EVALUATION OF CRUDE OIL CONTAMINATED SOIL AFTER BIOREMEDIATION AND BIOCEMENTATION USING *BACILLUS LICHENIFORMIS* FOR USE IN WASTE CONTAINMENT APPLICATIONS

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AUGUST, 2021

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DEPARTMENT OF CIVIL ENGINEERING,
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ZARIA, NIGERIA

AUGUST, 2021

DECLARATION

I declare that the work in this Dissertation entitled Evaluation of Crude Oil Contaminated Soil after Bioremediation and Biocementation using *Bacillus licheniformis* for use in Waste Containment Applications has been performed by me in the Department of Civil Engineering. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this dissertation was previously presented for another Degree or Diploma in this or any other institution.

Musa Kurgwi Alhassan _____ _____

Name of Student **Signature** **Date**

CERTIFICATION

This Dissertation entitled EVALUATION OF CRUDE OIL CONTAMINATED SOIL AFTER BIOREMEDIATION AND BIOCEMENTATION USING *BACILLUS LICHENIFORMIS* FOR USE IN WASTE CONTAINMENT APPLICATIONS by MUSA KURGWI ALHASSAN meets the regulations governing the award of the degree of Masters of Science of the Ahmadu Bello University Zaria, and is approved for its contribution to knowledge and literary presentation.

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Dedication

This work is dedicated to my son Late Alhassan M. Alhassan who died while I was waiting for the external examination.

Abstract

A study was conducted in the laboratory on a crude oil contaminated soil treated with *Bacillus licheniformis* to investigate the suitability of the material for use as a liner material in waste containment applications. An indigenous hydrocarbon utilizing bacteria (HUB) was identified as *Bacillus licheniformis* and isolated from crude oil contaminated soil (COCS) collected from the Nigerian National Petroleum Corporation (NNPC) Kaduna, Nigeria, at a depth of 0.5m from the surface. A bio-stimulation strategy of bio-remediation was adopted using chicken droppings (CD) in stepped concentrations of 0, 10, 20, 30 and 40% CD content as a source of nutrients only, to activate the indigenous bacteria for a 12 weeks period of remediation. At the end of the remediation, it was observed that *Bacillus licheniformis* removed 89.8% of crude oil content out of the initial 98g/kg of total petroleum hydrocarbon concentration at 30% CD dosage. The bio-remediated soil was further subjected to microbial activity with a view to improve the engineering properties of the soil using the *Bacillus licheniformis* suspension in stepped concentrations of 0, $1.5x10^8$, $6.0x10^8$, $1.2\ x10^9$, $1.8\ x10^9$ and $2.4\ x10^9$ cell/ml suspension densities of one-third of the pore volume (1/3), mixed with soil for 12hours and two-third (2/3) of the pore volume of the cementation reagent injected by gravity flow in four cycles. Preparation of samples were done at optimum moisture content (OMC) using three compactive efforts namely; British Standard light (BSL), West African Standard (WAS) and British Standard heavy (BSH) and the following tests were carried out: Index properties, compaction characteristics, unconfined compressive strength (UCS), volumetric shrinkage strain (VSS) and hydraulic conductivity. The results showed that there was increase in liquid and plastic limits with increase in *Bacillus licheniformis* dosage. The plasticity index decreased with increase in the microbial flora. The hydraulic conductivity value reduced to $9.92\ x\ 10^{-11}$m/s for samples treated with $2.4\ x10^9$cell/ml of *Bacillus* licheniformis suspension and compact ed using BSH compactive effort. Void ratios consistently decreased with higher doses of microbial flora in the bio-remediated and bio-cemented crude oil contaminated soils. Minimum void ratio values obtained at $2.4\ x\ 10^9$ cell/ml of *B. licheniformis* of 0.83, 0.74 and 0.62 respectively, when compacted using BSL WAS and BSH compaction energies. There was reduction in porosity with increasing microbial content. Similarly, samples containing higher doses of the microbial flora attained higher degree of saturation for all the compactive efforts considered. The UCS values of the crude oil contaminated soil were 119.56, 202.82 and 253.15 kN/m^2 while for samples treated with $1.5\ x10^8$ cell/ml of *B. licheniformis* suspension, the UCS values were 330.64, 415.57 and 460.78$kN/\ m^2$, when compacted using BSL, WAS and BSH compactive efforts, respectively. For VSS, only higher doses of the bacterial specie of up to $2.4\ x\ 10^9$cell/ml of *B. licheniformis* yielded the VSS values of 3.98, 3.78 and 3.06 % respectively which satisfied the maximum permissibl e value of 4%.

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CHAPTER ONE

INTRODUCTION

1.1 Preamble

Crude oil contaminated soil (COCS) is an unwanted material (US EPA, 2005). The Colorado Department of Public Health and Environment defined Crude Oil contaminated soils (COCS) as "any earthen material or artificial fill that has human or natural alteration of its physical, chemical, biological or radiological integrity resulting from the introduction of crude oil, any fraction or derivative thereof (such as gasoline, diesel or used motor oil)" (CDPHE,2005). Ijimdiya (2011) defined crude oil contaminated soils (COCS) as the waste material obtained by cleaning up petroleum spills or due to oil spillage.

Crude oil consists of hydrocarbon mixture of different molecular weights and structure, comprising of four classes, namely: saturate (pentane, hexadecane, octacosane, cyclohexane,) aromatic (naphthalene, phenanthrene, benzene, pyrene), aliphatic (paraffinic) and naphthenic (alicyclic). There are other chemical compounds that exist in crude oil with different specific gravity, volatility and viscosity (Speight, 2006). Oil contamination is widespread in Nigeria, especially in the Niger Delta and other countries endowed with oil resulting to devastating effect in the environments (Ezeji *et al*., 2007 and Ijimdiya 2010a, b).

There are several methods of removing crude oil contaminants from soils like soil excavation, soil washing, vapour extraction, thermal desorption and incineration. However, these methods are very expensive and environmentally unfriendly (Adams *et al.*, 2015; Ezeji *et al*., 2007; Ijah *et al*., 2008; Bento *et al*., 2003). Negative effects of crude oil contaminants on geotechnical properties of soil such as shear strength, hydraulic

conductivity, consolidation, volume change and Atterberg limits are well documented in literature (Habeb-ur-Rahman *et al.*, 2007; Rahman *et al.*, 2010; Ijimdiya, 2010a,b; Ijimdiya and Igboro, 2012; Ijimdiya, 2013; Pandey and Bind,2014 and Taha and Taha, 2015). A more recent and environmentally sustainable technique of remediating crude oil contaminated soils is by utilizing bacterial species otherwise referred to as bio-remediation (Al- Sanad *et al*., 2007 and Ijimdiya, 2017).

Standard regulatory agency such as US EPA (1991) recommended that the maximum permissible value of hydraulic conductivity of the material to be used as a hydraulic barrier in waste containment systems should be $\leq 1 \times 10^{-9}$ m/s. Similarly, researchers like Daniel and Wu (1993) suggested that material to be used for liner and cover should have a minimum unconfined compressive strength (UCS) value of 200kN/m^2 so that it can withstand load that may be placed on it when used as a liner material. Benson *et al*., (1999), recommended that the value of the volumetric shrinkage strain (VSS) of material to be used as liner in waste containment system should be $\leq$ 4%. Another important consideration in the design of liner that designer(s) must consider is the compatibility of the material with leachate which should not affect the engineering properties of material (Shackelford and Daniel, 1991).

According to Burbank *et al*., 2011, resident bacteria specie could be utilized to form calcite in order to strengthen liquefiable soil. Previous researchers have shown the possibilities of using microbial induced calcite precipitation (MICP) in reducing hydraulic conductivity and increased in stiffness and strength of soils (Dejong *et al.*, 2013; Wei *et al.*,2012 and Ivanov *et al.*, 2009).

The Federal Environmental Protection Agency, Nigeria (FEPA, 1991), reported on the violation of rules guiding the disposal of municipal solid waste (MSW). Previous researchers have investigated the suitability of materials for use as engineered liner to mitigate the egress of leachate from the landfill into underground water. Daniel and Wu, 1993; Rowe *et al.*, 1996; Benson, 2000; Osinubi and Eberemu, 2006; Eberemu, 2008; Ijimdiya, 2010 and Ochepo, 2017, studied various materials for use as landfill liner in waste containment applications. The choice of liner materials depends on some basic engineering properties such as hydraulic conductivity, unconfined compressive strength and volumetric shrinkage strain.

Therefore, this research considers the use of microbes as a sustainable means of removing the hydrocarbons in the crude oil contaminated soils. The bio-remediated soil was then treated using bacterial specie predominant in a crude oil contaminated soils known as *Bacillus licheniformis* (*B. licheniformis*) using the microbial induced calcite precipitation in waste containment application.

1.2 Statement of Research Problem

In an area where there is oil contamination like the Niger Delta of Nigeria, sourcing for soil materials for civil engineering works become difficult. This may necessitate travelling to a far distance to get the materials which may increase the cost of construction. Also, contamination by oil affects the geotechnical properties of soil like bearing capacity of foundation, permeability, consolidation, Atterberg limits and volume change (Habeb-ur-rahman *et al.*, 2007, Rahman *et al.*, 2010; Ijimdiya, 2010a,b; Pandey and Bind,2014 and Taha and Taha, 2015). Several methods of treating such soils exist but not without some negative environmental impacts. However, previous studies by Ijimdiya (2010a,b;

Ijimdiya, 2011) have been conducted in Civil Engineering Department of Ahmadu Bello University (ABU) , Zaria which dwelled on bioremediation using cow dung to remediate soil contaminated by oil. The result indicated that animals waste have the potentials to activate microorganisms capable of degrading hydrocarbon in bio-remediation. Similarly, Al- Wahaibi *et al*. (2016) studied the potentialities of utilizing *B. licheniformis* in remediation of heavy crude oil. This study therefore, utilized chicken droppings as a source of nutrients for *B. licheniformis* in remediation of soil contaminated by oil and used the same bacteria with cementation agents to bio-cement the soil for use in waste containment applications.

`1.3 Justification for the Study

Alexander *et al*. (1999) reported that microorganisms depend on nutrients particularly carbon, nitrogen and phosphorus for their enzymatic activities. McCall (1980) carried out analysis of composition of chicken manure and reported that it contained higher nutrients like nitrogen, phosphorus and potassium among the domesticated poultry manure. Also, the National Veterinary Research Institute, Vom (NVRI, 2015) reported that there were over 180 million chicken population in Nigeria. Furthermore, McCall (1980) opined that; 1000 hens are capable of producing 25 tons/year of chicken droppings (on dry basis).

Previous studies have shown the potentialities of utilizing chicken droppings as a nutrient in the bioremediation of crude oil contaminated soils (Ijah *et al*., 2008; Ijimdiya and Musa, 2017). However, there is little or no documented report in the literature that mentioned bio-remediated crude oil contaminated soil was treated using microbial induced calcite precipitation (MICP) with a view to improving the engineering properties of soil for use in waste containment applications.

1.4 Aim and Objectives

The aim of this study is to investigate the engineering (geotechnical) properties of the bio-remediated and bio-cemented soil using *B. licheniformis* for use in waste containment application.

The specific objectives of the study are:

(i) Determination of the optimum amount of chicken dropping required in the bio-remediation of crude oil contaminated soil.

(ii) Determination of the index properties of the bio-remediated and bio-cemented soil using *B. licheniformis*

(iii) Determination of the moisture- density relationships of the bio-remediated and bio-cemented soil using *B. licheniformis* compacted using varying compactive efforts of Britiush Standard light (BSL), West African Standard (WAS) and British Standard heavy BSH) compactive efforts.

(iv) Determination of the effect of *B. licheniformis* on the hydraulic conductivity, unconfined compressive strength and volumetric shrinkage strain of the bio-remediated and bio-cemented soil.

(v) Determination of the amount of calcite formed during the MICP process.

1.5 Scope of the Study

The study was limited to evaluation of properties of contaminated and bio-remediated and bio-cemented crude oil contaminated soil using B. *licheniformis* for use as liner and cover material. Contaminated soil was mixed thoroughly with 10, 20, 30 and 40% chicken droppings to stimulate the resident microbes (*B. licheniformis*) over the period of three

months and placed in microcosms. Three compactive efforts namely British Standard light (BSL), West African Standard (WAS) and British Standard heavy BSH) were considered.

CHAPTER TWO

LITERATURE REVIEW

2.1 Oil Contaminated Soil

Crude oil contaminated soil is that soil which contains oil such that the amount of carbon is high due to hydrocarbon. Hydrocarbon is an organic material with different individual hydrocarbon compounds. Crude oil contamination of soil can be described as a process whereby crude oil comes in contact with the natural soil either through natural seepage from the wells or deliberate or accidental spills. This led to building of a slim deposit of the absorbed material on the surface of soil particle that could fill the voids which eventually make the soil particles to develop hydrophobic behavior (Pandey and Bind, 2014 and Speight, 2006). Previous authors have reported the negative effect of crude oil contamination of soil on geotechnical properties such as strength characteristics (shear strength), hydraulic conductivity, compressibility, volume change and Atterberg limits (Habeb-ur-Rahman *et al*, 2007, Rahman *et al.*, 2010; Ijimdiya, 2013; Pandey and Bind, 2014 and Taha and Taha, 2015). Similarly, Shin and Das, (2001) studied the negative effect of oil contamination on shallow foundation of building structures and reported a reduction of the ultimate bearing capacity drastically. This may ultimately increase the cost of construction.

2.1.1 Historical background of oil contamination

Ijimdiya (2013) and Itsetekhale *et al.* (2013) explained that exploration contributes to oil contamination of land and water ways. In other words, it is difficult to carry out oil exploration without incidence of oil contamination of soil and water bodies. Etkin (2001)

defined oil spill as discrete event in which oil is discharged through neglect, by accident, or with intent over a relatively short time.

The history of oil spillage is synonymous with the origin of oil contamination. Table 2.1 indicates some incidences of oil spillage in Nigeria (Kadafa, 2012). It was observed that oil contamination constituted a big problem in the Niger Delta of Nigeria for a very long time. Another means through which oil spillage occurs include: material defect, pipeline corrosion, ground erosion, oil theft, sabotage, militancy and bunkering. The first oil spillage occurred at Araromi in Ondo state in 1908**.** According to United Nation Development Programme (2006, Report), from 1979- 2001 alone, there were 6,817 incidences of oil spillage recorded with huge loses of quantity of oil spilled in the Niger Delta environment**.**

Table 2.1: Some Incidences of Petroleum Spill in Nigeria (1979-2001)

S/No	Year	Location	Quantity(Million Gallons)
01	1972	Obagi	23000
02	1979	Echoma-Brass	500
03	1979	Forcados	560000
04	1980	Faniwa	421000
05	1980	Oyakama	30000
06	1982	Abudu	18818
07	1983	Oshika	5000
08	1991	Ogoniland	618000
09	1995	Ogada-Brass	24000
10	1998	Eket	40000
11	2011	Bonga	40000

(Source: Kadafa, 2012)

In developed countries, oil spills occur during exploration, storage, transportation and pipeline leakages (Habib-ur-Rahman *et al*, 2007).

Table 2.2 shows some notable incidences of oil spillage in other parts of the world. The invasion of Kuwait by Iraq in 1990 was one of the biggest environmental disasters that has to do with crude oil where 3000 hectares of land was spread with crude oil when 660 million barrel of crude oil was released which affected 40 million tons of contaminated soil. Mexico and U S also witnessed oil spillage in1979, 1989 of 0.3, 0.2million barrel, respectively.

Table 2.2 Largest crude oil releases from 1978-1992 worldwide

S/No	Year	Location	Quantity (Million Gallons)
1	1967	Cornwall England	35.4
2	1968	South Africa	78
3	1972	Gulf of Oman	31
4	1976	La corona Spai	20
5	1978	Portsall, Brittany, France	68
6	1978	France	68.67
7	1979	Mexico	180
8	1979	Trinida and Tobago	48.8
9	1980	Libya	42
10	1980	Greece	36.6
11	1981	Kuwait	31.17
12	1983	Arabian Gulf	240
13	1983	CapeTown,SouthAfrica	75
14	1983	Persian Gulf	180
15	1983	South Africa	11
16	1989	Alaska, U.S. (Valdiz)	10.8
17	1989	Prince William Sound, off Alaskan Coast	10.9
18	1990	Kuwait	27720
19	1991	Arabian Gulf	240
20	1992	Shetland Islands, Scotland	39
21	1992	Uzbekistan (USSR)	78

(Source: Etkin, 1997)

2.1.2 Some negative effect of crude oil contamination in the environment

Crude oil contamination reduces soil nitrates, extractable phosphorus, cation exchange capacity (CEC), porosity, pH, soil moisture and water holding capacity. It also destroys soil structures, depletes the nutrients availability for microorganisms; it increases carbon and damages the microorganism, thus affects the ecosystem (Habeb-ur-rahman *et al.*, 2007; Barua *et al.*,2011 and Ameh *et al.*, 2013). According to Thapa *et al.*(2012), oil contamination of soil is a worrisome issue that receives wider attention because of its negative effect such as economic loss, environmental problems and reduction in fertility of the soil for agricultural development.

2.2 Crude Oil

Crude oil or petroleum is a hydrocarbon substance formed in a natural way and in liquid form with the presence of compounds of sulphur, nitrogen and metals (Speight, 2006). Petroleum is formed from two words, petra and oleum which refers to oil from rock thus, it implied to hydrocarbons occurred in sedimentary rocks in the form of gases, liquids, semi solids or solids. Generically, crude oil is known as fossil energy resources because it is carbon based and formed from organic material that decayed hundred million years ago. Giovanni and Simon (1997) explained that some compounds that are linear in nature including C30 could be removed from crude oil (hydrocarbon) in 4 weeks however, others take about 17 weeks. Table 2.3 indicates the characteristics of a type of crude oil known as Bonny light.

Table 2.3: Some Specifications of Bonny crude oil.

Property	Quantity
Specific gravity (API)	35.2-37.5
Density at 20°C	0.85

Color	Dark Brown
Carbon Content (%)	86.2
Nitrogen Content (%)	0.1
Water Content (%)	0.2 (by vol)
Sediment Content(%)	0.01
Max KinematicsViscosityat20°C	5.572
Salt Content (%)	2.1
Flash point (°C)	> 25
Gross Heating Value, Kcal/kg	10.771

(Source: NNPC Kaduna, 2017)

2.3 Remediation Techniques

It is suggested that remediation should be done to minimize the quantity of oil to 1% (1% TPH concentration) (US EPA, 2005). Remediation can be differentiated based on place of treatment. Based on place of treatment, two types of soil remediation exist. They include in place (*in situ*) and out of place (*ex situ*) remediation.

(i) *in- situ* remediation is a type of remediation whereby soil treatment is carried out in place where contamination occur. It is a field remediation of a contaminated site in which there has been no excavation or disturbance of the soil. Here the original soil to be remediated received the treatment(s) directly without disturbances of the soil in order not to alter or distort the characteristics of land hence, change the result. Its merits sides include (1) the natural environment of the site is maintained. (2) No money needed for excavation and conveying the soil.

(ii) *ex-situ* soil treatment or remediation is another type in which treatment is given to the soil outside the site of contamination. This is a type of remediation in which the soil is taken from the site of contamination and transported to another place for treatment.

However, according to Ezeje *et al*. (2007) and Hamby (2009), remediation can be categorized based on method of treatment used hence, based on that the following types of remediation exist.

2.3.1 Physico-chemical method

Physicochemical methods of soil treatment require large equipment and tend to add more toxic to the environment during remediation.

2.3.1.1 Soil excavation

It is a soil treatment where contaminated soil is removed from the site of contamination for the purpose of burying or burning in another location. High priced method due to excavation and the excavated land could be a source of erosion or environment degradation.

2.3.1.2 Soil washing

This method is carried out outside the site of contamination and could be applied to organic, inorganic and radioactive contaminants. It requires the use of water or suitable solvents together with chemical additives to remove hazardous contaminants thereby lowering the concentration of the contaminants. However, only less than 80% removal efficiency could be achieved except when hot water is used thus, it is a pre-treatment method that requires further cleaning up.

2.3.1.3 Stabilization/Solidification

Ba-Naimoon and Hamid (2016) used this method in treating soil contaminated by oil where the soil was mixed with additives like Portland cement, lime/fly ash to contain the

contaminants. However, this method does not remove the contaminants completely from the soil.

2.3.1.4 Electro-kinetic

This treatment is applied to anionic contaminants by using electrode placed in soil to be treated and allows current (DC) to the electrode so that migration of ionic species and charged particles in the soil -water mixture could go towards one of the electrode.

2.3.1.5 Soil vapour extraction

This is a physical method that is applicable to volatile contaminants where the oil is removed in form of vapor by using a sophisticated system. It employs the use of vacuum through a system of underground wells when air is pumped and then sucked the contaminants in form of vapour or gas.

2.3.1.6 Oxidation

It is the movement of contaminants into a more stable environment which achieved through photolysis.

2.3.2 Thermal method

Heating effect is needed for this method to be carried out, therefore, it is energy required method and is expensive. The following are the examples of thermal method:

2.3.2.1 Thermal desorption

It is a method that involved heating crude oil contaminated soil at temperature such that contaminants with low boiling point vaporizes and physically separated (desorb) from the soil. This method does not require excavation or using sophisticated equipment and 90% removal efficiency can be achieved if remediation is carried using thermal method,

however, the method has the following disadvantage: it is expensive, time consuming and hazardous.

2.3.2.2 Incineration

Incineration simply means burning off the contaminants from the surface thus, high temperature is required to destroy hazardous waste and reduce toxic element to basic elements which react with oxygen to form water, carbon dioxide and nitrogen oxide. However, there could be rapid loss of flammable and volatile components through evaporation. Some of the demerits of this method include: high operational cost due to high energy demand, large space requirement as well as environmental pollution.

2.3.3 Biological Method

2.3.3.1 Biodegradation

Hamby (2009) defines biodegradation as the decomposition of organic material by microorganism that will result to formation of simpler substance like carbon dioxide and water or gas (methane). It occurs when microorganism attack a specific molecular site. Bacteria, yeast, filamentous fungi and algae have been identified as crude oil degrading microorganisms

2.3.3.2 Bioremediation

According to Obasi *et al*. (2013), Bento *et al*. (2003) and Ijah *et al*. (2008), remediation of contaminated soil by using microbes is known as bioremediation and it is widely accepted because it improves the biological, physical and chemical properties of soil by increasing the soil nutrients that can enhance microbial growth and proliferation so that properties of the soil could be restored. It is simple, cheap, easy to maintain, totally destroyed contaminants, environmentally friendly as well as possibility of up-scaling. Ijimdiya

(2013) defined bioremediation as a cleanup method, aimed at improving the natural processes of degradation by utilizing bacteria specie which would lead to eventual disappearance of oil from the environment.

Strategies in achieving bioremediation include bio-augmentation which was explained by Adams *et al.* (2015), as a process that involved an addition or introduction of microorganism that is not inhabitant in the environment capable of detoxifying contaminants. In this case, there is a deliberate attempt to increase population of microbial flora in order to initiate the process of bioremediation. A process known as seeding i.e. inoculation of capable mixed microbial consortium into the soil contaminated with crude oil to increase its degradation capacity can also be used (Riskua-Shehu and Ijah, 2016). Similarly, they mentioned another strategy known as bio-stimulation which was described as the injection or addition of nutrients to enhance the capacity of microorganisms. Here utilization of the dwelling soil organism population to remediate contaminated soil is encouraged; through a direct introduction of the substances to promote the growth of the organism. Natural attenuation is a bioremediation strategy which takes place without human effort. Therefore, it depends upon the environmental conditions and behavior of organisms residing in the soil.

It is worth to mention that before choosing any strategy for bioremediation, consideration should be given to the following three principles. First is biochemistry which refers to how the contaminant will react to biological transformation when changing to less toxic substance**.** Another principle is bioavailability which refers to accessibility of the contaminant to the microorganisms. Bioactivity is the third principle which refers to

provision of the necessary condition for biological process to occur unhindered (Ezeji *et al*., 2007).

Ezeji *et al*. (2007) and Syke (2003) explained some of the technologies employed in treatment of contaminated soil in large scale. This include windrowing technique whereby constructed windrow mixed with composting materials (such as fresh green waste) are placed in an elongated piles known as bio-piling. Land farming is another bioremediation technology used in a large form whereby the soil is constantly tilled to provide aeration of the soil, controlled moisture content and addition of nutrients to facilitate microbial degradation of the contaminants. Similarly, compost bioremediation is a technology that involves using microbes in a well prepared compost in order to breakdown contaminants in water or soil.

Kumari *et al*. (2013) and Thapa *et al*. (2012) reported that there are some critical factors in bioremediation and opined that environmental conditions such as effects of temperature, oxygen, soil moisture, nutrients, pH, porosity and type of soil contaminant played key role in bioremediation. According to them the pH of the medium is important because enzymes are actively involved in catalyzing bioremediation and biochemical reactions. Usually enzymatic reactions take place under a favourable pH level since microbes have sensitivity to pH changes; therefore, pH monitoring is important in bioremediation. Researchers studied the influence of temperature in determining the efficiency of bioremediation of contaminated soil. Hesnawi and Mogadami (2013) observed that most hydrocarbon degraders are mesophilic and conducted a bioremediation study using 25°C (mesophilic) and 45°C (thermophilic). They concluded that moderate temperature favours

bioremediation. It has been observed that at 30°C, total hydrocarbon petroleum percentage (THP) was reduced by 80% while, at 20°C the removal efficiency was 60%.

2.3.3.3 Phytoremediation

Vegetative plants are used in treating soil at contaminated site when contaminated by different hazardous contaminants. Generally, phytoremediation is environmentally friendly, cheap and physically attractive. Syke (2003) reported four different types of phytoremediation. They include phytoextraction which involves the natural ability of the plant to take up certain substances like lead (heavy metal) in the soil and decompose in cells until such a time when the plant can be harvested. Another type is phytostabilization which is the release of certain chemicals by the plants that would be attached to the contaminants and make it less bioavailable and less mobile. Also, phytovolitization is a biological process in which plants are able to take out contaminants from the soil and change them into gas for onward release to the environment and phytodegradation is the conversion of organic contaminant into non- toxic ones by the plants.

2.4 Chicken Droppings

Poultry refers to domesticated birds like chickens, ducks, geese and turkeys. Chicken droppings or chicken drop is the faeces of chickens. Chicken droppings contain high content of nitrogen, phosphorus and potassium when compared with manure of other animals such as pig, cow and ox (McCall, 1980). (Ijimdiya and Musa, 2017; Nwaogu *et al*., 2016 and Ijah *et al*., 2008) studied bioremediation of crude oil contaminated soil using chicken droppings. According to McCall (1980) factors that affect the composition of chicken droppings include water content, time spent by manure, type and quantity of litter, storage and handling practice. He also reported that uric acid and urea change to ammonia

rapidly in the first two weeks when soil is moist and warm, however, nitrogen in organic form changes to a useable form slowly in the first four weeks after injection. At six weeks about 60% of the nitrogen becomes available in the soil. Phosphorus is organic in nature and becomes available as chicken droppings decompose. Table A2.4 shows the composition of chicken droppings

The Food and Agricultural Organization (FAO, 2008) and National Veterinary Research Institute (NVRI, 2015) Vom estimated that there were 137. 679, 180 million chicken population in Nigeria, respectively. Magagula *et al*. (2010) mentioned that chicken droppings addition improves biological activities, water retention capacity of soil and observed an improvement in nitrogen, phosphorus and potassium contents in the amended soil. Chijioke-Osuji *et al*. (2014) investigated bioremediation using chicken droppings and other organic manure such as goat dung, sawdust and chemical fertilizer for bioremediation of contaminated soil by crude oil and concluded that nutrient addition would improve remediation of crude oil. In bioremediation of palm oil mill effluent polluted soil, Okwute and Ijah (2014) used animal wastes (chicken manure and cow dung) in 10, 20 and 30% dosage of each organic waste and the findings showed that the reduction was most effective at 20% of the organic waste. Burghal *et al.* (2015) studied the potentiality of sawdust together with cow and sheep dung in bio-stimulation of autochtonous microbial flora in bioremediation of hydrocarbon for 90 days, the result showed that there was microbial growth capable of degrading hydrocarbon.

2.5 Microbial Induced Calcite Precipitation (MICP)

Microbial induced calcite precipitation (MICP) is a new idea and a sustainable technique being applied to geotechnical engineering by using microbial specie in changing the

engineering property of soil. It is a technology that uses metabolism by microorganism to form calcite ($CaCO_3$) capable of binding soil together (Mujah, *et al.,* 2016 and Wei *et al*., 2012). Similarly, Sel *et al*. (2015) explained that other conventional methods of soil improvement like solution/grout tends to change the ground pH level and bring harmful chemicals (toxic) in the soil. MICP is useful in bringing down the cost of construction due to the possibility of producing microbial cement at lower cost with environmental benefits (Wei *et al.,* 2015; Al- Thawadi, 2013; Whiffin *et al.,* 2007 and Ivanov *et al.,* 2009). Little or no investigation has been conducted on the bio-remediated crude oil contaminated soil using MICP with a view to enhance the engineering properties of soil.

2.5.1 Factors influencing calcite formation

According to Wei *et al*. (2012) and Khadhim and Zheng (2016), MICP is facilitated by:

2.5.1.1 Type of bacteria

Urea positive bacteria like the *genera* of *Bacillus*, *Sporosarcina, Spoloactobacillus, Desulfotomaculum* capable of hydrolysis of urea are suitable for MICP. *Bacillus sp* is more applicable in MICP owing to its ability in speeding up reaction in changing urea to ammonia and carbon dioxide hence, the amount of calcite formed depends on the type of bacillus strain. Also size of the bacteria should be consistent with the soil. Komala and Khun (2013) reported that four natural ways by which microorganisms could form calcite in the soil exist; bacteria must be sulphate reducer, nitrate reducer, calcite anhydrous and urea positive bacteria. Sel *et al*. (2015) suggested that the number of bacteria present and interactions of the augmented bacteria with other bacteria present in the environment should be considered. Mitchel and Santamarina (2005) reported that there are more than

10^{12} microbes in one kilogram of soil close to the ground and the number decrease with increase in depth

2.5.1.2 Concentration of bacteria solution

The concentration of bacteria solution determines the amount of calcite that may be formed. This implies that the more the bacteria cell concentration, the greater the rate of urea hydrolysis which would lead to higher calcite deposition as long as the cementation reagents are enough (Wei, *et al*., 2012). The amount of bacteria solution supplied determines the presence of nucleation site that would be readily available for urease activity to take place when the bacteria fully attached themselves in the soil matrix, the nucleation site also affect the size of calcite crystal that may be formed. For higher nucleation site, small crystal or small nucleation site, larger crystal was observed by previous researchers (Mujah *et al.*, 2016).

2.5.1.3 Concentration of cementation reagent

Concentration of cementation reagent and salinity has their influence on MICP process. High cementation reagent concentration increases the amount of calcite deposition. High salinity inhibits microbial activity and calcite precipitation (Wei *et al*., 2012). However, some bacteria have the ability to resist the effect of salinity (Umar *et al*., 2016). Mujah *et al.* (2016) reported that the quality of calcite crystal formation depends largely on the type of cementation solution concentration used during the MICP process. It has been observed that lower cementation solution concentration tend to give homogenous calcite crystal however, at higher concentration, the crystal are randomly formed owing to faster precipitation inherent in addition of higher cementation solution concentration. According to Kadhim and Zeng (2016) the quantity of cementation solution supply to the sample

distinguishes bio-clogging from bio-cementation. When the concentration is lower, the precipitation of calcite will be found to be at particle contact (i.e. bio-cementation) while higher concentration of cementation solution leads to deposition of calcite in the pore spaces (i.e. bio-clogging).

2.5.1.4 pH

When urease enzyme decomposed the urea then calcite precipitated produced by microbial metabolic activities at suitable pH level thus, the hydrolysis of urea is favoured around the cell environment (Wei *et al*., 2012). Mujah *et al.* (2016) reported that environment that is alkaline (6.5< pH<9.3) favours MICP and affects the distribution of calcite crystal in a homogenous manner which control the strength in the sample. Kadhim and Zeng (2016) stated that the best pH range for MICP take place 8.3-9.0. Although Stock- Fisher *et al*., (1999) gave a pH range of 7.5- 8.0 and said urea decomposition become high at pH 8.0 but reduced slowly at the higher pH. However, good urease decomposition could occur at 9.0 pH value. Other benefits of pH in MICP include calcium speciation and carbonate solubility.

2.5.1.5 Injection methods

Injection utilizes pressure, flow and hydraulic gradient condition. There are two methods of injection of cementation fluid, stopped and continuous flow injection method. Injection method of both the chemical and bacteria solution is good in MICP process. This is because Injection method enables the bacteria to be fixed on soil. Mujah *et al.* (2016) mentioned that in large scale experiment, vertical and horizontal method of injection was used successfully. Sel *et al*. (2015) explained that injection cycles of bacteria solution and cementation reagent affects the amount of calcite formation. However, researches in MICP

continue to adopt different strategies in injection method so that the problem of lack of uniform in distribution of bacteria and cementation solution which resulted in producing non uniform calcite content in the specimens treated could be addressed (Mujah *et al*., 2016).

2.5.1.6 Type of soil

The amount of calcite precipitated in MICP process depends on soil type and its particle size. Soil particle size of 50 – 400μm enable the bacteria to move freely within the soil matrix (Kadhim and Zeng, 2016 and Sel *et. al*., 2015). Mujah *et al*. (2016) mentioned that MICP is very good for sand having the particle size of 0.5 – 3mm and suggested that silt and clay should be subjected to further research using MICP with a view to enhance their engineering properties.

2.5.1.7 Temperature

Soil temperature affects the microbial activity and growth of microorganisms, it has been established that 20°C-30°C is a range of temperature that is less sensitive for microbes. In the tropics, this range of temperature is achievable The optimum temperature for urease enzymes activity increase with increase in temperature from 10°C up to 60°C being the highest however, such temperature is practically not possible for field treatment of soil or in laboratory work (Wei *et al.,* 2012).

2.5.1.8 Nutrients

For calcite to be formed, it is important to ensure that the bacteria receive proper and enough nutrients during culturing and application to the soil. This will go a long way in keeping the bacteria active in the process of calcite formation as well as multiplication of the bacteria capable of bringing the necessary changes in the soil (Wei *et al.,* 2012).

According to Kadhim and Zeng (2016) there is a strong relationship between the ability of bacteria to bring changes in hydraulic conductivity of soil and nutrient availability.

2.5.2 Bio-cementation

Bio-cementation involves the deliberate use of microorganisms to strengthen granular soil. It has to do with improvement of shear strength in soil due to formation of calcite which serves as soil particle binding material using MICP techniques (Wei *et al*., 2012). According to Ivanov *et al*. (2009), facultative anaerobes are the most suitable microorganisms for bio-cementation. It has been recommended that a low concentration of the cementation reagent should be used to obtain the desired strength in the sample (Mujah *et al*., 2016).

2.5.3 Bio-clogging

This is important in permeability studies. It is a process where the pore space in the soil is occupied by calcite due to microbial activity (Wei *et al*., 2015). According to Umar, *et al.* (2016), bio-clogging aims at reduction of pore spaces of a porous material by a calcium carbonate formed during MICP process which restricts the flow of water through the soil hence decreases hydraulic conductivity. High concentration of the cementation reagent is recommended in order to reduce permeability of the sample (Mujah *et al.*, 2016).

2.6 *Bacillus licheniformis*

Bacillus *licheniformis* are not pathogenic or toxigenic bacteria and they are available in soil. It is part of the subtilis group along with *Bacillus subtilis* and *Bacillus pumilus*. However, they differ in the amount and location of prophages, transposable elements and extracellular enzymes. B. *licheniformis* is a rod-shaped, Gram- positive, motile, halophilic, facultative anaerobe and has 1.5 to 3.0 by 0.6-0.8 μm diameter. It has a growth temperature

from 15°C to 55°C. It tends to form endospores in soil in order to survive harsh environmental condition such as heat and desiccation. It can be isolated from soil as well as other sources since it can produce highly resistant endospores that are spread around with dust (Nabti *et al*., 2012). It is this characteristic that makes the bacteria suitable for degrading hydrocarbon, although, bacteria of the same specie do have different capability to degrade hydrocarbon petroleum (Riskuwa-Shehu and Ijah, 2016). Barua *et al*. (2011) and Al-Wahaibi *et al*. (2016), reported on the potential use of *B. licheniformis* to degrade oil. *B. licheniformis* is used in fermentation industry for making detergent, dehairing in leather industry, protease, alpha- amylases from *B. licheniformis* were used in textiles industry for dyeing. It is also used in making antibiotics (such as Bacitracin) by producing cyclic polypeptides as well as in treating ornamental plants to protect them from fungal pathogens (Al-Disi *et al*., 2016).

2.7 Municipal Solid Waste

Municipal solid wastes (MSW) are generated from places like homes, market places, and factory areas. MSW are found in various forms and shapes and they could be durable and nondurable goods like containers, packages and food waste. Things like disposable diapers, newspapers, boxes, and clothing are some of the example of MSW. The following are methods of disposing MSW.

2.7.1 Waste Disposal Methods

Generally, waste disposal are removed from the environment using what is referred to as *Surface impoundment* method which involved placing liquid or semi-liquid waste in unlined pits, in which the waste is kept in long term storage but not as a final method of disposal. Another way of getting rid of waste is by *Deep wells* method which means

disposal of waste in which about 8% of waste is injected into the deep well. *Landfills* method is a method of waste disposal whereby a large unlined pit is excavated in order to accommodate waste. The excavated soil serves as a support for the waste, so 21% enters the landfills as a final place for disposal. No doubt this creates another environmental problem. *Incinerator* is a method that involved burning of garbage. Although this method provides the opportunity of converting waste to energy (steam and electricity), it requires less land and do not pollute underground water. However, using incinerators induce emission of toxic air while disposal of the ash that is left after burning contains toxic metal like lead, mercury and cadmium that could not be burnt in raw garbage so they become concentrated in the ash which finally finds its way to landfills and ultimately ends in underground water. This means there is possibility of the toxins from the landfill in leachate and contaminate groundwater. For these reasons containment systems equipped with good and affordable liners to prevent leachate should be devised (Ojuri, 2012).

2.7.2 Leachate

Leachate is a liquid substance formed when municipal solid waste mixes with moisture. Table 2.4 shows the constituents of a typical municipal landfill leachate.

Table 2.4 Municipal landfill leachate Minimum and Maximum limit

Municipal Landfill leachate concentration (ppm)		
Indicator Parameter	Minimum	Maximum
Alkalinity	470	57,850
Ammonia	0.39	1200
BOD	7	29200
Calcium	95.5	2100
COD	42	50450
Chlorine	31	5475

Flouride	0.11	302
Iron	0.22	2280
Phosphorus	0.29	117.18
Potassium	17.8	117.5
Sulfate	8	1400
Sodium	12	2574
Total Dissolve Solid	390	31800
Total Suspended Solid	23	17800
Total Organic Carbon	20	14500
	Inorganic compound (ppm)	
Aluminum	0.01	5.8
Antimony	0.0015	47
Arsenic	0.0002	0.982
Barium	0.08	5
Berylium	0.001	0.01
Cadmium	0.0007	0.15
Chromium	0.0005	1.9
Cobalt	0.004	0.13
Copper	0.03	2.8
Cyanide	0.004	0.3
Lead	0.005	1.6
Magnesium	74	79
Manganese	0.03	927
Mercury	0.0001	0.0098
Nickel	0.02	2227
Vanadium	0.09	0.029
Zinc	0.03	350

(Source: US EPA, 1991)

2.8. Engineered Liner

Ab Aziz (2015) reported on the necessity of designing a modern landfill in the base of waste containment system as engineered hydraulic barrier in order to prevent leachate from contaminating underground water. Sabat and Nayak (2015) defined engineered landfills as designed physical facilities put in place in order to dispose waste materials and ensure safe environment. The US EPA (1991), Daniel and Wu, (1993) and Benson *et* al., (1999) explained that for a material to be used as a lining material in waste containment systems, it must have a low hydraulic conductivity (k) $\leq 1x10^{-9}$ m/s, Unconfined Compressive Strength (UCS) $\geq 200kN/m^2$ and Volumetric Shrinkage Strain (VSS) (resistance shrinkage cracking) $\leq 4\%$.

2.8.1 Types of engineered liners

Engineered landfills are physical facilities designed for the environmentally safe disposal of waste material. Liner is the most important component of the engineered liner landfills (Ojuri, 2012). According to Rowe and Fraser (2007) liner can be grouped into three groups: (a) Compacted clay liner (b) Synthetic liner (c) Composite liner. Low hydraulic conductivity, high contaminant attenuation and less cost of construction as compared to other types of liners, compacted clay liner are widely used in engineered liner (Ojuri, 2012)

CHAPTER THREE

MATERIALS AND METHODS

3.1 MATERIALS

3.1.1 Crude oil contaminated soil

The crude oil contaminated soil (COCS) used in this study was collected by method of disturbed sampling based on BS 1377 part 1 (1990) from a depth of 0.5m below the ground from Kaduna Refinery and Petrochemical Cooperation (KPRC) (latitude 10° 27'16.85'' N and Longitude 7° 24'46.58''E).

3.1.2 Chicken dropping (CD)

The CD used in this study` was collected in an unsterilized polyethylene bag from Al-Amin Farms along Jos road in Zaria, Kaduna state- Nigeria. The chicken dropping was used as a source of nutrients for the microorganism with the necessary nutrients nitrogen, phosphorus and potassium (N P K) only to activate the microorganism to carry out the needed microbial activity.

3.1.3 Bacteria

The bacteria used in this study was *B. licheniformis* residing in the crude contaminated soil. It is classified based on American Type Culture Collection as ATCC 14580 and used throughout the study. The microbe was activated with CD (i.e. bio-stimulation) to bio-degrade the hydrocarbons and were cultured in liquid media and sterilized by autoclaving for 15 minute at 121°C.

3.1.4 Cementation reagent

The cementation reagent has the following constituents of 20 g of urea, 2.8 g of calcium chloride, 3 g nutrient broth, 10 g ammonium chloride and 2.12 g sodium bicarbonate per

litre of distilled water as described by Stocks-Fischer *et al.,* 1999; Stoner *et al.*, 2005; Dejong *et al.,* 2006 and Al-Qabany *et al.,* 2011.

3.1.5 Municipal solid waste (MSW) leachate

Leachate samples labeled $W_{1,}$ W_2 and W_3 for the study were collected at a refuse dump site behind the Catholic Church Samaru, Zaria. The site was opened therefore the wastes are susceptible to rainfall and sunlight.

3.1.6 Water

Tap water and distilled water were collected from Civil Engineering Department, ABU, Zaria and Nitrogen Laboratory, Institute of Agricultural Research, Samaru respectively for this work.

3.2 METHODS

3.2.1 Sample preparation for bio-remediation

The air- dried chicken droppings were ground to powder in stepped concentration of 10, 20, 30 and 40% by dry weight of soil as described by Antizar-Ladislao *et al*., (2006). The ground chicken dropping was dissolved in water in a ratio 1:10 to form bio- slurry and kept in the laboratory for three days in order to extract nutrients as described by Bonten *et al*. (2014). The bio slurry formed was filtered using No 200 sieve. The liquid filtrate obtained was used to hydrate the soil sample containing the indigenous bacteria. The mixture was blended thoroughly to obtain a homogenous mix. The blended samples were kept in plastic containers (microcosms) for 12 weeks in order to allow a complete bio-remediation of the hydrocarbons through microbial activity.

3.2.2. Identification, enumeration and isolation of microorganism

Soil samples (contaminated and uncontaminated) were collected in a new polyethylene bags (unsterilized) properly labeled. 10 g of each sample was weighed using a digital weighing balance out of which 1g was used in serial dilution using five (5) dilution bottles with 9 ml each of distilled water. The 1g of soil was transferred into the first bottle to form a stock solution of each sample. The samples were allowed to soak for few minutes; the mixtures were then shaken thoroughly. 1ml was transferred aseptically into the other four bottles having 9 ml of distilled water which was serially diluted until CO^{-3} dilution was obtained to produce soil suspension. Viable cell counts of the samples were determined using Spread Nate Method. After that 1ml of 10^{-5} dilution of each sample was aseptically transferred into nutrient agar separately, a bent spreader was used to spread the inoculants on the surface of the culture media. Cultured bacteria were inoculated at 37°C for 24 hours.

After the inoculation period, plates were observed for growth. The growth on nutrient agar (colonies of bacteria) were enumerated and results were expressed as colony forming unit per gram (Cfu/g) using the formula expressed by Equation (3.1):

$$cfu/g = \frac{total\ number \times dilution\ factor}{Volume\ of\ inoculation} \quad (3.1)$$

The B. *licheniformis* was first characterized using biochemical method and then identified with the Microgen *Bacillus* – ID 24 Test kit.

3.2.3 Total petroleum hydrocarbon (TPH) test

The total petroleum hydrocarbon percent was determined according to US EPA 418 (1993) by gravimetric method as described by Ameh *et al.*, (2012). The test was conducted in Chemical Engineering department, ABU Zaria. 10g untreated sample was air-dried at room

temperature for 72hours; out of that, 5g of soil was placed in a beaker of 200ml capacity, then 150ml v0lume of toluene was added and stirred the mixture continuously for 30 minutes using glass stirrer and allowed to dissolve for 2hours in a fume cupboard, filtering the mixture was carried out using Whatman No 42 filter paper. The residue was oven-dried at 50°C for 24 hours. The Equations (3.2) and (3.3) were used in calculating TPH and Removal Efficiency:

$$\text{TPH (g/kg)} = \frac{(initial\ weight\ of\ soil - weight\ of\ soil\ after\ soil\ extraction) in\ g}{initial\ weight\ of\ soil\ in\ kg} \quad (3.2)$$

$$Removal\ Efficiency(\%) = \frac{initial\ TPH - Remediated\ TPH}{Initial\ TPH} \times 100 \quad (3.3)$$

3.2.4 pH test

The sample's pH determination was performed according to BS 1377-3: 1990 using electrometric method with pH meter (PHS-25; Techmel & Techmel, USA) in ratio of 1: 2.5 (w/v) soil/water mixture. A 30g of sample collected from microcosms, air- dried and sieved using BS No 8 sieve (2mm aperture). The soil was then mixed with distilled water of 75ml capacity put into a container (100ml capacity beaker) then stirred the mixture using glass stirrer for 30 minutes for proper and homogeneous mixture, after that the mixture was covered with a glass for 8hours. The suspension was stirred before inserting the electrode into the suspension. The electrode was washed with distilled water after calibration with buffer solution and inserted into the suspension. Similarly, The effluent's pH of *Bacillus licheniformis* suspension and cementation reagent was collected during unconfined compressive strength test and measured directly using pH meter (PHS-25; Techmel & Techmel, USA) by inserting the probe in the effluent collected in a plastic container during MICP.

3.2.5 Temperature measurement

The temperature measurement was carried out using temperature meter (PHS-25 by Techmel & Techmel, USA).The probe was inserted into the microcosms at three different positions and allowed for 5minutes. Temperature values were obtained from the digital display of the temperature meter.

3.2.6 Sample preparation for bio-cementation

Bio-remediated crude oil contaminated samples were kept at a constant moisture corresponding to the optimum moisture content of the soil for bioremediation by constant moisture addition at 7days interval. The moisture of the microcosms was monitored at 2days interval using VG-200 soil moisture meter and maintained corresponding to the optimum moisture of the soil. Distilled water was used for the moisture adjustment when necessary and the sample was tilled prior to moisture addition by hand sprinkling. The volume of water used throughout the period of bio-remediation were 1525, 870, 550, 255 ml for 10, 20, 30 and 40% CD, respectively. This was done to ensure the survival of the microbes and maintain the optimum moisture content of 20-60% recommended by Thapa *et al.* (2012). After remediation, the soil sample was air-dried for three days and later pulverized using rubber-headed pestle and sieved through sieve No 4 (4.75mm aperture) in accordance with BS 1377 (1990).

3.2.7 Bacteria culture and growth medium

The bacteria were cultured in liquid media consisting of 3g Nutrient Broth (NB), 20g of Urea, 10 g of Ammonium Chloride (NH_4Cl), 2.12g $NaHCO_3$ per liter of distilled water, with a pH measured at 9.0 using NaOH for adjustment. Liquid media were sterilized by

autoclaving for 15 minutes at 121°C. Cultured bacteria were inoculated at 37°C for 24 hours. After inoculation, plates were observed for growth.

3.2.8 Bacteria solution

The Bacillus *licheniformis* suspension was used in stepped concentration of *Bacillus licheniformis* concentration of 0, 0.5, 2, 4, 6, 8 on McFarland scale which is equivalent to 0 /ml, 1.5 x 10^8 /ml, 6.0 x 10^8 /ml, 12.0 x 10^8 /ml, 18.0 x 10^8 /ml and 24.0 x 10^8 cell/ml. These were determined based on suspension density. The maximum quantity of bacteria used in mixing for compaction was one-third (i.e. 1/3) of the pore volume which was determined using degree of saturation which form part of the optimum moisture content as recommended by Rowshanbakhta *et al.*,(2016). The pore volume of BSL, WAS and BSH compactive efforts were determined in the study as 3.69, 3.21 and 2.25 respectively.

3.2.9 Natural moisture content

Oven- drying method is the standard method of determining moisture content in the laboratory based on BS 1377-2: 1990. The portion of soil collected right from the site was put in three labeled polyethylene bags (plastic bag) and transferred into cans covered with lid and taken to laboratory. Samples were emptied into empty containers having measured their weight and recorded in the data sheet. Similarly weight of containers with samples were measured and recorded accordingly. Thereafter the samples were put in the oven set at 105°C - 110°C thermostatically controlled and allowed for 24 hours. Thereafter weight of dry samples were measured after cooling the specimen in desiccators and recorded in the data sheet. The natural moisture content was calculated as average of the three oven dried samples from Equation (3.5a) and (3.5b)

Moisture content (w) = $\frac{moiture\ loss}{dry\ mass} \times 100$ (3.5a)

$$w = \frac{m_2 - m_3}{m_3 - m_1} \times 100\% \quad (3.5b)$$

Where m_1 is weight of container

m_2 is weight of container and soil

m_3 is weight of container and dry soil

3.2.10 Specific gravity

The test for specific gravity was carried out according to BS1377 (1990) test method for fine grained soils. The bio-remediated soil sample was first mixed with the bacteria solution and the cementation reagent at optimum moisture content and then dried at room temperature for three days prior to carrying out test.

The bottle with cork was weighed to the nearest 0.1g and the weight was noted as (m_1). The prepared air dried soil sample was poured into the bottle and then the whole was weighed and the weight noted as (m_2). Distilled water was then added enough which covered the soil and was gently shaken to dispel any void. The bottle was filled up to the mark level, corked and surfaced dried properly. The whole was then weight and noted m_3. The mixture in bottle was then emptied, washed and filled with water. The bottle filled with water was weighed and noted m_4 to the nearest 0.001g. The specific gravity (G_S) was computed using Equation (3.6)

$$(G_S) = \frac{m_2 - m_1}{(m4 - m1) - (m3 - m2)} \quad (3.6)$$

where m_1 = weight of bottle

m_2 = weight of bottle and soil

m_3 = weight of soil, bottle and water

m_4 = weight of bottle and water

3.2.11 Particle size distribution

The particle size distribution of the crude oil contaminated soil and bio-remediated and bio-cemented soil was determined according to BS 1377-1: 1990. The bio-remediated sample was treated with bacteria solution and cementation reagent at optimum moisture content (OMC) and dried at room temperature for three days. Mechanical sieve and hydrometer test were carried out. Sample of 200g was soaked for 24 hours, and then washed using BS No. 4 and 200 sieves. The particles retained were oven dried for 24 hours and dry sieving was carried out on the dried sample using a set of sieves and percentage of soil passing through the sieve was also analyzed. Also, sedimentation test was done on the solution kept in 1000 ml cylinder by using hydrometer analysis. In this analysis, a dispersing agent (Sodium Hexa-metaphosphate) was used in the soil suspension so that separation of discrete particles of the soil can be ensured. The percentage of fines was calculated to establish particle size distribution (grading curve) on a semi log graph.

3.2.12 Atterberg limits

For Atterberg limit tests soil passing through (BS) sieve No 40 sieve (425 μm aperture size) was used in accordance with BS 1377 (1990). The Atterberg limit test consists of liquid limit, plastic limit and plasticity index. The bio-remediated soil samples were treated with bacteria solution at 25, 50 and 75% levels and cementation reagent at 75, 50

and 25% level at optimum moisture content for British Standard light (BSL) and allowed to dry at laboratory temperature of 25 ± 2°C for three days before performing Atterberg limit tests.

3.2.12.1 Liquid limit

The liquid limit was determined in accordance with BS 1377-2: 1990 procedure. For the treated soil, the sample, bacteria solution and the cementation reagent were mixed together at optimum moisture content and then dried in laboratory before the test was conducted. About 200g of the representative sample passed through a BS No 4 sieve (425 μm). The soil was placed on flat glass mixing tray and thoroughly mixed with water in order to form a homogenous paste. A portion of the paste was placed in the cassagrande apparatus leveled off parallel to the base and divide it into two equal halves by moving the grooving tool along the diameter through the centre of the hinge. The crank handle of the machine was turned at a steady rate of two revolutions per second, so that the bowl is lifted and dropped until the two part of the soil come in contact at the bottom of the groove along distance of 13mm. The number of blows at which this occurs was recorded and a portion of the sample was taken for moisture content determination. The mix in the bowl was removed and the bowl was cleaned with cloth. Some of the prepared dry soil sample was added (depending on target number of blow) to the paste mixed thoroughly again and subjected to blows. This process was repeated until two consecutive runs give around the same number of blows for closure. The value of the moisture content (determined) and the corresponding number of blows was plotted on a semi-logarithmic graph and the liquid limit was determined as the moisture content corresponding to 25 blows.

3.2.12.2 Plastic limit

According to BS 1377-2: 1990, the plastic limit test is aimed at determining the lowest moisture content at which the soil is plastic. For treated soil, the sample was first mixed together with the bacteria solution and the cementation reagent at optimum moisture content and then dried in the laboratory before the test was conducted. A sample weighing about 20g from the paste prepared for liquid limit test was collected and placed on a glass tray. The soil was then allowed on the mixing tray to dry partially until it turns plastic enough to be kneaded and shaped into a ball. The ball of soil was molded between the palms of hands. This continued until the diameter of the thread was reduced to about 3mm in five to ten forward and backward movements of the hand. The movement continued until the thread sheared both longitudinally and transversely. The crumbled soil was then put in the moisture container and the moisture content determined.

3.2.12.3 Plasticity index

The plasticity index (PI) of the soil and crude oil contaminated soil is the difference between the liquid limit (LL) of the natural and crude oil contaminated soil and their corresponding plastic limit. The calculation of plasticity index of the sample was done using equation (3.7)

$$PI = LL - PL \quad (3.7)$$

3.2.13 Compaction characteristics

3.2.13.1 Maximum dry density

The compaction test was conducted for natural, crude oil contaminated and bio-remediated and bio-cemented soils in accordance with BS 1377- 4: 1990 using British standard light, British Standard heavy and West African Standard. The pulverized, air-dried soil samples

which passed through BS No 4 sieve (4.76 mm aperture) were used. The bio-remediated soil samples were treated with bacteria solution and cementation reagent using 50% each of the optimum moisture content and compacted using three compactive efforts, namely British Standard light (BSL), West African Standard (WAS) and British Standard heavy (BSH). The bacteria constituted 1/3 of the pore volume while the remaining being distilled water. This was thoroughly mixed with 3000g of the sample and kept in polyethene in the laboratory for 12 hours, for the bacteria to be well distributed in the soil before compaction in accordance with BS 1377, 1990. The 1000cm^3 mould was used in compacting the sample in three layers of approximately equal part with each layer receiving 27 blows from a 2.5kg rammer falling through 300mm for BSL compactive effort; samples compacted using WAS compaction energy were compacted in five layers each receiving 10 blows from a 4.5 kg rammer while samples compacted for BSH compactive effort were compacted in five layers receiving 27 blows from a 4.5 kg rammer.

The bulk density (ρ) in Mg/m^3 calculated for each compacted mix is given by Equation (3.8)

$$\rho = \frac{(m2 - m1)}{100} \qquad (3.8)$$

Where;

m_1= mass of mould with base

m_2= mass of mould with base and soil

And the dry density (ρ_d) is computed using Equation (3.9a) and (3.9b), respectively

$$\rho_d = \frac{100 \times bulk\ density}{100 + moisture\ content\ (\%)} \quad (3.9a)$$

$$\rho_d = \frac{100\rho}{(100+w)} \quad (3.9b)$$

3.2.13.2 Optimum moisture content

The optimum moisture content (O.M.C) corresponds to the maximum dry density (MDD) from the graph of dry density against moisture content.

3.2.14 MICP treatment

The soil samples were saturated with *Bacillus licheniformis* in stepped concentrations of 1.5 x 10^8 /ml, 6.0 x 10^8 /ml, 1.2 x 10^8 /ml, 1.8 x 10^8 /ml and 2.4 x 10^8 /ml and properly kept in an air-tight material (polythene) for 12hours in the laboratory at room temperature 25 ± 2 °C for the bacteria to fix themselves onto particles of soil. The soil samples were compacted into the 1000cm^3 compaction moulds at optimum moisture contents compacted using three compactive efforts namely BSL,WAS and BSH in accordance to BS 1377 (1990). The compacted specimens were saturated with cementation reagent in four circles for 24 hours as described by Yasuhara (2016). The maximum quantity of cementation reagent used in treating the specimen was 2/3 of the pore volume and was allowed to flow by gravity using top – bottom approach in order to allow microbial induced calcite precipitation to occur.

3.2.15 Hydraulic conductivity

A falling head permeameter apparatus was used as described by Head (1994) and in accordance to BS 1377-6: 1990 to carry out permeability test. The compacted and microbially treated samples using *Bacillus licheniformis* were saturated in a rectangular steel tank (water pool) of 60 x50cm for 24hours. The fully saturated test specimens were

then connected to a standpipe, which provided both the head of water flowing through the sample. Tests were discontinued when steady flow was established by comparing two consecutive readings with insignificant difference. The initial height (h_0) was recorded at t = t_0, after three hours (t_3) the drop in height was recorded as h_1. These procedures were repeated throughout the experiment. To determine dry density for determination of hydraulic characteristics (porosity, void ratio, and degree of saturation), sample was taken from top and bottom of the specimen.

Hydraulic conductivity k was determined from Equation (3.10)

$$k = 2.303 \frac{aL}{100\ At} log10(\frac{h1}{h2}) \quad (3.10)$$

k- Hydraulic conductivity (or coefficient of permeability) in m/s, a- cross sectional area of the stand pipe, L-the height of specimen, A- Cross sectional area of the sample, t- Time taken for the drop from height h_0 to h_1 in sec, h_1- initial height of the liquid in stand pipe, h_2- final height of the liquid in stand pipe in mm after time t

3.2.16 Compatibility test

A compatibility test was carried out on the samples in which the hydraulic conductivity test procedures described earlier were used.

3.2.16.1 Long term hydraulic conductivity

Long term hydraulic conductivity was carried out for the three bio-remediated and bio-cemented soil treated with 1.5 x10^8, 1.8 x10^9 and 2.4 x10^9/ml *Bacillus licheniformis* suspension using MSW leachate as the permeant liquid. Standard Proctor, West African

Standard and Modified Proctor respectively were used to find out the interaction of the permeant that flow through the samples.

At the beginning a period of 22 days was observed before imposing MSW leachate for a period 69days. The readings were taken daily for 91 days of the test and data obtained were used in calculating hydraulic conductivity.

3.2.17 Unconfined compressive strength

The unconfined compressive strength (UCS) test was conducted on both the treated and untreated samples in accordance to BS 1377-2: 1990. The compacted and microbially treated samples using *Bacillus licheniformis* were cured for 24hours and then extruded from the cylindrical mould measuring 38.1mm diameter and 76.2mm length. The specimens were extruded and air-dried at room temperature of 25±2°C in the laboratory, then cured for 24 hours before curing it in polyethylene bags for another 24 hours. After the curing time (i.e. third day), a compression testing machine was used for the three cylindrical specimens which were placed centrally on the lower platen and a compressive force was applied to the specimen with the strain control at 0.01mm. Reading was taken simultaneously of the axial deformation and axial force at regular intervals until failure occurred. The UCS of the sample was determined at the point on strain – stress curve at which failure occurred. The UCS was calculated from Equation (3.11)

$$Compressive\ strength = \frac{failure\ load}{Surface\ Area\ of\ specimen} \quad (kN/m^2) \qquad (3.11)$$

3.2.18 Volumetric shrinkage strain

The volumetric shrinkage strain (VSS) tests were carried out on both the untreated and treated samples in accordance to BS 1377-2: 1990 and BS 1924: 1990. The specimens for volumetric shrinkage strain were compacted and microbially treated using *Bacillus licheniformis*. The compacted cylindrical specimens were extruded from lubricated compaction mould; air- dried at room temperature (25 ± 2 °C) for 30 days. The mass and height of the VSS specimens were measured using a digital weighing balance and a Vernier caliper, respectively, at an interval of five days. The height and diameter of the specimens were taken at three different positions to the nearest 0.01mm the average height and diameter were used in calculating the volume (V), while the VSS was calculated using Equation (3.12a), other important Equations are (3.12a) and (3.12b)

$$V = \pi r^2 h \tag{3.12}$$

$$\text{VSS} = \frac{Change\ in\ Volume}{Original\ volume} \times 100 \quad (\%) \tag{3.12a}$$

$$\text{VSS} = \frac{V_i - V_f}{V_i} \times 100 \tag{3.12b}$$

Where V_i is the initial volume

V_f is the final volume

3.2.19 Calcite content determination

The calcite content was estimated using acid washing method as reported by Burbank *et al*., (2011) and Choi *et al*., (2017). 5g of treated sample used in volumetric shrinkage strain test was cut off from surface to core of the specimen and weighed (denoted as W_1) and placed in a 50ml beaker; 20ml of 2M of hydrochloric acid (HCl) was introduced into a

container (beaker) and stirred using glass rod until the calcium carbonate dissolved in the acid, it was then transferred to Whatman filter paper in sieve No 200 and washed with distilled water for 10 min. The filtrate was dried in oven at 105°C. The dry weight (W_2) was recorded. Calcite content was computed using Equation (3.13)

$$W_D\ (g) = W_1 - W_2 \qquad (3.13)$$

Where, W_D is weight of the calcite content (CC)

W_1 is sample weight before acid washing

W_2 is sample weight after acid washing

3.2.20 Statistical analysis

The two way analysis of variance ANOVA was used to determine the effect of B. *licheniformis* and compactive efforts on volumetric shrinkage strain and hydraulic conductivity, unconfined compressive strength of the bio-remediated and bio-cemented crude oil contaminated soil. The calculated F value (Fcal) from the results was obtained from the ratio of the samples sum of squares to the error sum of the square. The letter n and k represents the number of observations and number of sample mean, respectively. The critical F value (F critical) is the upper limit of observations, the p-value is the tail probability for a given distributions. The p- value will be less than 0.05 for (5 % level of significance) whenever the calculated F value is greater than the critical F-value which is an indication of the existence of statistical significance in relation to the contribution of a given variable (Osinubi and Nwaiwu, 2006).

3.2.21 Micrograph

The imagery of both treated and untreated samples by scanning Electron Microscope (SEM) imagery was conducted to reveal the calcite formation in the soil specimens. The images were taken using **Phenom proX, made by Phenonworld Eindhoven,** the Netherland which was used in examining the structural arrangement of sample prepared at OMC with magnification x 1500. The soil sample used was the one treated with $1.5x10^{8}$/ml *Bacillus licheniformis* which gave the optimum results in this study

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Properties of Materials Used in the Study

4.1.1 Crude oil contaminated soil

Preliminary test conducted on the crude oil contaminated soil before remediation showed that the soil has very low natural soil moisture content of 5.52% due the fact that the sampling was done in dry season (April) when the rain has not set in proper. Thus, the soil was in a dry condition. The initial quantity of crude oil (TPH) found in the contaminated soil was 98g/kg, which is regarded as heavily contaminated (Sayara *et al.,* 2010).

The index properties of the crude oil contaminated (untreated) and bio-remediated (treated) soil using *B. licheniformis* are summarized in Table 4.1. The soil is classified as A-2-5(5) according to AASHTO (1986), which belongs to the SM group in the Unified Soil Classification System (ASTM, 1992). The percentage passing BS No 200 sieve for the crude oil contaminated soil (untreated) and natural soil were 13.01% and 46.5% ,respectively, with liquid and plastic limits of 42.2% and 35.44%, respectively, for the crude oil contaminated soil and 45.06% and 37.09% for natural soil respectively. It was observed from Fig. 4.1 that the untreated soil has percentage of fine and coarse sand fraction of 36% and 52%, respectively. This may be due to the presence of crude oil in the soil as contaminants which adhere to silt and clay sized particles and agglomerated them to form coarse sand particles. Similarly, the natural soil has 27% fine sand fraction with 43% coarse sand fraction. Similar findings were reported by Habeb-ur-Rahman *et al,* 2007; Rahman *et al.*,2010; Ijimdiya, 2010a,b; Ijimdiya and Igboro, 2012; and Ijimdiya 2017.

Table 4.1: Physico-chemical Properties of the crude oil contaminated soil and treated soil

Property	TREATED *B. licheniformis suspension* (cell/ml)						
	COCS	0	$1.5x10^8$	$6x10^8$	$1.2x10^9$	$1.8x10^9$	$2.4x10^9$
Natural moisture content (%)	5.6						
Percentage Passing B.S sieve No 200 (%)	13.01	46.5	34.55	37.10	38.5	40.45	44.45
Liquid Limit (LL) (%)	42.2	45.06	42	46.2	45.7	45.2	46.7
Plastic Limit (PL) (%)	35.44	37.09	32.12	43.12	35.89	32.73	38.69
Plastic Index (PI) (%)	6.76	8.97	9.88	3.08	8.7	10.87	6.31
AASHTO Classification	A-2-5	A-2-5	A-2-5	A-2-5	A-2-5	A-2-5	A-2-5
USCS	SM	SM	SM	SM	SM	SM	SM
Specific gravity (Gs)	2.13	2.53	2.6	2.23	2.15	2.17	2.17
pH value	6.9						

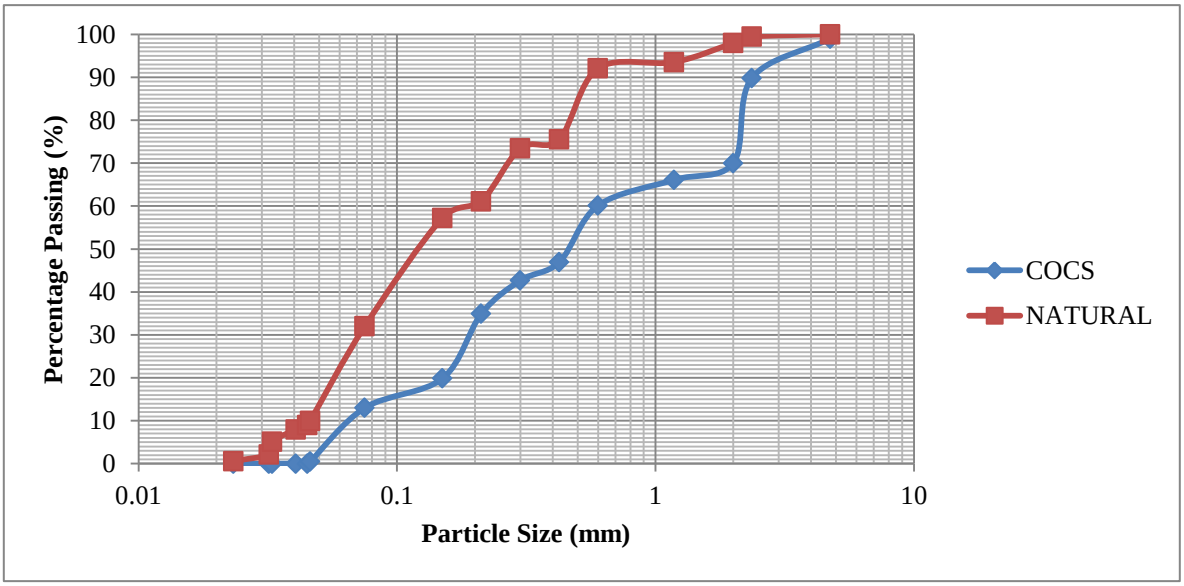


Fig.4.1: Particle Size Distribution of crude oil contaminated and Natural soil

4.2 Characterization of Bacteria

The bacteria species in the crude oil contaminated soils were characterized based on their reaction with Gram's staining reagent. The bacteria was identified to be a gram positive bacteria having a rod like shape found either in chain or in pairs. Table 4.2 shows the result of the bacteria characterization.

Table 4.2: Bacteria characterization

Sample Label	Gram Reaction	Cell Morphology	Cell Arrangement	Inference
A1	Positive	Large motile	Chain	*Bacilli sp*
A2	Positive	Cocci	Clustered	*Staphylococus*
A3	Positive	Rod	Pairs	*Bacilli sp*
A4	Positive	Rod	Single	*Clostridium*

4.2.1 Bacteria Identification

Bacteria was identified and classified according to American Type Culture Collection as ATCC 14580. The results of the motility test, reaction to stains and action on coagulase show that *Bacilli* were suspected in the sample A1 and A3. Similar findings were made by Fawole and Oso (2007).

The results of cultured media prepared for those samples and identified by using Microgen *Bacillus* – ID 24 Test Kit for first and second reaction after 24 and 48 hours, respectively, were obtained which gave the octal code. The number submitted to GENMAT software identified the bacteria. Table 4.3 shows the predominant bacteria resident in crude oil contaminated soil (A1) and uncontaminated soil (A3). This is in agreement with findings made by Barrow and Feltham, (1999).

Table 4.3: Bacteria identified in the contaminated and uncontaminated soil

Sample Code	Profile No. (Octal code)	Final Identified Bacteria
A1	70772036	*Bacillus licheniformis*
A2	50672004	*Bacillus megaterium*

4.3 Bioremediation of the crude oil contaminated soil

Fig. 4.2 shows the Variation of Total Petroleum Hydrocarbon (TPH) with Time of bioremediation for various CD contents. The initial total petroleum hydrocarbon dosage in contaminated soil was 98g/kg and microbial population was $5.3x10^7$ cfu/g. However, when chicken droppings (CD) were added in stepped concentrations of 10, 20, 30, and 40% of the total sample by dry weight. It was observed that the microbial population increased with higher CD contents and a gradual decrease in crude oil content from 2 through 12 weeks period of bioremediation occurred while monitoring moisture, temperature, pH and oxygen contents.

It was observed that crude oil content in the contaminated soil decreased from 4.5 g/kg to 4.0, 3.5, 2.5, and 2.3 g/kg when treated with 0, 10, 20 30 and 40% CD, respectively, for a period of 12 weeks as shown in Fig. 4.2

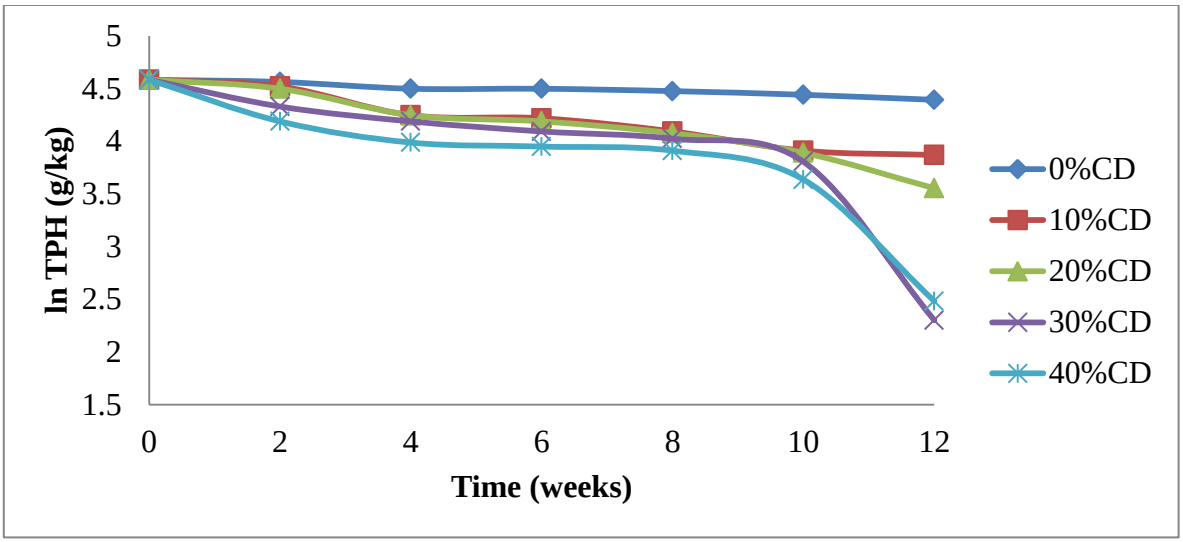


Fig. 4.2: Variation of TPH with time of bioremediation for various CD content

Similarly, Figure 4.3 shows the results of the removal efficiency of the bio-stimulation strategy adopted. It was observed that the removal efficiency increased with increase in time or period of remediation. The removal efficiency of crude oil from the contaminated soil when treated with 0, 10, 20, 30 and 40 % CD contents were 5, 45, 60, 89.8 and 85 %, respectively. The samples treated with 30% CD recorded higher removal efficiency probably due to the temperature and pH of the mixture. According to Nabti *et al.*, (2012), *B. licheniformis* has a growth temperature range of 15°C to 55°C and pH range of 7.17-8.76 (Ijah *et al*., 2008). This conform with findings made by previous researchers who studied bioremediation of crude oil contaminated soil using chicken droppings (Ijah *et al*.,2008 and Ijimdiya and Musa, 2017) and under mesophilic condition (Hesnawi and Mogadimi 2013). Detailed test results are given in Table A4.1 of the Appendix

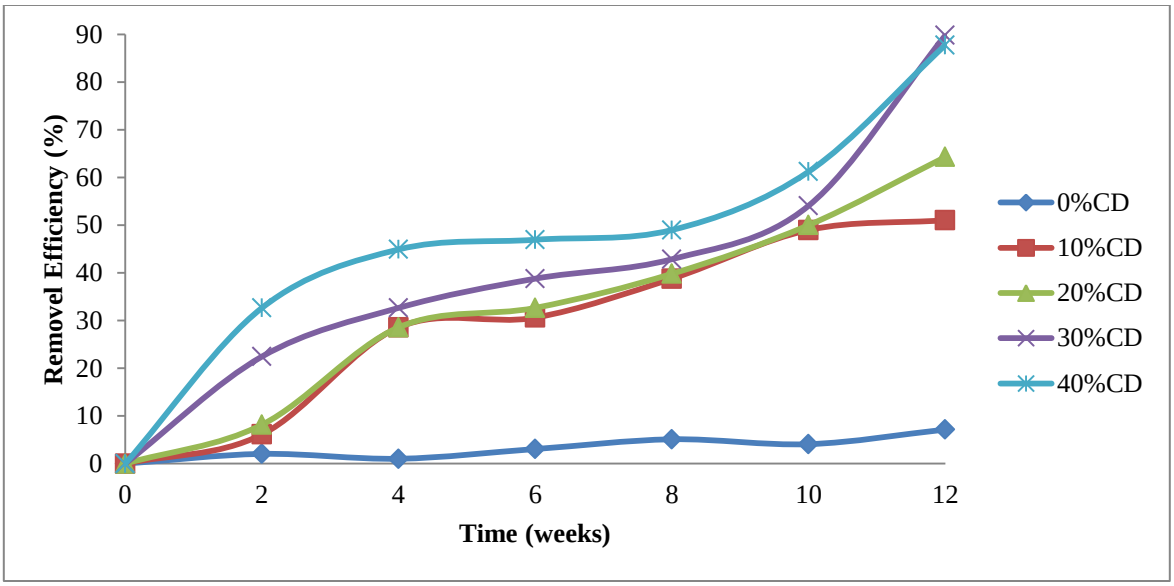


Fig. 4.3: Variation of Removal efficiency with time of bio-remediation for various CD content

The variation of temperature with time of bio-remediation for varying CD contents are shown in Fig.4.4. It was observed that the temperature of the bio-remediated samples slightly increased with increase in time of bio-remediation for all the CD content from 27.1, 26.8, 26.8, 26.9 and 26.2°C to 28.5, 27.18, 27.9, 28.68 and 27.28°C when treated with 0, 10, 20, 30 and 40% CD content, respectively for a period of 12 weeks. The highest temperature was recorded for samples containing 30% CD content. The probable reason for the slight increase in temperature could be due to the availability of adequate oxygen content that led to increased microbial activity that facilitated the hydrocarbon degradation. This is in agreement with the findings of Hesnawi and Mogadimi 2013 and Ijah *et al.*, 2008. Detailed test results are given in Table A4.2 of the Appendix.

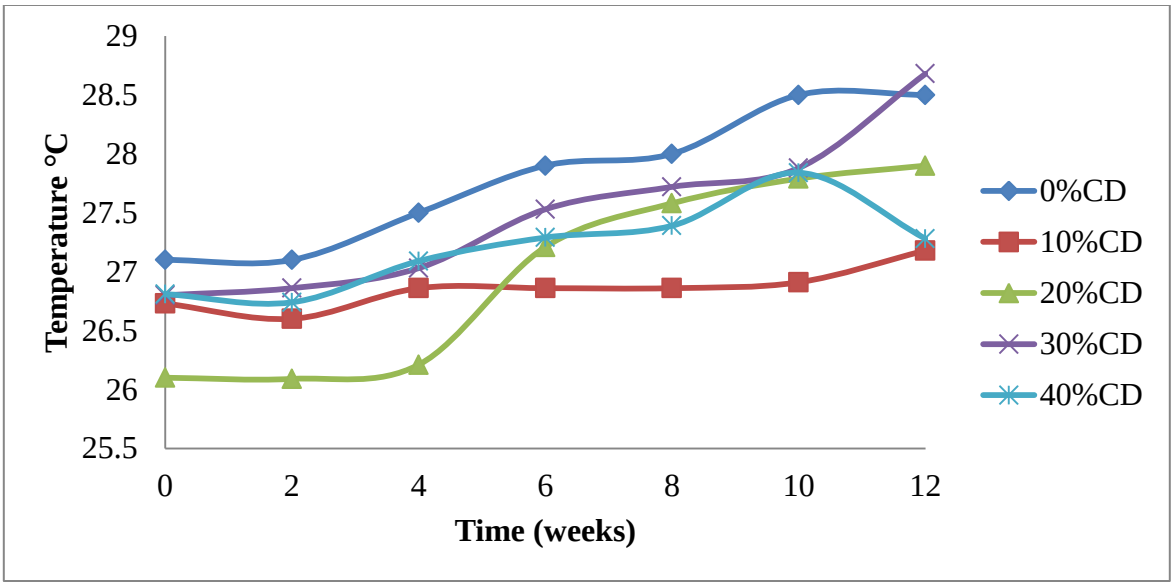


Fig. 4. 4: Variation of Temperature with time of bio-remediation for various CD content

Fig. 4.5 shows the variation of pH with time of bio-remediation for various CD contents. It was observed that the pH of samples increased from 6.9, 7.1, 7.2, 7.3, and 7.5 to 6.9, 7.50, 7.71, 7.93 and 7.80 when treated with 0, 10, 20, 30 and 40% CD content, respectively, from 2 through 12 weeks. This may be attributed to degradation of the crude oil by the microbial specie. Similar finding was made by Ijah *et al*. (2008)**.** Detailed test results are given in Table A4.3 of the Appendix

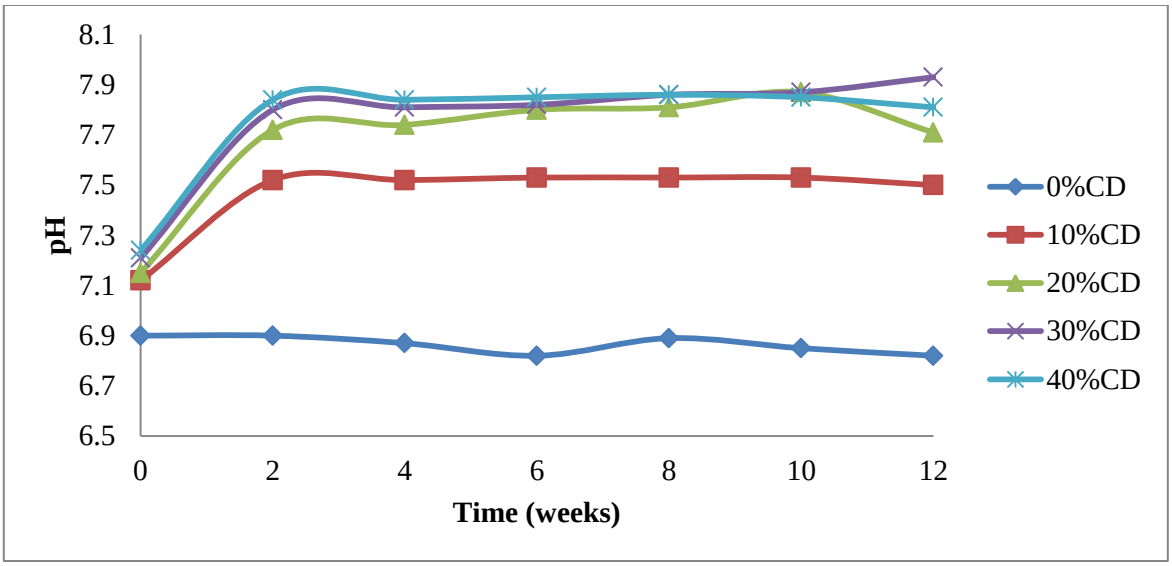


Fig. 4.5: Variation of pH with time of bioremediation for various CD content

The variation of microbial population with CD content is shown in Fig.4.6. It was observed that the microbial content increased to 4.97 x 10^7, 5.26x 10^8, 6.41x10^8, 7.24x10^8 and 6.67 x10^8 CFU/g when treated with 0, 10, 20, 30 and 40% CD contents, respectively. The probable reason for the optimum microbial count at 30% CD content may be attributed to the alkaline nature of the sample as well as the temperature which was more conducive for the bacterial species as reported by Wei *et al.*, 2012. The author stated that alkaline environment favours microbial activity. .Detailed test results are given in Table A4.4 of the Appendix.

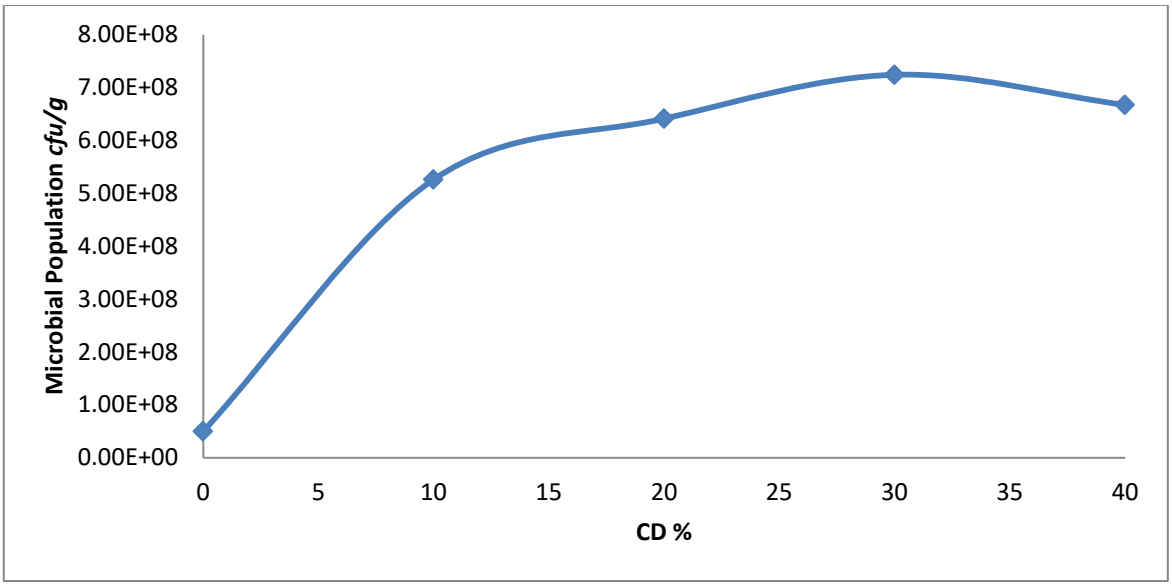


Fig.4 6: Variation of Microbial population with various CD content

4.4 Bio-cementation

Bio-cementation of the treated samples was achieved when cementation reagent was injected into the compacted soil- bacteria solution mixture using MICP techniques (Wei *et al*., 2012). The geotechnical properties of the bio-remediated soil such as Atterberg limits, particle size distribution, unconfined compressive strength, volumetric shrinkage and hydraulic conductivity were obtained when bio-cementation was carried out.

4.5 Atterberg limits

4.5.1 Liquid limits

Fig.4.7 shows the variation of liquid limit (LL) of the bio-remediated soil treated with cementation reagent at 1.5 x 10^8, 6.0 x 10^8, 1.2 x 10^9 , 1.8 x 10^9 , 2.4 x 10^9 cell/ml of *B. licheniformis*. The liquid limit of the sample treated with 1.5 x 10^8 cell/ml suspension density initially decreased from 4.22 to 4.18%. The probable reason for the initial decrease in liquid limit may be low microbial activity in the sample. However, with higher doses of the microbial flora, LL increased to a peak value of 47% when treated with 2.4 x 10^9

cell/ml suspension density. This may be as a result of the cell solution reacting with cementation reagent which brought about the production of calcite. Therefore, the calcite produced may coat the soil particle which may facilitate stronger surface charge that allowed more absorption of water leading to increase in the liquid limit. This result agrees with Habeb-ur-Rehman (2007) who reported that as the specific surface increases, more water molecule is attracted thus, liquid limit increases.

The one-way analysis of variance (ANOVA) test on the effect of *B. licheniformis* on liquid limits of the bio-remediated and bio-cemented crude oil contaminated soil is shown in Table 4.4. The results indicate that the effect of B. *licheniformis* on liquid limit for the bio-remediated and bio-cemented crude oil contaminated soil was not statistically significant as the F-calculated is less than the F-critical, that is F-cal=0.7162<Fcrit=3.1059. Detailed test results are given in Table A4.5 of the Appendix

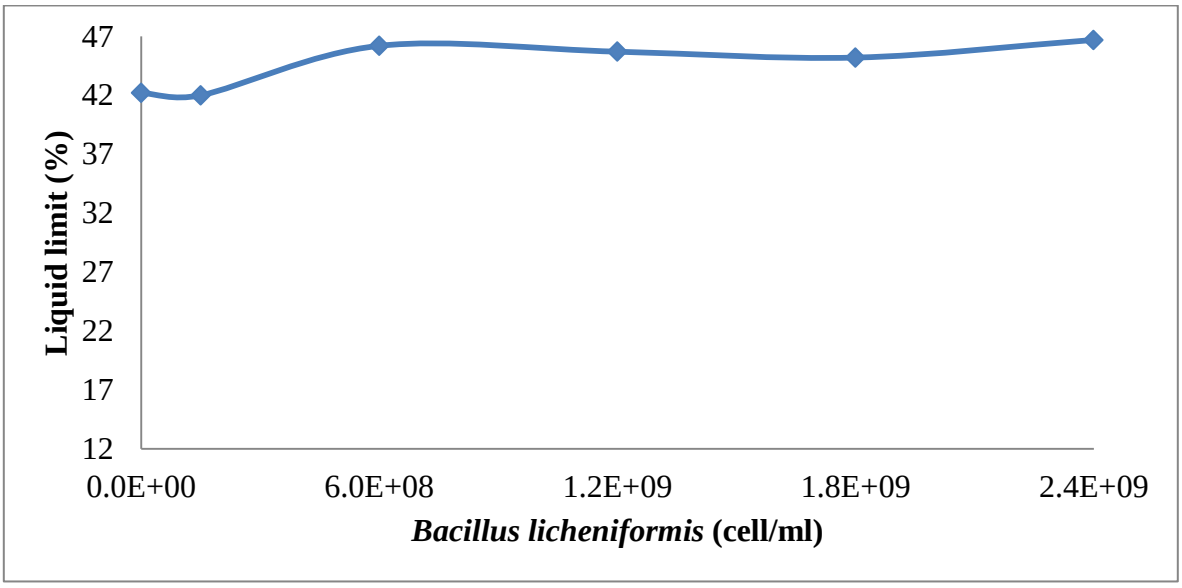


Fig. 4.7: Variation of liquid limit with *B. licheniformis* suspension

Table 4.4: One- way analysis of variance for Atterberg limits of the bio-remediated and bio-cemented soil

Property	Sources of Variation	Degree of Freedom	F_{CAL}	P-Value	F_{CRIT}	Remarks
Liquid limit	*B. licheniformis*	5	0.7162	0.6234	3.1059	Fcal<Fcrit Not statistically significant
Plastic limit	*B. licheniformis*	5	0.6049	0.6979	3.1059	Fcal<Fcrit Not statistically significant
Plasticity Index	*B. licheniformis*	5	0.6570	0.4828	3.1059	Fcal<Fcrit Not statistically significant

4.5.2 Plastic limit

The variation of plastic limit of the bio-remediated and bio-cemented crude oil contaminated soil is shown in Fig. 4.8. It was observed that the plastic limit initially decreased from 37.09% to 32.12% when treated with 1.5 x 10^8 cell/ml suspension density. However, with higher doses of *B. licheniformis*, plastic limit increased to a peak value of 38.69% when treated with 2.4 x 10^9 cell/ml suspension density. The probable reason for the observed decrease in plastic limit may be attributed to the presence of deposited calcites within the soil pores while the observed increase in plastic limit may be due to microbial urease hydrolysis of urea that formed dissolved ammonium, inorganic carbon and carbon dioxide which led to accumulation of calcium trioxocarbonate (IV). This is in agreement with the finding made of Wei *et al.*., (2012)

The one-way analysis of variance (ANOVA) test on the effect of *B. licheniformis* on plastic limits of the bio-remediated and bio-cemented crude oil contaminated soil is shown in Table 4.5. The results indicate that the effect of *B. licheniformis* on plastic limit for the bio-remediated and bio-cemented crude oil contaminated soil was not statistically significant as the F-calculated is less than the F-critical,(i.e. Fcal=0.6049 < Fcrit=3.1059). Detailed test results are given in Table A4.6 of the Appendix

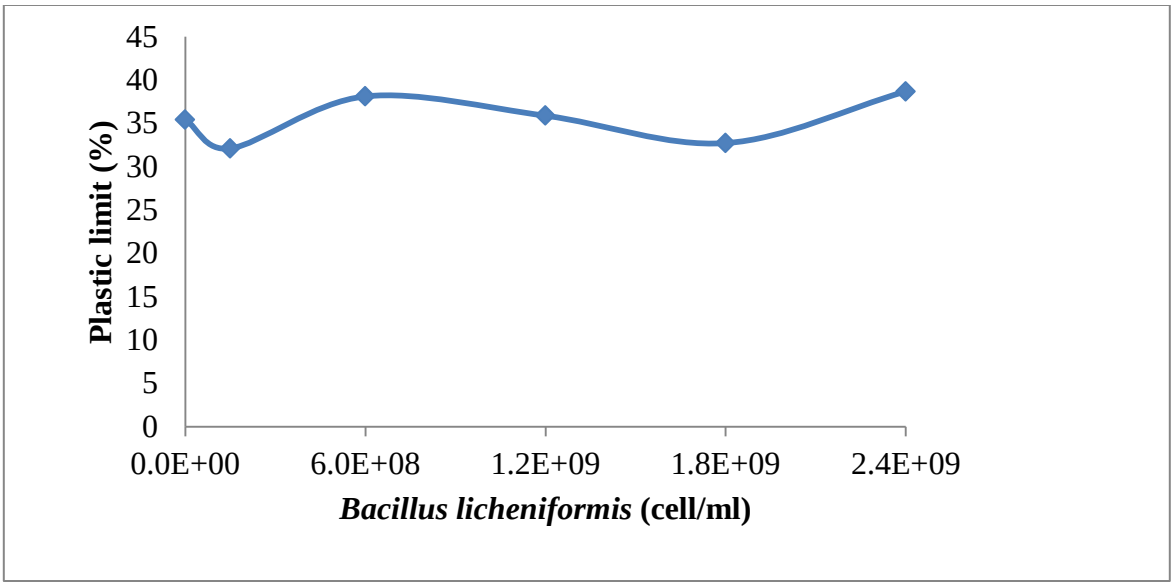


Fig. 4.8: Variation of plastic limit with *B. licheniformis* suspension

4.5.3 Plasticity index

The variation of plasticity index with *B. licheniformis* suspension is shown in Fig.4.9. Generally, it was observed that plasticity index increased from 6.76% for the crude oil contaminated soil (COCS) to a peak value of 10.87% at 1.8 x 10^9cell/ml of *B. licheniformis*. However, with further increase in the microbial population, the plasticity index decreased to a value of 7.8% at 2.4 x 10^9 cell/ml suspension densities. The probable reason for the increase in plasticity is the agglomeration of the soil particles by the calcites formed while the observed decrease in plasticity may be due to nutrient unavailability for the microbial flora (Yengejeh *et al*., 2014).

The one-way analysis of variance (ANOVA) test on the effect of *B. licheniformis* suspension on plasticity index of the bio-remediated and bio-cemented crude oil contaminated soil is shown in Table 4.4. The results indicate that the effect of *B. licheniformis* on plasticity index for the bio-remediated and bio-cemented crude oil contaminated soil was not statistically significant as the F-calculated is less than the F-

critical,(i.e. Fcal=0.6570<Fcrit=3.1059). Detailed test results are given in Table A4.7 of the Appendix

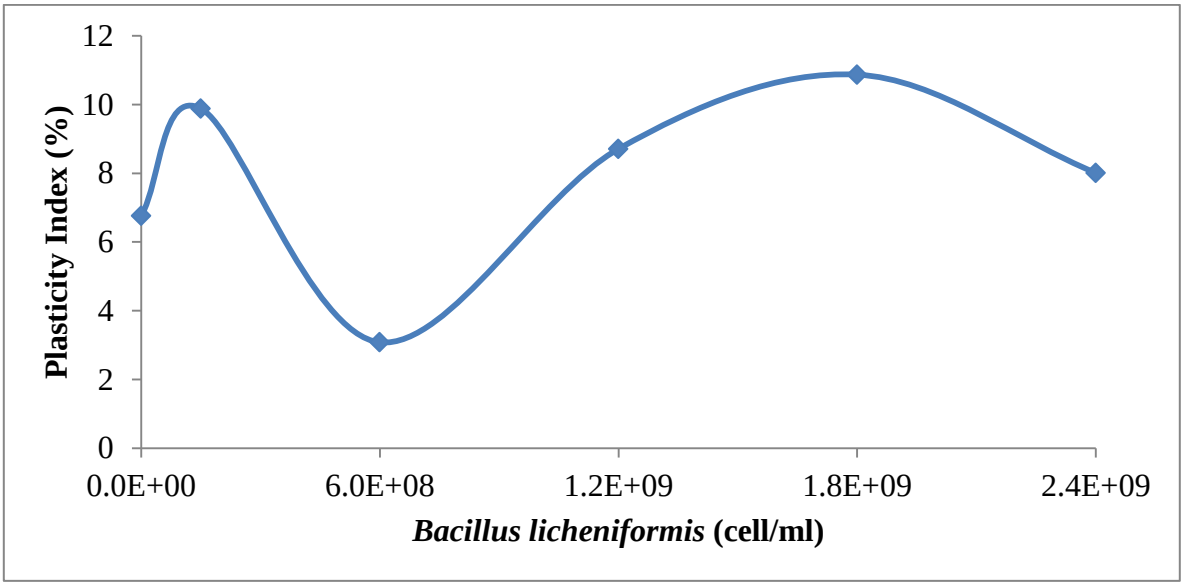


Fig. 4.9: Variation of plasticity index with *B. licheniformis* suspension density

4.6 Particle Size Distribution

The crude oil contaminated soil was observed to contain more aggregates, clods or lumps otherwise referred to as crumbs. The crumbs may have been due to the presence of crude oil in the soil that glued silt and clay sized particles together and agrees with the findings of Habib-ur-Rehman *et al*., (2007). However, when the indigenous bacteria were activated with chicken droppings as a source of nutrients only, it was observed that the crude oil contaminant was removed (bio-remediated) as discussed earlier. Fig. 4.10 shows the particle size distribution of the bio-remediated soil treated with varying *B. licheniformis* and cementation reagent. It was observed that the percentage passing BS No 200 sieve of the bio-remediated and bio-cemented soil decreased with increase in *B. licheniformis* suspension densities from 46.5% for the bio-remediated soil to 34.6, 37.1, 38.5, 40.5 and 44.5%, when treated with 1.5 x 10^8, 6.0 x 10^8, 12.0 x 10^8, 18.0 x $10^8$and 24.0 x 10^8 cell/ml

of *B. licheniformis* suspension densities, respectively. The decrease in percentage passing BS No 200 sieve of the bio-cemented soil may be due to the deposition of calcites by the microbial flora which bound the silt and clay-sized particles. This result agrees with the findings of Ijimdiya and Musa (2017), who reported on the reduction in the proportion of silt or fine fraction probably due to the bonding of both the silt sizes to form pseudo- sand sizes and of the sand sizes to form larger sand or clog sizes. Detailed test results are given in Table A4.8 of the Appendix.

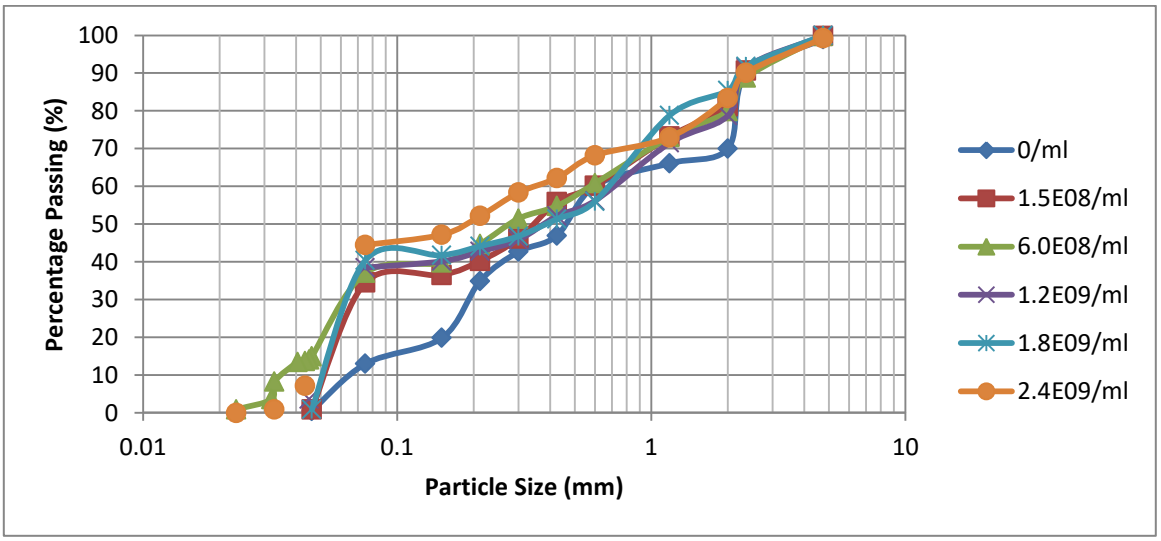


Fig. 4.10: Particle size distribution curve of the bio-remediated and bio-cemented soil using *B. licheniformis* suspension

4.7 Specific Gravity

Fig. 4.11 shows the variation of specific gravity with *B. licheniformis* suspension densities and cementation reagent. The specific gravity of bio-remediated COCS increased from 2.13 (no treatment) to 2.6, 2.23, 2.15, 2.17 and 2.17 when treated with 1.5 x 10^8, 6.0 x 10^8, 12.0 x 10^8, 18.0 x $10^8$and 24.0 x 10^8 cell/ml. of *B. licheniformis* suspension densities and cementation reagent, respectively. The sharp increase in specific gravity observed when

treated with 1.5 x 10^8 cell/ml. of *B. licheniformis* suspension density may be attributed to be due to the calcium bicarbonate (calcite) deposition that made the soil become denser as evidently observed in the results of particle size analysis. The amounts of calcites formed decreased with higher bacterial population and cementation reagent. Detailed test results are given in Table A4.9 of the Appendix.

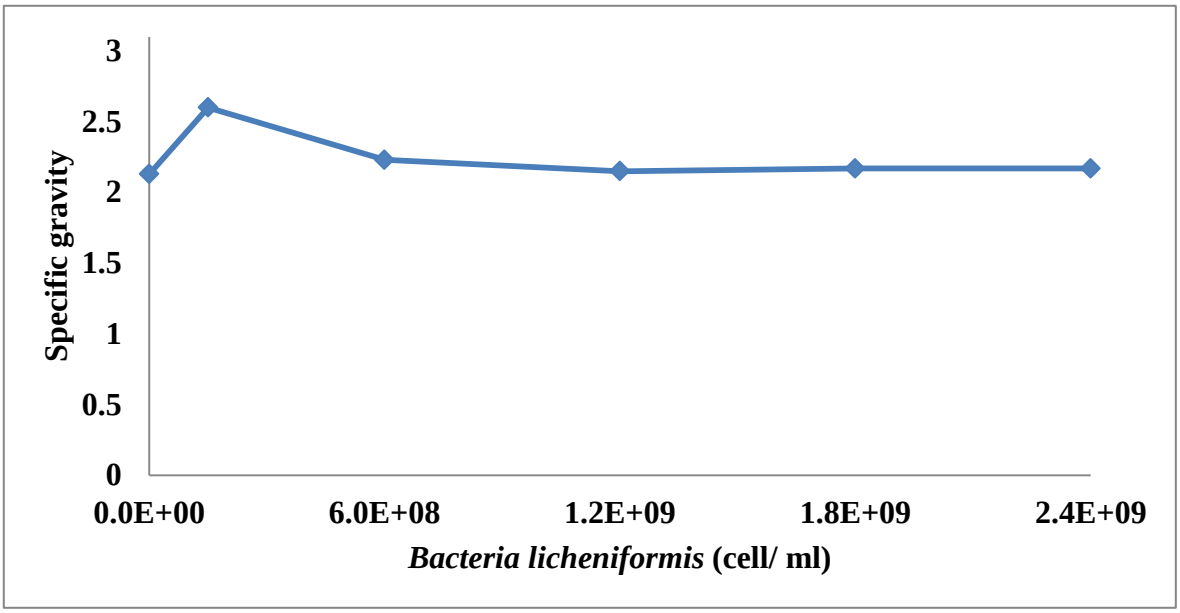


Figure 4.11: Variation of specific gravity with *Bacillus licheniformis* suspension

4.8 Compaction Characteristics

4.8.1 Maximum dry density

Fig.4.12 shows the variation of MDD of the bio-remediated and bio-cemented soil at various compactive efforts namely BSL, WAS and BSH. Generally, MDD decreased with increase in *B. licheniformis* suspension density as shown in Fig. 4.12. There was increase in MDD of the bio-remediated and bio-cemented soil from 1.67, 1.72, and 1.83 to 1.76, 1.81 and 1.93 Mg/m^3 when compacted using BSL, WAS, and BSH compactive efforts, respectively. However, it was observed that for samples bio-remediated and bio-cemented using varying microbial flora, MDD values decreased with higher doses of *B. licheniformis*

suspension densities. The MDD of the bio-remediated and bio-cementated soil increased from 1.67, 1.73 and 1.83 Mg/m^3 for the untreated soil to peak values of 1.76, 1.81 and 1.93 Mg/m^3 , respectively when treated with $1.5x10^8$ cell/ml of *B. licheniformis* suspension density. However, with higher doses of *B. licheniformis,* the MDD values decreased continuously for all the treatments considered. The peak values of MDD obtained when treated with $1.5x10^8$ cell/ml of B. *licheniformis* suspension density may be attributed to the amounts of calcites formed by the bacterial species that bridged the particle to particle structure to form coarser particles in the bio-cemented soil, hence making the soil denser (Mujah *et al*., 2016).

The two-way analysis of variance (ANOVA) test on the effect of B. *licheniformis* and compactive efforts maximum dry density of the bio-remediated and bio-cemented crude oil contaminated soil is shown in Table 4.5. The results show that the effect of B. *licheniformis* on maximum dry density for the bio-remediated and bio-cemented crude oil contaminated soil were statistically significant as the F-calculated is greater than the F-critical (i.e. F-cal=21.189>3.3259). Similarly, the effect of compactive efforts on the maximum dry density was statistically significant as the F-calculated is greater than F-critical (i.e. F-cal=315.401>4.1028). A detailed test result is given in Table A4.10a and A4.10b.

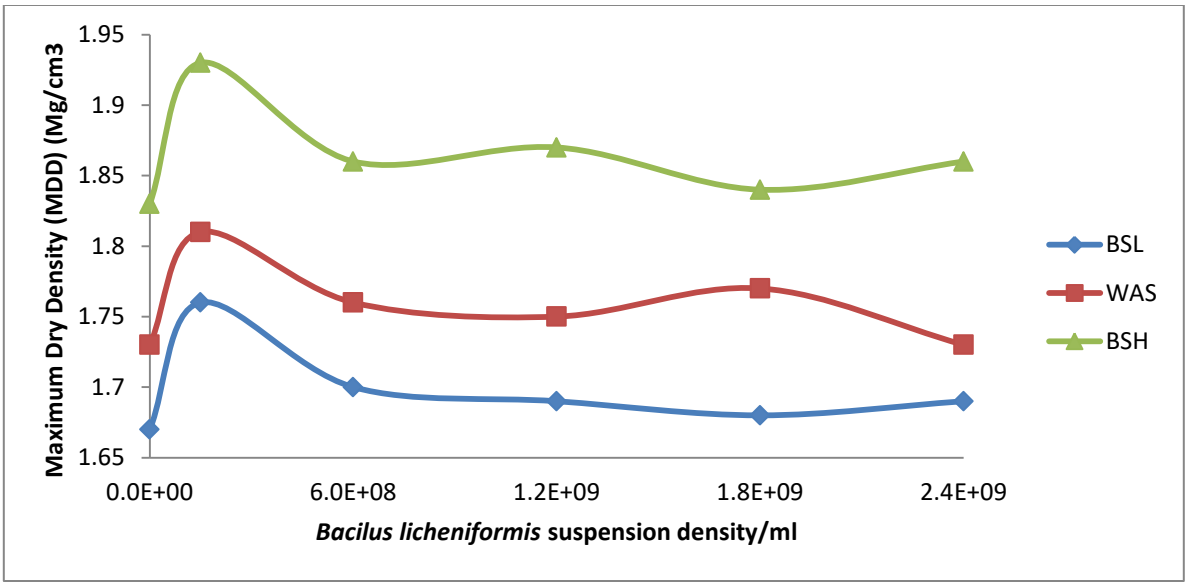


Fig. 4.12: Variation of MDD with *Bacillus licheniformis* suspension

4.8.2 Optimum moisture content

Fig. 4.13 shows the variation of OMC of the bio-cemented soil with increasing *B. licheniformis* suspension densities. The OMC of the bio-remediated soil increased from 14.3, 13.3 and 12.8 % with no treatment, to peak values of 15.8, 14.1 and 13.7 %, respectively when treated with 24.0 x 10^8 cell/ml. of *B. licheniformis* suspension density and compacted using the three compactive efforts of BSL, WAS and BSH compactive efforts, respectively. The probable reason for the increased in OMC may be due to higher amounts of water required by the microbial activity.

The two-way analysis of variance (ANOVA) test on the effect of *B. licheniformis* and compactive efforts on optimum moisture content of the bio-remediated and bio-cemented crude oil contaminated soil is shown in Table 4.5. The results show that the effect of *B. licheniformis* on optimum moisture content for the bio-remediated and bio-cemented crude oil contaminated soil were statistically significant as the F-calculated is greater than the F-critical (i.e. F-cal=10.6303>3.3259). Similarly, the effect of compactive efforts on the

optimum moisture content was statistically significant as the F-calculated is greater than F-critical (i.e. F-cal=101.5126>4.1028). Detailed test results are given in Table A4.10a and A4.10b.

Table 4.5: Two- way analysis of variance for compaction characteristics of the bio-remediated and bio-cemented soil

Property	Sources of Variation	Degree of Freedom	F_{CAL}	P-Value	F_{CRIT}	Remarks
Maximum Dry Density	*B. licheniformis*	5	21.1885	5.0E-05	3.3259	Fcal>Fcrit Statistically significant
	Compactive efforts	2	315.4098	1.0E-05	4.1028	Fcal>crit Statistically significant
Optimum Moisture Content	*B. licheniformi*	5	10.6303	9.5E-04	3.3259	Fcal>Fcrit Statistically significant
	Compactive efforts	2	101.5126	1.0E-05	4.1028	Fcal>Fcrit Statistically significant

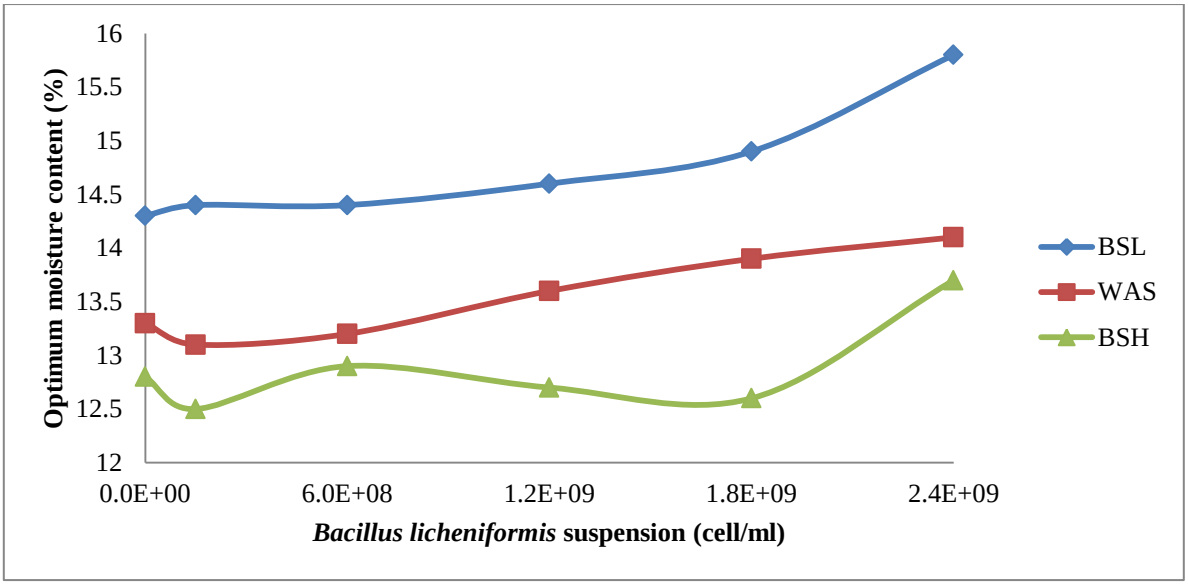


Fig. 4.13: Variation of OMC with *Bacillus licheniformis* suspension

4.9 Physical and chemical composition of the leachate used

Table 4.6 shows the physical and chemical composition of the leachate used in compatibility test. The values for biochemical oxygen demand (BOD) and chemical oxygen demand (COD) were 441.67 and 662.5mg/l, respectively. It was observed that the concentration of BOD and COD of the leachate was high as the values obtained were more than the minimum limits of 7 and 42mg/l, respectively. Similarly, the pH value obtained was 5.2 thus, the leachate was acidic. The probable reason for the high concentration of the leachate may be attributed to exposure of the open by dumped refuse. This is in agreement with findings by Amadi, 2006; Eberemu, 2008 and Ijimdiya, 2010

Table 4.6 Physical and Chemical characteristics of Municipal Solid Waste (MSW) leachate used

Parameter		Quantity
Biochemical Oxygen Demand, BOD	(mg/l)	441.67
Chemical Oxygen Demand, COD	(mg/l)	662.5
Total suspended Material	(mg/l	2000
Hardness	(mg/l)	1056.0
Chloride	(mg/l)	582.2
pH		5.6
Turbidity	NTU	28.05
Sulphate	(mg/l)	410.23
Potassium	(mg/l)	110
Calcium	(mg/l)	275
Sodium	(mg/l)	235
Magnesium	(mg/l)	524

4.10 Hydraulic Conductivity

Fig 4.14 shows the variation of hydraulic conductivity of the bio-remediated and bio-cemented soil at various compactive efforts namely BSL, WAS and BSH. The hydraulic conductivity decreased with increased in *B. licheniformis* suspension density as shown in Fig.4.14. There was increase in hydraulic conductivity of the bio-remediated and bio-cemented soil from 3.62 $x10^{-10}$, 1.8 $x10^{-10}$, 9.92 $x10^{-11}$ m/s for the untreated to 6.88 x 10^{-10}, 1.11 x 10^{-9} , and 1.50 x 10^{-9} m/s, 1.5 $x10^{8}$, 1.2 $x10^{9}$ and 2.4 $x10^{9}$ cell/ml of *B. licheniformis* suspension density compacted at OMC using BSL, WAS and BSH energy respectively. The reduced hydraulic conductivity values obtained when treated with higher doses of *B. licheniformis* may be attributed to bio- clogging of the pore spaces as a result of increased microbial population in the treated sample. This finding is similar to that of Wei *et al*., (2012) who explained that *Bacilli species* has potential utilization in MICP process and Mujah *et al.* (2016) who reported the reduction in hydraulic conductivity values of bio-cemented sand from $5x10^{-5}$.to 1.4x 10^{-9} m/s.

The two-way analysis of variance (ANOVA) test on the effect of *B. licheniformis* on hydraulic conductivity of the bio-remediated and bio-cemented crude oil contaminated soil is shown in Table 4.7. Indication by the results show that the effect of *B. licheniformis* on hydraulic conductivity for the bio-remediated and bio-cemented crude oil contaminated soil was not statistically significant because the calculated F-value is less than the F-critical that is F-cal=2.1378<Fcrit=3.3259. Test results are given in Table A4.11 of the Appendix.

Table 4.7: Two- way analysis of variance for hydraulic conductivity of the bio-remediated and bio-cemented soil

Property	Sources of Variation	Degree of Freedom	F_{CAL}	P-Value	F_{CRIT}	Remarks

	B. licheniformis	5	2.13784	0.1435	3.3259	Fcal<Fcrit
Hydraulic Conductivity						Not statistically significant
	Compactive efforts	2	0.9931	0.4042	4.1028	Fcal<Fcrit
						Not statistically significant

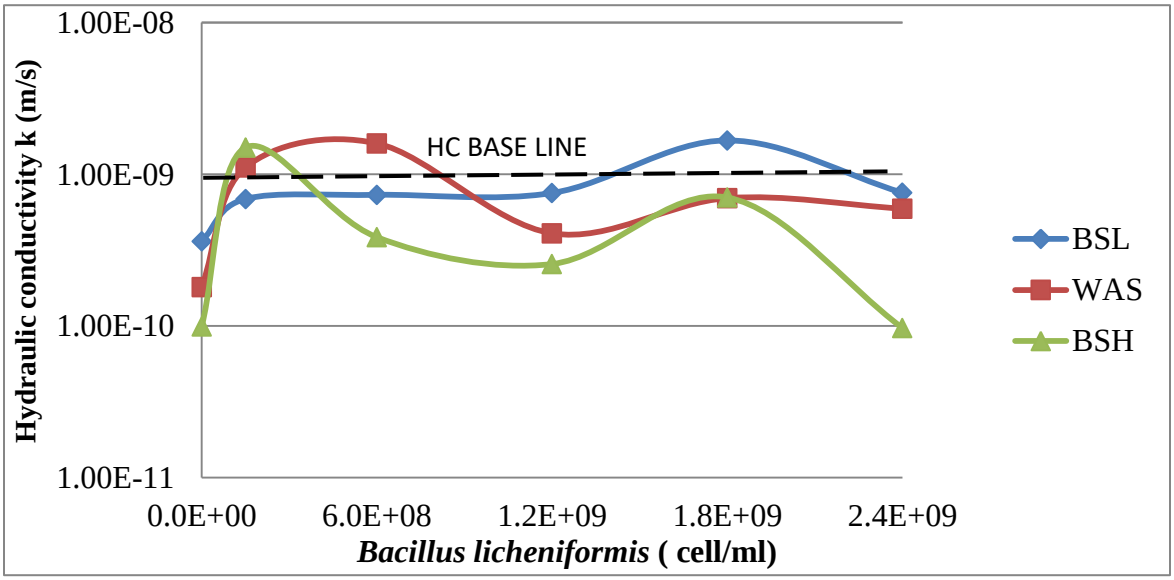


Fig. 4.14: Variation of hydraulic conductivity with *Bacillus licheniformis* suspension

The effect of compactive effort on the hydraulic conductivity of bio-remediated crude oil contaminated soil

The variation of hydraulic conductivity of the bio-remediated and bio-cemented soil with varying compactive efforts when compacted at OMC is shown in Fig.4.15. The hydraulic conductivity values of samples containing 1.2 x 10^{9} cell/ml of *B. licheniformis* suspension and compacted using BSL, WAS and BSH were 7.53 x 10^{-10}, 4.08 x 10^{-10} and 2.25 x 10^{-10} m/s when compacted using BSL, WAS and BSH compactive efforts, respectively. It was observed that hydraulic conductivity decreased with higher compaction energies. Similar results were obtained at higher doses of the microbial species. The decrease in hydraulic conductivity values with increasing compaction energy and *B. licheniformis* suspension densities may be attributed to the calcite produced by the microbes, which filled in the pore

spaces as well as the mechanical effect of compaction on the surface of the soil that made particles of the soil become denser and hence affecting the arrangement of individual particles. The consequence of this is reduction in the values of hydraulic conductivity (Ola,1980; Acar and Oliveri, 1989). Similar explanation was offered by (Lambe, 1958, Olsen, 1962; Mitchell *et al*.,1965; Garcia- Bengochea *et al.*, 1979; Benson and Daniel 1990), who observed that the soil particles were randomly flocculated into aggregates or clusters that formed prior to compaction. The resistance to deformation of such clusters was expected to decrease with increasing moisture content and consequently decreased the hydraulic conductivity.

The two-way analysis of variance (ANOVA) test on the effects of compactive efforts on hydraulic conductivity of the bio-remediated and bio-cemented crude oil contaminated soil is shown in Table 4.7. The results show that the effect of compactive efforts on the hydraulic conductivity of the bio-remediated and bio-cemented crude oil contaminated soil was not statistically significant as the calculated F-value is less than the F-critical (i.e. Fcal=0.9931 < Fcrit=3.3258). Test results are given in Table A4.11 of the Appendix.

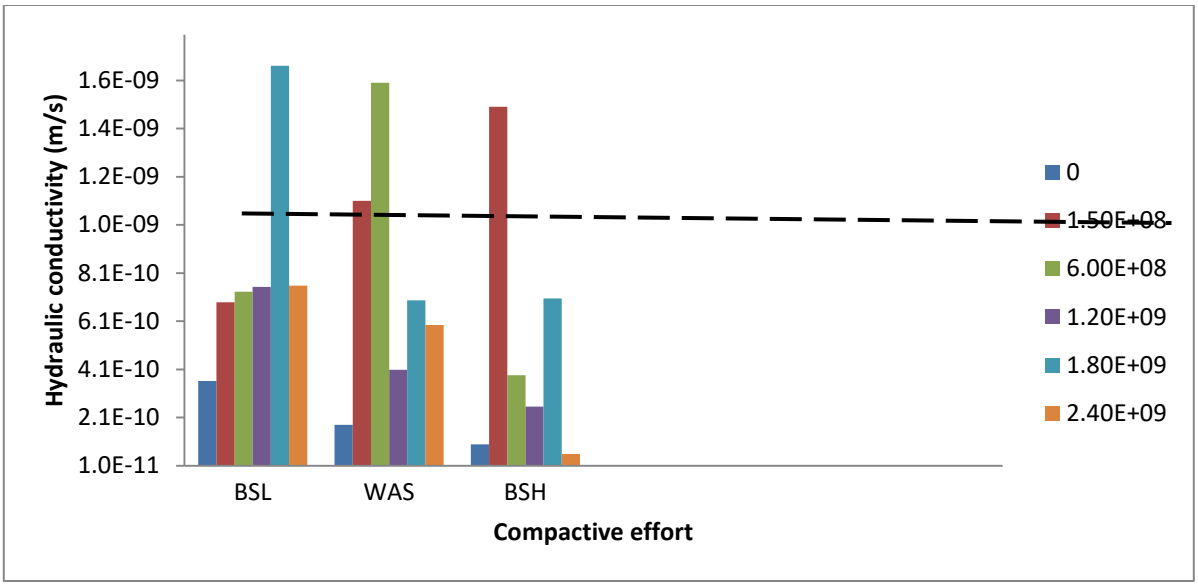


Fig.4.15: Variation of hydraulic conductivity of bio-remediated crude oil contaminated soil - *B. licheniformis* mixtures with compactive efforts

4.10.1 Void ratio

The variation of void ratio of the bio-remediated crude oil contaminated soil using *B. licheniformis* suspension density with varying compactive efforts at optimum moisture content (OMC) is shown in Fig. 4.16. The void ratio of the bio-remediated soil increased from 0.62, 0.58 and 0.56 for the untreated soil to 0.92, 0.88 and 0.71 when treated with 1.5 x 10^8 cell/ml of *B. licheniformis* suspension densities and compacted using BSL, WAS and BSH compactive efforts, respectively. However, it was observed that for samples bio-remediated and bio-cemented using varying microbial flora, void ratio values decreased with higher doses of *B. licheniformis* suspension densities The observed low values of void ratio for untreated soil may be to the occupation of voids by crude oil (Ijimdiya, 2010).

The two-way analysis of variance (ANOVA) test on the effect of *B. licheniformis* suspension and compactive efforts on void ratio of the bio-remediated and bio-cemented crude oil contaminated soil is shown in Table 4.8. The results show that the effect of *B.*

licheniformis on void ratio for the bio-remediated and bio-cemented crude oil contaminated soil was statistically significant as the F-calculated is greater than the F-critical (i.e. F-cal=15.5141>3.3259). Similarly, the effect of compactive efforts on the void ratio was statistically significant as the F-calculated is greater than F-critical (i.e. F-cal=40.3579>4.1028). Test results are given in Table A4.13 of the Appendix.

Table 4.8: Two- way analysis of variance for void ratio of the bio-remediated and bio-cemented soil

Property	Sources of Variation	Degree of Freedom	F_{CAL}	P-Value	F_{CRIT}	Remarks
Void ratio	*B. licheniformis*	5	15.5141	1.96E-04	3.3259	Fcal>Fcrit Statistically significant
	Compactive efforts	2	40.3579	1.6E-05	4.1028	Fcal>Fcrit Statistically significant

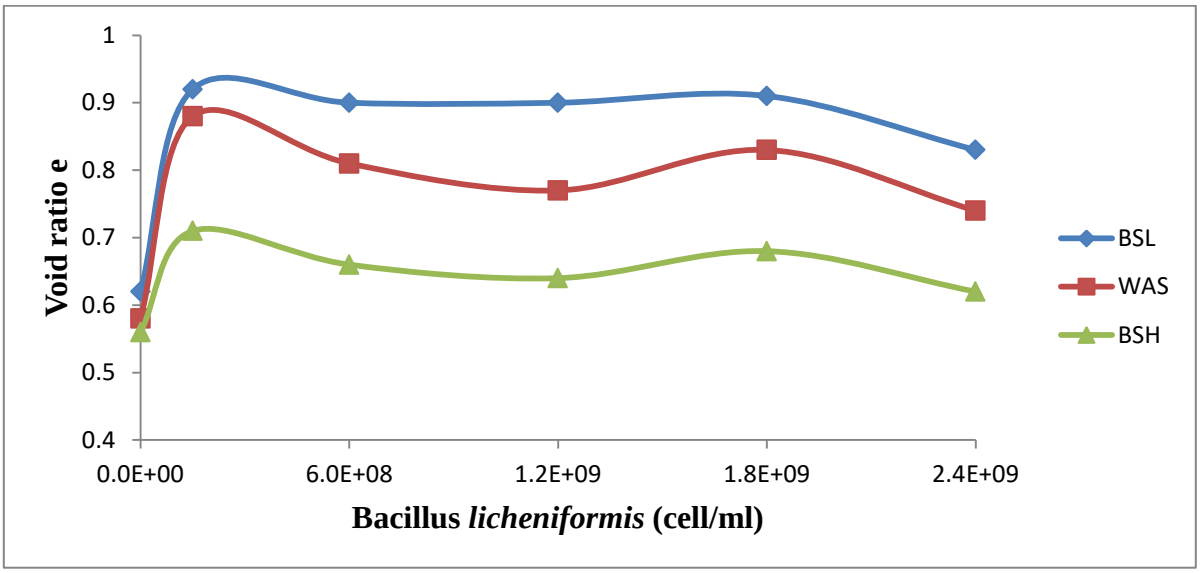


Fig. 4.16: Variation of void ratio *e* with *Bacillus licheniformis* suspension

4.10.2 Porosity

The variation of porosity of the bio-remediated and bio-cemented soil using *B. licheniformis* at varying compactive efforts when compacted at OMC is shown in Fig.

4.17. The porosity values of the untreated or crude oil contaminated soil were 38.22, 33.01 and 35.9% respectively, when compacted using BSL, WAS and BSH compactive efforts.

However, for samples containing 1.5 x10^8 cell/ml of *B. licheniformis* suspension, the porosity values were 47.22, 41. 72 and 41.37% respectively, when compacted using BSL, WAS and BSH compactive efforts. Similar trends were obtained for samples treated with higher doses of the microbial species. The probable reason for the decrease in porosity with increasing microbial content may be attributed to the presence of calcite formed by the bacteria which occupied the void spaces within the soil matrix as earlier observed. The finding is in agreement with that of Mujah *et al*., (2016) who reported a reduction of 25% of porosity of the biocemented sand.

The two-way analysis of variance (ANOVA) test on the effect of *B. licheniformis* suspension and compactive efforts on porosity of the bio-remediated and bio-cemented crude oil contaminated soil is shown in Table 4.9. The outcome reveal that the effect of *B. licheniformis* on porosity for the bio-remediated and bio-cemented crude oil contaminated soil was statistically significant as the F-calculated is greater than the F-critical (i.e. F-cal=10.9964>3.3259). Similarly, the effect of compactive efforts on the porosity was statistically significant as the F-calculated is greater than F-critical (i.e. F-cal=10.9576>4.1028). Test results are given in Table A4.14 of the Appendix

Table4.9: Two- way analysis of variance for porosity of the bio-remediated and bio-cemented soil

Property	Sources of Variation	Degree of Freedom	F_{CAL}	P-Value	F_{CRIT}	Remarks
	B. licheniformis	5	10.9964	8.24E-03	3.3259	Fcal>Fcrit Statistically significant
Porosity	Compactive	2	10.9567	3.02E-03	4.1028	Fcal>Fcrit

efforts	Statistically significant

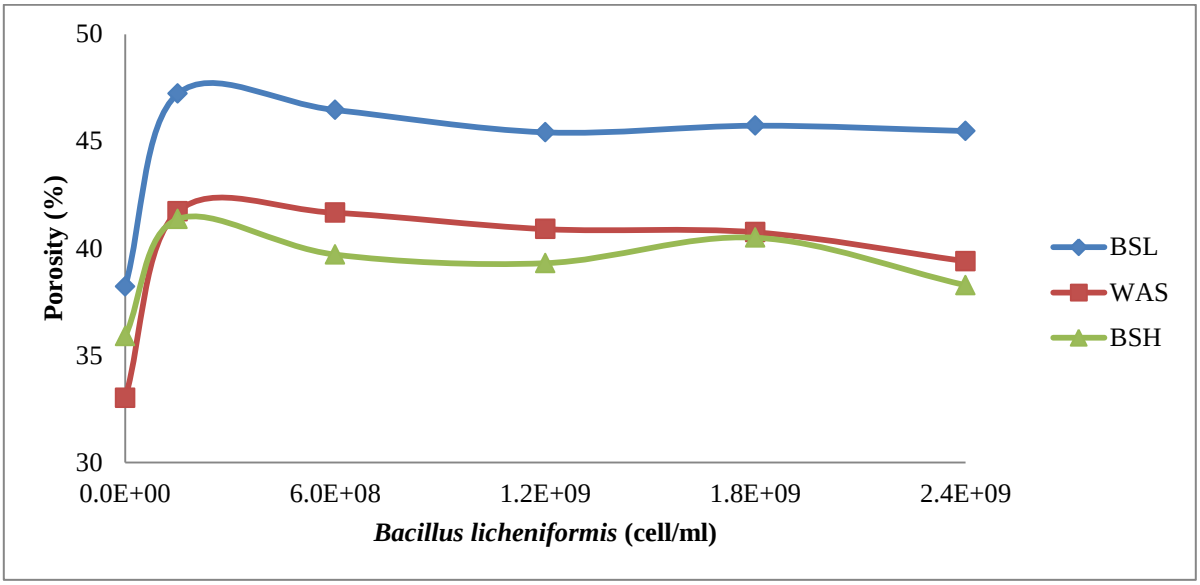


Fig. 4.17: Variation of porosity with *Bacillus licheniformis* suspension

4.10.3 Degree of Saturation

Fig.4.18 shows the variation of degree of saturation of the bio-remediated and bio-cemented soil at varying compactive efforts namely BSL, WAS and BSH energy level. Generally, degree of saturation values of the bio-remediated and bio-cemented soil decreased with increase in *B. licheniformis* suspension density as shown in Fig. 4.18. There was decrease in degree of saturation of the bio-remediated and bio-cemented soil from 84.05, 89.28 and 94.11 % for the untreated soil, to 78.59, 69. 94 and 79.05% when compacted using BSL WAS and BSH compactive efforts, respectively. However, it was observed that for samples bio-remediated and bio-cemented using varying microbial flora, the degree of saturation values decreased with higher doses of *B. licheniformis* suspension densities. The probable reason for the decreased in the degree of saturation with higher *B. licheniformis* suspension may be the presence of to the voids produced by the microbial flora thus, facilitating the absorption of water molecules (Ijimdiya and Musa, 2017).

The two-way analysis of variance (ANOVA) test on the effect of *B. licheniformis* suspension and compactive efforts on degree of saturation of the bio-remediated and bio-cemented crude oil contaminated soil is shown in Table 4.10. The results show that the effect of *B. licheniformis* on degree of saturation for the bio-remediated and bio-cemented crude oil contaminated soil was not statistically significant as the F-calculated is less than the F-critical (i.e. F-cal 0.3148=<3.3259). Similarly, the effect of compactive efforts on the degree of saturation was not statistically significant as the F-calculated is less than F-critical (i.e. F-cal=0.4168<4.1028). Detailed test results are given in Table A4.15 of the Appendix.

Table 4.10: Two- way analysis of variance for degree of saturation of the bio-remediated and bio-cemented soil

Property	**Sources of Variation**	**Degree of Freedom**	**F_{CAL}**	**P-Value**	**F_{CRIT}**	**Remarks**
Degree of Saturation	*B.* licheniformis	5	0.3148	0.8930	3.3259	Fcal<Fcrit Not statistically significant
	Compactive efforts	2	0.4168	0.6701	4.1028	Fcal<Fcrit Not statistically significant

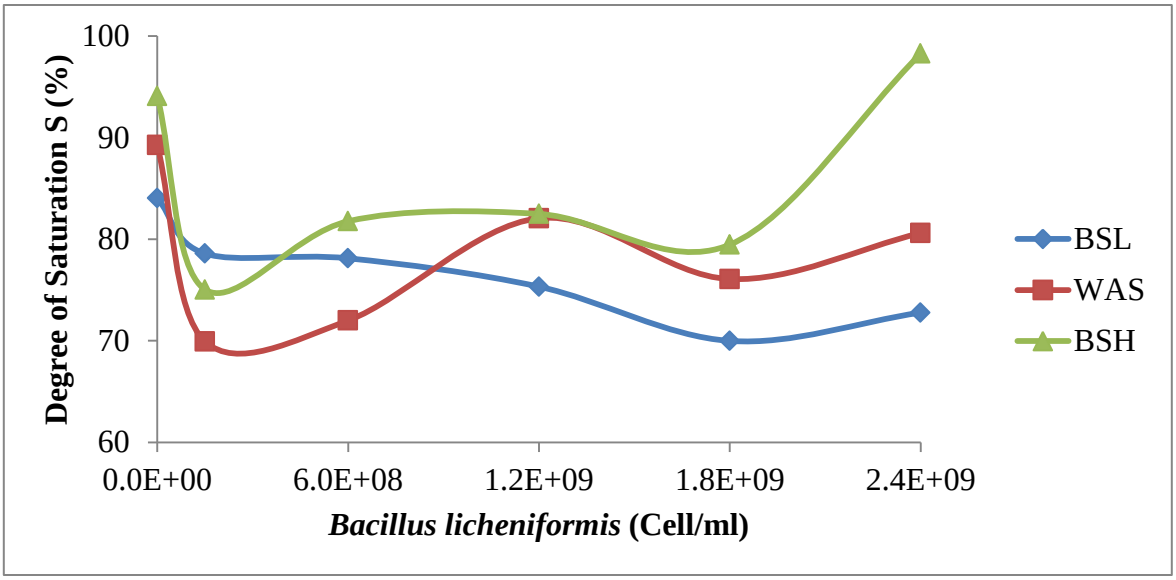


Fig. 4.18: Variation of Degree of Saturation with *Bacillus licheniformis* suspension

4.11 Long term Hydraulic Conductivity

Compatibility test was performed on specimens prepared at OMC moulding water content with *B. licheniformis* suspension of 0 cell/ml, 1.5 x 10^8 cell/ml, 1.2 x 10^9 cell/ml and 2.4 x 10^9 cell/ml compacted at BSL, WAS and BSH energy, respectively. Specimens were permeated with tap water for 21 days in order to achieve full saturation so that the effect of first time exposure could be examined (Quaranta *et al.*, 1997; Eberemu, 2008, Ijimdiya, 2010 and Ochepo, 2017). After 21 days, specimens were permeated with leachate for the 69days. Fig. 4.19 shows the variation of hydraulic conductivity of bio-remediated and bio-cemented crude oil contaminated soil permeated with tap water and leachate. It was observed that the hydraulic conductivity increased for specimen treated with 1.5 x 10^8 cell/ml up to 21 day, it then decreased continuously up to 69 days. However, for specimen treated with 1.2 x 10^9 cell/ml and 2.4 x 10^9 cell/ml there was decreased in hydraulic conductivity throughout the 90days. The changes in hydraulic conductivity may be attributed to the formation of mineral clogs that is bio-clogging. This is in agreement with findings by Shackelford, (1994). Detailed test results are given in Table A4.12.

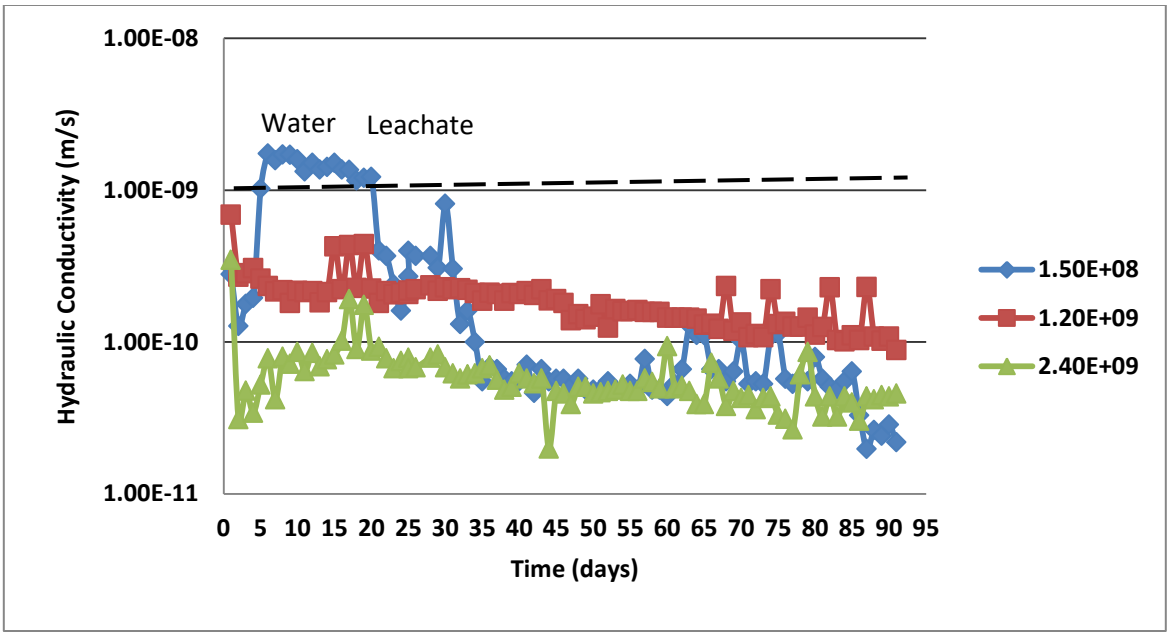


Fig.4.19: Variation of hydraulic conductivity of bioremediated and biocemented soil permeated with tap water and leachate

4.12 Unconfined Compressive Strength

Fig. 4.20 shows the variation of unconfined compressive strength (UCS) of the bio-remediated and bio-cemented soil at varying compactive efforts namely BSL, WAS and BSH and at their respective optimum moisture contents (OMCs). Generally, UCS increased with increase in *B. licheniformis* suspension density as shown in Fig. 4.20. The UCS values of the bio-remediated and bio-cemented soil increased from 119.56, 202.82 and 253.15 kN/m^2 for the untreated soil to peak values 330.64, 415.57 and 460.78 kN/ m^2 when compacted using BSL, WAS and BSH compactive efforts, respectively, when treated with 1.5 x10^8 cell/ml of *B. licheniformis* suspension density. The increased UCS observed when treated with 1.5 x10^8 cell/ml of *B. licheniformis* suspension density may be due to the amounts of calcites deposited as a result of the microbial activity. However, the decreasing UCS with increasing *B. licheniformis* suspension may be attributed to the unavailability of nutrients with increased bacteria population as explained by Dejong *et*

al.,(2010). They reported that availability of nutrients at the grain contact is one of the reasons that attract microbes to the particle to particle contact which eventually leads to the production of $CaCO_3$.

The two-way analysis of variance (ANOVA) test on the effect of *B. licheniformis* on unconfined compressive strength of the bio-remediated and bio-cemented crude oil contaminated soil is shown in Table 4.11. The results reveal that the effect of B. *licheniformis* on unconfined compressive strength for the bio-remediated and bio-cemented crude oil contaminated soil was statistically significant as the calculated F-value is greater than the F-critical, that is F-cal=199.72>Fcrit=3.3259. Detailed test results are given in Table A4.16 of the Appendix.

Table 4.11: Two- way analysis of variance for unconfined compressive strength of the bio-remediated and bio-cemented soil

Property	**Sources of Variation**	**Degree of Freedom**	**F_{CAL}**	**P-Value**	**F_{CRIT}**	**Remarks**
Unconfined Compressive Strength	*B. licheniformis*	5	199.72	1.0E-05	3.3259	Fcal>Fcrit Statistically significant
	Compactive efforts	2	320.07	1.E-06	4.1028	Fcal>Fcrit Statistically significant

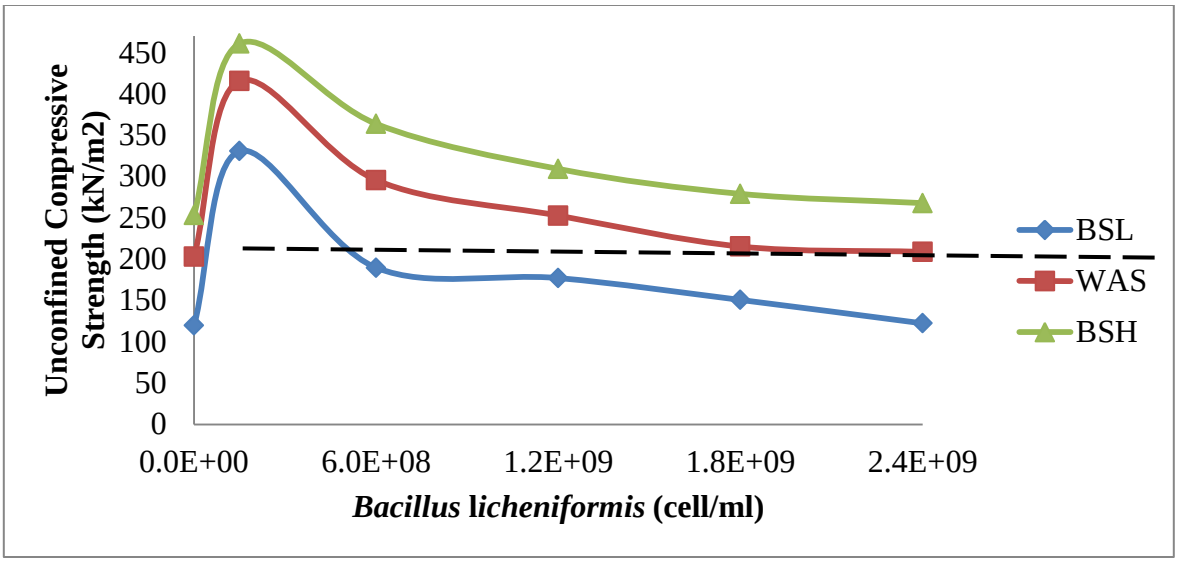


Fig.4.20: Variation of UCS with *Bacillus licheniformis* suspension

The effect of compactive effort on unconfined compressive strength (UCS) of treated crude oil contaminated soil

The variation of UCS of bio-remediated and bio-cemented soil using *B. licheniformis* suspension mixtures with varying compactive effort at OMC is shown in Fig.4.21. The UCS of the untreated samples were 119.56, 202.82 and 253.15kN/m^2 when compacted using BSL, WAS and BSH compactive efforts, respectively. It was observed that only samples compacted using WAS and BSH compaction energy satisfied the minimum required compressive strength of 200kN/m^2. The UCS values of samples containing 1.5 x10^8 cell/ml of *B. licheniformis* suspension were 330.64, 415.57 and 460.78kN/m^2 when compacted using BSL, WAS and BSH compactive efforts, respectively. It was observed that there were increased UCS values with increased compactive efforts. However, lower UCS values were obtained with higher doses of the bacterial population. The probable reason for the increased UCS values with higher compactive efforts may be attributed to the compaction energy provided which led to a more compact and dense structure with higher density. This findings conform with that of Cheng, and Cord-Ruwisch (2013) and

Rowshanbakhta *et al.* (2016). The decreased UCS values with higher doses of the microbial flora may be due to the decreased nutrients available which agrees with the findings of Dejong *et al.*,(2010).

The two-way analysis of variance (ANOVA) test on the effect of compactive efforts on unconfined compressive strength of the bio-remediated and bio-cemented crude oil contaminated soil is shown in Table4.11. The results show that the effect of compactive efforts on the unconfined compressive strength of the bio-remediated and bio-cemented crude oil contaminated soil was statistically significant as the calculated F-value is greater than the Fcal=320.07 > Fcrit=4.1028). Detailed test results are given in Table A4.16.

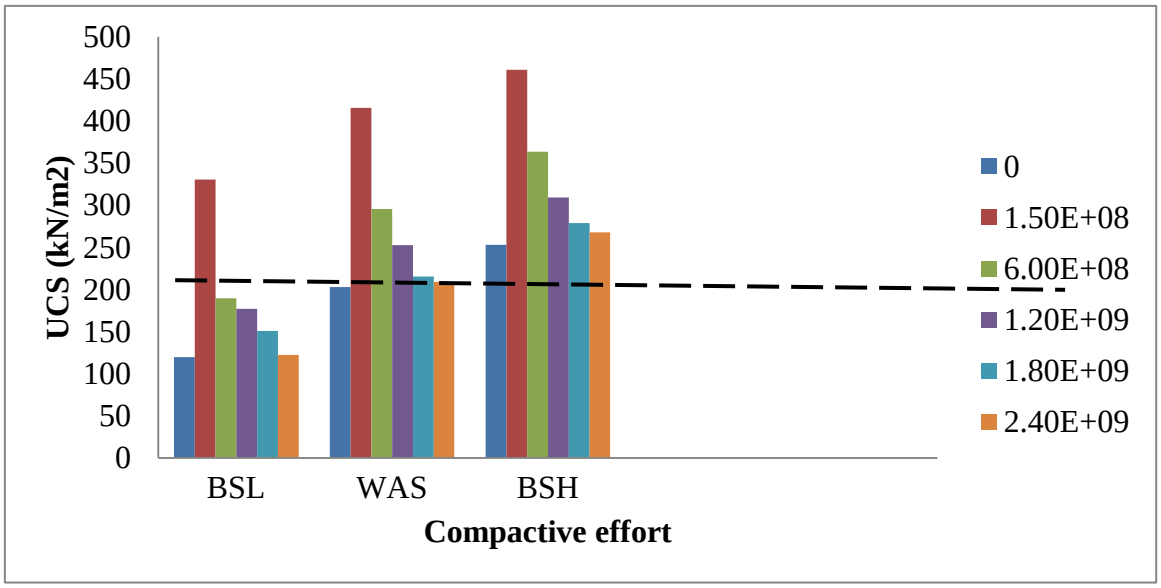


Fig. 4.21: Variation of UCS with compactive effort

4.13 Volumetric Shrinkage Strain

The variation of volumetric shrinkage strain (VSS) of the bio-remediated and bio-cemented soil at various compactive efforts namely BSL, WAS and BSH compactive efforts at optimum moisture content (OMC) is shown in Fig. 4.22a-f. Generally, the VSS values decreased with increase in *B. licheniformis* suspension density as shown in Fig.

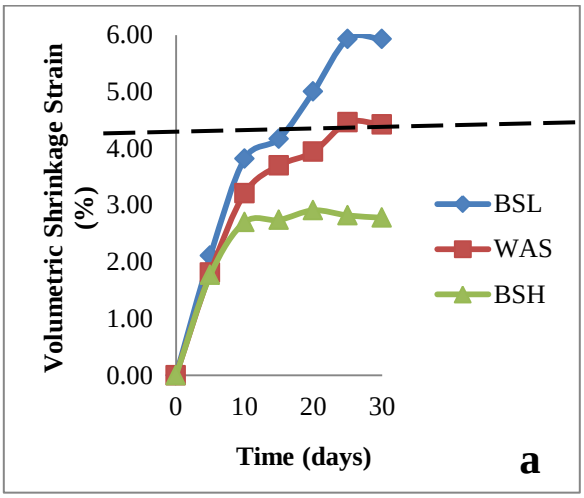
4.22a-f. The VSS values of bio-remediated and bio-cemented soil decreased from 5.93, 4.42 and 2. 78% for the untreated soil to 3.98, 3.78 and 3.06 % when compacted using BSL, WAS and BSH compactive efforts, respectively, when treated with 2.4 x 10^9cell/ml of *B. licheniformis* suspension density. However, with higher doses of the bacterial specie of up to 2.4 x 10^9cell/ml, the VSS obtained satisfied the maximum permissible value of 4%. The probable reason for the decreased VSS may be attributed to calcite formation that occurred during MICP that filled up the void spaces between the soil particles. This finding is in agreement with that of Mitchell and Santamarina (2005), Mujah *et al.*(2016), Taha and Taha (2015) and Oluremi *et al*., (2014).

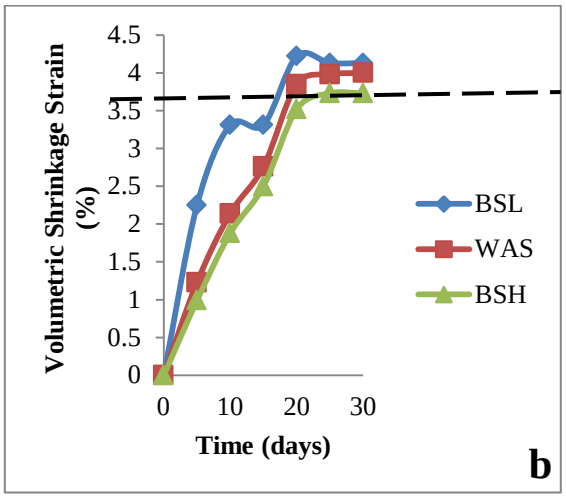
The two-way analysis of variance (ANOVA) test on the effect of *B. licheniformis* suspension and compactive efforts of the bio-remediated and bio-cemented crude oil contaminated soil is shown in Table4.12. The results indicate that the effect of *B. licheniformis* on volumetric shrinkage strain for the bio-remediated and bio-cemented crude oil contaminated soil was statistically insignificant as the calculated F-value is less than the F-critical, that is F-cal=1.4980<Fcrit=3.3259. Also, the results show that the effect of compactive efforts on the volumetric shrinkage strain of the bio-remediated and bio-cemented crude oil contaminated soil was statistically significant as the calculated F-value is greater than the F-critical (i.e. Fcal=10.9357 >Fcrit=4.1028). Detailed test results are given in Table A4.17 of the Appendix.

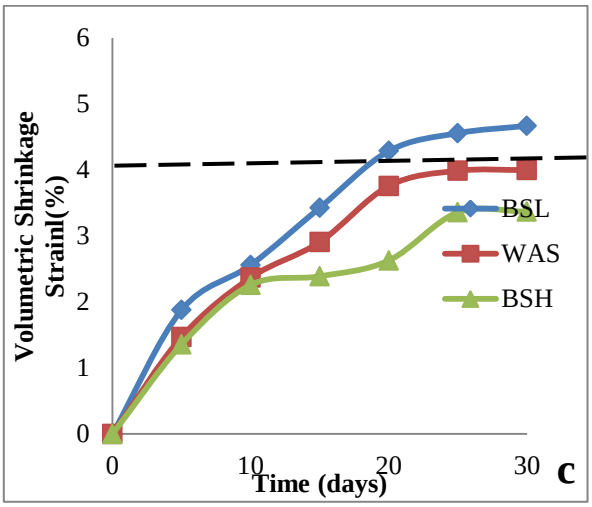
Table 4.12: Two- way analysis of variance for volumetric shrinkage strain of the bio-remediated and bio-cemented soil

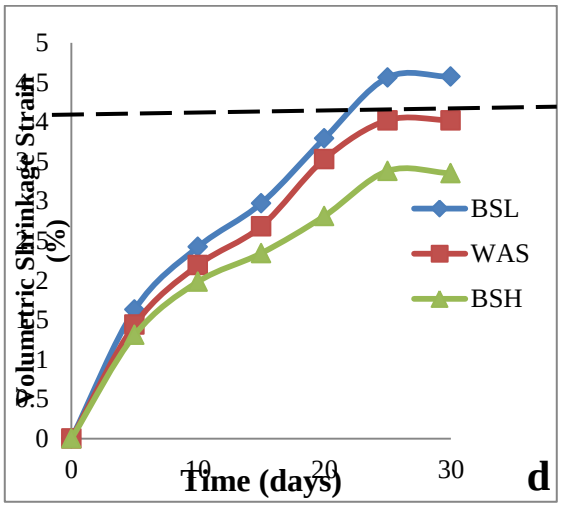
Property	Sources of Variation	Degree of Freedom	F_{CAL}	P-Value	F_{CRIT}	Remarks
Volumetric Shrinkage	*B. licheniformis*	5	1.4980	0.2739	3.3259	Fcal<Fcrit Not tatistically significant

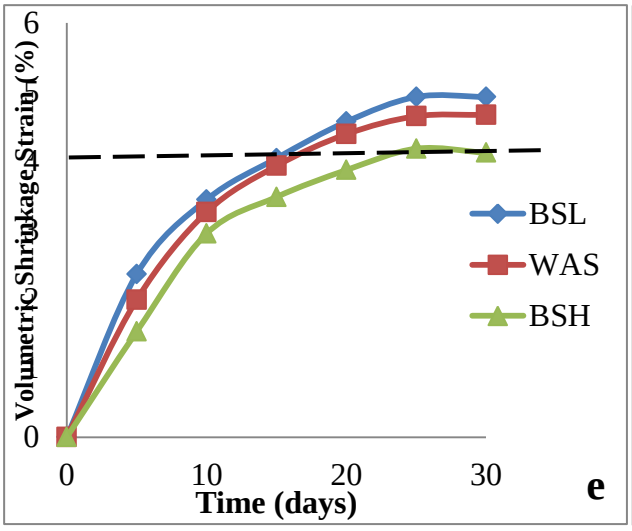
Strain	Compactive efforts	2	10.936	3.0E-03	4.1028	Fcal>Fcrit Statistically significant











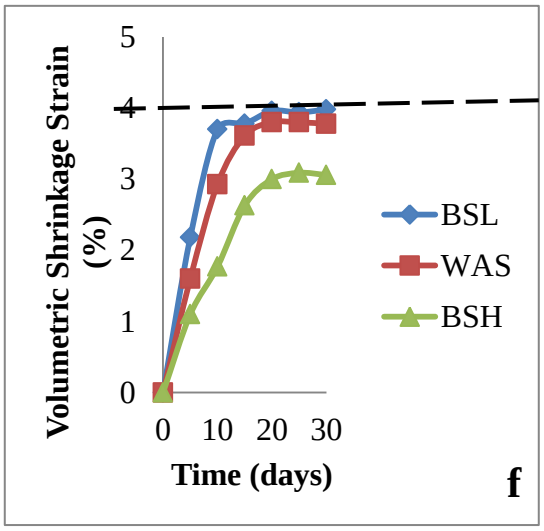


Fig.4.22: Variation of volumetric Shrinkage Strain of bio-remediated and bio-cemented soil at optimum moisture content for various *Bacillus licheniformis* suspension (a) 0 cell/ml (b) 1.5 x 10^8 cell/ml (c) 6.0 x 10^8 cell/ml, (d) 1.2 x 10^9 cell/ml, (e) 1.8 x 10^9 cell/ml (f) 2.4 x 10^9 cell/ml

4.14 Effect of pH on the bio-cementation of the bio-remediated soil

The variation of pH of the bio-remediated and bio-cemented soil using *B. licheniformis* suspension is shown in Fig. 4.23. The pH value of the natural and the crude oil contaminated soil were 7.8 and 6.2, respectively.

The pH of samples treated with 1.5 x 10^8 cell/ml of *B. licheniformis* suspension were 8.31, 8.35 and 8.37 when compacted at BSL, WAS and BSH respectively. The pH of the soil containing up to 2.4 x 10^9 cell/ml of *B. licheniformis* were 8.24, 8.25 and 8.27, respectively. It was observed that the pH values increased with higher bacterial population. The increased pH with higher *B. licheniformis* suspension may be attributed to the urealytic activity which increased the ammonia production as an end product in formation of calcium carbonate ($CaCO_3$). This is in line with findings made by Al- Thawadi (2013). Also Cheng and Cord-Ruwisch (2013) reported that pH of 9.5 (alkaline) or 3.5 (acidic) are harmful for strength development in MICP.

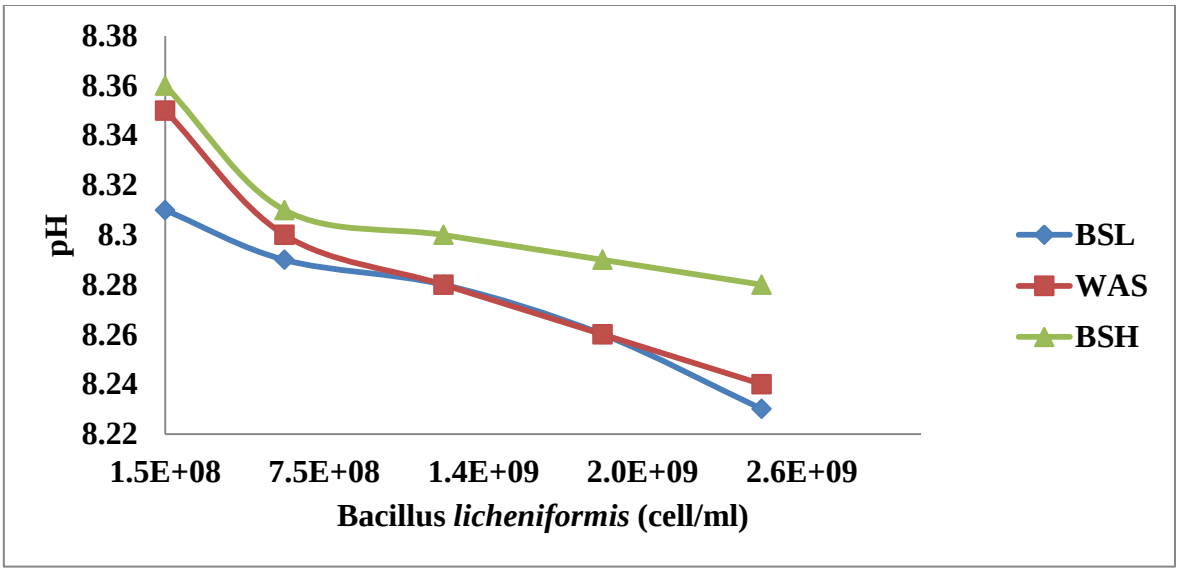


Fig. 4.23: Variation of pH of the bio-remediated and bio-cemented soil with *Bacillus licheniformis* suspension

The two-way analysis of variance (ANOVA) test on the effects of *B. licheniformis* suspension and compactive efforts on pH of the bio-remediated and bio-cemented crude oil contaminated soil is shown in Table 4.13. The results indicate that the effect of *B. licheniformis* on pH for the bio-remediated and bio-cemented crude oil contaminated soil was statistically significant as the calculated F-value is greater than the F-critical, that is F-cal=35.67294>Fcrit=3.3259. Also, the results show that the effect of compactive efforts on the pH of the bio-remediated and bio-cemented crude oil contaminated soil was statistically significant as the calculated F-value is greater than the F- critical (i.e. Fcal=7.4235> Fcrit=4.1028). Detailed test results are given in Table A4.18 of the Appendix.

Table4.13: Two- way analysis of variance for pH of the bio-remediated and bio-cemented soil

Property	Sources of Variation	Degree of Freedom	F_{CAL}	P-Value	F_{CRIT}	Remarks
pH	*B. licheniformis*	5	7.4235	1.0E-05	3.3259	Fcal>Fcrit

					Statistically significant
Compactive efforts	2	35.6729	0.0156	4.1028	Fcal>Fcrit Statistically significant

4.15 Calcite Content

Fig. 4.24 shows the variation of calcite content of the bio-remediated and bio-cemented soil at varying compactive efforts namely BSL, WAS and BSH energy. Generally, calcite content decreased with increase in *B. licheniformis* suspension density as shown in Fig.4.24. The calcite content of the bio-remediated and bio-cemented soil increased from 0g for all the compactive efforts for the untreated soil to peak values of 1.1, 1.3 and 2.4g when compacted using BSL, WAS and BSH energy, respectively, when treated with 1.5 x 10^8 cell/ml of *B. licheniformis* suspension density. However, with higher doses of *B. licheniformis* suspension density, the calcite content values decreased continuously for all the treatment. The peak values of calcite obtained when treated with 1.5 x 10^8 cell/ml of *B. licheniformis* suspension density may be attributed to availability of nutrients for microbes during calcite formation and the calcite content with higher doses of the bacterial population due to the unavailability of nutrients. Similar finding was made by Yengejeh *et al*. (2015).

The one-way analysis of variance (ANOVA) test on the effect of *B. licheniformis* suspension density on calcite content of the bio-remediated and bio-cemented crude oil contaminated soil is shown in Table4.14. The results indicate that the effect of *B. licheniformis* on calcite content for the bio-remediated and bio-cemented crude oil contaminated soil was statistically significant as the F-calculated is greater than the F-critical, that is F-cal=9.94>Fcrit=2.7109. Detailed test results are given in Table A4.19 of the Appendix

Table 4.14: One- way analysis of variance for calcite content of the bio-remediated and bio-cemented soil

Property	Sources of Variation	Degree of Freedom	F_{CAL}	P-Value	F_{CRIT}	Remarks
Calcite content	*B. licheniformis*	5	9.9366	8.0E-04	3.3259	Fcal>Fcrit Statistically significant

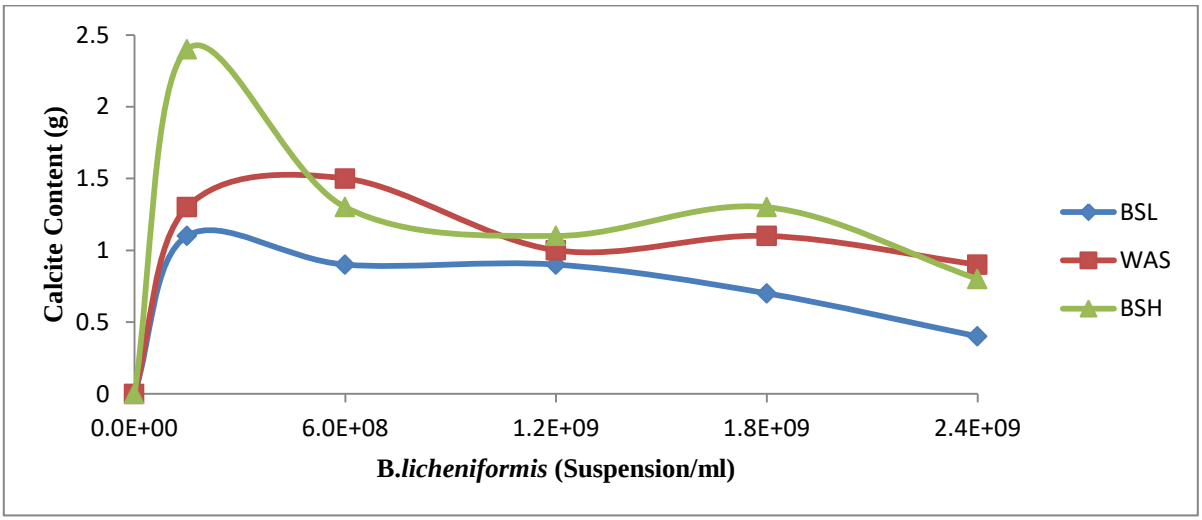


Fig. 4.24: Variation of calcite content with *Bacillus licheniformis* suspension

4.16 Micrograph

The image of the bio-remediated and bio-cemented soil treated with 1.5 x 10^8 cell/ml of B. *licheniformis* compacted using BSL compactive effort at OMC and used in the compatibility test is shown in Plate I while the image of the crude oil contaminated soil compacted using BSL compactive effort at OMC is shown in Plate II. The micrograph of the treated sample with magnification of 1500x shown in plate I showed a relatively rough surface with bonding in the soil matrix. The crystals were generally dispersed and have a coating effect on the solid particle. In addition, some whitish patches are observed on the soil which may be indicative of the calcites formed during the MICP process. The calcite crystals that agglomerated the irregular crystal precipitate on both particle to particle contact point and grain surface bridging the pore spaces. The micrograph of the untreated

sample with magnification of 1500x in plate II showed a smooth surface with fissure/joint formed on the surface of the sand particle with an irregular crystal shape. This phenomenon could further explain the reason for the observation made for unconfined compressive strength treated with 1.5 x 10^8 cell/ml of B. *licheniformis*. This finding is in agreement with the one by Wei *et al.,* (2012 and 2015) ; Cheng, and Cord-Ruwisch, (2013) and Hanifi *et al*., (2015) .

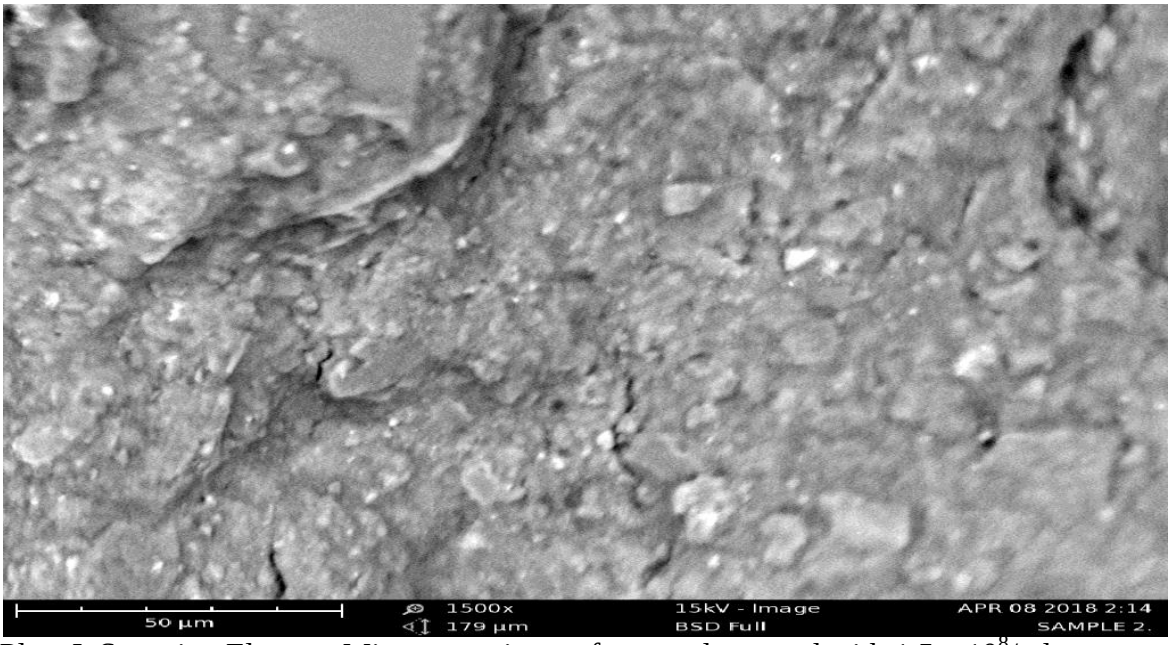


Plate I: Scanning Electron Microscopy image for sample treated with 1.5 x 10^8/ml *B.licheniformis*

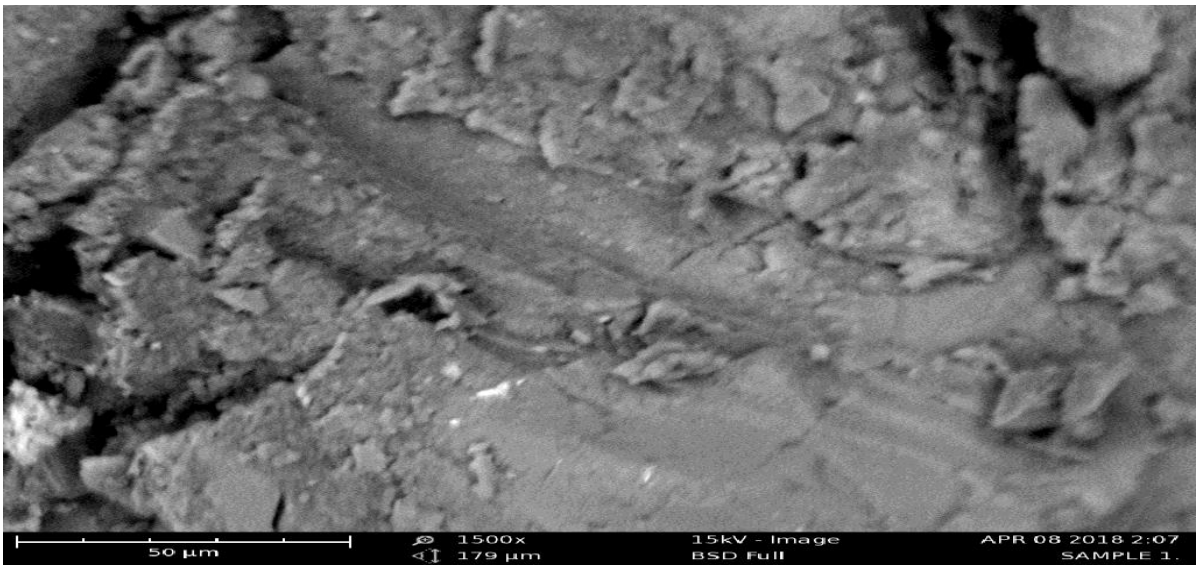


Plate II: Scanning Electron Microscopy image for untreated sample

CHAPTER FIVE

CONCLUSION AND RECOMMENDATION

5.1 Conclusion

A laboratory study was conducted to determine the geotechnical properties of bio-remediated crude oil contaminated soil microbially treated with *B. licheniformis* for use in waste containment system. From the results of the tests conducted, the following conclusions are drawn:

1 Bioremediation of crude oil contaminated soil reduced the quantity of crude oil to 10g/kg from the untreated sample which had 98g/kg of total petroleum hydrocarbon (TPH) concentration for sample treated using *B. licheniformis* activated with 30% CD which gave the optimal result. This translates to 89.8% removal efficiency of TPH. Therefore bio-stimulation is an achievable bioremediation strategy in crude oil clean up.

2. The index properties such as liquid limit, plastic limit and plasticity index of crude oil contaminated soil classified as A-2-5 (5) using AASHTO classification or SM using USCS prepared at OMC and compacted using three energy levels (British Standard light, West African Standard and British Standard heavy) showed improvement when treated with the five levels of *B. licheniformis* suspension densities.

3. Generally, values of the maximum dry density decreased with increased in B. *licheniformis* suspension. The highest MDD values of 1.76, 181 and 1.93 Mg/cm^3 compacted at BSL, WAS and BSH energy, respectively, were achieved at 1.5 $x10^8$ cell/ml of *B. licheniformis* suspension density while the OMC increased with increase in B. *licheniformis* suspension.

4. The lowest value of hydraulic conductivity of 5.86 x 10^{-11}m/s which satisfied the maximum permissible value of hydraulic conductivity of 1 x 10^{-9} m/s recommended by regulatory agency was obtained at 2.4 x10^{9} cell/ml of *B. licheniformis* suspension for compacted specimen prepared at optimum moisture content using BSH compaction energy. The sample was found to be compatible with the municipal solid waste leachate used as the hydraulic conductivity did not increase with the duration of permeation. The UCS of the crude oil contaminated soil treated with B. *licheniformis* suspension significantly improved to a peak value of 460.82 kN/m^2 when treated with 1.5 x 10^8 cell/ml B. *licheniformis* suspension density, compacted using BSH compactive effort.. The maximum permissible volumetric shrinkage strain values of 3.98, 3.78 and 3.06% were obtained for samples treated with 2.4 x10^{9} cell/ml of B. *licheniformis* suspension density when compacted using BSL, WAS and BSH compaction energy, respectively which satisfied the maximum permissible value of 4%.

5. The highest calcite content of 52% was produced with sample treated with 1.5 x 10^8 cell/ml B. *licheniformis* suspension density, compacted using BSH compaction energy, while the lowest calcite content concentration of 12% was obtained when treated with 2.4 x 10^9 cell/ml of B. *licheniformis* suspension density and compacted using BSL compaction energy.

5.2 Recommendation

1. It is recommended that 1.5 x 10^8 cell/ml of B. *licheniformis* suspension treatment of A-2-5 or SM crude oil contaminated soil prepared at moulding water content of 14.3% and compacted with British Standard light (BSL) compaction energy level is recommended for use as a liner material in waste containment applications

2.Further research should be conducted on crude oil contaminated soil to include Reduced energy level and more moulding water contents (-2,+2 and +4) for use in waste containment system.

5.3 Contribution to Knowledge

1. The 30% chicken droppings activated the *B. licheniformis* that was identified in the crude oil contaminated soil as the predominant bacteria and the bacteria removed 89.8 % of the crude oil thus, CD was found to be useful in management of environmental degradation.

2. 52% calcite (bio-cement) was produced by the *B. licheniformis* with a microbial population of 1.5 x 10^8 compacted using BSH compaction energy during the biocementation for use in waste containment applications thus *B. licheniformis* is a calcite induced bacteria

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APPENDICES

Table A 2.1: Composition of Chicken Droppings (%)

	Dry Basis			
Age	**Nitrogen**	**Phosphorus**	**Potassium**	**Organic matter**
Fresh	6.7	4.5	2.0	79.6
6 weeks	3.6	3.6	2.7	64.1
10months	3.3	3.8	2.1	61
1 year	1.4	1.2	0.8	29.4

(McCall, 1980)

Table A4.1a: Test results of Total Petroleum Hydrocarbon (TPH) concentration over the remediation period

CD content (%)	**Time (weeks)**						
	0	**2**	**4**	**6**	**8**	**10**	**12**
	TPH (g/Kg)						
0	98	96	97	95	93	94	91
10	98	92	70	68	60	50	48
20	98	90	70	66	59	49	35
30	98	76	66	60	56	45	10
40	98	66	54	52	50	38	12

Table A4.1b: Test results of Removal Efficiency of crude oil contaminated soil over the remediation period

CD content (%)	**Time (weeks)**						
	0	**2**	**4**	**6**	**8**	**10**	**12**
	Removal Efficiency (%)						
0	0	1.02	2.04	3.06	4.08	5.1	7.14
10	0	6.12	28.57	30.61	38.78	48.98	51.02
20	0	8.16	28.57	32.65	39.8	50	64.29
30	0	22.45	32.65	38.78	42.86	54.08	89.8
40	0	32.65	44.9	46.94	48.98	61.22	87.76

Table A4.2: Test results of Natural Moisture Content for Crude Oil Contaminated soil

Container No	K2	BQ	S4
Weight of empty can (M_1) g	31.5	30.2	31.1
Weight of empty can + Wet Soil (M_2)g	83.1	50.1	99.3
Weight of empty can + dry soil (M_3) g	80.4	49.1	95.6
Weight of wet soil $M_w=M_2-M_1$	51.6	19.9	68.2
Weight of Dry soil $Ms=M_3-M_1$	48.9	18.9	64.5
$M_w = (M_2 – M_1)- (M_3-M_1)$	2.7	1.0	3.7
Moisture Content $w= M_w/M_s$ x100	5.52	5.29	5.74
Av. Moisture Content (%)		5.52	

Table A4.3: Test results of Temperature (°C) of the microcosms

CD content (%)	**Time (week)**						
	0	**2**	**4**	**6**	**8**	**10**	**12**
0	27.1	27.1	27.5	27.9	28.0	28.5	28.5
10	26.73	26.6	26.86	26.86	26.86	26.91	27.18
20	26.1	26.09	26.21	27.21	27.58	27.79	27.9
30	26.8	26.86	27.03	27.53	27.72	27.88	28.68
40	26.81	26.74	27.09	27.29	27.39	27.84	27.28

Table A4.4: Test results of pH of samples treated with chicken droppings

CD content (%)	**Time (week)**						
	0	**2**	**4**	**6**	**8**	**10**	**12**
0	6.9	6.9	6.87	6.82	6.89	6.85	6.82
10	7.12	7.52	7.52	7.53	7.53	7.53	7.5
20	7.15	7.72	7.74	7.8	7.81	7.87	7.71
30	7.21	7.8	7.81	7.82	7.86	7.87	7.93
40	7.24	7.84	7.84	7.85	7.86	7.85	7.81

Table A4.5: Test results of B. *licheniformis* (Microbial Population) in sample collected at 12 weeks

CD content (%)	Microbial Population *(cfu/g)*
0	4.97E+07
10	5.26E+08
20	6.41E+08
30	7.24E+08
40	6.67E+08

Table A4.6: Test results of Liquid Limit of bio-remediated and bio-cemented soil

Mixture of Bacteria(B)/ Cementation(C) agent	TREATED *B. licheniformis* suspension (cell/ml)					
	0	**1.5×10^8**	**6.0×10^8**	**1.2×10^9**	**1.8×10^9**	**2.4×10^9**
50%B50%C	42.2	42	46.2	45.7	45.2	46.7
75%B25%C	42.2	32.38	36.86	37.09	35.91	38.6
25%B75%C	42.2	27.22	27.45	26.99	25.47	27.1

Table A4.7: Test results of Plastic Limit of bio-remediated and bio-cemented soil

Mixture of Bacteria(B)/ Cementation(C) agent	TREATED *B. licheniformis* suspension (cell/ml)					
	0	**1.5×10^8**	**6.0×10^8**	**1.2×10^9**	**1.8×10^9**	**2.4×10^9**
50%B50%C	34.44	32.12	43.12	35.89	32.73	38.69
75%B25%C	34.44	25.41	29.12	32.45	30.76	31.23
25%B75%C	34.44	22.68	23.05	21.98	20.18	23.76

Table A4.8: Test results of Plasticity Index of bio-remediated and bio-cemented soil

Mixture of Bacteria(B)/ Cementation(C) agent	TREATED *B. licheniformis* suspension (cell/ml)					
	0	**1.5×10^8**	**6.0×10^8**	**1.2×10^9**	**1.8×10^9**	**2.4×10^9**
50%B50%C	6.76	9.88	3.08	8.71	10.87	6.31
75%B25%C	6.76	6.95	7.74	4.64	5.15	7.37
25%B75%C	6.76	4.54	4.4	5.01	5.29	3.34

Table A4.9: Test results of Wet Sieve Analysis of bio-remediated and bio-cemented soil

BS Sieve Size	TREATED *B. licheniformis* suspension (cell/ml)					
	0	**1.5 x 10^8**	**6.0 x10^8**	**1.2x 10^9**	**1.8 x 10^9**	**2.4 x 10^9**
4.75	100	99.8	99.85	99.9	100	99.25
2.36	97.48	90.7	88.8	91.4	91.8	90.1
2	87.4	81.2	79.95	78.7	85.4	83.35
1.18	86.23	73.2	73.05	71.5	78.8	73
0.6	80.35	60.2	60.8	56.2	55.98	68.2
0.425	77.7	55.8	54.8	52.15	51.35	62.2
0.3	67.1	46.2	51.45	45.85	46.8	58.35
0.212	65.53	40.25	44.75	42.75	44.15	52.15
0.15	59.25	36.55	39.8	40.15	41.75	47.2
0.075	46.5	34.55	37.1	38.5	40.45	44.5
0.04619	0.3	0.9	1.5	0	0.9	0
0.04505		0	14.1	3.59	0	0
0.0435	0	0	13.7	0	0	7.17
0.0406	0	0	13.45	0	0	0
0.0329	0.2	0	8.32	0	0	0.9
0.032	0	0	3.59	0	0	0
0.02327		0	0.9	0	0	0

Table A4.10: Test results of specific gravity of bio-remediated and bio-cemented soil

Bacteria (B)/ agent	**Cementation(C)**		**TREATED** *B. licheniformis* **suspension (cell/ml)**			
	0	**1.5 x 10^8**	**6.0 x10^8**	**1.2x 10^9**	**1.8 x 10^9**	**2.4 x 10^9**
50%B50%C	2.13	2.6	2.23	2.15	2.17	2.17

Table A4.11a: Test results of Maximum dry density of bio-remediated and bio-cemented soil

Compactive efforts	TREATED *B. licheniformis* suspension (cell/ml)					
	0	**1.5 x 10^8**	**6.0 x10^8**	**1.2x 10^9**	**1.8 x 10^9**	**2.4 x 10^9**
BSL	1.67	1.76	1.7	1.69	1.68	1.69
WAS	1.73	1.81	1.76	1.75	1.77	1.73
BSH	1.83	1.93	1.86	1.87	1.84	1.86

Table A4.11b: Test results of Optimum moisture content of bio-remediated and bio-cemented soil

Compactive efforts	TREATED *B. licheniformis* suspension (cell/ml)					
	0	**1.5 x 10^8**	**6.0 x10^8**	**1.2x 10^9**	**1.8 x 10^9**	**2.4 x 10^9**
BSL	14.3	14.4	14.4	14.6	14.9	15.8
WAS	13.3	13.1	13.2	13.6	13.9	14.1
BSH	12.8	12.5	12.9	12.7	12.6	13.7

Table A4.12: Test results of hydraulic conductivity of bio-remediated and bio-cemented soil

Compactive efforts	TREATED *B. licheniformis* suspension (cell/ml)					
	0	**1.5 x 10^8**	**6.0 x10^8**	**1.2x 10^9**	**1.8 x 10^9**	**2.4 x 10^9**
BSL	3.62E-10	6.88E-10	7.32E-10	7.53E-10	1.67E-10	7.57E-10
WAS	1.8E-10	1.11E-09	1.6E-09	4.08E-10	6.97E-10	5.94E-10
BSH	9.92E-11	1.5E-09	3.85E-10	2 56E-10	7.04E-10	5.86E-11

Table A4.13: Test results of hydraulic conductivity k (m/s) with time of sample permeated with water and MSW leachate as the permeant liquid with time at 0 % OMC

Time (days)	*B. licheniformis* suspension (cell/ml)		
	1.5 x 10^8	**1.2x 10^9**	**2.4 x 10^9**
1	2.7982E-10	6.8801E-10	3.4700E-10
2	1.2717E-10	2.6902E-10	3.1000E-11
3	1.7759E-10	2.8300E-10	4.7800E-11

4	1.9475E-10	3.0600E-10	3.4200E-11
5	1.0194E-09	2.6100E-10	5.2400E-11
6	1.7414E-09	2.3400E-10	7.7800E-11
7	1.5751E-09	2.1500E-10	4.1800E-11
8	1.7182E-09	2.1941E-10	7.9800E-11
9	1.7074E-09	1.8085E-10	7.1900E-11
10	1.5909E-09	2.1705E-10	8.5700E-11
11	1.3270E-09	2.1234E-10	8.4700E-11
12	1.5119E-09	2.1587E-10	6.9000E-11
13	1.3625E-09	1.8201E-10	7.6800E-11
14	1.4182E-09	2.1234E-10	8.2700E-11
15	1.5119E-09	4.2628E-10	1.0200E-10
16	1.3625E-09	2.2178E-10	1.9100E-10
17	1.3593E-09	4.3429E-10	8.9700E-11
18	1.1576E-09	2.2651E-10	1.7500E-10
19	1.2007E-09	4.3965E-10	8.7700E-11
20	1.2194E-09	2.2414E-10	9.2600E-11
21	4.0014E-10	1.8085E-10	7.7800E-11
22	3.6840E-10	2.1587E-10	6.7000E-11
23	2.3959E-10	2.0529E-10	7.4800E-11
24	1.6090E-10	2.1116E-10	7.8800E-11
25	2.7016E-10	2.0646E-10	6.7000E-11
26	3.9769E-10	2.1823E-10	6.8000E-11
27	3.6846E-10	2.2178E-10	7.8800E-11
28	3.6888E-10	2.3603E-10	8.2700E-11
29	3.0816E-10	2.1705E-10	6.8000E-11
30	8.1151E-10	2.2889E-10	6.2100E-11
31	3.0339E-10	2.2178E-10	5.7300E-11
32	1.3128E-10	2.2533E-10	6.1100E-11
33	1.6099E-10	2.2059E-10	6.2100E-11
34	9.9673E-11	2.0998E-10	6.7000E-11
35	5.5015E-11	1.8664E-10	6.9900E-11
36	6.8352E-11	2.1116E-10	5.6300E-11
37	6.6125E-11	2.0646E-10	4.8500E-11
38	5.7235E-11	1.8664E-10	5.0500E-11
39	5.5015E-11	2.0998E-10	6.2100E-11
40	5.5015E-11	2.0646E-10	5.7300E-11
41	7.0579E-11	2.1587E-10	5.3400E-11
42	4.6153E-11	2.0412E-10	5.7300E-11
43	6.6125E-11	2.2178E-10	1.9800E-11
44	5.5015E-11	1.8780E-10	4.7600E-11
45	5.7235E-11	1.9128E-10	4.5600E-11
46	5.7235E-11	1.8085E-10	3.8900E-11
47	5.0582E-11	1.3862E-10	5.0500E-11
48	5.7235E-11	1.5220E-10	5.0500E-11
49	4.8368E-11	1.4088E-10	4.5600E-11

50	4.8368E-11	1.4653E-10	4.6600E-11
51	4.8367E-11	1.7739E-10	4.7600E-11
52	5.5015E-11	1.2402E-10	4.8500E-11
53	4.8367E-11	1.6475E-10	5.2400E-11
54	4.8368E-11	1.5904E-10	4.7600E-11
55	5.2798E-11	1.6018E-10	4.7600E-11
56	4.8367E-11	1.6246E-10	5.7300E-11
57	7.7271E-11	1.5790E-10	5.4300E-11
58	4.8367E-11	1.5790E-10	4.9500E-11
59	4.8367E-11	1.5790E-10	9.3600E-11
60	4.3942E-11	1.4427E-10	4.9500E-11
61	5.2798E-11	1.4427E-10	5.1400E-11
62	6.6125E-11	1.4427E-10	4.7600E-11
63	1.2902E-10	1.4427E-10	3.8900E-11
64	1.1093E-10	1.4201E-10	3.8900E-11
65	1.1093E-10	1.2962E-10	7.2900E-11
66	6.6125E-11	1.3187E-10	5.7300E-11
67	6.6125E-11	1.2179E-10	3.7900E-11
68	5.5015E-11	2.3365E-10	4.7600E-11
69	6.3904E-11	1.1844E-10	4.2700E-11
70	1.0192E-10	1.3411E-10	4.4700E-11
71	5.2798E-11	1.0733E-10	3.6000E-11
72	5.5015E-11	1.1288E-10	4.2700E-11
73	5.2798E-11	1.0733E-10	4.3700E-11
74	1.0867E-10	2.2178E-10	3.3100E-11
75	1.1318E-10	1.3074E-10	3.1200E-11
76	5.7235E-11	1.3524E-10	2.6500E-11
77	5.2798E-11	1.2626E-10	6.1100E-11
78	5.7235E-11	1.2626E-10	8.5700E-11
79	5.5015E-11	1.4427E-10	4.3700E-11
80	7.9505E-11	1.1177E-10	3.2200E-11
81	5.7235E-11	1.2514E-10	4.3700E-11
82	5.0582E-11	2.2889E-10	3.2200E-11
83	4.8367E-11	1.0291E-10	4.3700E-11
84	5.5015E-11	1.0070E-10	3.9900E-11
85	6.3900E-11	1.1066E-10	3.0300E-11
86	3.2903E-11	1.0291E-10	4.3700E-11
87	1.9704E-11	2.3008E-10	4.1800E-11
88	2.6297E-11	1.0844E-10	4.4700E-11
89	2.4098E-11	1.0180E-10	4.3700E-11
90	2.8498E-11	1.0844E-10	4.5600E-11
91	2.1900E-11	8.8608E-11	3.6100E-11

***elapsed time for using water permeant**

Table A4.14: Test results of void ratio of bio-remediated and bio-cemented soil

Compactive efforts	TREATED *B. licheniformis* suspension (cell/ml)					
	0	**1.5 x 10^8**	**6.0 x10^8**	**1.2x 10^9**	**1.8 x 10^9**	**2.4 x 10^9**
BSL	0.62	0.92	0.9	0.9	0.91	0.83
WAS	0.58	0.88	0.81	0.77	0.83	0.74
BSH	0.56	0.71	0.66	0.64	0.68	0.62

Table A4.15: Test results of Porosity of bio-remediated and bio-cemented soil

Compactive efforts	TREATED *B. licheniformis* suspension (cell/ml)					
	0	**1.5 x 10^8**	**6.0 x10^8**	**1.2x 10^9**	**1.8 x 10^9**	**2.4 x 10^9**
BSL	47.22	46.46	45.42	45.73	45.48	47.22
WAS	41.72	46.88	40.9	40.75	39.4	41.72
BSH	41.37	39.71	39.31	40.5	38.28	41.37

Table A4.16: Test results of Degree of saturation of bio-remediated and bio-cemented soil

Compactive efforts	TREATED *B. licheniformis* suspension (cell/ml)					
	0	**1.5 x 10^8**	**6.0 x10^8**	**1.2x 10^9**	**1.8 x 10^9**	**2.4 x 10^9**
BSL	84.05	78.59	78.12	75.33	69.99	72.78
WAS	89.28	69.94	72.0	82.07	76.08	80.61
BSH	94.11	75.05	81.77	82.49	79.47	98.27

Table A4.17: Test results of Unconfined compressive strength of bio-remediated and bio-cemented soil

Compactive efforts	TREATED *B. licheniformis* suspension (cell/ml)					
	0	**1.5 x 10^8**	**6.0 x10^8**	**1.2x 10^9**	**1.8 x 10^9**	**2.4 x 10^9**
BSL	119.56	303.64	189.63	177.11	150.63	122.27
WAS	202.82	415.57	295.59	252.7	215.27	208.85
BSH	253.15	460.78	363.66	309.17	278.99	267.8

Table A4.18: Test results of Volumetric shrinkage strain of bio-remediated and bio-cemented soil

Compactive efforts	TREATED *B. licheniformis* suspension (cell/ml)					
	0	**1.5 x 10^8**	**6.0 x10^8**	**1.2x 10^9**	**1.8 x 10^9**	**2.4 x 10^9**
BSL	5.93	4.13	4.67	4.57	4.93	3.98
WAS	4.42	4.0	4.0	4.2	4.67	3.78
BSH	2.78	3.73	3.37	3.35	4.12	3.06

Table A4.19: Test results of pH monitored during MICP of bio-remediated and bio-cemented soil

Compactive efforts	TREATED *B. licheniformis* suspension (cell/ml)					
	0	**1.5 x 10^8**	**6.0 x10^8**	**1.2x 10^9**	**1.8 x 10^9**	**2.4 x 10^9**
BSL	8.19	8.37	8.32	8.31	8.29	8.27
WAS	813	8.35	8.3	8.29	8.28	8.25
BSH	8.06	8.31	8.29	8.29	8.27	8.24

Table A4.20: Test results of Calcite Content of bio-remediated and bio-cemented soil

Compactive efforts	TREATED *B. licheniformis* suspension (cell/ml)					
	0	**1.5 x 10^8**	**6.0 x10^8**	**1.2x 10^9**	**1.8 x 10^9**	**2.4 x 10^9**
BSL	0	1.6	1.3	1.1	1.0	0.9
WAS	0	1.3	1.5	1.1	1.2	1.1
BSH	0	2.6	1.3	1.1	1.3	0.8

Table A4.21a: Analysis of variance for Liquid Limit of bio-remediated and bio-cemented soil

SUMMARY	***Count***	***Sum***	***Average***	***Variance***
0.00E+00	3	126.6	42.2	0
1.50E+08	3	101.6	33.86667	56.269733
6.00E+08	3	110.51	36.83667	87.891033

1.20E+09	3	109.78	36.59333	87.701033
1.80E+09	3	106.58	35.52667	97.428433
2.40E+09	3	112.4	37.46667	97.003333

Table A4.21b: Analysis of variance for Plastic Limit of bio-remediated and bio-cemented soil

SUMMARY	*Count*	*Sum*	*Average*	*Variance*
0.00E+00	3	1.06E+02	3.5440E+01	0.00E+00
1.50E+08	3	8.02E+01	2.6743E+01	2.36E+01
6.00E+08	3	9.53E+01	3.1763E+01	1.06E+02
1.20E+09	3	9.03E+01	3.0107E+01	5.25E+01
1.80E+09	3	8.37E+01	2.7890E+01	4.56E+01
2.40E+09	3	9.37E+01	3.1227E+01	5.57E+01

Table A4.21c: Analysis of variance for Plasticity Index of bio-remediated and bio-cemented soil

SUMMARY	*Count*	*Sum*	*Average*	*Variance*
0.00E+00	3	2.03E+01	6.7600E+00	1.18E+03
1.50E+08	3	2.14E+01	7.1233E+00	7.15E+00
6.00E+08	3	1.52E+01	5.0733E+00	5.77E+00
1.20E+09	3	1.84E+01	6.1200E+00	5.07E+00
1.80E+09	3	2.13E+01	7.1033E+00	1.06E+01
2.40E+09	3	1.70E+01	5.6233E+00	4.36E+00

Table A4.22a: Analysis of variance for Maximum Dry Density for bio-remediated and bio-cemented soil

SUMMARY	*Count*	*Sum*	*Average*	*Variance*	*SUMMARY*	*Count*	*Sum*	*Average*	*Variance*
0.00E+00	3	5.23	1.743	0.005222	BSL	6	10.19	1.6983	0.565429
1.50E+08	3	5.5	1.833	0.005913	WAS	6	10.55	1.7583	0.627449
6.00E+08	3	5.32	1.773	0.005222	BSH	6	11.19	1.865	0.678225
1.20E+09	3	5.31	1.77	0.006613					

1.80E+09	3	5.29	1.763	0.00544
2.40E+09	3	5.28	1.76	0.00596

Table A4.22b: Analysis of variance for Optimum Moisture Content for bio-remediated and bio-cemented soil

SUMMARY	*Count*	*Sum*	*Average*	*Variance*	*SUMMARY*	*Count*	*Sum*	*Average*	*Variance*
0.00E+00	3	40.4	13.4667	0.34722	BSL	6	88.4	29.4667	260.80
1.50E+08	3	40	13.3333	0.56889	WAS	6	81.2	27.0667	219.94
6.00E+08	3	40.5	13.5	0.405	BSH	6	77.2	25.7333	198.848
1.20E+09	3	40.9	13.6333	0.46722					
1.80E+09	3	41.4	13.8	0.605					
2.40E+09	3	43.6	14.5333	0.80222					

Table A4.23: Analysis of variance for Hydraulic Conductivity for bio-remediated and bio-cemented soil

SUMMARY	*Count*	*Sum*	*Average*	*Variance*	*SUMMARY*	*Count*	*Sum*	*Average*	*Variance*
0.00E+00	3	6.41E-10	2.1373E-10	1.81E-20	BSL	6	4.96E-09	8.27E-10	1.94E-19
1.50E+08	3	3.30E-09	1.099E-09	1.65E-19	WAS	6	4.59E-09	7.648E-10	2.63E-09
6.00E+08	3	2.72E-09	9.0567E-10	3.92E-19	BSH	6	3.00E-09	5.0047E-10	2.95E-09
1.20E+09	3	1.42E-09	4.723E-10	6.49E-20					
1.80E+09	3	3.07E-09	1.0237E-09	3.13E-19					
2.40E+09	3	1.41E-09	4.6987E-10	1.33E-19					

Table A4.24: Analysis of variance for Void ratio for bio-remediated and bio-cemented soil

SUMMARY	*Count*	*Sum*	*Average*	*Variance*	*SUMMARY*	*Count*	*Sum*	*Average*	*Variance*
0.00E+00	3	1.76E+0	5.8667E-01	9.33E-04	BSL	6	5.08E+0	.6933E+0	3.45E+00
1.50E+08	3	2.51E+0	8.3667E-01	1.24E-02	WAS	6	4.61E+00	1.5367E+0	2.86E+00
6.00E+08	3	2.37E+0	7.9000E-01	1.47E-02	BSH	6	3.87E+00	1.2900E+00	2.08E+00
1.20E+09	3	2.31E+0	7.7000E-01	1.69E-02					
1.80E+09	3	2.42E+0	8.0667E-01	1.36E-02					
2.40E+09	3	2.19E+0	7.3000E-01	1.11E-02					

Table A4.25: Analysis of variance for Porosity for bio-remediated and bio-cemented soil

SUMMARY	Count	Sum	Average	Variance	SUMMARY	Count	Sum	Average	Variance
0.00E+00	3	107.13	35.71	6.8131	BSL	6	268.53	44.755	10.72159
1.50E+08	3	130.31	43.43667	10.76583	WAS	6	237.44	39.57333	11.04287
6.00E+08	3	127.83	42.61	12.0675	BSH	6	235.07	39.17833	3.680937
1.20E+09	3	125.63	41.87667	10.04843					
1.80E+09	3	126.98	42.32667	8.702633					
2.40E+09	3	123.16	41.05333	15.01013					

Table A4.26: Analysis of variance for Degree of Saturation for bio-remediated and bio-cemented soil

SUMMARY	Count	Sum	Average	Variance	SUMMARY	Count	Sum	Average	Variance
0.00E+00	3	2.67E+02	89.14667	2.53E+01	BSL	6	458.86	76.47667	24.31583
1.50E+08	3	2.24E+02	74.52667	1.89E+01	WAS	6	469.98	78.33	50.9224
6.00E+08	3	2.32E+02	77.29667	2.44E+01	BSH	6	511.16	85.19333	81.03543
1.20E+09	3	2.40E+02	79.96333	1.61E+01					
1.80E+09	3	2.26E+02	75.18	2.31E+01					
2.40E+09	3	2.52E+02	83.88667	1.70E+02					

Table A4.27: Analysis of variance for Unconfined Compressive Strength for bio-remediated and bio-cemented soil

SUMMARY	Count	Sum	Average	Variance	SUMMARY	Count	Sum	Average	Variance
0.00E+00	3	575.53	191.8433	4551.937	BSL	6	1089.84	181.64	6125.14
1.50E+08	3	1206.99	402.33	4365.578	WAS	6	1590.8	265.1333	6650.10
6.00E+08	3	848.88	282.96	7691.248	BSH	6	1933.55	322.2583	6137.49
1.20E+09	3	738.98	246.3267	4390.425					
1.80E+09	3	644.89	214.9633	4119.143					
2.40E+09	3	598.92	199.64	5358.363					

Table A4.28: Analysis of variance for Volumetric Shrinkage Strain for bio-remediated and bio-cemented soil

SUMMARY	Count	Sum	Average	Variance	SUMMARY	Count	Sum	Average	Variance
0.00E+00	3	13.13	4.37667	2.482	BSL	6	28.21	4.7017	0.485377
1.50E+08	3	11.87	3.95667	0.042	WAS	6	24.93	4.155	0.10619

6.00E+08	3	12.07	4.02333	0.422.	BSH	6	20.41	3.4017	0.226137
1.20E+09	3	11.94	3.98	0.3733					
1.80E+09	3	13.72	4.5733	0.171033					
2.40E+09	3	10.82	3.6067	0.234133					

Table A4.29: Analysis of variance for pH for bio-remediated and bio-cemented soil

SUMMARY	*Count*	*Sum*	*Average*	*Variance*	*SUMMARY*	*Count*	*Sum*	*Average*	*Variance*
0.00E+00	3	24.38	8.12667	0.004233	BSL	6	49.75	8.291667	0.003617
1.50E+08	3	25.03	8.343	0.000933	WAS	6	49.6	8.266667	0.005547
6.00E+08	3	24.91	8.303	0.000233	BSH	6	49.46	8.243333	0.008627
1.20E+09	3	24.89	8.296	0.000133					
1.80E+09	3	24.84	8.28	0.000040					
2.40E+09	3	24.76	8,25	0.000233					

Table A4.30: Analysis of variance for Calcite Content for bio-remediated and bio-cemented soil

SUMMARY	*Count*	*Sum*	*Average*	*Variance*	*SUMMARY*	*Count*	*Sum*	*Average*	*Variance*
0.00E+00	3	0	0	0	BSL	6	4	0.666667	0.162667
1.50E+08	3	4.8	1.6	0.125	WAS	6	5.8	0.966667	0.270667
6.00E+08	3	3.7	1.23	0.055	BSH	6	6.9	1.15	0.611
1.20E+09	3	3	1	0.05556					
1.80E+09	3	3.1	1.0333						
2.40E+09	3	2.1	0.7						