titration methods, as the latter would require skills and expensive equipment. The titration test for COD utilised a few chemicals for example acid, chromium, silver and mercury which resulted in the production of liquid hazardous waste which have to be properly disposed. It has been found that the mercuric ion combines with the chloride ion to form a poorly ionised mercuric chloride complex, which causes threat to human health and environment (World Health Organization (WHO), 2007).
This research proposes that the Hg-free COD vials provide the likely solution to minimise risk to environmental and disposal expenses compared with traditional mercury containing COD vials. As discussed in (Section 3.4.8.1), a highly significant linear correlation ($r^2 = 0.939$) were determined between COD values using Hg and Hg-Free vials for artificial seawater (4.500%), artificial seawater (2.250%) and artificial seawater (1.125%). Furthermore, a highly significant linear correlation ($r^2 = 1$) were found as well for oily wastewater (1:100), oily wastewater (1:50) and treated water. The COD test which is free from mercury has yet to be officially endorsed such that it can be taken in terms of the mercury containing test. Therefore, mercury free COD vials could be successfully employed to in this project test treated water, oily wastewater and artificial seawater (4.5%).

5.5 Engineering Properties of Treated Sand

This study discusses the effects in examining the durability and workability of treated sand (TPH < 10,000 mg/kg) concrete mixes which are considered as environmental hazard. The treated sand was employed to substitute fine aggregate and the properties of the concrete blocks were calculated using guidelines from British Standards.

5.5.1 Characterisation of Oil Contaminated Sand

5.5.1.1 Soil properties

In order to determine the physical properties of the contaminated soil, a soil permeability test was conducted using a constant head permeameter (BS 1377, Part 5, 1999) on the Kuwaiti oil contaminated sand sample as shown in Figure (5-44). This test was conducted to simulate the field conditions and provide hydraulic flow for the sample.
It was found that average coefficient of permeability (k) for the contaminated soil was 0.064 mm/s. This value represents a typical sandy soil (Bodó and Jones, 2013). Based on British Standards (BS 1377: Part 2:1990), as explained in Section 3.6.1.2, the wet sieving was required as the sample tended to agglomerate during dry sieving.

A mechanical sieve was carried out to eliminate the coarse fraction as well as to ensure homogeneity for the oil contaminated soil. The results from particle size distribution, wet sieving (see Figure 5-45) showed that these particles are responsible for important chemical and physica properties, as explained in Section 5.1.3.12. Therefore, the sample must not be altered during the wet sieving.
5.5.2 Results

5.5.2.1 Slump test

The slump test was used to measure the workability, the outcomes exhibit the mean values of the slump gathered from the slump test for the contaminated specimens and clean specimens were 1.36 cm (SD = 0.18) and 1.48 cm (SD = 0.15), respectively. It was noted that for all mixes, the slump was a “true slump”. No major change was noted while using the sand contaminated with TPH < 10,000 mg/kg or clean sand.

5.5.2.2 Compressive strength of concrete cubes

Oil contamination can affect the compressive strength of concrete (Ejeh and Uche, 2009; Ajagbe et al., 2012). Compressive strength tests were performed on three concrete cubes after 7, 14 and 28 days upon curing, as shown in Figure (5-46).
As illustrated in the average result in Figure (5-46), the cubes compressive strength showed a slightly increase with curing age. This is expected since the strength of properly cured cement based products increases with age. The compressive strength of concrete made of Kuwaiti oily sand also increases however at a slower rate. The average of compressive strengths for contaminated sand after 7, 14 and 28 days of curing were 24.6, 31.0 and 31.7 MPa, respectively. Paired comparison analysis revealed that the compressive strength significantly increased within the first two weeks (Paired test, $p = 0.030$), however, no-significant different was found after two weeks (Paired test, $p = 0.184$). Moreover, the average compressive strength for the control samples (clean sand) after 7, 14 and 28 days of curing increased from 29.5, 35.7 and 36.6 (MPa), respectively.
Paired comparison analysis revealed that the compressive strength significantly increased within the first two weeks (Paired test, \( p = 0.009 \)), however, no-significant different was found after two weeks as well (Paired test, \( p = 0.225 \)).

The variation in results obtained from the contaminated sample can be contributed by the chemical reaction that occurred because of the oil residue (< 1%) in the concrete cubes. The slightly increase in strength may be caused by the changes in the quantity of oil residue (< 1%) within the concrete which may result in the loss of water/moisture content, thus preventing water absorption by the cubes when soaked in water while curing. This phenomenon may be affected the cement hydration within the concrete cubes. The contamination of sand by the crude oil forming part of the concrete matrix microstructure have resulted in dilation of the gel thus deteriorating the cohesive force within the mix (Ajagbe et al., 2012) and therefore effecting concrete strength of Kuwai oily sand.

5.5.2.3 Rebound hammer test

Type NR-10 (47027) (Schmidt Hammer, Proceq, Switzerland) was used in this study as shown in Figure (5-47), which is able to measure range 10 to 70 N/mm² compressive strength with impact energy of 2,207 Nm. Rebound values were recorded as a bar chart on a paper strip. The result is shown in Figure (5-48).
Figure 5-47: Hammer test for contaminated sand after 7 days.

Figure 5-48: Mean of rebound hammer test for (A) contaminated sand, and (B) clean sand. Note: The data are expressed as mean ± SD (n = 3).
It can be seen in Figure (5-48), the average of compressive strengths for contaminated sample after 7, 14 and 28 days of curing were 21.7, 26.3 and 31.2 MPa, respectively. This shows that the compressive strength significantly increased within the first two weeks (Paired test, $p = 0.005$), also, significant different was found after two weeks (Paired test, $p = 0.008$).

Furthermore, the average compressive strength for control samples (clean sand) after 7, 14 and 28 days of curing increased from 26.5, 33.7 and 35.5 (MPa), respectively. It has been found that the compressive strength significantly increased within the first two weeks (Paired test, $p = 0.001$), also, significant different was found after two weeks as well (Paired test, $p = 0.007$).

This investigation concludes that the rebound hammer test concurs with the results of compressive strength, while the slightly decreasing in compressive strength of Kuwait oily sand cubes due to the effect of hydrocarbon on concrete.

5.5.2.4 Concrete water absorption test result

The water absorption test aims to estimate the total pore volume of the concrete, during this research the water absorption test was investigated for oil contaminated sand (TPH < 10,000 mg/kg). The experimentally generated result after curing for 28 days, the obtained result is average value for 3 replicate as shown in Figure (5-49).
As illustrated in Table (5-8), the water absorption capability decreases by increasing the percentage of oil residue to contaminate the sample cubes.

Table 5-8: The average of the water absorption test after 28 day (n=3).

<table>
<thead>
<tr>
<th>Type of samples</th>
<th>Wet Weight (g)</th>
<th>Dry Weight (g)</th>
<th>Measured absorption (%)</th>
<th>Correction factor</th>
<th>Corrected absorption (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Contaminated sample</td>
<td>1455.4 ±6.7</td>
<td>1446.3 ±16.50</td>
<td>0.63</td>
<td>1.17</td>
<td>0.73 ±0.006</td>
</tr>
<tr>
<td>Clean sample</td>
<td>1461.4 ±5.20</td>
<td>1429.4 ±7.00</td>
<td>2.19</td>
<td>1.17</td>
<td>2.56 ±0.010</td>
</tr>
</tbody>
</table>

On average, the quantity of water absorbed by the specimens for the contaminated sample and clean sample were 9.1 g and 32.0 g whilst, the quantity of water absorbed by the specimens was 0.63 and 2.19 %, respectively.
Based on previous Table (5-8), it can be seen that the water absorption was reduced with the contaminated sample around three times than the clean sample. Using treated sample in concrete cube may be able to enhance resistance to the penetration of water, chloride ions, alkali ions and other. It was evident that the oil residue (<1%) presented in the concrete cubes encourage to use treated sand in the coastal construction.

Both these characteristics hugely improve the concrete durability by means of protecting the steel from corrosion and other substances that have some impacts towards the concrete durability. As such, the application of oily sand concrete can be spread to the production of concrete products where the water absorption properties are crucial to the aesthetics. Some examples of this are the roof tiles and liquid retaining structures for instance water and septic tanks, in both cases water passage has to be minimized.

The treated sand concrete may be has low resistivity to fire, however, this has no effect towards the concrete durability when used in damp proof courses and liquid retaining structures as previously discussed. In addition, the oily sand concrete demonstrates excellent durability thus concurred with the recommendations by Al-mutairi and Eid (1997) that these materials are suitable to be applied in the production of asphalt concrete mixes in road bases, secondary roads, impermeable layers for landfills and containment structures or in stabilising steep embankments.
5.6 Direct Detection of Saponins in Soapnuts

5.6.1 Introduction
The principal aim of this experiment was to characterize the saponin extracted from soapnuts by various methods. Several methods were used to analyse the saponins in the plant kingdom (Roy et al., 1997; Kareru et al., 2008; Saidu et al., 2012). These techniques entail abstraction of plant matrix using aqueous or organic solvents before performing the analyses. Nevertheless, saponins present in plants can be directly identified by the high precision infrared absorption spectra of their sapogenins without the need of expensive and time-consuming extraction or purification techniques. By performing characterisation FTIR, the time taken to detect these valuable saponins present in plants can be shortened. UPLC-QTOF-MS/MS were also performed for qualitative analysis of samples to support the detection of triterpenoid saponins by FTIR.

5.6.2 Results and Discussion
5.6.2.1 Phytochemical tests:
i) Froth test
The froth test showed that the aqueous extract and alcoholic extract contain saponins (Figure 5-50) on observation of foam stable for more than ten minutes.
Figure 5-50: Observation of froth test with a) aqueous extract b) 95% ethanol extract c) standard saponin as positive control and d) water as negative control.

ii) Hemolysis test

The results of hemolytic test demonstrates that the aqueous extract and alcoholic extracts contain saponins which can be observed from the distinct hemolytic zone of clearance around the site of application of the respective samples on the blood agar plate as depicted in Figure 5-51. The measurement of hemolytic zones along with standard and negative control are given in Table 5-9.
Figure 5-51: Observation of hemolysis test with a) aqueous extract of soapnuts b) 95% ethanol extract of soapnuts c) standard Quillaja saponin as positive control and d) water as negative control.

Table 5-9: Hemolytic zones observed in hemolysis test.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Haemolytic zone* (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aqueous extract</td>
<td>9</td>
</tr>
<tr>
<td>95% Ethanol extract</td>
<td>9</td>
</tr>
<tr>
<td>Standard saponin (Fisher)</td>
<td>10</td>
</tr>
<tr>
<td>Sterile water (control)</td>
<td>0</td>
</tr>
</tbody>
</table>

*Hemolysis zone is indicated by the distance from the edge of the cup to the farthest end of the zone (in mm)

5.6.2.2 FTIR

The presence of saponins in the aqueous and 95% ethanol extract were confirmed by infrared absorption spectrum and compared with that of the standard. Saponins showed characteristic infrared absorbance of the hydroxyl group (OH) ranging from 3,407 cm$^{-1}$ in the aqueous extract, 3,419 cm$^{-1}$ in the 95% ethanol extract and ranging from 3,525 cm$^{-1}$ to 3,281 cm$^{-1}$ in the standard Quillaja saponin.
C-H absorption was ranging from 2,931 cm\(^{-1}\) in the aqueous extract, 2931 cm\(^{-1}\) 95 % ethanol extract and ranging from 2,973 cm\(^{-1}\) to 2,932 cm\(^{-1}\) in standard Quillaja saponin. C=C absorbance were observed ranging from 1,613 cm\(^{-1}\), 1,636 cm\(^{-1}\) and 1,609 cm\(^{-1}\) in the aqueous extract, 2,931 cm\(^{-1}\) 95 % ethanol extract and standard Quillaja saponin respectively.

Whereas C=O absorbance was found to be at 1,727 cm\(^{-1}\) in the aqueous extract and 1,726 cm\(^{-1}\) in the 95% ethanol extract, while standard saponin showed 1,724 cm\(^{-1}\). Oligosaccharide linkage absorptions to sapogenins, that is C-O-C, were evident between 1,074 cm\(^{-1}\) in the standard, 1,045 cm\(^{-1}\) in 95 % ethanol extract and 1,046 cm\(^{-1}\) in aqueous extract. The abovementioned infrared functional group absorptions characteristic of saponins has been referred to in literature such as Toshiyuki et al. (2001) and Da Silva et al. (2002). Owing to the oleanolic acid/ester, these oleanane-type triterpenoid saponins are characterised by the C=O infrared absorbance. According to Toshiyuki et al. (2001); Kirmizigul et al. (2002); and Natori et al. (1981), these triterpenoid saponins tend to be bidesmosides as they have two attachments of glycones to the sapogenin namely glycosidic and ester groups. In this study, saponins detected in soapnuts, was likely to be bidesmosidic, oleanane-type triterpenoids.

However, these observations need clarification using nuclear magnetic and mass spectroscopy. The FTIR spectra of standard Quillaja saponin (Fisher Scientific, UK) demonstrated triterpenoid saponin absorptions characteristics. It can be seen from Figure (5-52), that saponins are clearly detectable in the crude aqueous and alcoholic soapnut extracts even before the purification of saponins.

The similarity in the FTIR spectrum of the crude soapnut extracts and the standard Quillaja saponin shows the importance of detection of saponins right after the extraction and freeze
drying step thereby eliminating the need of further extensive purification steps. The significance of this observation was that saponins are detectable in crude aqueous and alcoholic extracts directly using FTIR spectroscopy, therefore shortening the time and the necessity for purification steps before performing analysis.

![Figure 5-52: Fourier Transform Infrared (FT-IR) absorption spectrum data with organic extracts (KBr disc), cm⁻¹. A) Standard Quillaja saponin B) 95% ethanol extract, and C) aqueous extract.](image)

5.6.2.3 UFLC

The UFLC chromatograms of aqueous and 95% ethanolic extract of soapnuts and standard Quillaja saponin is shown in Figure (5-53). The comparison of retention times of the separated compounds are tabulated in Table (5-10). UFLC separation of compounds in all samples was achieved within 6 minutes.
It can be inferred from the chromatograms that an earlier onset of separation of Quillaja saponin than the soapnuts extracts under the same chromatographic conditions is consistent with the study by Shiau et al (2009). However, the shorter retention times in this study is due to the difference in technique as described in experimental section. Chromatograms of Both the aqueous extract and 95% ethanol extract of soapnuts showed close similarity. By comparing the retention times of peaks on the chromatograms of the soapnut extracts and Quillaja saponin it can be inferred that peaks corresponding to retention times 2.8, 3.5, 4.1, 4.8 and 5 min correspond to common saponin constituents.

Figure 5-53: UFLC chromatograms of a) aqueous extracted soapnut saponin, b) 95% ethanol extracted soapnut saponin, c) Quillaja saponin (Fisher) on a Shim Pack XR ODS (3mm id × 50mm, 2.2μm) column; gradient elution with solvents water (A)and  acetonitrile(B) at 5-1
<table>
<thead>
<tr>
<th></th>
<th>Aqueous extract (a)</th>
<th>95% ethanol extract (b)</th>
<th>Standard Quillaja saponin (c)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>ND</td>
<td>ND</td>
<td>0.61</td>
</tr>
<tr>
<td>2</td>
<td>ND</td>
<td>ND</td>
<td>1.072</td>
</tr>
<tr>
<td>3</td>
<td>ND</td>
<td>ND</td>
<td>1.654</td>
</tr>
<tr>
<td>4</td>
<td>ND</td>
<td>ND</td>
<td>2.142</td>
</tr>
<tr>
<td>5</td>
<td>ND</td>
<td>ND</td>
<td>2.437</td>
</tr>
<tr>
<td>6</td>
<td>2.827</td>
<td>2.82</td>
<td>2.702</td>
</tr>
<tr>
<td>7</td>
<td>3.025</td>
<td>3.024</td>
<td>ND</td>
</tr>
<tr>
<td>8</td>
<td>3.36</td>
<td>3.361</td>
<td>ND</td>
</tr>
<tr>
<td>9</td>
<td>3.535</td>
<td>3.532</td>
<td>3.494</td>
</tr>
<tr>
<td>10</td>
<td>3.785</td>
<td>3.787</td>
<td>ND</td>
</tr>
<tr>
<td>11</td>
<td>4.112</td>
<td>4.112</td>
<td>4.141</td>
</tr>
<tr>
<td>12</td>
<td>ND</td>
<td>ND</td>
<td>4.633</td>
</tr>
<tr>
<td>13</td>
<td>4.87</td>
<td>4.85</td>
<td>4.803</td>
</tr>
<tr>
<td>14</td>
<td>5.042</td>
<td>5.023</td>
<td>4.981</td>
</tr>
<tr>
<td>15</td>
<td>5.341</td>
<td>5.115</td>
<td>ND</td>
</tr>
<tr>
<td>16</td>
<td>ND</td>
<td>5.334</td>
<td>ND</td>
</tr>
</tbody>
</table>

*ND= Not detected.*
5.6.2.4 UPLC Q-TOF-MS/MS

The mass spectroscopic data of the aqueous extract, 95% ethanolic extract was carried out to substantiate the presence of triterpenoid saponins by screening for characteristic molecular masses, based on similar cases of reports of other researchers. However, a study at the level of detailed molecular identification was not intended at this stage. The uniformity in appearance of 381 m/z, 723 m/z, 1065 m/z and 1407 m/z demonstrates the presence of disaccharide moiety in the glycone element of saponins. This is in agreement with the results of Wong (2014). Another study by Heng et al (2014) refers to compounds molecular weights of 1206, 1074, 882, 750, 924 and 966 were identified as triterpenoid saponins, primarily the oleanolic acid type, the outcome is presented in Table (5-11).

Table 5-11: Comparison of characteristic molecular mass fragments in the mass spectrum of the soapnut extracts and standard Quillaja saponin.

<table>
<thead>
<tr>
<th>Characteristic Molecular mass of triterpenoid saponins</th>
<th>Aqueous extract of soapnuts</th>
<th>95% Ethanol extract of soapnuts</th>
<th>Standard Quillaja saponin</th>
<th>Reference *</th>
<th>Type of saponin from reference *</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mass (m/z)</td>
<td>RT** (min)</td>
<td>Mass (m/z)</td>
<td>RT (min)</td>
<td>Mass (m/z)</td>
<td></td>
</tr>
<tr>
<td>ND*</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>1076</td>
<td>0.47</td>
</tr>
<tr>
<td>881</td>
<td>6.63</td>
<td>881</td>
<td>6.60</td>
<td>895</td>
<td>6.41</td>
</tr>
<tr>
<td>757</td>
<td>17.63</td>
<td>757</td>
<td>17.59</td>
<td>751</td>
<td>21.28</td>
</tr>
<tr>
<td>1024</td>
<td>5.10</td>
<td>1024</td>
<td>5.10</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>1148</td>
<td>5.90</td>
<td>1148</td>
<td>5.90</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>1206</td>
<td>0.64</td>
<td>1207</td>
<td>0.64</td>
<td>1206</td>
<td>15.93</td>
</tr>
<tr>
<td>ND</td>
<td>ND</td>
<td>947</td>
<td>30.3</td>
<td>ND</td>
<td>947</td>
</tr>
</tbody>
</table>

*Heng et al. (2014); **RT= Retention time; *ND= Not detected.

Based on this report the LC-MS data of the aqueous extract, 95% ethanol extract and standard saponin was examined for the compounds with the above masses which are characteristic for triterpenoid saponins. The findings are depicted in Figure (5-54).
Figure 5-54: UPLC-MS/MS chromatogram a) Quillaja saponin (Fisher) b) aqueous extracted soapnut saponin, c) 95% ethanol extracted soapnut saponin.
5.7 Project summarization

Many of Kuwait’s oil wells were destroyed by the Iraqi armed forces during the Iraqi invasion. The resulting oil spills created oil lakes characterised by sludge, overlying a thickness of oil contaminated soil, which in turn overlies soil with no visible oil contamination. This project involves developing and evaluating more towards implementing environmentally friendly techniques for removing the oil residue to minimise the risk to human health and groundwater.

This research was conducted using lab-scale apparatus that had been developed in the laboratory of the School of Civil Engineering and Surveying. In this way the experimentation was made safer, more cost effective and environmentally friendly. In view of the catastrophic environmental situation, it is recommended that the following scheme (Figure 5-55) be established in order to select a remediation technique for Kuwaiti contaminated soil. The figure illustrates the outline of this research which has already been discussed in the previous sections.
Figure 5-55: Decision points is giving in subsequent chapters.
Chapter 6. Conclusion

Various types of soil remediation techniques were evaluated and developed to rehabilitate the current issue. This project attempts to contribute to knowledge by using the combination technique of ultrasonication and mechanical stirrer to deal with Kuwaiti weathered oil sand. The enhanced soil-washing technique demonstrated greater efficiency of oil removal and more economical in comparison with the individual methods.

It was found that the combination of ultrasonication and mechanical stirrer using saponin is one of the most suitable technique for the remediation of Kuwait oily sands. The main conclusions drawn from the studies are:

• Mechanical stirrer was able to keep the sandy particles in circulation during the washing process, and prevent the particles from settling.

• Ultrasonic waves produced cavitation bubbles, these bubbles collapse to split the oil from fine particles with contaminants from larger parent constituents to water as oily colloidal suspension.

• This research suggests the optimum condition for the highest oil removal from oil contaminated soils by using saponin with artificial sea water at a temperature of 50°C with 0.5 wt% of saponin, soil/solution ratio of 1:3 (mass/volume), an stirrer speed of 1000 rpm and washing time of 20 min.

• The percentage of oil removed from soil at 20°C, 50°C and 70°C were about 43, 54 and 65 %, respectively. Increasing the washing temperature will activate the oil, forming a good environment for its isolation from the soil.

• Saponin solution may have no impact on the oil removal properties at concentration higher than their CMC level.
• The percentage of oil removal from soil with ratios of soil/volume (1:6) and (1:3), were 54 and 66 %, respectively. These experiments indicate clearly that the greatest rate of oil removal from samples was achieved with a ratio of 1:3 (mass/volume).

• The percentage of oil removal by mechanical stirrer, ultrasonication and combination technique after the third wash were 75, 80 and 90 %, respectively. The combined techniques of ultrasonication and mechanical stirrer demonstrated greater efficiency of oil removal and more economical in comparison with the individual methods.

❖ Oily wastewater generated by washing of Kuwait oil sand has a negative impact on human health and environment. Therefore, various wastewater treatments were performed during this research. It has been concluded that the most suitable treatment method for oily wastewater is the combination of aeration with coagulation/flocculation. The main conclusions drawn from the studies are:

• The optimum concentrations of coagulants were 1.53 mg/l and 0.27 mg/l for Al and Fe, respectively. However, the coagulation/flocculation process showed better results when alum was utilised as a coagulant.

• The combination of anoxic with coagulation/flocculation was capable of lowering the COD values from 48,215 to 14,110 mg/l and producing 0.61 g of floc weight.

• The combined treatment system of aeration with coagulation/flocculation was capable of lowering the COD values from 48,215 to 4,784 mg/l as well as increasing the floc weight to 0.69 g.

• It has been concluded that the mean values of COD for saponin was removed about 69 % after 5 days of aeration.
• The biodegradability test suggested that microbial populations were not capable of degrading petroleum hydrocarbon.

• It was noted that the BOD/COD ratios for all samples were 0.4 or lower. This provides an indication that the constituents in the samples wastewater were somewhat non-biodegradable and cause high toxicity in the samples that could affect the aquatic living.

• The microbial seed was able to degrade saponin compounds in the oily wastewater, which allow the stability of oil emulsion to be broken down and released easily.

❖ Based on the experimental work, engineering properties were investigated by using treated sand (TPH < 10,000 mg/kg). The following observations were concluded:

• Sands containing less than 10,000 mg/kg crude oil contaminations did not affect the compressive strength of the concrete. The average compressive strength for contaminated sample after 7, 14 and 28 days of curing increased from 24.6, 31.0 and 31.7 (MPa), respectively.

❖ Direct detection of saponins in soapnuts (Sapindus mukorossi) using FTIR spectroscopy is investigated in this project. The following observations were concluded. The main conclusions drawn from the studies are:

• FTIR spectra of Quillaja saponin (standard) also demonstrated triterpenoid saponin absorptions characteristics and the saponin powder extracted by distilled water and ethanol (95 %) displayed 97 % similarity in absorbance to the standard Quillaja saponin.

• This study recommends extracting saponins from soapnuts by distilled water for safety, ecofriendliness and economic reasons.
6.1 Future Work

This research forms a section of a larger project which focusses on developing a solution to the oil-contaminated soil in Kuwait. KISR is set to sponsor the development of the work, in this theses pilot scale tests will be start on June 2015. The pilot project will be conducted prior to the commercial scale to decide whether the selected method can reliably obtain the desired results. If the pilot test verifies the remedial efficiency, the suggested method will be implemented at the commercial scale, schematic plan is shown in Figure (6-1).

Figure 6-1: Simplified process flow diagram for remediation of Kuwait oil sand.

At the moment, only one of 17 pre-qualified firms is permitted to participate in the tender for clean-up work. Pre-qualification entails a stringent analytical process between international establishments and KOC with only a handful companies have been successful. The proposed technique is predicted to become the leader in contracting new projects.
6.1.1 Pilot Scale

The real challenge is not only to investigate the most effective method to wash Kuwait oil sand but to also ascertain further understanding with respect to the practical applicability of soil washing. The required size of ultrasonication bath has proven very difficult to obtain as numerous manufacturers contacted have little or no expertise on the provision of an equipment of this magnitude. Hilsonic Ultrasonication Cleaners which is a leading company in the production of large scale ultrasonication cleaning devices provided a price quotation of £7,000 for a (300 l) unit which exceeds the budgetary allowance of this research (see Figure 6-2). Therefore, KISR is sponsoring the pilot scale study.

Figure 6-2: Pilot scale test machine.
It is possible to estimate the amount of soil could be processed in the suggested reactor (300 l), the selected ratio in this project was (1:3). Consequently, 100 kg of soils was treated in the suggested reactor. While, the surfactant needs to be employed in every washing process is 0.5 wt% x 300 l = 1.5 kg of surfactant, for each wash. Then, contaminated sand and saponin solution were mechanically mixed and sonified. Subsequent, the saponin dissolved petroleum hydrocarbons solution will pump out to the collected tank. When sand sediments, the oily wastewater will be transferred to the treatment tank, and then to the washing tank, schematic diagram of fabrication is shown in Figure (6-3). The process will be repeated three times. A prominent attribute is that the suggested technology can be transported, and implementing on-site treatment.

Figure 6-3: Schematic representation of the soil remediation for pilot study.
6.1.1.1 General construction of soil cleaning system.

Wash Tank with cylindrical bath with internal dimensions of 600 mm dia X 900 mm deep. Constructed from 2 mm high quality 316 L stainless steel with all joints double TIG welded, liquid connections are to include a 1” British Standard Pipe (BSP) drain. This system is encased in a high finish stainless steel insulated console with 50 mm foil backed insulation to increase energy efficiency. The pump will be mounted under the cleaning station and the filter is to be positioned to the side of the station for ease of maintenance. This system is encased in a high finish stainless steel insulated console with 50 mm foil backed insulation to increase energy efficiency. The pump will be mounted under the cleaning station and the filter is to be positioned to the side of the station for ease of maintenance. All electrical control and are housed within the main body of the system. The user interface incorporates on/ off controls and indicators for all operations, a digital temperature controller will provide temperature indication /control and a timer provides process control times.

6.2 Recommendation

This research recommends to determine the mass balance calculations as part of the process. In calculating the mass balance, it is important to know the quantity of contaminated soil and this has sparked huge interest. The target of TPH < 10,000 mg/kg has been recommended by KEPA. Therefore, the concentration of TPH in the soil after each washing technique needs to be determined. Throughout the washing, a total of 300 kg of soil will be used for each washing time. Besides, it is presumed that the usage of surfactant for three washing stages is 2,700 l x 0.005 kg/l surfactant = 13.5 kg of saponin in every washing. Each surfactant solution is used three times, it can be deduced that the consumption of saponin in every wash is 4.5 kg. A total of 1,200 l of
water was employed resulted in a total of 300 kg of oil contaminated sand, unit weight of a typical soil (20 kN/m³). Based on conventional method to calculate the cost of soil washing which includes but not limited to labour, materials, pumps and pipe works, drainage construction, storage tanks and cost of saponin, an estimate of £116/m³ of oil contaminates sand can be assumed, as presented in Table (6-1).

Table 6-1: Finical of operation factors to estimate the project cost.

<table>
<thead>
<tr>
<th>Critical Operation Factors</th>
<th>Cost/ day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Operation cost ( Power)</td>
<td>£10/ day</td>
</tr>
<tr>
<td>Operating workers</td>
<td>£10/ day</td>
</tr>
<tr>
<td>Soil washing system price (£7,000)*</td>
<td>£14/ day</td>
</tr>
<tr>
<td>Saponin (purity 60 %)</td>
<td>£10/ day</td>
</tr>
<tr>
<td>Aeration system and coagulation/flocculation price (£4500)*</td>
<td>£9/ day</td>
</tr>
<tr>
<td>Coagulant cost (alum)</td>
<td>£5/ day</td>
</tr>
<tr>
<td>Total cost for 1000 kg</td>
<td>£58 for tonne</td>
</tr>
</tbody>
</table>

*504 working days per two year.

As stated by Lowe et al. (1999), the estimated cost of flushing technologies can range between £55 and £679/m³. It should be noted that the costs for washing procedure are within the range of the average in-situ flushing methodologies costs. These recommendations are based on limited testing at lab scale. Additional research should be carried out to enhance the effectiveness and applicability of soil washing technology. In general, the role of saponin in the remediation of oil contaminated soil play significant role, additional studies will be conducted to use grind soapnuts as washing agent. Also, further testing is needed in order to obtain the desire results in the pilot scale and to resolve the difficulties seen in lab scale. Continuous research will be undertaken on development and testing of the wastewater treatment in pilot scale.
6.3 Scale up of Stirrer

Design of industrial-scale process is typically dependent upon the small-scale prototypes’ performance. It is costly and takes longer time to determine the optimum operating conditions at production scale, as such it is advantageous to learn whether a particular process will work properly prior to its construction in full scale. Preferably, scale-up ought to be undertaken such that the conditions in the large vessels are representative to those producing required results in the small vessels. Mixing is a key function of reactors, therefore it would be appropriate to maintain the mixing time constant on scale-up. It is necessary to provide sufficient shear conditions in reactors for the dispersion of bubbles, droplets and cell flocs. All these are essential factors in the mixing process which can be determined through an amalgamation of two physical processes:

1. Distribution is the process whereby materials are being circulated through all areas within the vessel by bulk circulation currents
2. Dispersion is the process whereby bulk flow is broken up into reduced sizes and smaller eddies

In order to find for the optimum parameters in implementing the appropriate procedure, it is important to determine and select the correct type of mixer to be used. The process to select the particular parameters includes

Step 1 – To identify the suitable type of mixing to be carried out
Step 2 – To prepare the inventory of the properties of the materials to be mixed
Step 3 – To identify the global characteristics of the mixing rotors
Step 4 – To select the appropriate type of rotors
Step 5 – To compute the various mixing parameters (tank – rotors)
The energy transferred by the mixer to the fluid can be in two forms namely flow and shear in accordance with the following relationship:

\[ P = Q \times S \times S.G. \]  

**E. q(6 – 1)**

P=Power  
Q=Flow  
S = Shear (head)  
S.G. = Specific Gravity

As mentioned by Brawn Mixer, Inc, (BMI, 2003), a mixer can be designed for any particular power level in order that either the shear component or the flow component consumes most of the power applied. Typically, a mixer with a small diameter rotating at a high speed, most of the applied power is lost as shear. Moreover, a low-speed mixer having a larger diameter impeller will manage a larger volume of fluid which results in high flow. The most suitable ratio of these components is decided based upon the application constraints.

Doran (2013) reported that there are many types of impellers which can be used for mixing applications as shown in Figure (6-4). However, there are several factors to be considered to select the best impeller such as turbulent shear stress, liquid viscosity and eddy currents. As shown in Figure (6-5), a number of impellers were recommended for a wide range of viscosity. During this project propeller was used for both the lab and pilot scale. Which is recommended for low viscosity fluids and generate rapid mixing. It has been noticed in this project that the propeller was able to generate circulation current to carry the mixture of contaminated sand and water with sufficient velocity.
Figure 6-4: Impeller design (Doran, 2013).

Figure 6-5: Different impeller for various fluid range (Holland and Chapman, 1996).
The purpose of conducting small-scale experiments (Figure 6-6) is to confirm operating parameters under conditions that can actually maintain both physical and economical perspectives at commercial scale. Based upon this approach, provided that the flow regime is consistent in the small- and large-scale reactors, it is highly likely that results obtained in the small-scale units will be replicated in the larger scheme.

Figure 6-6: Examples of prototype designed by the author used in proofing trials to be scaled up
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8 Appendix (A): Preliminary Work

8.1 Adsorption and Separation Methods

Adsorption process have been studied for the removal of hydrocarbons from water through sorbents material such as, activated carbon and woodchips, furthermore, the centrifuging was used to evaluate the potential of separate the contamination residual from water. All the selected technologies have been widely proved as a highly efficient method to treat the oily wastewater.

8.1.1 Treatment using powdered activated carbon (PAC)

PAC was used to remove organic compounds from oily wastewater. 150 ml glass funnel was used for preparation process, whereas fiber was packed into the glass tube of conical flask as shown in Figure (8-1). 5 g of PAC was weighted and then placed into the conical glass funnel for adsorption process. Subsequently, 50 ml of oily wastewater was decanted into the graduated mark of the glass funnel. When the process completed, the stock solution were analysed immediately. COD values were measured in the filtrate water, (Section 3.4.7).

Figure 8-1: Treatment of oily wastewater by PAC.
8.1.2 Treatment using woodchips

50 ml of oily wastewater decanted into a 250 ml glass beaker, and 5 g of woodchips were added. The flask was placed into a magnetic stirrer (CB302). The mixture of oily wastewater with woodchips was mechanically stirred for 15 min at a constant speed of 100 rpm to provide a uniform distribution of the woodchips during process, and then stand for 30 min, whereby the aggregation of oil residue was floated on the water surface, see in Figure (8-2). The chips that floated on water surface were removed with a spoon, while the aggregates of oil residue that sank in the bottom of the glass beaker were taken out with a spoon after decanting the supernatant water. All the aggregates mixture that removed manually from the beaker was allowed to filter for one hour using weighed filter paper (Whatman Grade No. 2) while the COD values were measured in the filtrate water, as explained in Section 3.4.7. After filtration process completed, the filter paper and the aggregates mixture were dried overnight at 45°C and finally they were weighed, as explained in Section 3.4.2.

Figure 8-2: Treatment of oily wastewater using woodchips.
8.1.3 Treatment using centrifuging
Eppendorf centrifuge (model 5804 R, Eppendorf, UK) was used to separate the dispersion samples based into fractions weight. The oily wastewater is decanted into graduated 50 ml glass vials, during this experiment 3 replicates were carried out, as shown in Figure (8-3). Then, the resolution of the organic phase was separated from solution by centrifuging at 3000 rpm for 10 min, while the supernatants were pipetted out to determine the COD values (Section 3.4.7). During the process of withdraw the top emulsion phase, a good care was taken to prevent distributing settled layers.

Figure 8-3: Treatment of oily wastewater by using centrifuge technique.

8.1.4 Adsorption removal
Removal of emulsified oils was determined by using different types of sorbents such as activated carbon (Section 4.4.1.1) and woodchips (Section 4.4.1.2), while a centrifuge (Section 4.4.1.3) was used to investigate the physical isolation efficiency, see Table (5-4).
Table 8-1: Preliminarily treatments of oily wastewater by centrifuging and adsorption (n= 3).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>COD (mg/l) Before</th>
<th>COD (mg/l) after</th>
<th>TSS (g)</th>
<th>pH</th>
<th>Turbidity (NTU)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAC</td>
<td>48,215 ± 2,720</td>
<td>13,133</td>
<td>-</td>
<td>8.55</td>
<td>18 ± 1.5</td>
</tr>
<tr>
<td>Woodchips</td>
<td>48,215 ± 2,720</td>
<td>41,067</td>
<td>0.22</td>
<td>7.85</td>
<td>144 ± 5.5</td>
</tr>
<tr>
<td>Centrifuging</td>
<td>48,215 ± 2,720</td>
<td>25,600</td>
<td>0.44</td>
<td>7.28</td>
<td>47 ± 3.0</td>
</tr>
</tbody>
</table>

Table (8-1) presents significant effective method for reduction of COD was as follows:
PAC (M = 13,133; SD = 351) > centrifuging (M = 25,600, SD = 458) > woodchips (M = 41,067; SD = 1,550), (ANOVA F (2, 6) = 644, Post-hoc Scheffe test, p = 0.00).

Further, the percentage reduction of COD by using PAC, centrifuging and woodchips were 72, 46 and 13 %, respectively. In the present research, pH of the oily wastewater was increased slightly after PAC treatment. Initial pH of oily wastewater was 8.27, which increased to 8.55 at 5 g /50 ml of PAC, there was slight reduction in pH to 7.82 by using centrifuge. However, there was no change in pH value, when woodchips was used. The results obtained in relation with turbidity showed that PAC has ability to reduce the turbidity value from 176 to 18 NTU, which considered the highest effective technique in colour removal than centrifuge or woodchips as well. The experiment found that the TSS for PAC was difficult to measure, since they stick together with wet PAC, while the centrifuging proved that was able to separate more suspended solid than woodchips. Wastewater treatment using a few inexpensive adsorbents and PAC had demonstrated that PAC was more effective in reducing COD compared with other pre-treated adsorbents such as bagasse, straw-dust, saw dust, coconut coir and fly ash (Rao and Bhole, 2000). It happens that many of these procedures, due to high contamination level and high cost, become impracticable. These results suggest that the adsorption and centrifuge treatments are not the appropriate method in this study.
9 Appendix (B) : DNA Extraction

The purification of Genomic DNA was performed from pure bacterial cultures using Wizard Genomic DNA purification kit, while the fluorometry model TK 100 fluorometer was used to quantify. The extracts of DNA were kept at −20 °C and used to amplify 16S rRNA from the extracted DNA aided by 27F and 1492 primers. All experiments were performed in volumes of 25 µl comprising 12.5 pmol of each primer, 200 µM of each deoxyribonucleoside triphosphate, 2.5 µl of 10x Polymerase Chain Reaction (PCR) buffer (100 mM Tris-HCl, 15 mM MgCl₂, 500 mM KCl; pH 8.3), and 0.5 U of Taq DNA polymerase (Applied Biosystems), and made up using sterile water to 25 µl volume. Based upon the following programme, PCR was conducted in a Thermocycler (Applied Biosystems, Warrington, UK): 5 min denaturation at 95 °C, followed by 30 cycles of 1 min denaturation at 95 °C, 1 min annealing at 55 °C, 1 min extension at 72 °C, and a final extension step of 5 min at 72 °C. Products from PCR were visualized by means of electrophoresis in 2% (wt vol⁻¹) agarose gels and stained using ethidium bromide (0.5 µg ml⁻¹). The purification of amplified DNA was performed with QIAQUICK PCR cleanup kit (Qiagen, Inc., Valencia, California, USA), and the determination of DNA concentrations was carried according to the previous description. Roughly 10 ng quantity of 16S rRNA were used as template in dye terminator cycle sequencing reactions (Applied Biosystems PRISM dye terminator cycle sequencing kit). The results from the 16S rRNA sequences were analysed by means of the National Center for Biotechnology Information (NCBI; Bethesda, Maryland, USA) BLAST.
### 10 Appendix (C): Engineering Application

Table C-1: Concrete mix design form, (Source of form: BRE, 1997)

<table>
<thead>
<tr>
<th>Stage</th>
<th>Item</th>
<th>Calculation</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.1 Characteristic strength</td>
<td>Specified</td>
<td>30 N/mm² at 28 days, Proportion defective 5%.</td>
</tr>
<tr>
<td></td>
<td>1.2 Standard deviation</td>
<td>Figure (C-1)</td>
<td>8 N/mm² or no data _N/mm² (k=1.64) 1.64 x 8 = 13.12 N/mm²</td>
</tr>
<tr>
<td></td>
<td>1.3 Margin</td>
<td>C₁ or specified</td>
<td>_N/mm²</td>
</tr>
<tr>
<td></td>
<td>1.4 Target mean strength</td>
<td>Specified</td>
<td>30 + 13.12 = 43.12 N/mm²</td>
</tr>
<tr>
<td></td>
<td>1.5 Cement strength class</td>
<td>C₂ specified</td>
<td>42.5/=2.5</td>
</tr>
<tr>
<td></td>
<td>1.6 Aggregate type: coarse</td>
<td>Table (C-2), Figure (C-2) Specified</td>
<td><strong>Crushed/uncrushed</strong></td>
</tr>
<tr>
<td></td>
<td>Aggregate type: fine</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.7 Free-water/cement ratio</td>
<td></td>
<td>0.48</td>
</tr>
<tr>
<td></td>
<td>1.8 Maximum free-water/ cement ratio</td>
<td></td>
<td>__</td>
</tr>
<tr>
<td>2</td>
<td>2.1 Slump or Vebe time</td>
<td>Specified</td>
<td>Slump 10-30 mm or Vebe time _s. 20 mm</td>
</tr>
<tr>
<td></td>
<td>2.2 Maximum aggregate size</td>
<td>Specified</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.3 Free-water content</td>
<td>Table (C-3)</td>
<td>160 kg/m³</td>
</tr>
<tr>
<td>3</td>
<td>3.1 Cement content</td>
<td>C₃</td>
<td>160 ÷ 0.48 = 333.33 kg/m³</td>
</tr>
<tr>
<td></td>
<td>3.2 Maximum cement content</td>
<td>Specified</td>
<td>__ kg/m³</td>
</tr>
<tr>
<td></td>
<td>3.3 Minimum cement content</td>
<td>Specified</td>
<td>__ kg/m³</td>
</tr>
<tr>
<td></td>
<td>3.4 Modified free-water/cement ratio</td>
<td>use 3.1 if &lt;3.2 use 3.3 if &gt; 3.1</td>
<td>333.33 kg/m³</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>__</td>
</tr>
<tr>
<td>4</td>
<td>4.1 Relative density of aggregate (SSD)</td>
<td>Figure (C-3)</td>
<td>2.6 known/assumed</td>
</tr>
<tr>
<td></td>
<td>4.2 Concrete density</td>
<td>C₄</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4.3 Total aggregate content</td>
<td></td>
<td>2400 kg/m³</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2400 – 333.33 – 160 = 1906.67 kg/m³</td>
</tr>
<tr>
<td>5</td>
<td>5.1 Grading of fine aggregate</td>
<td>Percentage passing 600 μm sieve Figure (C-4)</td>
<td>73 %</td>
</tr>
<tr>
<td></td>
<td>5.2 Proportion of fine aggregate</td>
<td>C₅</td>
<td>27 %</td>
</tr>
<tr>
<td></td>
<td>5.3 Fine aggregate content</td>
<td></td>
<td>1906.67 × 0.27 = 514.8 kg/m³</td>
</tr>
<tr>
<td></td>
<td>5.4 Coarse aggregate content</td>
<td></td>
<td>1906.67 – 514.8 = 1391.87 kg/m³</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Quantities</td>
<td>Cement kg</td>
<td>Water (kg or Liter)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Per m³ (to nearest 5kg)</td>
<td>333.33</td>
<td>160</td>
<td>514.8</td>
</tr>
<tr>
<td>Per trial mix of 0.001 m³</td>
<td>0.33</td>
<td>0.16</td>
<td>0.5148</td>
</tr>
<tr>
<td>No. of Scoopfuls is 9</td>
<td>3</td>
<td>1.44</td>
<td>4.6</td>
</tr>
<tr>
<td>Per trial mix of 0.003375 mm³</td>
<td>1.125</td>
<td>0.54</td>
<td>1.74</td>
</tr>
<tr>
<td>No. of Scoopfuls is 3</td>
<td>3.375</td>
<td>1.62</td>
<td>5.22</td>
</tr>
<tr>
<td>Total requirement</td>
<td>6.5</td>
<td>3.06</td>
<td>10</td>
</tr>
</tbody>
</table>

* _c = Calculation*
Figure (C-1): Relationship between standard deviation and characteristic (Source: BRE, 1997).

Table (C-2): Approximate compressive strength (N/mm²) of concrete mixes with a free-water/cement ratio of 0.5 (BRE, 1997)

<table>
<thead>
<tr>
<th>Cement strength class</th>
<th>Type of coarse aggregate</th>
<th>Compressive strengths (N/mm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Age (days)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
</tr>
<tr>
<td>42.5</td>
<td>Uncrushed</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>Crushed</td>
<td>27</td>
</tr>
<tr>
<td>52.5</td>
<td>Uncrushed</td>
<td>29</td>
</tr>
<tr>
<td></td>
<td>Crushed</td>
<td>34</td>
</tr>
</tbody>
</table>

Throughout this publication concrete strength is expressed in the units N/mm².
1 N/mm² = 1MN/m² = 1MPa. (N = newton; Pa = pascal.)
Figure (C-2): Relation between compressive strength and free water/ Cement ratio (Source: BRE, 1997).

Table (C-3): Approximate free contents (kg/m$^3$) required to give various level of workability (BRE, 1997).

<table>
<thead>
<tr>
<th>Slump (mm)</th>
<th>0-10</th>
<th>10-30</th>
<th>30-60</th>
<th>60-180</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vebe time (s)</td>
<td>&gt;12</td>
<td>6-12</td>
<td>3-6</td>
<td>0-3</td>
</tr>
<tr>
<td>Maximum size of aggregate (mm)</td>
<td>Type of aggregate</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>Uncrushed</td>
<td>150</td>
<td>180</td>
<td>205</td>
</tr>
<tr>
<td>Crushed</td>
<td>180</td>
<td>205</td>
<td>230</td>
<td>250</td>
</tr>
<tr>
<td>20</td>
<td>Uncrushed</td>
<td>135</td>
<td>160</td>
<td>180</td>
</tr>
<tr>
<td>Crushed</td>
<td>170</td>
<td>190</td>
<td>210</td>
<td>225</td>
</tr>
<tr>
<td>40</td>
<td>Uncrushed</td>
<td>115</td>
<td>140</td>
<td>160</td>
</tr>
<tr>
<td>Crushed</td>
<td>155</td>
<td>175</td>
<td>190</td>
<td>205</td>
</tr>
</tbody>
</table>
Figure (C-3). Estimated wet density of fully compacted concrete (Source: BRE, 1997).

Figure (C-4): Proportions of fine aggregate with percentage passing sieve (Source: BRE, 1997).
Figure (C-5): Graph for rebound hammer test.
**Form UPR16**

Research Ethics Review Checklist

Please include this completed form as an appendix to your thesis (see the Postgraduate Research Student Handbook for more information)

<table>
<thead>
<tr>
<th>Postgraduate Research Student (PGRS) Information</th>
<th>Student ID: 439803</th>
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<tbody>
<tr>
<td>PGRS Name: Meshari Saad Almutairi</td>
<td></td>
</tr>
<tr>
<td>Department: School of Civil Engineering and Surveying</td>
<td></td>
</tr>
<tr>
<td>First Supervisor: Muhammad Ali</td>
<td></td>
</tr>
<tr>
<td>Start Date: Sep 2011</td>
<td></td>
</tr>
<tr>
<td>Study Mode and Route:</td>
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<tr>
<td>Part-time</td>
<td>MPhil</td>
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<tr>
<td>Full-time</td>
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<tr>
<td></td>
<td>MD</td>
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<td>Professional Doctorate</td>
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<tr>
<td>Title of Thesis: Development and Evaluation of a Remediation Strategy for the Oil Lakes of Kuwait</td>
<td></td>
</tr>
<tr>
<td>Thesis Word Count: 65,670</td>
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</tr>
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If you are unsure about any of the following, please contact the local representative on your Faculty Ethics Committee for advice. Please note that it is your responsibility to follow the University's Ethics Policy and any relevant University, academic or professional guidelines in the conduct of your study. Although the Ethics Committee may have given your study a favourable opinion, the final responsibility for the ethical conduct of this work lies with the researcher(s).

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- [ ] a) Have all of your research and findings been reported accurately, honestly and within a reasonable time frame? **YES** [ ] **NO** [ ]
- [ ] b) Have all contributions to knowledge been acknowledged? **YES** [ ] **NO** [ ]
- [ ] c) Have you complied with all agreements relating to intellectual property, publication and authorship? **YES** [ ] **NO** [ ]
- [ ] d) Has your research data been retained in a secure and accessible form and will it remain so for the required duration? **YES** [ ] **NO** [ ]
- [ ] e) Does your research comply with all legal, ethical, and contractual requirements? **YES** [ ] **NO** [ ]

**Candidate Statement:**

I have considered the ethical dimensions of the above named research project, and have successfully obtained the necessary ethical approval(s)

**Ethical review number(s) from Faculty Ethics Committee (or from NRES/SCREC):**

If you have not submitted your work for ethical review, and/or you have answered 'No' to one or more of questions a) to e), please explain below why this is so:

Not applicable to this area of research
Direct detection of saponins in crude extracts of soapnuts by FTIR

Meshari Saad Almutairi & Muhammad Ali

School of Civil Engineering & Surveying, University of Portsmouth, Portland Building, Portland Street, Portsmouth PO1 3AH, UK

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To link to this article: http://dx.doi.org/10.1080/14786419.2014.992345
Direct detection of saponins in soapnuts using Fourier transform infrared (FTIR) spectroscopy is investigated in this project. Potassium bromide powder was mixed with extracted powder of soapnuts and compressed to a thin pellet for examination process. The outcome of the FTIR spectra of saponin demonstrated characteristic triterpenoid saponin absorptions of OH, C\(_{v}\)O, C\(_{Z}\)H, and C\(_{v}\)C, while the glycoside linkages to the sapogenins were indicated by the absorptions of C\(_{Z}\)O. The significance of this study is that saponin absorption peaks are directly detectable in crude aqueous and 95% ethanol extracts of soapnuts powder using FTIR spectroscopy, thereby eliminating the need of further expensive and exhaustive purification steps. The extracts of soapnuts were screened for saponins along with controls by phytochemical tests, and advanced spectroscopic techniques such as ultra fast liquid chromatography and ultra performance liquid chromatography quadrupole-time of flight-mass spectrometry were also implemented to validate the saponins.

Keywords: saponin; soapnuts; FTIR; extraction; phytochemical

1. Introduction

Saponins are a large assembly of glycosides classified as non-ionic bio-surfactants. They are extensively distributed in the territory of green plants (Roy et al. 1997), especially vegetables, soapnut pericarp, soybeans, peas, beans and herbs, whereas saponins are commercially produced from *Yucca schidigera* and *Quillaja saporina*. The name ‘saponin’ is originated from the word ‘sapo’ which in Latin means soap, owing to the foam-generating characteristic in aqueous solution by stirring action. The solutions are amphiphilic glycosides in which the polar components include sugars (pentoses, hexoses or uronic acids) which are connected covalently to a non-polar group which includes an aglycone known as sapogenin that can be in the form of either steroidal, alkaloidal or triterpene depending on the nature of the aglycone. Saponin’s
structure is in the form of lipid soluble polycyclic aglycones connected to single or multiple sugar chains and water-soluble sugar residues (Hong et al. 2002).

In general, the glycone elements of saponins are oligosaccharides. It is possible for oligosaccharides to be connected to the sapogenin by means of either ether or ester bond at one or two glycosylation sites resulting in the monodesmosidic or bidesmosidic saponins. The amphipathic nature of the compounds, which is the main reason for their detergent-like characteristics, is attributed to the uneven distribution of their hydrophobic (aglycone) and hydrophilic (sugar) moieties. The saponin’s unique foaming property resulted from the mixture of components within their non-polar (oil soluble) sapogenin and the polar (water soluble) sugar chain molecular structure (Zhou et al. 2013).

Soapnuts are well known to be rich in saponins and this study is aimed at direct determination of saponins in crude extracts of soapnuts. The initial screening for saponins on the extracts was conducted by phytochemical tests such as foam and hemolysis test. Ultra-fast liquid chromatography (UFLC) and ultra performance liquid chromatography quadrupole-time of flight-mass spectrometry (UPLC-QTOF-MS/MS) were also performed for qualitative analysis of samples to verify that the detection of triterpenoid saponins by Fourier transform infrared (FTIR) will suffice fast, reliable and cost-effective system for direct detection of saponins directly from the crude extracts. Despite the fact that UFLC and UPLC-QTOF-MS/MS are highly sensitive and reliable methods, they are expensive and time consuming and hence FTIR has an advantage over these methods in terms of quick, easy and direct detection of saponins from crude extracts.

2. Results and discussion
2.1. Phytochemical tests

The aqueous and alcoholic extracts were shown to be positive for saponins when subjected to phytochemical screening by froth test and hemolysis test as shown in supplementary Figures S1, S2 and Table S1.

2.2. Fourier transform infrared spectroscopy

The presence of saponins in the aqueous and 95% ethanol extract was confirmed by infrared absorption spectrum and compared with that of the standard. Saponins showed characteristic infrared absorbance of the hydroxyl group (OH) ranging from 3407 cm\(^{-1}\) in the aqueous extract, 3419 cm\(^{-1}\) in the 95% ethanol extract and from 3525 to 3281 cm\(^{-1}\) in the standard Quillaja saponin. Carbon–hydrogen (C–H) absorption ranged from 2931 cm\(^{-1}\) in the aqueous extract, 2931 cm\(^{-1}\) in 95% ethanol extract, while in standard Quillaja saponin it ranged from 2973 to 2932 cm\(^{-1}\). The C–C absorbance was observed at 1613, 1636 and 1609 cm\(^{-1}\) in the aqueous extract, 95% ethanol extract and standard Quillaja saponin respectively. Whereas C–O absorbance was found to be at 1727 cm\(^{-1}\) in the aqueous extract and 1726 cm\(^{-1}\) in the 95% ethanol extract, while standard saponin showed a value of 1724 cm\(^{-1}\). Oligosaccharide linkage absorptions to sapogenins, that is C–O–C, were evident between 1074 cm\(^{-1}\) in the standard, 1045 cm\(^{-1}\) in 95% ethanol extract and 1046 cm\(^{-1}\) in aqueous extract.

The aforementioned infrared functional group absorptions, characteristic of saponins, have been referred to by researchers such as Toshiyuki et al. (2001) and Da Silva et al. (2002). Owing to the oleanolic acid/ester, these oleane-type triterpenoid saponins are characterised by the C–O infrared absorbance. According to Toshiyuki et al. (2001), Kirmizigul and Anil (2002), and Natori et al. (1981), these triterpenoid saponins tend to be bidesmosides as they have two attachments of glycones to the sapogenin namely glycosidic and ester groups. In this study, saponins detected in soapnuts were likely to be bidesmosidic, oleanane-type triterpenoids. The
FTIR spectra of standard Quillaja saponin (Fisher) demonstrated triterpenoid saponin absorption characteristics.

It can be seen from Figure 1, that saponins are clearly detectable in the crude aqueous and alcoholic soapnut extracts even before the purification of saponins. The similarity in the FTIR spectrum of the crude soapnut extracts and the standard Quillaja saponin shows the importance of detection of saponins right after the extraction and freeze-drying step thereby eliminating the need of further extensive purification steps. The significance of this observation is that saponins are detectable in crude aqueous and alcoholic extracts directly using FTIR spectroscopy, therefore shortening the time and the necessity for purification steps before performing analysis.

2.3. Ultra performance liquid chromatography quadrupole-time of flight-mass spectrometry

The mass spectroscopic data of the aqueous extract and 95% ethanolic extract were carried (Figure S3) out to substantiate the presence of triterpenoid saponins by screening for characteristic molecular masses, based on similar cases of reports of other researchers. However, a study at the level of detailed molecular identification was not intended at this stage. The uniformity in appearance of 381, 723, 1065 and 1407 \(m/z\) demonstrates the presence of disaccharide moiety in the glycone element of saponins. This is in agreement with the results of Wong (2014), while another study by Heng et al. (2014) refers to compounds of molecular weights 1206, 1074, 882, 750, 924 and 966 which were identified as triterpenoid saponins, primarily the oleanolic acid type. Based on this report, the LC-MS/MS data of the aqueous
extract, 95% ethanol extract and standard saponin were examined for the compounds with the above masses which are characteristic for triterpenoid saponin. The findings are depicted in Table S2.

2.4. Ultra fast liquid chromatography

The UFLC chromatograms of aqueous and 95% ethanolic extract of soapnuts and standard Quillaja saponin is shown in Figure S4. The comparison of retention times of the separated compounds is tabulated in Table S3. UFLC separation of compounds in all samples was achieved within 6 min. It can be interpreted from the chromatograms that an earlier onset of separation of Quillaja saponin than the soapnuts extracts under the same chromatographic conditions is consistent with the study by Shiau et al. (2009). However, the shorter retention times in this study is due to the difference in technique as described in experimental section (see supplementary material). Chromatograms of both the aqueous extract and 95% ethanol extract of soapnuts exhibited close similarity. By comparing the retention times of peaks on the chromatograms of the soapnut extracts and Quillaja saponin, it can be established that peaks corresponding to retention times 2.8, 3.5, 4.1, 4.8 and 5 min correspond to common saponin constituents.

3. Conclusions

This study has shown that the FTIR absorptions of oleanane-type triterpenoid saponin are characterised by the C=O, which tend to be bidesmosides as they have two attachments of glycones to the sapogenin namely glycosidic and ester groups. It was found that saponins detected in soapnuts were likely to be bidesmosidic, oleanane-type triterpenoids. This claim was verified by the characteristic mass spectrum of the soapnut extracts. Moreover, the FTIR spectra of Quillaja saponin (standard) also demonstrated triterpenoid saponin absorption characteristics. The saponin powder extracted by distilled water and ethanol (95%) displayed 97% similarity in absorbance to the standard Quillaja saponin. Moreover, to validate the presence of saponin in the soapnuts extract, advanced analytical methods such as UFLC and UPLC-QTOF-MS/MS were carried out for qualitative analysis. The results from this preliminary qualitative analysis of saponin showed that the FTIR absorption spectra of the crude would be sufficient, fast and easy.

Supplementary material

Experimental details relating to this paper are available online, alongside Tables S1–S3 and Figures S1–S4.

Funding

This work was supported by the Ministry of Higher Education, Kuwait. The authors are grateful to GFS and NUERS, Faculty of Science, Kuwait University, for FTIR analysis [GS 02/01], UFLC & QTOF LC-MS/MS [GS 02/10] and freeze-drying [SRUL01/13].

References


Wong YM. 2014. Tip spray ionization mass spectrometry and its analytical application in direct sample analysis. [dissertation]: The University of Hong Kong.
HONORABLE COMMISSIONER OF PATENTS
ALEXANDRIA, VA 22313-1450

SIR:

Transmitted herewith for filing is the utility patent application of:

First Named Inventor: MESHARI ALMUTAIRI

Applicant: MESHARI ALMUTAIRI, Inventor

for: SYSTEM AND METHOD FOR REMEDIATION OF OIL-CONTAMINATED SAND

Enclosed are:

1. Application Data Sheet (PTO/AIA/14 EFS-Web)
3. 4 sheets of Formal Drawings Containing 6 Figures
4. Combined Declaration and Power of Attorney, 1 sheet
5. Micro Entity Certification
6. Information Disclosure Statement
7. Form PTO-1449 w/1 reference
8. Filing fee in the amount of $400.00

The filing fee has been calculated as shown below:

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Respectfully submitted,

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IN THE APPLICATION

OF

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FOR A

SYSTEM AND METHOD FOR REMEDIATION OF OIL-CONTAMINATED SAND
SYSTEM AND METHOD FOR REMEDIATION OF OIL-CONTAMINATED SAND

BACKGROUND OF THE INVENTION

1. FIELD OF THE INVENTION

[0001] The present invention relates generally to the treatment of soil, sand and the like, and particularly to a system and method for remediation of oil-contaminated sand.

2. DESCRIPTION OF THE RELATED ART

[0002] Oil sands (also referred to as tar sands or bituminous sands) are a type of unconventional petroleum deposit. Although it is useful to extract usable oil and other petroleum products from the oil sands, the recovered sand is also of great use. If the sand can be effectively washed or cleaned of oil, and other oil-based materials, the sand could be used for a variety of industrial applications, such as, for example, the production of asphalt concrete mixes in road bases, secondary roads, permeable layers for landfills and containment structures, stabilizing steep embankments, enhancement of resistance to the penetration of water, chloride ions, alkali ions and the like in concrete, etc.

[0003] Although the usage of surfactants is common for the separation of the oil from the sand, the primary focus of such techniques is the remediation of oil, thus there has been little focus on further or additional processing for the sand. Thus, a system and method for remediation of oil-contaminated sand addressing the aforementioned problems is desired.
SUMMARY OF THE INVENTION

[0004] The system and method for remediation of oil-contaminated sand provides for washing and separation of sand from oil and oil-based contamination. The system for remediation of oil-contaminated sand includes a feed hopper for receiving a volume of oil-contaminated sand in communication with a cleaning tank for receiving the volume of oil-contaminated sand therefrom. The volume of oil-contaminated sand is fed to the feed hopper by a solid pump or the like. A conveyor belt preferably extends between an outlet of the feed hopper and the cleaning tank for selectively transporting the volume of oil-contaminated sand from the feed hopper to the cleaning tank.

[0005] A mechanical stirrer is provided in the cleaning tank for mixing the volume of oil-contaminated sand with a surfactant solution in the cleaning tank. An ultrasonicator in the cleaning tank ultrasonicates the volume of oil-contaminated sand and the surfactant solution in the cleaning tank to create a mixture of washed sand and oily wastewater. The cleaning tank is in communication with a collection tank for receiving the mixture. The collection tank has an open upper end and an outlet formed in a sidewall thereof. A band filter covers the open upper end of the collection tank, such that the mixture of washed sand and oily wastewater conveyed to the collection tank is filtered to separate out the washed sand, which may then be collected. The separated oily wastewater is then collected in the collection tank. A separate sand receptacle may be provided for receiving the washed sand filtered from the mixture by the band filter.

[0006] For treatment of the oily wastewater, an aeration tank is in communication with the outlet of the collection tank for receiving the oily wastewater for aeration thereof. The
aeration tank not only aerates the oily wastewater but decontaminates the oily wastewater by destroying and/or immobilizing any microorganisms therein. A coagulation tank is in communication with the aeration tank for mixing the oily wastewater, following aeration thereof, with at least one coagulant for separation of the oily wastewater into treated water and an oily sludge. The treated water may then be drawn off and stored in a treated water tank. The oily sludge may be collected for disposal or recycling thereof.

[0007] These and other features of the present invention will become readily apparent upon further review of the following specification and drawings.

**BRIEF DESCRIPTION OF THE DRAWINGS**

[0008] Fig. 1 diagrammatically illustrates a system for remediation of oil-contaminated sand according to the present invention.

[0009] Fig. 2 is a scanning electron microscope (SEM) micrograph (at 90X magnification) showing surfaces of oil-contaminated sand prior to treatment with the system and method for remediation of oil-contaminated sand according to the present invention.

[0010] Fig. 3A is a scanning electron microscope (SEM) micrograph (at 25X magnification) showing surfaces of oil-contaminated sand prior to treatment with the system and method for remediation of oil-contaminated sand according to the present invention.

[0011] Fig. 3B is a scanning electron microscope (SEM) micrograph (at 25X magnification) showing surfaces of oil-contaminated sand following a step of mixing thereof with a surfactant solution.

[0012] Fig. 3C is a scanning electron microscope (SEM) micrograph (at 25X magnification) showing surfaces of oil-contaminated sand following a step of ultrasonication thereof.
[0013] Fig. 3D is a scanning electron microscope (SEM) micrograph (at 25X magnification) showing surfaces of oil-contaminated sand following both mixing thereof with a surfactant solution and ultrasonication thereof, according to the present method for remediation of oil-contaminated sand.

[0014] Unless otherwise indicated, similar reference characters denote corresponding features consistently throughout the attached drawings.

**DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS**

[0015] The system and method for remediation of oil-contaminated sand (OCS) provides for washing and separation of sand from oil and oil-based contamination. As illustrated in Fig. 1, the system for remediation of oil-contaminated sand 10 includes a feed hopper 16 for receiving a volume of oil-contaminated sand. The volume of oil-contaminated sand is fed to the feed hopper 16 by a solid pump 12 or the like, and the flow into the feed hopper 16 may be selectively controlled by a valve 14. Preferably, the feed hopper 16 is provided with a filter for separating out relatively large particles (on the order of 10 mm in diameter or greater), such as coarse aggregate, metal pieces, etc.

[0016] The feed hopper 16 is in communication with a cleaning tank 24 for receiving the volume of oil-contaminated sand. The oil-contaminated sand may be selectively transferred to the cleaning tank 24 by any suitable method, such as, for example, a conveyer belt 18 extending between an outlet 26 of the feed hopper 16 and the cleaning tank 24. A mechanical stirrer 20 or the like is provided for mixing the volume of oil-contaminated sand with a surfactant solution in the cleaning tank 24. The soil solution ratio can be about 1:2 (mass/volume). Preferably, the surfactant solution is an environmentally-friendly surfactant,
such as saponin. About 0.25 wt% surfactant can be present for each batch wash. After or during the mixing by the mechanical stirrer 20, an ultrasonicator 22 ultrasonicates the volume of oil-contaminated sand and the surfactant solution in the cleaning tank 24 to create a mixture of washed sand and oily wastewater. Preferably, the washing of the sand, including both mixing and sonication, occurs at a temperature of approximately 60°C. For an aqueous saponin solution, the concentration of saponin in water can be approximately 0.25 wt%, with an oil-contaminated sand to surfactant solution ratio of approximately 1:2 (mass/volume). Mixing preferably takes place at an agitation speed of approximately 1,000 rpm, with an overall washing time of approximately 2 hours. It should be understood that any suitable type of sonicator may be used. Ultrasonication preferably takes place at a frequency of approximately 45 kHz.

[0017] It should be understood that cleaning tank 24 is shown in Fig. 1 for illustrative purposes only and may have any desired configuration. For example, cleaning tank 24 may have a cubical outer housing 50 cm long on each side, with a cylindrical chamber being formed therein for mixing and ultrasonication. It should be understood that cleaning tank 24 may be formed from any suitable material which will not degrade under the cleaning process, such as stainless steel or the like. Using the exemplary dimensions given above, an interior working/cleaning volume of the cleaning tank 24 would be approximately 41 cm X 41 cm X 36 cm, and the cylindrical chamber received therein would have approximately 24 conventional ultrasonic transducers evenly mounted in an array thereon for performing ultrasonication. Preferably, for such an exemplary system, the 24 ultrasonic transducers, each operating at approximately 45 kHz, would have an overall power output of 2,400 W.
It should be further understood that cleaning tank 24 may include any desired additional cleaning components, such as, for example, an under liquid jetting system. Such conventional under liquid jetting systems typically include a high capacity flow pump which re-circulates the cleaning solution from the main cleaning bath via an in-line strainer with a pair of stainless steel sparger pipes for increased flow agitation. Further, as noted above, the cleaning process preferably occurs at approximately 60°C. It should be understood that the internal temperature of cleaning tank 24 may be maintained and controlled by any suitable type of heating system, such as, for example, a 2 kW stainless steel immersion heater mounted near the base of cleaning tank 24. Preferably, such a heater is mounted in an offset position so as to not impede the overall height of the system. Separate heating control may include full power initial heat up, idle control, low level cut out, over temperature cut out and temperature control. It should be understood that any suitable type of mixer or stirrer may be used, e.g., a mechanical stirrer 20. For example, a 1,000 rpm motor may be attached to the lid of cleaning tank 24, with a removable paddle being fixed thereto.

The cleaning tank 24 is in communication with a collection tank 28 for receiving the mixture. The collection tank 28 has an open upper end and an outlet 42 formed in a sidewall thereof. A band filter 30 or the like covers the open upper end of the collection tank 28, such that the mixture of washed sand and oily wastewater conveyed to the collection tank 28 is filtered to separate out the washed sand WS, which may then be collected. The band filter 30 preferably has a filter size on the order of 0.04 mm for separating out the washed sand WS from the oily wastewater. The separated oily wastewater is collected in the collection tank 28. A separate sand receptacle 32 may be provided for receiving the washed sand WS filtered from the mixture by the band filter 30. The washed sand WS should have an
oil residue volume of less than 1% and, thus, may be collected and shipped for the desired purposes thereof. The washed sand WS can be used, for example, for the production of asphalt concrete mixes in road bases, secondary roads, impermeable layers for landfills and containment structures, stabilizing steep embankments, enhancement of resistance to the penetration of water, chloride ions, alkali ions and the like in concrete, etc.

[0020] For treatment of the oily wastewater, an aeration tank 36 is in communication with the outlet 42 of the collection tank 28 for receiving the oily wastewater for aeration thereof. The aeration tank 36 aerates the oily wastewater. For an exemplary aeration tank 36 having a volume of 1,200 L, approximately 1,000 L of the oily wastewater would be received therein. In addition to the oily wastewater, an additional volume of prime settled sludge (PSS), obtained from a wastewater treatment plant or the like, may also be added (on the order of 100 L in this example). During the aeration process, air is continuously supplied into the aeration reactors by a compressor 34 for a period of approximately 24 hours, providing aeration, acclimatization and immobilization for any microorganisms. The aeration is preferably controlled to a rate of approximately 5.5 m/s to avoid creation of froth, thus preventing wastewater from bubbling out of the aeration tank 36. As discussed in detail below, sodium chloride (NaCl) can also be added at this stage.

[0021] A coagulation tank 38 is in communication with the aeration tank 36 for mixing the oily wastewater, following aeration thereof, with at least one coagulant for separation of the oily wastewater into treated water and an oily sludge. It should be understood that any suitable coagulant may be used. For example, approximately 1.5 kg of aluminum in approximately 10.0 L of distilled water may be used as the coagulant. With this particular coagulant, the mixture of coagulant with aerated, oily wastewater preferably has a coagulant
concentration of approximately 5 wt%. Mixing of the coagulant and the aerated, oily wastewater in the coagulation tank 38 preferably occurs with an agitation speed of approximately 50 rpm for a period of approximately 45 minutes to ensure that the wastewater and coagulant are well mixed. The mixing speed may then be gradually reduced to 0 rpm, followed by an overnight settling period. The treated water may then be drawn off and stored in a treated water tank 40. The oily sludge OS may be collected for disposal or recycling thereof.

[0022] Figs. 2 and 3A are each scanning electron microscope (SEM) micrographs showing surfaces of oil-contaminated sand prior to treatment thereof. Fig. 3B is an SEM micrograph showing the surfaces of the oil-contaminated sand following mixing thereof with the surfactant solution alone. Fig. 3C is an SEM micrograph showing the surfaces of the oil-contaminated sand following ultrasonication alone. Fig. 3D is an SEM micrograph showing the surfaces of the oil-contaminated sand following both mixing thereof with the surfactant solution and ultrasonication, as in the present method. As shown, each method, taken alone (as in Figs. 3B and 3C) results in relatively insignificant particle breakup and removal of oil, whereas the combination of both methods (as in Fig. 3D) results in noticeable particle breakup and removal of oil residue.

[0023] The results show that the mechanical stirring enhances the ultrasonication effectiveness to the sand particles by agitating the sand matrix and aiding the dispersion of oil and sand. This improves contaminant desorption from the particle surface and effectively enhances the ultrasonic removal efficiency by generating internal air bubbles that can reach the surface of the sand. This leads to a high frequency of ultrasonic sound (on the order of 45 kHz), which produces cavitation by alternating high and low pressure waves. These bubbles
grow in a low pressure phase from micro-bubbles until they are formed and compressed at a high pressure phase. The bubbles then collapse to split the oil from the fine particles with contaminants from larger parent constituents. As such, homogenization occurs by breakup of agglomerates of oil residue from the sand. Further, it can be seen that there is damage to the surface of the sand particles, which is caused by sonophysical effects. This may improve the effectiveness of contaminant removal from the sand and, moreover, the cavitation may be used to maintain the water temperature during the process by releasing large quantities of energy.

[0024] It is well known that varying ionic strength is able to affect the efficiency of the coagulation process and then flocs weight. Saponins are categorized as non-ionic biosurfactants, but contain acidic and ionizable groups of glucuronic acid as part of its head group (i.e., the hydrophilic fraction). Further, some charged species would exist in saponin solutions due to the ionic strength of artificial seawater. Thus, the ionic strength of the saponin solution may have an effect on the destabilization of colloidal particles during the coagulation/flocculation process. Specifically, sodium chloride (NaCl) aids in double layer compression. The ions with an opposite charge appear to play a significant role in improving the flocculation process by bridging the particles and allowing the flocs to be formed. The applicability of the secondary treatment (i.e., the aeration) may be able to destabilize colloidal particles by breaking down the long chain of complex organic compounds in the oil emulsion, and convert this into small molecules by increasing the surface area colloid concentration.

[0025] As a result, the contact of any microorganisms with the oily wastewater is improved and the solubility of the oil is enhanced, allowing the saponin to be available for biodegradation. In experiments, the solution of saponin in artificial seawater was aerated for
24 hours, and the average values of chemical oxygen demand (COD) were found to be reduced by about 70%. This suggests that the reduction in the COD of the oily wastewater may be due to the biodegradation of the saponin, allowing the stability of the oil emulsion to be broken down and released easily. This may be due to the double layer compression and sweep flocculation. With the increase in sodium ion concentration in the solution, the double layer becomes thinner and aids in getting same-charge particles nearer. As such, the floc formation becomes quicker and better, thus improving the settleability of the colloid matter, which causes a quick decrease in turbidity.

[0026] The two methods by which a high coagulant dosage can improve the rate of coagulation are (a) increasing metal hydroxide precipitate concentration and, thus, the rate of aggregation, and (b) enmeshing particulates to enlarge the size of aggregates using sweep-floc coagulation. In experiments with the present method, it was found that adding a high dosage of coagulant precipitated a heavy, sticky and high quantity of flocs, with an accelerated settling time. The settling time for colloidal particles by adapting the oily wastewater sample under aeration for 5 days required 15 minutes, while a sample without aeration required 24 hours to settle down. This scenario suggests that the sweep-floc coagulation (whereby the colloidal particles are removed from the suspension) occurs via enmeshment into the aluminum hydroxide Al(OH)₃ precipitate. This process resulted in the creation of a suspension with smaller size and high quantity of flocs. Thus, the method eliminates higher quantities of organic particles in view of the increased surface area offered for adsorption. By contrast, low doses of coagulant boosts larger and fewer quantity flocs due to the faster rate of growth compared to the rate of nucleation. This causes a reduced surface area for the adsorption of organic compounds.
The experiments suggested that the destabilization improvement of the colloidal particles occurred due to the linkage of inter-particle interactions. This effect results in the generation of a large amount of precipitation which permits the colloidal particles to be enmeshed while settling.

It is to be understood that the present invention is not limited to the embodiments described above, but encompasses any and all embodiments within the scope of the following claims.
CLAIMS

I claim:

1. A system for remediation of oil-contaminated sand, comprising:
   a feed hopper for receiving a volume of oil-contaminated sand;
   a cleaning tank in communication with the feed hopper for receiving the volume of oil-contaminated sand from the feed hopper;
   a mechanical stirrer in the cleaning tank for mixing the volume of oil-contaminated sand with a surfactant solution in the cleaning tank;
   an ultrasonicator in the cleaning tank for ultrasonicating the volume of oil-contaminated sand and the surfactant solution in the cleaning tank to create a mixture of washed sand and oily wastewater;
   a collection tank in communication with the cleaning tank, the collection tank having an open upper end and an outlet formed in a sidewall thereof; and
   a band filter covering the open upper end of said collection tank, wherein the mixture of washed sand and oily wastewater is conveyed to said collection tank, the washed sand being filtered from the mixture by the band filter and the oily wastewater being collected in the collection tank.

2. The system for remediation of oil-contaminated sand as recited in claim 1, further comprising a solid pump for feeding the volume of oil-contaminated sand into said feed hopper.
3. The system for remediation of oil-contaminated sand as recited in claim 2, further comprising a conveyer belt extending between an outlet of the feed hopper and the cleaning tank for selectively transporting the volume of oil-contaminated sand from the feed hopper to the cleaning tank.

4. The system for remediation of oil-contaminated sand as recited in claim 3, further comprising a sand receptacle in communication with the collection tank, the sand receptacle for receiving the washed sand filtered from the mixture by the band filter.

5. The system for remediation of oil-contaminated sand as recited in claim 1, further comprising an aeration tank in communication with the outlet of the collection tank for receiving the oily wastewater for aeration thereof.

6. The system for remediation of oil-contaminated sand as recited in claim 5, further comprising a coagulation tank in communication with the aeration tank for mixing the oily wastewater, following aeration thereof, with at least one coagulant for separation of the oily wastewater into treated water and an oily sludge.

7. The system for remediation of oil-contaminated sand as recited in claim 6, further comprising a treated water tank for receiving the treated water.

8. A system for remediation of oil-contaminated sand, comprising:

   a feed hopper for receiving a volume of oil-contaminated sand;
a cleaning tank in communication with the feed hopper for receiving the volume of
oil-contaminated sand from the feed hopper;
a mechanical stirrer in the cleaning tank for mixing the volume of oil-contaminated
sand with a surfactant solution in the cleaning tank;
an ultrasonicator in the cleaning tank for ultrasonicating the volume of oil-
contaminated sand and the surfactant solution in the cleaning tank to create a mixture of
washed sand and oily wastewater;
a collection tank in communication with the cleaning tank, the collection tank having
an open upper end and an outlet formed in a sidewall thereof;
a band filter covering the open upper end of said collection tank, wherein the mixture
of washed sand and oily wastewater is conveyed to said collection tank, the washed sand
being filtered from the mixture by the band filter and the oily wastewater being collected in
the collection tank;
an aeration tank in communication with the outlet of the collection tank for receiving
the oily wastewater for aeration thereof; and
a coagulation tank in communication with the aeration tank for mixing the oily
wastewater, following aeration thereof, with at least one coagulant for separation of the oily
wastewater into treated water and an oily sludge.

9. The system for remediation of oil-contaminated sand as recited in claim 8, further
comprising a solid pump for feeding the volume of oil-contaminated sand into said feed
hopper.
10. The system for remediation of oil-contaminated sand as recited in claim 9, further comprising a conveyer belt extending between an outlet of the feed hopper and the cleaning tank for selectively transporting the volume of oil-contaminated sand from the feed hopper to the cleaning tank.

11. The system for remediation of oil-contaminated sand as recited in claim 10, further comprising a sand receptacle in communication with the collection tank for receiving the washed sand filtered from the mixture by the band filter.

12. The system for remediation of oil-contaminated sand as recited in claim 11, further comprising a treated water tank for receiving the treated water.

13. A method for remediation of oil-contaminated sand, comprising the steps of:
mixing a volume of oil-contaminated sand with a surfactant solution;
ultrasonicating the volume of oil-contaminated sand and the surfactant solution to create a mixture of washed sand and oily wastewater;
filtering the mixture of washed sand and oily wastewater to separate the washed sand from the mixture; and
collecting the oily wastewater.

14. The method for remediation of oil-contaminated sand as recited in claim 13, further comprising the steps of:
acrating the oily wastewater; and
mixing the oily wastewater with at least one coagulant for separation of the oily wastewater into treated water and an oily sludge.

15. The method for remediation of oil-contaminated sand as recited in claim 14, wherein the step of mixing the volume of oil-contaminated sand with the surfactant solution comprises mixing the volume of oil-contaminated sand with an aqueous saponin solution.

16. The method for remediation of oil-contaminated sand as recited in claim 14, wherein the step of mixing the volume of oil-contaminated sand with the aqueous saponin solution occurs at a temperature of approximately 60°C.

17. The method for remediation of oil-contaminated sand as recited in claim 16, wherein the step of mixing the volume of oil-contaminated sand with the aqueous saponin solution comprises mixing at approximately 1,000 rpm over an approximately two hour period.

18. The method for remediation of oil-contaminated sand as recited in claim 17, wherein the steps of mixing the volume of oil-contaminated sand with the surfactant solution and ultrasonicallyg the volume of oil-contaminated sand and the surfactant solution are performed at substantially the same time.
19. The method for remediation of oil-contaminated sand as recited in claim 18, wherein the step of ultrasonicating the volume of oil-contaminated sand and the surfactant solution comprises ultrasonication at a frequency of approximately 45 kHz.

20. The method for remediation of oil-contaminated sand as recited in claim 13, wherein a ratio of the oil-contaminated sand to surfactant solution is 1:2 (mass/volume).
ABSTRACT OF THE DISCLOSURE

The system and method for remediation of oil-contaminated sand provides for washing and separation of sand from oil and oil-based contamination. The system includes a feed hopper for receiving a volume of oil-contaminated sand in communication with a cleaning tank for receiving the volume of oil-contaminated sand therefrom. A mechanical stirrer mixes the volume of oil-contaminated sand with a surfactant solution in the cleaning tank. An ultrasonicator ultrasonicates the volume of oil-contaminated sand and the surfactant solution in the cleaning tank to create a mixture of washed sand and oily wastewater. A collection tank then receives the mixture. A band filter covers an open upper end of the collection tank, such that the mixture of washed sand and oily wastewater is filtered to separate out the washed sand, which may then be collected. The separated oily wastewater is then collected in the collection tank.
COMBINED DECLARATION AND POWER OF ATTORNEY

As the below named inventor, I hereby declare that:

My residence and post office address are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

SYSTEM AND METHOD FOR REMEDIATION OF OIL-CONTAMINATED SAND

the specification of which is attached hereto unless the following box is checked:

☐ was filed on ___________________ as United States Application Serial Number or PCT International Application Number_________________________ and was amended on ___________________ (if applicable).

The above-identified application was made or authorized to be made by me.

I believe that I am the original inventor of or an original joint inventor of a claimed invention in the application.

I hereby acknowledge that any willful false statement made in this declaration is punishable under 18 U.S.C. 1001 by fine or imprisonment of not more than five (5) years or both.

I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and transact all business in the Patent and Trademark Office connected therewith.

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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true, and further that these statements were made with the knowledge that willful false statements and the like made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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Signature: ___________________________ Date: 16/11/2015

Residence: ___________________________

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

IN THE APPLICATION OF:

FIRST NAMED INVENTOR : MESHARI ALMUTAIRI

SERIAL NO.     : Unassigned           ART UNIT: Unassigned
FIELD         : Herewith                EXAMINER: Unassigned
FOR           : SYSTEM AND METHOD FOR REMEDIATION OF OIL-CONTAMINATED SAND

COMMISSIONER FOR PATENTS
ALEXANDRIA, VA 22313-1450

Sir:

INFORMATION DISCLOSURE STATEMENT
35 U.S.C. 6; 37 C.F.R. 1.97; 1.98

Related art domestic patents, published U.S. patent applications, foreign patents and/or publications are identified on the attached PTO-1449 form. Copies of any identified foreign patents and/or publications are attached.

The discussion of these related art foreign language patents and/or publications, if any, in the attached specification and the identification of the related art on the PTO-1449 is believed to satisfy the duty to disclose requirements as set forth in the above identified statute and rules.

Respectfully submitted,

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