EFFECT OF MICROBIAL INDUCED CALCITE PRECIPITATE ON SHEAR STRENGTH PARAMETERS OF WEAK CLAYEY SOIL

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ABSTRACT

Soil improvement is a key factor when dealing with weak soils in civil engineering works. Conventional soil improvement methods have negative effects on human health and environment which results in the new innovation for soil improvement. Microbial induced calcite precipitation involves the use of bioactivity which involves the introduction of calcite forming microorganism and cementation reagent into a soil matrix to form a cement compound which improves the engineering properties of soil. The natural soil was treated with $1.5x10^8$, $3.0x10^8$, $9.0x10^8$, $18.0x10^8$ and $27.0x10^8$ of *Bacillus megaterium* suspension densities. The soil was classified as clayey sand (SC) according to USCS classification system and A-7-6(2) under the AASHTO classification system. The MDD increased by 0.56% while the OMC decreased by about 2.9% at $1.5x10^8$ and $3.0x10^8$ *B. megaterium* cell/ml respectively when compared with the natural soil. The cohesion was decrease by 53.97% while angle shearing resistance was increased by 36.99% at $3.0x10^8$ *B. megaterium* suspension densities. This result shows a great improvement in the soil since the clayey nature of the natural soil was reduced.

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CHAPTER ONE

1.0 INTRODUCTION

1.1 Background to the Study

Soil is a heterogeneous material which contains a mixture of gravel, sand, silt and organic matter, the classification of soil into groups with analogous behaviour provides guidance to a geotechnical engineer on its properties via accumulated experience (Aasimnaeem, 2015). There are soils that are good for construction in their natural state and there are also others that require improvement before they can be used for civil engineering works.

Soil improvement can be achieved either by modification or stabilization or both, while modification is defined as the improvement of soil by addition of a modifier such as cement or lime to improve its index properties, stabilization is the treatment of soil to enable improvement of their strength and durability such that it is suitable for construction (Alhassan and Mustapha, 2017a). Various methods of soil improvement have evolved as a result of the increasing demand for infrastructural development, occasioned by rapid industrialization and population growth. Some of these methods use conventional soil improving additives (cement, lime and natural pozzolanas), others use agricultural waste products (rice husk ash, bagasse ash, groundnut shell ash). Biological processes have also recently been introduced into the process of soil improvement. One of these biological processes of soil improvement is Microbial Induced Calcite Precipitation (MICP). MICP can be applied to ground improvement and bioremediation. It is referred to as a multi-field study which combines engineering, chemistry and microbiology (Whiffin *et al.*, 2007; DeJong *et al.*, 2010; Rajasekar *et al.*, 2017).

MICP is a bioactivity that involves introduction of calcite forming microorganism and cementing reagent into a soil matrix to form a cement compound that improves the engineering properties of soil (Ahmad, 2019). Fatima and Benoit (2018) defined MICP

(or calcification) as a biochemical process governed by microbial activity to induce precipitation of calcite between soil particles. MICP is applied in sand strengthening, concrete and bricks durability, and manufacturing of bio-concrete (Moneo, 2015).

Generally, conventional ground improvement techniques are either by mechanical compaction which involves high energy consumption or by injection of cement or other chemicals which involve the use of synthetic materials which could be risky to the natural environment and human health. Chemical grout is under research due to the detrimental effect it has on the environment (DeJong *et al.*, 2010). Example is a case in 1974 where acrylamide grout was leached into surrounding water sources in Japan leading to five cases of water poisoning (Karol, 2003). Certain enterprises in countries such as United States propose the ban of most synthetic grouting material (DeJong *et al.,* 2010). Ordinary Portland cement which is easy to use in stabilization and compactible to most soil type but contributes up to 7 percent of world carbondioxide emission (Ariyanti *et al.*, 2011), therefore, there is need to develop an alternative soil improvement technique which is economically feasible, environmentally suitable and can achieve optimum performance.

1.2 Statement of the Research Problem

Most of the known soil stabilization methods rely greatly on mechanical or manmade materials which requires substantial amount of energy for production or installation (Donovan *et al.*, 2016). Chemical stabilization is the most commonly used soil improvement method which is often costly and can cause environmental and health issues (Liang *et al.,* 2016). Stability and safety of structures are affected when founded on weak (or problematic) soil (Mousa *et al.,* 2019). These necessitate the need for new alternative approaches that would be environmentally friendly, sustainable and able to meet the increasing demands for ground improvement especially in Civil Engineering infrastructural development.

1.3 Aim and Objectives of the Study

The aim of this research is to evaluate the effect of microbial induced calcite precipitate on shear strength parameters of weak clayey soil. The objectives of this study are to;

i. Determine the index properties of the soil.
ii. Evaluate effect of microbial induced calcite precipitate on compaction characteristics of the weak clay treated with 0, 0.5, 1, 3, 6 and 9 McFarland standard (MFU) of bacteria suspension (equivalent to $1.5x10^8$, $3.0x10^8$, $9.0 x10^8$, $18.0 x10^8$ and $27.0 x10^8$ cell/ml respectively).
iii. Determine effect of the microbial induced calcite precipitate on shear strength parameters of weak clay soil treated with 0, 0.5, 1, 3, 6 and 9 McFarland standard (MFU) of bacteria suspension (equivalent to $1.5x10^8$, $3.0x10^8$, $9.0 x10^8$, $18.0 x10^8$ and $27.0 x10^8$ cell/ml respectively.

1.4 Justification for the Study

Major problems related to conventional methods of soil improvement are heavy equipment dependent which could disturb urban infrastructure, high pressures required and chemicals which may have significant environmental impact. Methods such as thermal improvement are costlier whereas vertical drains require skilled labour. In terms of environmental sustainability, microbial induced calcite precipitate has shown greater potential in geotechnical engineering applications. This work will help to further understand the geotechnical behaviour of the weak clay soil when treated with *Bacillus megaterium* through microbial induced calcite precipitation process.

1.5 Scope of the Study

This research work is limited to the laboratory investigation of an A-7 (weak clay) soil obtained from along Airport Road in Abuja. The clayey soil was treated with *Bacillus*

megaterium through microbial induced calcite precipitation which was obtained from Microbiology Department Laboratory, Ahmadu Bello University, Zaria, Kaduna State. The Index and Engineering properties of the soil were obtained from the test conducted at Terracorn Engineering Company Limited (Soil Laboratory) located at suite 14, behind Shema filling station along Airport Road, Abuja.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Soil

The term ‘soil’ is derived from a Latin word Solium meaning upper layer of earth crust which can be ploughed and it is used by geologists, agronomists, agriculturists, soil scientists and civil engineers. The product of disintegration and decomposition of rocks via action of physical and chemical agents leading to the breaking down of these rocks into smaller particles is referred to as soil (Abdulmannam, 2016). It is composed of

loosely bounded mineral particle of various sizes and shapes (Safiullah, 2015). Soil formation is affected by parent materials, time, climate, relief and organisms. The four processes of soil formations are addition (by organic matter input, soil from wind erosion), losses (by leaching, erosion), transformation (by weathering of primary particles) and translocation (by movement of organic and inorganic materials). There are different types of soil which are grouped based on origin and engineering consideration (grain sizes).

A. Based on origin: The origin of soils is used in grouping them and they are;

I. Residual soil: These are soils weathered with little or no propensity to move. They are found near the weathered parent rock and are governed by exposure time and climatic.

II. Transported soil: These types of soil are transported from the place of weathering to another location with the aid of transportation agents. Table 2.1 shows different soil types and their transportation agents.

Table 2.1: Different soil types and their transporting agents

Soil Type	Transporting Agent	Properties
Aeolian soils	Wind	Silt (low density & high compressibility)
Alluvial soil	Running water	Coarser and finer particles
Colluvial soil	Gravitational force	
Glacial soil	Glaciers	Finer particles, boulders
Lacustrine soil	Water (deposited at quiet lakes)	Coarser and finer particles
Marine soil	Sea water	Coarser and finer particles

(Source: Mishra, 2010)

III. Organic and inorganic soil: These soils are formed by the growth and decomposition of plants, shells of organisms and inorganic skeleton. Other types of soils used in practised are summarized in Table 2.2.

Table 2.2: Other types of soils used in practise

Soils	Formation and Properties
Bentonite	This is formed by the decomposition of volcanic ash. It exhibit high clay properties such as hydration, swelling and water adsorption. It contains high amount of montmorillonite
Boulder clay	This is a combination of glacial clay, pulverised rocks and unsorted rocks
Kaolin	It is a pure form of white clay. It is also known as China clay and used mainly in clay industries
Loam	This consist of silt, clay and sand
Marl	This consist of clay, loam and calcareous sand
Peat	This is fibrous aggregates with decomposed vegetable matter. It is very compressible
Shale	This material have a state between clay and slate
Varved clays	This consist of layers of silts and flat clays

(Source: Mishra, 2010)

B. Engineering consideration (based on grain sizes): It depends greatly on particle sizes. They includes;

i. Clay (less than 0.002mm): Clay comprises of very fine particles which are flaky in shape. Surface of clay possess electrical charges which help in determining engineering properties of the soil. Clays are commonly brown in colour.

ii. Silt (0.002-0.06mm): Silt possesses slight friction and cohesion. It has high capillarity and mostly brown colour.

iii. Sand (0.06-2mm): Sand has no plasticity but has high strength. It also has high permeability and low capillarity. It is mostly grey in colour.

iv. Gravel (2-60mm): Gravel has high frictional resistance and is a good material for foundation. Gravel can be angular in shape (crushed from rock) or rounded (taken from river beds).

v. Cobbles and boulders: Cobbles are usually between 60-200mm while above 200m particle size is termed boulders.

vi. Organic matter: Plant and animal remains are the main sources of organic matter. They are found at the top surface of the soil.

Based on their performance under Civil Engineering construction, soil can be competent or deficient.

2.1.1 Deficient soil

Soils that do not meet the essential criteria to function (such as base courses in road construction, embankments in dams, subsoil base for foundation and liners for containment of leachates) as a geotechnical structure is referred to as a deficient soil (or weak soil), (Alhassan and Mustapha, 2015). These deficient soils have to be improved in terms of stability and strength in other to make them suitable for construction works (Alhassan and Mustapha, 2017b).

2.2. Soil Improvement

Some soil found at construction site may not be suitable for supporting structures in its natural form therefore, it will need to be improved in other to increase its bearing capacity and reduce settlement (Gaafar *et al.,* 2015). Soil improvement is the alternation of soil properties for the purpose of improving its engineering properties which can be strength, permeability or ground water condition (Mishra, 2010). There are various methods of soil improvement

2.2.1 Conventional soil improvement methods

2.2.1.1 Surface compaction

This is the oldest form of soil improvement and it is used when the depth to be compacted is small. Smooth wheel, rubber-tyred, sheep foot, vibratory and grid rollers are examples of equipment that can be used to achieve surface compaction (Gopal, 2021).

2.2.1.2 Drainage method

During excavation for any construction work, ground water is one of the major problems encountered which increases pore pressure and reduces shear strength. Dewatering can

be achieved by well points systems, deep well drainage, dewatering by electro-osmosis and pumping by open sump (Gopal, 2021).

2.2.1.3 Vibration method

This method is applicable to non-cohesive soils. This is used for densifying loose sands to create stable foundation soils. Vibration and shock causes liquefaction and densification of loosed materials (soils) which result in dissipation of excess pore water pressures. Examples of equipment used in vibratory method includes vibratory rollers, vibro-displacement compaction piles, vibrating probe and blasting, Vibrofloatation (Gopal, 2021).

2.2.1.4 Grouting method

This is the injection of materials (grouts) into a soil or rock formation to improve the properties of the soil or rock. Grouting increases soil strength, rigidity and reduces ground movement. Permeation, compaction, fracture, jet grouting are examples of grouting method used in soil improvement. This method controls ground water, prevents excessive settlement, and strengthens the soil and adjacent foundation (Gopal, 2021).

2.2.1.5 Preloading (pre-compression) method

This is a process which involves the placement of additional vertical stress on a compressible soil to remove pore water over time. Pore water dissipated reduces the total volume causing settlement. Preloading reduces secondary compression and improves bearing capacity (Gopal, 2021).

2.2.1.6 Chemical stabilization method

Soils are also improved by addition of different chemicals which can improve strength, increase bearing capacity and decrease settlement. Chemical stabilization is more

expensive than other types of stabilization techniques. Chemicals used for soil stabilization are lime, cement and fly ash (Gopal, 2021).

2.2.1.7 Mechanically stabilized earth structures

This involves the use of metallic (strip or bar mat) or geosynthetic (geogrid or geotextile) reinforcement which is connected to a precast concrete or prefabricated metal facing panel to create a reinforced soil mass. Geotextiles are primarily petroleum products and they are described as a porous fabric manufactured from synthetics materials. Geotextiles can be used as separators, filters, drains, geomembranes and reinforcement (Gopal, 2021).

2.2.1.8 Soil reinforcement method

This is when a weak soil is reinforced by a high-strength thin horizontal membrane. Varieties of material such as rubber, aluminium and thermoplastics have been used (Gopal, 2021).

2.2.1.9 Heating (vitrification) method

Soil particles are broken down to form crystalline or glass product. It involves the use of electric current to heat the soil and modify its physical characteristics. The temperature of heating ranges from 300 – 1000°C. Vitrification of soil causes immobility of radioactive or contaminated soil (Gopal, 2021).

2.2.1.10 Ground freezing method

This is an example of drainage method and it can be used as a temporary underpinning. It prevents ground water from flowing into an excavated area. This involves the process of refrigerating which convert in-situ pore water to ice and acts as a cement or glue, bonding together adjacent particles of soil or blocks of rock to increase their combine strength thus making them impervious (Gopal, 2021).

2.2.1.11 Soil nailing

This involves the process of reinforcing the ground by passive inclusions which are closely spaced to create in-situ soil and restrain its displacement. Soil nailing is used in railroad and highway cut slopes. It is also used in funnel portals in steep and unstable stratified slopes (Gopal, 2021).

2.2.1.12 Micro-piles

These are small diameter piles (usually 300mm) which have the ability of sustaining high loads. Micro-piles are used for structural support, stability and foundation. It is also used to prevent movement as well as soil strengthening (Gopal, 2021). Some of the advantages and disadvantages of conventional soil improvement methods are summarized in Table 2.3.

Table 2.3: Summary of advantages and disadvantages of conventional soil improvement method

Methods	**Advantages**	**Disadvantages**
Dewatering	Effective as a primary step in waterlogged areas	Expensive as continuous pumping is necessary
Earthquake drains	Effective dissipation of pore pressure	Difficulty in treating a single liquefiable layer
Vibratory	Applicable to granular soils. Stone column tend to dilate as they get sheared during an earthquake providing better anchorage	Costly if deep single liquefiable layers need to be treated. Cannot be used in cohesive soils
Deep dynamic compaction	Economic site improvement technique for arrange porous soil type	Existing structures acts as hindrance
Permeation set compaction	Deep isolated liquefiable layers can be treated	Underground utilities such as pipeline may prove to be a hindrance
Removal and replacement	Reliable measure as tendency of liquefaction is eliminated	Costly method to be practised. Practicably only above ground water table
Pre-compression	Cost is comparatively low as it uses conventional earth moving equipment	It can have unacceptable environmental impact like dust, nose, and traffic on adjacent

	areas. Cannot be used for confined sited

(Source: Muhammed *et al.,* 2018)

2.2.2 Biological soil improvement method

The concept of using biological process in improvement of soil is known as biomediated soil improvement technique. This technique involves the use of biochemical reactions that occur in a soil mass which produces calcite precipitation which modifies engineering properties of soils (DeJong *et al.,* 2010). It is also referred to as a biological process involving chemical reaction whose by-products have the ability to improve the geotechnical properties of soil (Muhammed *et al.,* 2018). This method utilizes microbial processes known as Microbial Induced Calcite Precipitation (MICP) which precipitates calcium carbonate in a soil matrix.

2.2.2.1 Microbial metabolic processes involved in MICP

Microbial metabolic process in MICP includes autotrophic metabolic process (which are photosynthesis and methane oxidation) and heterotrophic metabolic process (which are urea hydrolysis (Ureolysis), ammonification of amino acid, dissimilatory sulphate reduction and denitrification). Osinubi *et al.* (2019) investigated the strength of compacted lateritic soil improved with MICP and reported a decrease in unconfined compressive strength values with increasing *S. pasteurii* suspension density and moulding water content, relative to optimum for the compactive effort used. The research suggested that for construction of liners and covers in municipal solid waste containment systems, a suspension density of $1.20x10^9$ cells/ml can be used with lateritic soils.

Sanchita *et al.* (2019) carried out an investigation on soil stabilization using MICP. The shear strength of the black cotton soil was increased, while the hydraulic conductivity was reduced on the addition of *Bacillus megaterium* microorganism. Geotechnical application of MICP includes cementation of sands to enhance the bearing capacity and liquefaction resistance, soil erosion control, crack healing in concrete and masonry and remediation of radionuclide and metal contaminated soils (Mark *et al.*, 2019).

Sani and Bala (2021) carried out a research on microbial induced modification of silty soil using *Bacillus coagulans* for building foundation and proved that the bearing capacity for square footing increased from 977.90 to 1289.90 kN/m^2 (approximately 31.59%) when compared with the natural soil at $18.0x10^8$ *B. coagulan* cell/ml.

Soon *et al.* (2013) studied the optimum condition for improving engineering properties and concluded that, improvement was achieved for undrained shear strength when residual soil was treated using MICP process. Lee *et al.* (2012) researched on the effect of microbial induced calcite precipitation on shear strength and hydraulic conductivity of sandy and residual soils (sandy silt). The study concluded that MICP increased shear strength and decrease hydraulic conductivity for both soils.

Van *et al.* (2010) carried out a research on the fixation and distribution of bacterial activity in sand to induce carbonate precipitation for ground reinforcement and suggested that urea hydrolysis possess the highest calcite conversion rate compared to other processes. Urea hydrolysis refers to a chemical reaction where urea $\{CO(NH_2)_2\}$ is decomposed by urease enzyme that can be either supplied externally or produced in-situ by urease-producing microorganisms (Greene *et al.*, 2003; DeJong *et al.*, 2006).

Osinubi *et al.* (2018) studied the strength of tropical residual lateritic soil treated with *Sporosarcina pasteurii* and stated that at $1.2x10^9$ cell/ml *S. pasteurii* suspensions, a peak

unconfined compressive strength was obtained as an optimal treatment for the lateritic soil. It was stated that MICP occurs through extracellular means though studies have shown intracellular precipitation of calcium carbonates in cyanobacteria, (Cam *et al.,* 2015). In an investigation carried out by Xu *et al.,* (2019) on the precipitation of calcite and aragonite using virus induced lysis of cyanobacteria cells, it was concluded that this is a new mechanism in expanding the calcium carbonate bio-mineralization process. MICP is generally achieved by the following six processes.

a) Hydrolysis of urea (Ureolysis)

Naturally urea is found in the environment but artificially, it can be injected comprising mainly of synthetic nitrogen fertilizers which is used globally (Glibert *et al.,* 2006). Urease activity in soil is as a result of heterotrophic microorganisms (Soon *et al.,* 2013). It has been demonstrated that some chemoautotrophic ammonium oxidizing bacteria have the capability to grow on urea which can serve as the only carbon, nitrogen and energy source (Marsh *et al.,* 2005). Ureases are useful in biomineralization since it promotes the formation of calcium carbonate (Anbu *et al.,* 2016).

Microbial urease catalyzes the hydrolysis of urea into ammonium and carbonate. One mole of urea is hydrolyzed intercellularly to one mole of ammonia and one mole of carbamic acid (Okyay and Rodriguez, 2015).

$$CO(NH_2)_2 + H_2O \longrightarrow NH_2COOH + NH_3 \quad (2.1)$$

Carbamic acid hydrolysis to form one mole of ammonia and carbonic acid

$$NH_2COOH + H_2O \longrightarrow NH_3 + H_2CO_3 \quad (2.2)$$

Ammonium and carbonic acid reacts with water to form bicarbonates and two moles of ammonium and hydroxide ions.

$$2NH_3 + 2H_2O \longrightarrow 2NH^+_4 + 2OH^-$$

(2.3)

$$H_2CO \longrightarrow HCO_3^- + H^+$$

(2.4)

The production of hydroxide ion leads to an increase in pH which then results in bicarbonate equilibrium leading to the formation of carbonate ions.

$$HCO_3^- + 2NH_4^+ + 2OH^- \longleftrightarrow CO_3^{2-} + 2NH_4^+ + 2H_2O$$

(2.5)

In the presence of calcium ions, the carbonate ions precipitate to produce calcium carbonate crystals.

$$Ca^{2+} + CO_3^{2-} \longleftrightarrow CaCO_3$$

(2.6)

The formation of monolayer of calcite further increases the affinity of the bacteria to the soil surface resulting in the production of multiple layers of calcite. Urea hydrolyzing bacteria are *Escherichia coli*, *Sporosarcina pasteurii*, *Bacillus megaterium*, *Bacillus firmus*, *Myxococcus species*, *Methlocystis parvum*, *Brucella*, *Proteus mirabilis*, *and Pseudomonas dentificans* (Dhami *et al.*, 2014).

b) Ammonification of amino acids

Microbial activity produces carbondioxide and ammonia during the metabolism of amino acids

$$\text{Amino acids} + O_2 \longrightarrow NH_3 + CO_2 + H_2O$$

(2.7)

When ammonia is hydrolyzed, it produces ammonium and hydroxide ion which results in super-saturation which favours precipitation of calcium carbonate (Zhu and Dittrich, 2016).

$$NH_3 + H_2O \longrightarrow NH_4 + OH^-$$

(2.8)

$$CO_2 + OH \longrightarrow HCO_3^-$$

(2.9)

$$Ca^{2+} + HCO_3^- \longrightarrow CaCO_3 + H^+$$

(2.10)

Myxococcus xanthus precipitates uranium as meta-autunite used to protect concrete structures exposed to radioactive waste (Turick and Berry, 2016).

c) Denitrification

MICP results from the oxidation of organic matter using NO^{3+} as the final electron acceptor. The process produces Nitrogen dioxide, Carbon dioxide and Hydroxide. The bacteria create an alkaline microenvironment by the consumption of H^+ in the presence of soluble calcium ion (Zhu and Dittrich, 2016).

$$(CH_3COOH)_2Ca + NO_3 \longrightarrow CaCO_3 + 45NO_2 + 3CO_2 + 3H_2O + OH$$

(2.11)

Denitrification process is limited by the accumulation of toxic by product generated such as nitrate and nitrous oxide.

d) Dissimilatory sulfate reduction

In anaerobic environment rich in organic matter, calcium present induces the formation of calcium carbonate minerals indirectly by sulfate reducing bacteria (SRB) due to dissimilatory sulfate reduction process.

$$6CaSO_4 + 4H_2O + 6CO_2 \longrightarrow CaCO_3 + 4H_2S + 11O_2$$

(2.12)

Perito and Mastromei, (2011) showed that *Desulfovibrio species* has the ability to precipitate calcium carbonate through the removal of sulfates from gypsum ($CaSO_4.H_2O$) using a combination of these three mechanisms: dissolution, diffusion and calcium carbonate. Calcium ion released by gypsum dissolution reacts with carbondioxide under an alkaline pH environment due to sulfide removal which forms calcium carbonate precipitation.

e) Photosynthesis

In aquatic environment, cyanobacteria and microalgae are the main microorganism responsible for MICP. Calcium carbonate precipitations by photosynthetic microorganism occur due to bicarbonate and carbon trioxide exchange.

$$Ca^{2+} + 2HCO_3^- \longrightarrow CaCO_3 + CO_2 + H_2O$$

(2.13)

Bicarbonates are diffused through the membrane and dissociates in cytosol of the cells into carbondioxide and hydroxide ion. The reaction is catalyzed by carbonic anhydrase resulting to an increase in pH due to hydroxide ion generation which along with calcium ion present in the microenvironment induces calcite precipitation.

$$Ca^{2+} + HCO_2^- \longrightarrow CaCO_3 + 2H_2O$$

(2.14)

$$2HCO_2^- \longleftrightarrow CO_2 + CO_3^{2-} + H_2O$$

(2.15)

Seifan *et al.* (2016) reported that the use of photosynthetic microorganisms as an agent of bioconcrete is achieved only when structures are exposed to carbondioxide and sunlight which are the basic constituents of photosynthetic process.

f) Methane oxidation

Carbondioxide concentration is driven by methane oxidizing bacteria under both aerobic and anoxic conditions in marine and freshwater sediments. In aerobic conditions, this process is initiated with the conversion of methane to methanol by methane mono-oxygenase activity in the presence of oxygen (Ersan, 2019).

$$CH_4 + O_2 \longrightarrow CH_3OH + H_2O \quad (2.16)$$

Methanol is converted to formate through enzymatic processes. When formate is at equilibrium with formic acid, methane mono-oxygenase oxidizes formic acid to carbondioxide (Ersan, 2019).

$$CH_3OH \longrightarrow CHOH \quad (2.17)$$

$$CHOH + H_2O \longrightarrow HCOO^- + OH^+ \quad (2.18)$$

$$HCOO^- + H_2O \longrightarrow HCOOH + OH^- \quad (2.19)$$

$$HCOOH \longrightarrow CO_2 \quad (2.20)$$

The carbondioxide produced turns into carbon trioxide and calcium carbonate is precipitated in the presence of calcium ion (Ersan, 2019).

$$Ca^{2+} + CO_2 + 2OH^- \longleftrightarrow CaCO_3 + H_2O \quad (2.21)$$

In the presence of calcium ion, anaerobic methanotrophic bacteria produce bicarbonates as a result of methane anaerobic oxidation with sulfate as the final electron acceptor (Seifan *et al.,* 2016).

$$CH_3^{2-} + H_2O \longleftrightarrow HCO_3^-$$

(2.22)

$$CH_4 + SO_4^{2-} \longrightarrow HCO_3^- + HS^- + H_2O$$

(2.23)

2.2.2.2 Factors affecting microbial induced calcite precipitation process

a) Reagent concentration

There is a decrease in the efficiency of MICP at higher concentration of reagent. DeMuynck *et al.* (2010) concluded from one of their research that the efficiency of calcite formation ratio dropped from 0.66 to 0.56 and 0.33 as the concentration of urea calcium chloride increased from 0.25 to 0.5M and 1.0M respectively.

b) Potential of hydrogen (pH)

Urease enzyme is active at a certain pH. The change in pH level, which is due to the formation of the hydroxyl ions (OH^-) generated from the production of ammonium ions (NH_4^+) helps to create an alkaline environment suitable for calcite precipitation (DeJong *et al.,* 2010). In soil bio-cementation, variability of the pH values can influence the bacterial transport and adhesion, which is an important factor affecting homogeneity in the distribution of calcite crystals precipitation. *Bacillus megaterium* had an optimum pH of 7-9 and urease activity peaked at pH of 7 (Khan *et al.,* 2011). The precipitation of calcite is influenced by pH because urease enzymes are only active at specified pH values for urea hydrolysis. Many researchers reported an optimum pH value of 8.0 for urease (Gorospe *et al.,* 2013).

c) Bacteria cell concentration

A high bacterial concentration supplied to the soil sample increases the amount of calcite precipitate from MICP process (Okwadha and Li, 2010). The rate of urea hydrolysis has a direct relationship with the bacterial cell concentration as long as there is adequate amount of cementitious reagent. High concentration of bacteria produced more urease per unit volume to commence the urea hydrolysis (Soon *et al.,* 2012).

d) Nutrients

Bacteria obtain energy from nutrients therefore, it is important to provide sufficient and adequate nutrient for urease-producing bacteria. Common bacteria nutrient are carbondioxide, nitrogen, potassium, phosphorus, magnesium, calcium and lead. Bacteria need nutrient to sustain long calcite precipitation process in order to achieve the desired improvement level (Soon *et al.,* 2014).

e) Type of bacteria

Bacteria types are usually urease positive bacteria. Bacteria used in microbial induced calcite precipitation must be able to catalyse urea hydrolysis. Aerobic bacteria are preferable as they release carbondioxide from cell respiration. Carbondioxide production is paralleled by the pH rise due to ammonium production (Soon *et al.,* 2014: Umar *et al.,* 2016).

f) Temperature

The rate of microbial induced calcite precipitation is affected by urease activity in which temperature is of significant influence. The effect of temperature on microbial induced calcite precipitation is complex as it affects the urease activity of microorganisms, growth and nucleation rate of calcite crystals and solubility of calcite (Donovan *et al.,* 2016).

g) Degree of saturation

Lower degree of saturation gives higher strength at even lower calcite precipitation within a soil matrix (Cheng *et al.,* 2013). Degree of saturation of bio-cemented soil during MICP treatment controls and restricts the distribution of calcium carbonate crystals to interparticle contact point (Cheng and Cord-Ruwish, 2012).

2.2.2.3 Application of microbial induced calcite precipitation

i. Remediation for heavy metal and radionuclide contamination

MICP technique is used for containment of various contaminants and heavy metals (Cuningham *et al.,* 2009). Lead in soil can be reduced by chelation which involves the use of microbial induced calcite precipitation products to immobilize the lead. It is also applied to achieve sequestration of heavy metals and radionuclides (Donovan *et al,* 2016). Microbial induced calcium carbonate precipitation of radionuclide and contaminant metals into calcite is a competitive co-precipitation reaction in which suitable divalent cations are incorporated into calcite lattices (Hamdan *et al.*, 2011).

ii. Material science

Jagadeesha *et al.* (2013) stated that microbial induced calcite precipitation can be used as a long-lasting remediation technique as it has high crack cementation potential for structural formation such as concrete and granite.

iii. Liquefaction prevention

Microbial induced calcite precipitation has been proposed as an alternative cementitious technique in improving the properties of potentially liquefiable sand (Mortensen *et al.,* 2011). It has been shown in research that there is a linear relationship between amount of carbonate precipitation and the increase in strength and porosity (Soon *et al.*, 2014).

iv. Treatment of concrete

Due to the calcium carbonate precipitation, Microbial induced calcite precipitation has been observed to have prolonged concrete service life (Chen *et al.,* 2016). The cracking in concrete is healed by the calcium carbonate solidifying at the surface of the crack (Jagadeesha *et al.,* 2013). This treatment results in an increase in strength and durability of the concrete.

v. Bricks

The metropolis next generation design competition held in 2010 was won by architect Ginger Krieg Dosier that used microbial induced calcite precipitation to produce brick while lowering the carbondioxide emission (Suzzane, 2010). BioMASON incorporation founded by this architect uses microorganisms and chemical processes to manufacture building materials.

vi. Filler for rubber, plastics and ink

Microbial induced calcite precipitation technique can be utilized in the production of material that can be used as filler in rubber and plastics, fluorescent particles in stationary ink and fluorescent marker for biochemistry application such as protein immunoblot (Yoshida *et al.,* 2010). Protein immunoblot which is also known as western blot is used to detect specific proteins in a sample tissue (Soon *et al.,* 2013).

2.3 *Bacillus megaterium*

Bacillus megaterium is a rod-like, gram positive, mainly spore forming bacterium found in widely diverse habitat (Vos *et al.,* 2009). It is one of the biggest known bacteria having a cell length of up to 5µm and a diameter of 1.5µm (Lee *et al.,* 2012). It grows at a temperature range of 3 to 45°C with 30°C as it optimum. *B. megaterium* as an important industrial organism produces penicillin amidase which is used in making synthetic

penicillin, amylases used in baking industry, used in drugs manufacturing such as vitamin B_{12} and pyruvate (Vary *et al.,* 2007). *B. megaterium* is a common soil bacterium, an endophyte and it is shown in Plate I. It can be found in variety of surfaces such as clinical specimens, leather, paper, and stone (Mohammad *et al.,* 2020).

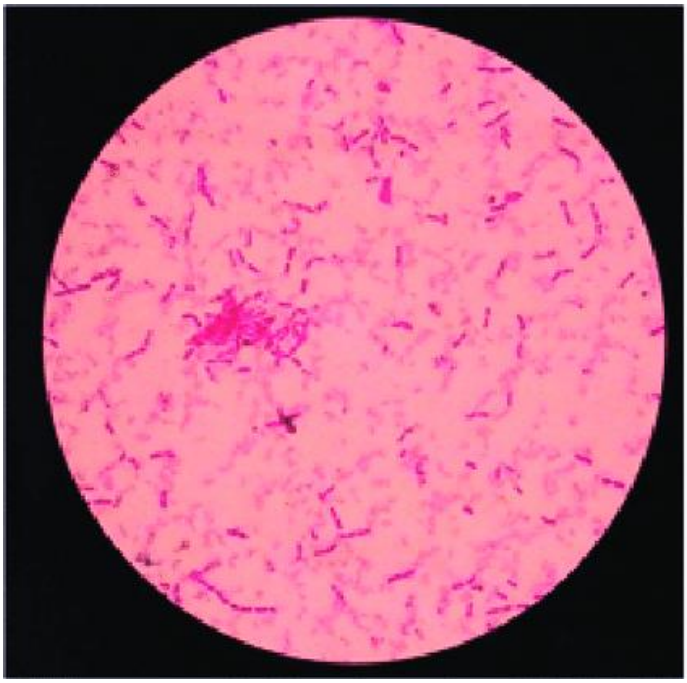

Plate I: *Bacillus megaterium* (Source: Andriani *et al.*, 2017)

2.4 Microbial Culture and Application

Microbial culture is a process of multiplying microorganisms by allowing them reproduce in predetermined culture medium under controlled laboratory condition. The main purpose of microbial culture is to determine the type of microorganism and its abundance in a sample under study. Other purposes of bacteria culture according to (Ahmad, 2016) are:

a) Isolation bacteria.

b) To know the properties of bacteria.

c) To maintain stock culture.

d) To enable estimation of viable count.

e) To test for antibiotic sensitivity.

f) To create antigens for laboratory uses.

Table 2.4: Culture methods

Culture Method	Uses
Streak culture	Bacteria isolation in pure culture from clinical specimens
Lawn culture	Provides uniform surface growth of the bacterium. It is used in preparing bacterial antigens and vaccines
Stroke culture	Provide a pure growth of bacterium for slide agglutination and other diagnostics tests
Stab culture	It is used in demonstrating gelatin liquefaction and also to maintain stock culture
Pour plate culture	It gives an estimation of the viable bacterial count in suspension and quantitative urine cultures
Broth culture	It help scientist grow large amounts of bacteria for variety of application
Agar plate culture	It is used when creating engineered strain of bacteria containing antibiotic-resistance gene
Solid plate culture (thermophilic microorganism)	It is used for microorganism having a growing temperature of 50-70^{0}C

(Source: Samira 2015; Ahmad 2016; Shinde 2019)

2.4.1 Culture media

Culture media (growth media) is referred to as a solid, liquid or semi-solid medium designed to support the growth of microorganisms or cell through a process of cell proliferation (Hadeler *et al.*, 1995). It is composed of water, nutrients, mineral salt and suitable pH of between 7.2 - 7.4 (Samira, 2015). Different types of culture media and their respective uses are summarized in Table 2.5.

Table 2.5: Bacteria Culture Media

Classification	Types	Function / Uses
Consistency	Solid media	For isolating bacteria and determining colony characteristics of the isolate
	Semisolid media	Cultivation of microaerophilic bacteria and determination of bacterial mobility
	Liquid (broth) media	Fermentation studies and propagation of large number of organisms
Nutritional components	Simple media	Peptone water, nutrient agar are used to fastidious bacteria
	Complex media	Special ingredient like yeast are used in microorganism growth
	Synthetic or chemically defined media	It is used in research purpose where the composition of every component is well known
Purpose or functional use	Enriched media	It is used to grow nutritionally exacting bacteria and to isolate pathogen from a mix culture. For example Chocolate agar, blood agar
	Selective and enrichment media	It is designed to inhibit unwanted commensal or contaminating bacteria and help recover pathogen from a mixture of bacteria. For example campylobacter agar
	Differential (or indicator) media	It recognizes different bacteria based on colony colour. For example MacConkey agar
	Transport media	It is used in clinics to prevent drying of specimen during transportation and also to inhibit overgrowth of unwanted bacteria. For example, Cary Blair, Alkaline peptone water
	Assay	It is used for testing for Vitamins, amino acids and antibiotics
	Anaerobic media	The media usually have supplement and nutrients like hemin and vitamin K. For example, Thioglycollate medium

(Source: Samira 2015; Ahmad 2016; Shinde 2019)

2.5 Biomineralization

Biomineralization is a natural process aided by living organisms and it is referred to as a process whereby living organisms produced minerals which eventually hardens the existing tissues (Vert *et al.,* 2012). Examples of minerals obtained from these organisms are silicates in algae and diatoms, carbonates in invertebrates, calcium, phosphate and carbonates from vertebrates. Achal *et al.* (2012) stated that biomineralization is divided into three different mechanisms: Biologically Controlled Mineralization (BCM), Biologically Induced Mineralization (BIM) and Biologically Mediated Mineralization (BMM).

2.5.1 Biologically controlled mineralization

Metabolic activity of the microorganism controls growth, morphology and location for deposition of the mineral. This mechanism could be intracellular, intercellular or extracellular with the precipitation of organic macromolecule exopolysaccharides or vesicles (Sherma and Vincent, 2015).

2.5.2 Biologically induced mineralization

Modification of chemical environment like change in pH leads to mineralization. The reaction between metabolic byproducts of microorganisms and ions present in the environment results in mineral precipitation. Minerals generated in biological induced mineralization are characterized by wide range in particle size, poor crystallinity and morphology (Vermai and Geerat, 2013).

2.5.3 Biologically mediated mineralization

Mineral formation is due to the interaction between an organic matrix and an organic and/or inorganic compound without the necessity of extracellular or intracellular biological activity (Achal *et al.,* 2015).

2.6 Bioclogging and Biocementation

Bioclogging is a process where soil voids is filled by the product from microbial induced biochemical process. Bioclogging of soil restrict water flow through soil and permeable rock leading to a reduction in hydraulic conductivity. It can be used to close leaky construction pit, landfills or dike (Osinubi *et al.,* 2020). Example of the application of bioclogging is in landfill liners where the hydraulic conductivity of a compacted clay liner is lowered due to the microorganisms in the leachate occupying the pores spaces of the clay (Aldaeef and Rayhani, 2015: Tang *et al.,* 2015).

Biocementation on the other hand, is one of the common processes of achieving MICP. It is a branch of geotechnical engineering that deals with the application of microbial activity to improve the engineering properties of soil (Osinubi *et al.,* 2020). Biocementation improves shear strength of soil through the production of soil-particle binding material with the use of a bacteria and cementing reagent in the soil. Cementitious reagents are mostly carbonates, silicates, sulphides and hydroxide (Ivanov and Chu, 2008). Different possible microbial process that may lead to bioclogging and biocementation are summarized in Tables 2.6 and 2.7 respectively.

Table 2.6: Microbial process that can lead to potential bioclogging

Physiological Group of Microorganism	Mechanism of Bioclogging	Essential Condition for Bioclogging	Potential Geotechnical Application

Algae and cyanobacteria	Formation of impermeable layer of biomass	Light penetration and presence of nutrients	Reduction of water infiltration into slopes and seepage control
Aerobic and facultative anaerobic heterotrophic bacteria	Production of slime in soil	Presence of Oxygen and medium with ratio of C:N<20	Avoid cover for soil erosion control and slope
Oligotrophic microaerophilic bacteria	Production of slime in soil	Low concentration Oxygen and medium with low concentration of Carbon source	Reduce drain channel erosion and seepage control
Nitrifying bacteria	Production of slime in soil	Presence of Ammonium and Oxygen in soil	Reduce drains channel
Sulphate reducing bacteria	Production of undissolved sulphides of metals in soil	Anaerobic conditions, presence of Sulphite and Carbon source in soil	Form grout curtains to reduce the migration of heavy metals and organic pollutants
Ammonifying bacteria	Formation of undissolved carbonates of metals in soil	Presence of Urea and dissolved metal salt	Prevent piping in earth dams and dikes

(Source: Ivanov and Chu, 2008)

Table 2.7: Microbial processes that can lead to potential biocementation

Physiological Group of Microorganism	**Mechanism of Biocementation**	**Essential Condition for Biocementation**	**Potential Geotechnical Application**
Sulphate-reducing bacteria	Production of undissolved sulphides of metals	Anaerobic condition, presence of sulphate and carbon source in soil	Enhance stability for slopes and dams

Ammonifying bacteria	Formation of undissolved carbonates of metals in soil due to increased pH and release of carbondioxide	Presence of urea and dissolved metal salt	Mitigate liquefaction of potential sand. Enhance stability for retaining walls, embankment and dam. Increase bearing capacity of foundations
Iron-reducing bacteria	Production of ferrous solution and precipitation of undissolved ferrous and ferric salt and hydroxides in soil	Anaerobic condition change for aerobic condition, presence of ferric minerals	Densifying soil on reclaimed land sites and prevents soil avalanching. Reduce liquefaction potential of soil

(Source: Ivanov and Chu, 2008)

2.7 McFarland Standards

McFarland standards are standards which help to adjust approximately the number of bacteria present in a liquid suspension. It is made up of mixing specified amount of barium chloride ($BaCL_2$) and sulfuric acid reagent which forms barium sulfate precipitate used in turbidity formation in a solution (Cockerill *et al.,* 2012). McFarland standard is used as a reference to produce solutions which contains approximately similar number of bacteria which is used in standardized microbial testing. This is carried out by matching the turbidity (cloudiness) of McFarland standard with that of the test solution, (Pakpour and Horgan, 2021). This means that if the bacterial suspension is too turbid, it can be diluted and if it is not turbid enough, more bacteria can be added. Table 2.8 shows different turbidity and their respective bacteria suspension densities.

Table 2.8: Guidelines for the preparation of McFarland standard

McFarland standard (ml)	1% $BaCL_2$	1%H_2SO_4	No of bacteria (10^8)/ml represented
0.5	0.5	99.5	1.5
1.0	1.0	99.0	3.0
2.0	2.0	98.0	6.0
3.0	3.0	97.0	9.0
4.0	4.0	96.0	12.0
5.0	5.0	95.0	15.0
6.0	6.0	94.0	18.0
7.0	7.0	93.0	21.0
8.0	8.0	92.0	24.0
9.0	9.0	91.0	27.0
10.0	10.0	90.0	30.0

(Source: Chapin and Lauderdale, 2003)

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Materials

3.1.1 Sample collection

The soil used for this study was obtained using disturbed sampling method. It was obtained at a depth of about 0.5-1.5m below the earth surface at Latitude 9˚1′25″ N and

Longitude 7°25′49″ E along Airport Road, Abuja. The soil sample was air dried as shown in Plate II.

Plate II: Air-drying of the sample after collection

3.1.2 Isolation of microorganisms

The pure isolated bacterium (*Bacillus megaterium*) was cultured and classified from another soil. It was obtained from Microbiology department, Ahmadu Bello University Zaria, Kaduna state. The bacteria suspension densities used includes 0.5, 1, 3, 6 and 9 McFarland standards (that is $1.5x10^8$, $3.0x10^8$, $9.0 x10^8$, $18.0 x10^8$ and $27.0 x10^8$ cell/ml respectively).

3.1.3 Cementation reagents

The cementation reagent was mixed according to Stock-Fischer *et al*., (1999); Park *et al*., (2014). The reagent contained 3g of nutrients broth, 10g of ammonium chloride (NH_4Cl), 20g of Urea, 2.12g of Sodium bicarbonate ($NaHCO_3$) and 2.8g of Calcium Chloride ($CaCl_2$) per litre of distilled water. Cementation reagents for microbial induced calcite precipitation process consist of urea and calcium which serves as the major ingredients in promoting calcite precipitation.

3.2 Methods (Laboratory Tests)

3.2.1 Natural moisture content

The natural moisture content was determined in accordance with BS 1377-2(1990). Four labelled weighing cans were cleaned and weighed to the nearest 0.01g as M_1. The sample was gently crushed and placed loosely in the cans. The cans and the sample were weighed together to the nearest 0.01g as M_2. The weighed samples were placed in the oven to dry at a temperature of about 105°C - 110°C for 24hours. The samples were then removed and allowed to cool before weighing to the nearest 0.01g as M_3. An average of all four samples was taken and the moisture content was computed using equation 3.1. Results are presented in Appendix A (Table A1).

$$w = \frac{m2-m3}{m3-m1} x\ 100\% \qquad (3.1)$$

where,

W is the moisture content (%)

M_1 is weight of empty cans (g)

M_2 is weight of cans and wet soil (g)

M_3 is weight of can and dry soil (g)

3.2.2 Consistency limits

Consistency limit is the water content at which soil changes from one state to another. The test liquid limit and plastic limit were done in accordance to BS 1377-2(1990). The liquid limit was determined using the cone penetrometer apparatus. The natural soil was crushed and then passed through BS No 40 sieve (0.425mm aperture). The sample was weighed, water was added and the sample was mixed thoroughly on a tray. A cup (50mm diameter and a height of 50mm) was filled with the sample ensuring there was no entrap air. The excess soil was removed ensuring there was a levelled surface. The cup was placed below the cone and the cone was gradually lowered until it touches the soil surface. The graduated scale was adjusted to zero. The cone was released and allowed to penetrate the soil for 30 seconds. The readings were recorded to the nearest 0.01mm. Part of the soil was placed in the moisture content can for moisture content determination. The cone was gently cleaned and the soil in the cup was removed and placed on the tray. The process was repeated three times. The results are presented in Appendix B (Table B1).

The plastic limit is the lowest moisture content which the soil is plastic and it was determined in accordance to BS 1377-2(1990). The natural soil was crushed and then passed through BS No 40 sieve (0.425mm aperture). The sample was weighed, water was added and the sample was mixed thoroughly on a tray. 20g of the soil paste was divided into smaller parts which were then rolled gently between palm and the top of the glass plate. The rolling continued until the sample is rolled to 3mm diameter or when the soil begins to crumble. A 3mm diameter rod is often used to measure the thickness of the thread when conducting the test. The 3mm diameter soil or the crumbled soil is then placed in the moisture contents cans. The cans were weighed, placed in the oven to dry and then weighed again after drying. The moisture content was computed. Results are

presented in Appendix B (Table B2). Plate III shows the liquid limit and plastic limit soil samples for natural and treated oil.

Plasticity Index (PI) is obtained numerically by calculating the difference between the liquid limit and the plastic limit of the soil sample as shown in equation 3.2 and results are presented in Appendix B (Table B3).

$$PI = LL - PL \tag{3.2}$$

where,

PI is plasticity index, LL is the liquid limit and PL is the plastic limit

Plate III: Liquid limit and plastic limit test

3.2.3 Specific gravity

The test was carried in accordance to BS 1377-2(1990). The empty density bottle and stopper was weighed as M_1. Part of the air-dried sample was placed the bottle and weighed as M_2. Water was added to the soil, just enough to cover the soil and the stopper was placed. The soil and water was mixed thoroughly by shaking the bottle to remove air bubbles. The stopper was removed and water was added to the mark of the density bottle.

The density bottle was weighed as M_3. The density bottle was emptied, cleaned and filled with water alone and weighed as M_4. The specific gravity was computed using equation 3.3. The process was repeated and the results are presented in Appendix C (Table C1 and C2).

$$Gs = \frac{m2-m1}{(m4-m1)-(m3-m2)}$$

(3.3)

where,

Gs is specific gravity

M_1 is weight of the empty density bottle and stopper (g)

M_2 is weight of the density bottle, stopper and soil (g)

M_3 is weight of the density bottle, stopper, soil and water (g)

M_4 is weight of the density bottle filled with water only (g)

3.2.4 Compaction characteristics of test soil

Compaction test was carried out using British Standard Light (BSL) compactive effort and was in accordance to BS 1377-4(1990). 3000g of the air dried soil sample passing through sieve 0.425mm was weighed. The sample was moisturized with eight (8) percent of the total mass of the soil and mixed thoroughly. Three (3) percent increment of water was used for each compaction experiment (that is, 8, 11, 14 and 17%). The mould was measured as M_1 and the base and collar were properly placed. The sample was shared into nearly three equal portions and each was poured into the mould and compacted with 27 numbers of blows with a 2.5kg rammer falling through a height of 300mm. It was ensured that the blows were applied uniformly across the surface of each layer. The collar was removed as well as the excess soil and the top surface was levelled. The base plate

was also removed and the mould with sample was weighed as M_2. Part of the sample from top and bottom was taken for moisture content determination. The sample in the mould was removed, pulverized and the process was repeated until four other sample values were obtained. For each compaction, bulk density and dry density were computed using equations 3.6 and 3.7 respectively. Results are presented in Appendix D (Table D1-D6).

$$\rho = \frac{m2-m1}{v} \tag{3.4}$$

$$\rho_d = \frac{\rho}{(1+w)} \tag{3.5}$$

where,

M_1 is mould and base plate (g) M_2 is mould with base plate and sample (g)

V is volume of mould (cm^3) W is moisture content (in decimal)

ρ is bulk density (g/cm^3) ρ_d is the dry density (g/cm^3)

The values for the dry densities were plotted graphically against their different moisture contents and the Maximum Dry Density (MDD) and Optimum Moisture Content (OMC) were obtained.

3.2.5 Particle size distribution (sieve analysis)

Sieve analysis is carried out to estimate the particle sizes in the soil sample and it was carried out in accordance to BS 1377-2(1990). 500g of the soil was weighed as M_1 (total weight) and the sample was washed and oven dried at a temperature of about 105 - 110°C for 24 hours. The set of sieves were cleaned and arranged in descending order (5.00, 4.75, 3.35, 2.00, 1.18, 0.600, 0.425, 0.300, 0.212, 0.150, and 0.075 mm with pan at the bottom). The oven-dried sample was gently poured into the sieve and the sieve cover was placed. The sieves were allowed to vibrate for 10minutes and then allowed to settle before removing the cover. Soil retained on each sieve was weighed as M_2. Percentage retained

and percentage passing each sieve was computed using equation 3.4 and 3.5 respectively. Results are presented in Appendix E (Table E1). Plate IV shows the sieve analysis test.

$$\%\ retained\ on\ sieve = \frac{m2}{m1} * 100\ \% \quad (3.6)$$

where,

M_1 is the total weight of sample (g) M_2 is the weight retained on sieve (g).

$$Percentage\ \ passing\ sieve = 100 - \%\ retained\ on\ the\ sieve \quad (3.7)$$

Plate IV: Particle size distribution test

3.2.6 Pore volume

Water infiltration method was used to determine the pore volume for the soil under study. The soil was compacted using British Standard Light compactive effort. The soil was mixed with water until the water was uniformly distributed. The mixed soil was kept tied in a nylon bag for 24 hours before compacting it in a mould. The weight of the mould was taken before the soil was compacted into it and the weighed again. The mould was cured for 24 hours again in a water tank. The mould was then removed and kept for a while.

The weight of the mould after saturation was taken. Bulk density, dry density, void ratio, porosity and degree of saturation were computed using respective equations. The change in the degree of saturation before and after curing is expressed in percentage. Results are presented in Appendix F (Table F1)

$$Bulk\ density\ (\rho) = \frac{m}{v} \quad (3.8)$$

$$Dry\ density\ (\rho d) = \frac{\rho}{(1+w)} \quad (3.9)$$

$$Void\ ratio\ (e) = SG\frac{1}{\rho d} - 1 \quad (3.10)$$

$$Porosity\ (n) = \frac{e}{(1+e)} \quad (3.11)$$

$$Degree\ of\ saturation = (\frac{SG * AMC}{e}) \quad (3.12)$$

where,

m is mass of the soil (g) v is volume of mould (cm^3)

w is moisture content (in decimal) SG is specific gravity

ρ is bulk density (g/cm^3) ρ_d is the dry density (g/cm^3)

e is void ratio n is porosity

AMC is average moisture content (in decimal)

3.2.7 Direct shear

The shear box was cleaned and the shear box cutter was greased. The soil sample was compacted at optimum moisture content and was extruded out of the proctor mould using

an extruder machine. The extruded sample was place on a clean tray. A spatula was used to divide the compacted sample horizontally into three (3) portions. The shear box cutter was driven gently into the one part and all excess soils were trimmed. The cutter was placed on a weighing balance to get the mass of the cutter and soil. The direct shear text apparatus was set up and the cutter and soil was placed in the shear box machine. At every sixty seconds, the horizontal force was recorded until there was no longer an increase in with increasing displacement or a decrease was observed with increasing displacement. The sheared sample was removed and the test was repeated for the remaining two portions. The results were computed, tabulated and linear graph was plotted. For each *B. megaterium* suspension, these processes were repeated. The machine constant results and test results are presented in Appendix G (Table G1 and G2-G7 respectively). The mass for each soil sample in the shear box cutter is presented in Appendix G (Table G8). Linear graphs for both natural and treated soil are presented in Appendix H-M and direct shear box samples are shown in Plate V. Table G9 in Appendix G, shows a summary of the direct shear test.

(a)

(b)

(c)

Plate V: Cutting of direct shear test specimens

(a) is the compacted soil sample

(b) is the placement of the sample in the shear box

(c) is the sheared samples

3.3 MICP Treatment Procedures

The natural soil was treated by mixing the bacteria and cementation reagent with the soil and then it was allowed to air dry for 24 hours. The consistency test, specific gravity test, compaction test and direct shear test were carried out in accordance to BS 1377 (1990) standards as explained above. The test was repeated for each bacteria cell concentration (that is, 0.5, 1, 3, 6 and 9 McFarland standard corresponding to $1.5x10^8$, $3.0x10^8$, $9.0\ x10^8$, $18.0\ x10^8$ and $27.0\ x10^8$ cells/ml respectively). The bacteria and cementation reagent (Plate VI) amounts used in this soil treatment are shown in equations 3.13 – 3.18.

a). Consistency test and specific gravity

$$\text{Bacteria amount} = \frac{25\ of\ liquid\ limit\ for\ the\ natural\ soil}{100}\ x\ 400g \tag{3.13}$$

$$\text{Cementation reagent amount} = \frac{75\ \ of liquid\ limit\ for\ natural\ soil}{100}\ x\ 400g \tag{3.14}$$

b). Compaction characteristics

$$\text{Bacteria amount} = \frac{25\ of\ OMC\ for\ the\ natural\ soil}{100}\ x\ 3000g \tag{3.15}$$

$$\text{Cementation reagent amount} = \frac{75\ \ of\ OMC\ for\ natural\ soil}{100}\ x\ 3000g \tag{3.16}$$

c). Direct shear

Bacteria amount = $\frac{1}{3}$ $pore\ volume$

(3.17)

Cementation reagent amount = $\frac{2}{3}$ $pore\ volume$ (in 4 cycles, after every six hours, that is 6, 12, 18 and 24)

(3.18)

The results for all treated soil samples are presented in Appendix B1 (liquid limit test), Appendix B2 (plastic limit test) Appendix B3 (plasticity index), Appendix C2 (specific gravity test), Appendix D2-D6 (Compaction test) and Appendix G3 – G7 (direct shear test). The linear graphs for the soil samples (both natural and treated) are presented in Appendix H-M.

Plate VI: Bacteria and cementation reagent (MICP treatment)

CHAPTER FOUR

4.0 DISCUSSION OF RESULTS

4.1 Properties of Natural Soil

The index properties of the natural soil are summarized in Table 4.1. The natural soil is greyish in colour and it has 38.74% passing through sieve no 200mm. Based on Unified Soil Classification System (USCS), the natural soil was classified as Clayey sand (SC) (ASTM, 1992) and as an A-7-6 (2) under the American Association of State Highway and Transportation Official (AASHTO, 1986) System of soil classification. Particle size distribution curve of the natural soil is shown in Figure 4.1.

Table 4.1: Index properties of the natural soil

S/No	Properties	Remarks/Quantities
1	AASHTO Classification	A-7-6 (2)
2	USCS Classification	Clayey sand (SC)
3	Natural moisture content (%)	25.70
4	Specific gravity	2.55
5	Liquid limit (%)	41.90
6	Plastic limit (%)	28.60
7	Plasticity index (%)	13.30
8	Percentage passing sieve No 200 (%)	38.74
9	Colour	Greyish

10	Maximum dry density, (MDD) (g/cm^3)	1.77
11	Optimum moisture content, (OMC) (%)	14.00

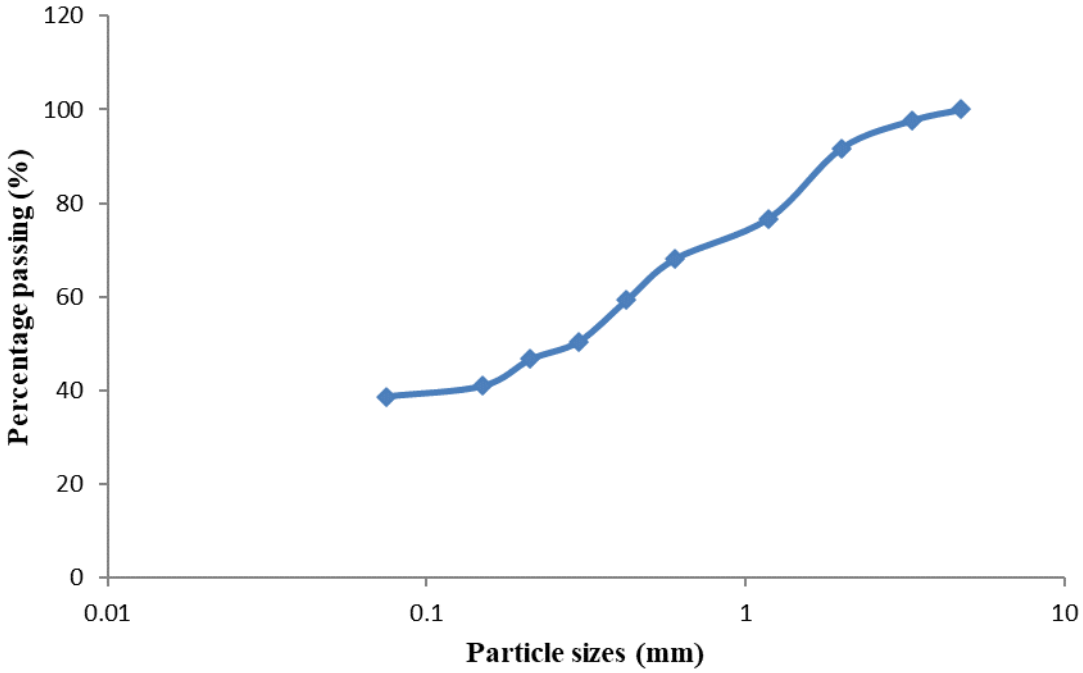

Figure 4.1: Sieve analysis graph for the natural soil

4.2 Effect of *B. megaterium* on Consistency Limits

Consistency limit test which include liquid limit, plastic limit and plasticity index are used to assess the plastic behaviour of the soil in relation to the amount of water content as it transits from solid to liquid phases. Table 4.2 show the consistency test results for both natural and treated soil.

Table 4.2: Consistency test result for natural and treated soil

Bacteria suspension densities (cell/ml)	0	$1.5x10^8$	$3.0x10^8$	$9.0x10^8$	$18.0x10^8$	$27.0x10^8$
Liquid limit (%)	41.7	44.2	45.8	49.7	47.8	51.6
Penetration (mm)	20.0	20.4	21.2	25.6	21.2	25.0
Plastic Limit (%)	28.6	26.1	24.5	33.1	23.8	44.7

Plasticity Index (%)	13.3	18.1	21.3	16.6	24.7	6.9

4.2.1 Liquid limit

The variation of liquid limit with *B. megaterium* suspension is presented in Figure 4.2. From the Figure, it can be observed that liquid limit of the soil increases with increase in *B. megaterium* suspension density. This could be attributed to the formation of calcite when the cementation reagent and bacteria suspension was mixed with the soil. Similar findings were reported by Moses and Afolayan (2011), Salahudeen *et al.*, (2014), Osinuibi *et al.*, (2017).

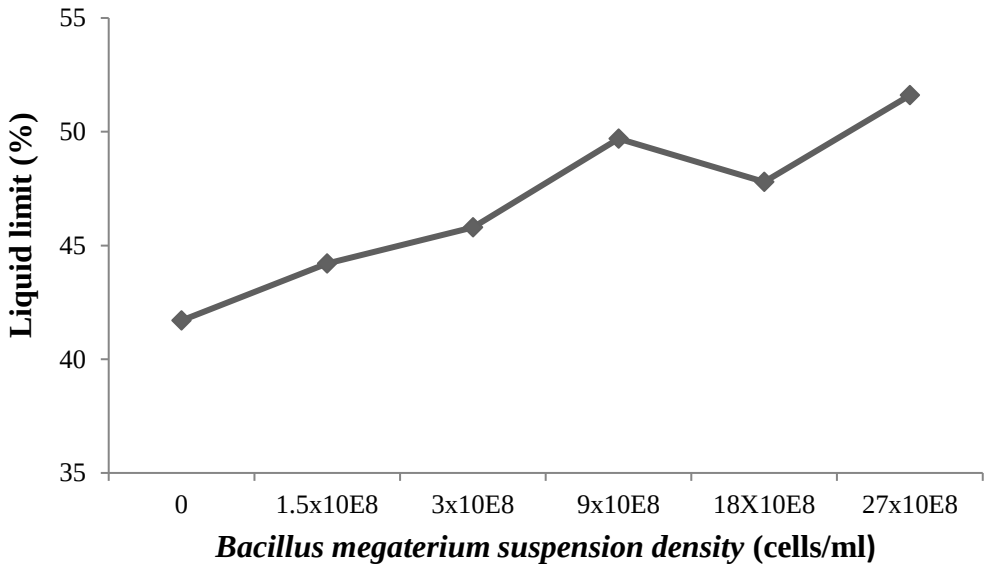

Figure 4.2: Variation of liquid limit with *B. megaterium* suspension densities

4.2.2 Plastic limit

The variation of plastic limit with *B. megaterium* suspension is presented in Figure 4.3. The observed trend could be due to the flocculation and agglomeration of clay particles which produces calcium ion from the MICP process which react with ions of lower valence in clay structures. The result pattern is similar to the study reported by Sani *et al.* (2020a).

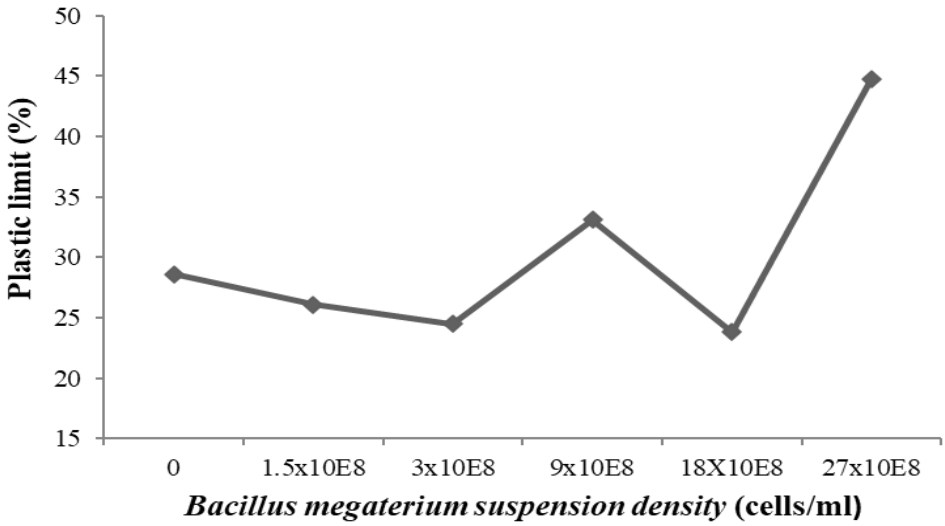

Figure 4.3: Variation of Plastic limit with *B. megaterium* suspension densities

4.2.3 Plasticity index

The variation of plasticity index with *B. megaterium* suspension is presented in Figure 4.4. There was a decrease from 13.3% for natural soil to 6.8% at $27.0x10^8$ bacteria suspension density. It is appropriate to state that decrease in plasticity index value is desirable for any method adopted for improving the engineering properties of a soil, Amadi and Eberemu, (2013); Sani *et al.*, (2020b).

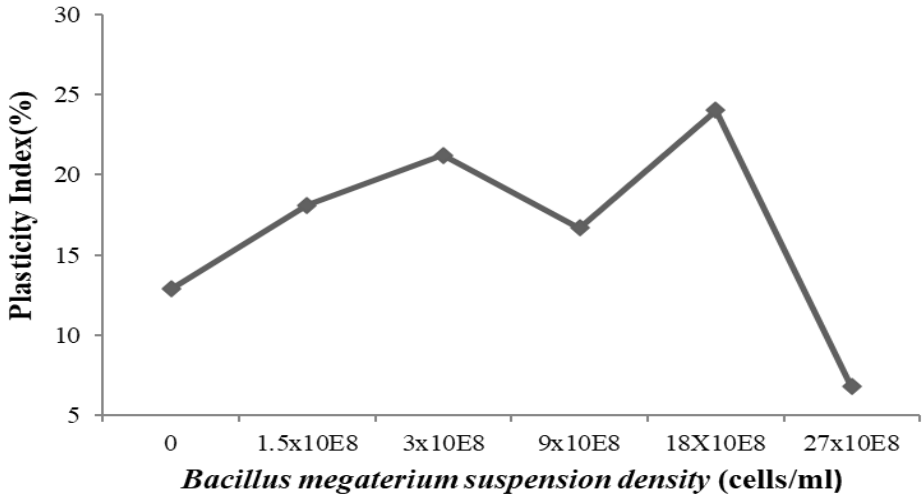

Figure 4.4: Variation of Plasticity index with *B. megaterium* suspension densities

4.3 Specific Gravity

Figure 4.5 shows the variation of specific gravity and *B. megaterium* suspension densities. The treated sample recorded the highest specific gravity as 2.77 at $18.0x10^8$ *B. megaterium* and the lowest is 2.19 at $3.0x10^8$ *B. megaterium* suspension densities. This result pattern could be due to the calcite formed within the soil matrix during the MICP process and it is similar to the results obtained by Osinuibi *et al.,* (2017), Osinubi *et al.,* (2018), Sani and Bala (2021).

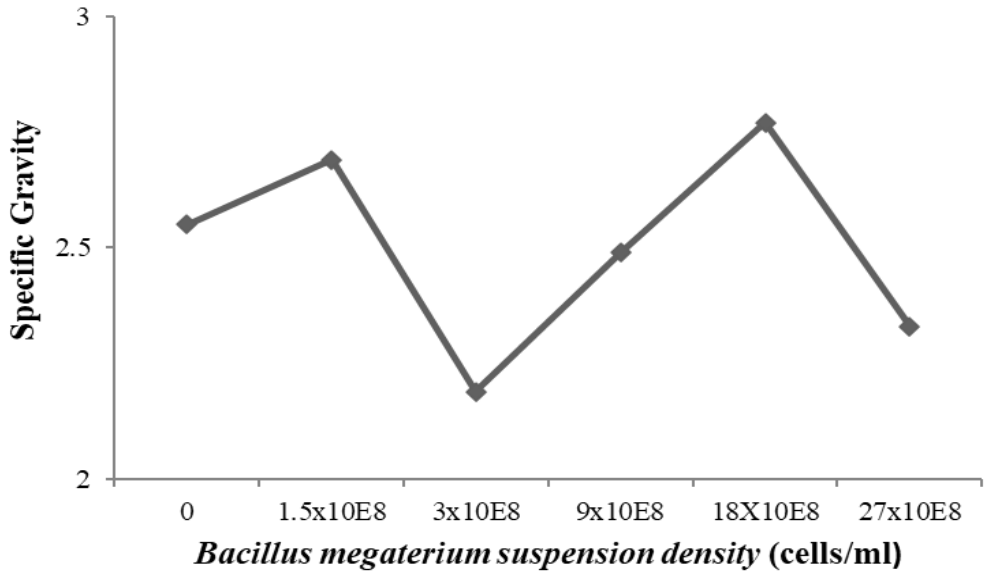

Figure 4.5: Variation of specific gravity with *B. megaterium* suspension densities

4.4 Compaction Characteristics

The variation of Optimum Moisture Content (OMC) and Maximum Dry Density (MDD) is shown in Figures 4.6 and 4.7 respectively for the natural and treated soils. With increasing *B. megaterium* suspension density, the MDD showed a slight fluctuation in its values. This fluctuation could be as a result of the reduction in specific gravity of the treated soil (2.19) against that of the natural soil (2.55). Similar trend was reported by Sani and Bala (2021).

OMC generally decreased with increase in *B. megaterium* cell concentration up to $9.0x10^8$ bacteria concentration and then an increased was observed. This increase may be as a result of soil particles been bounded together by the calcite (formed when the soil was treated) leading to the formation of larger surface area which has greater affinity for water resulting in higher moisture. Similar trend was reported by Abo-El-Enein *et al.,* (2012), Osinuibi *et al.,* (2018), Sani *et al.,* (2020a), Sani and Bala (2021).

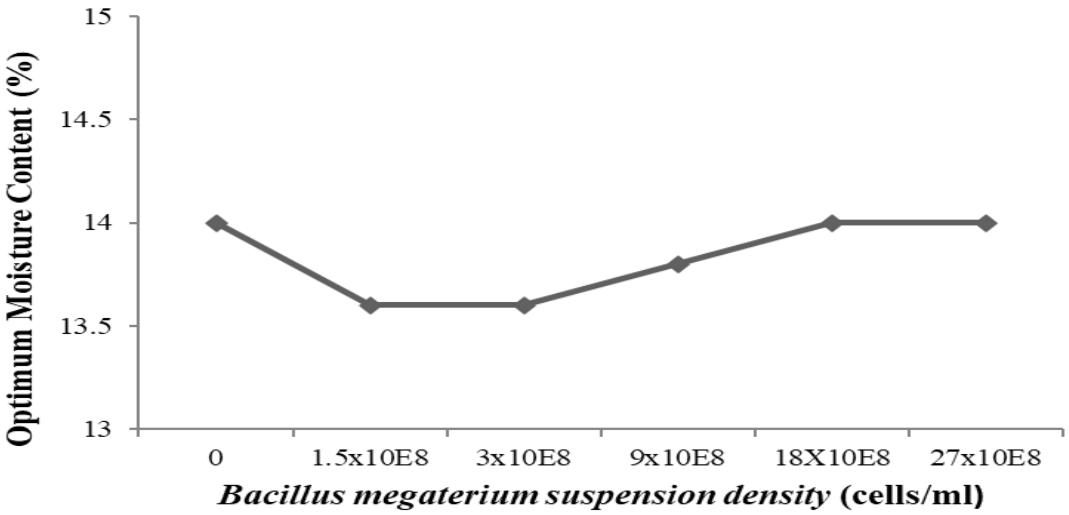

Figure 4.6: Variation of OMC with *B .megaterium* suspension densities

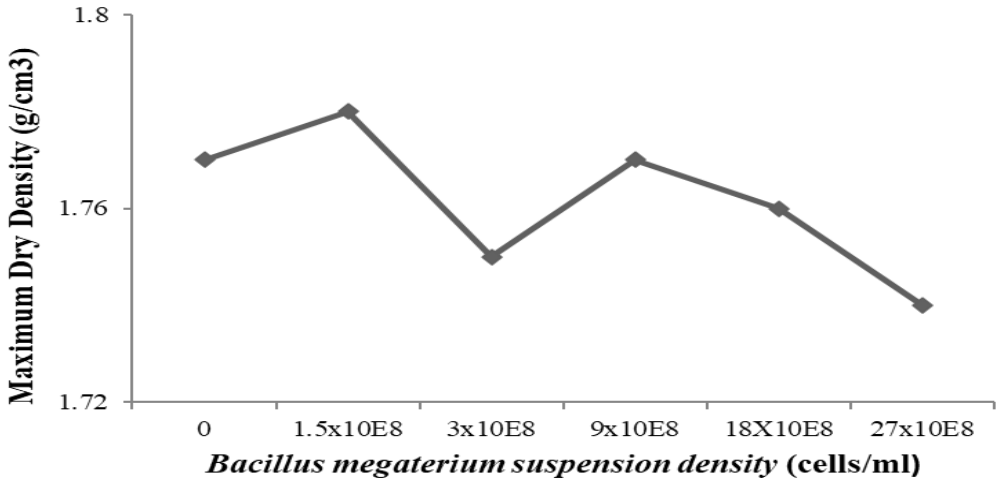

Figure 4.7: Variation of MDD with *B. megaterium* suspension densities

4.5 Direct Shear Test

4.5.1 Loadings (from kg to kN/m^2)

This test was carried out to determine the shear strength parameters of the soil under vertical Loads of 2, 4 and 9kg (which is equivalent to 54.40, 108.97 and 245.18 kPa respectively). Figure 4.8 - 4.13 shows displacement (mm) versus Shear stress (kN/m^2) graphs for the different *B. megaterium* cell concentration and also, shear stress of the soil shows direct proportionality with vertical stress. Peak shear stress was chosen as shear stress failure point.

For 2kg, $\sigma_2 = \frac{(2x9.81x0.001x10)}{(0.0036)} = 54.50$ kN/m^2

For 4kg, $\sigma_2 = \frac{(4x9.81x0.001x10)}{(0.0036)} = 109.00$ kN/m^2

For 9kg, $\sigma_2 = \frac{(9x9.81x0.001x10)}{(0.0036)} = 245.20$ kN/m^2

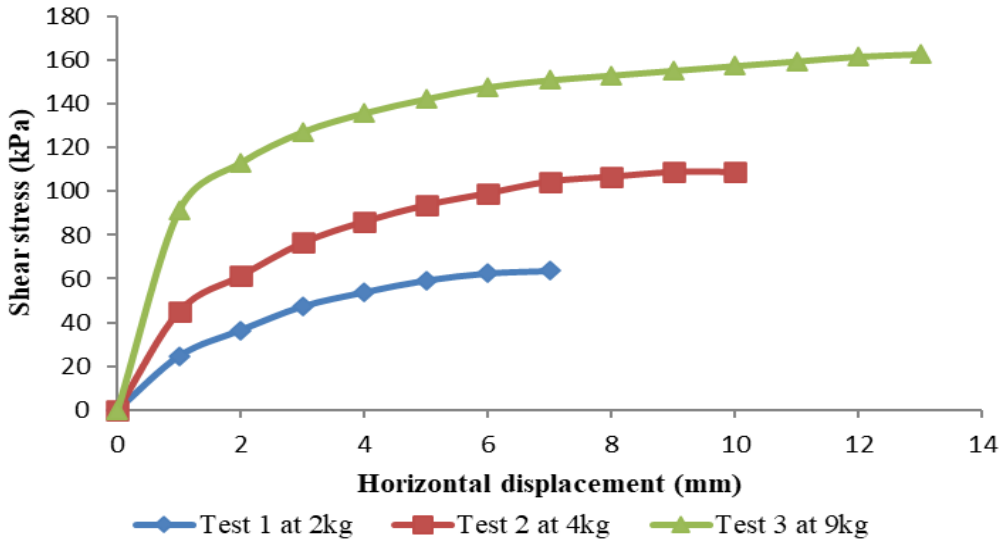

Figure 4.8: Stress-strain curve for natural soil

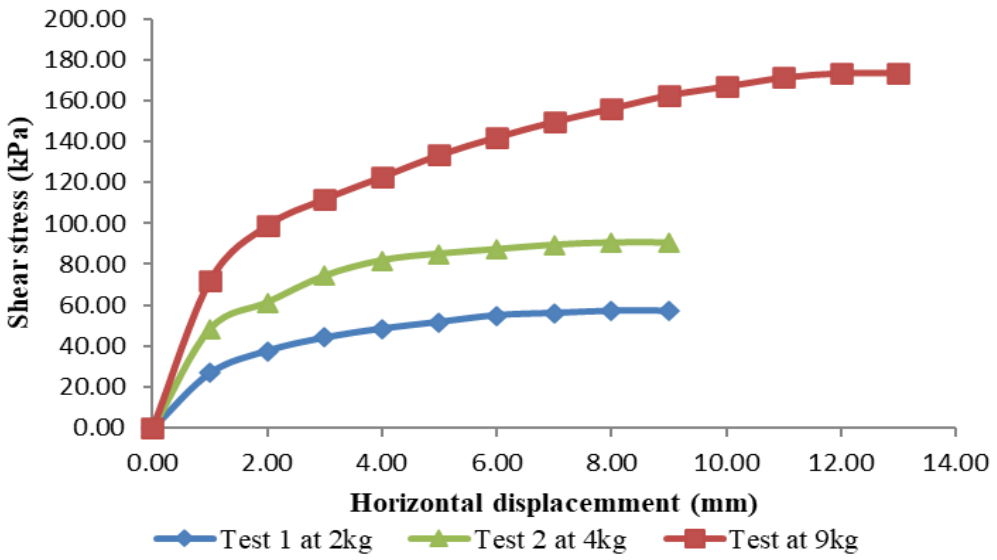

Figure 4.9: Stress-strain curve for $1.5x10^8$ *B. megaterium* suspension densities

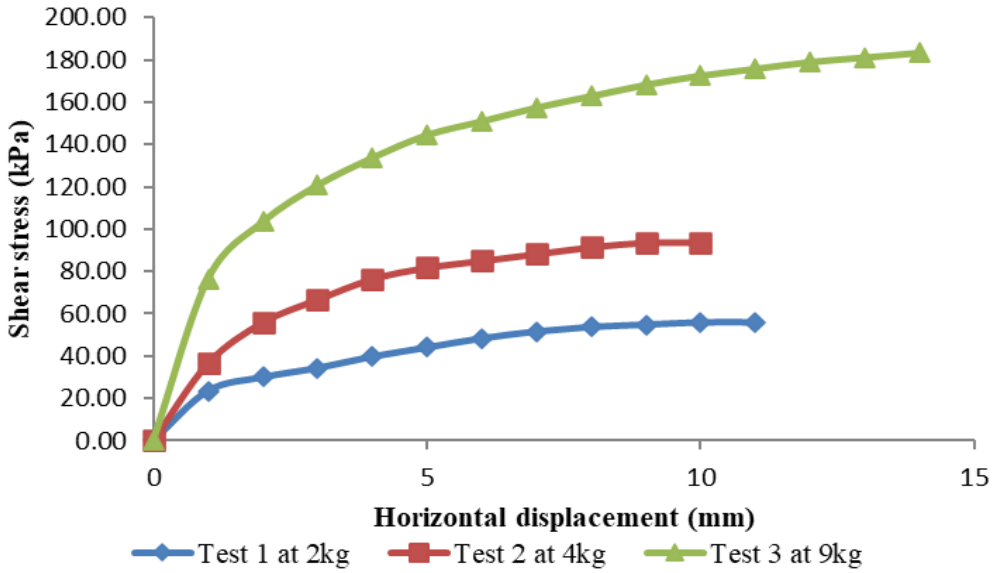

Figure 4.10: Stress-strain curve for $3.0x10^8$ *B. megaterium* suspension densities

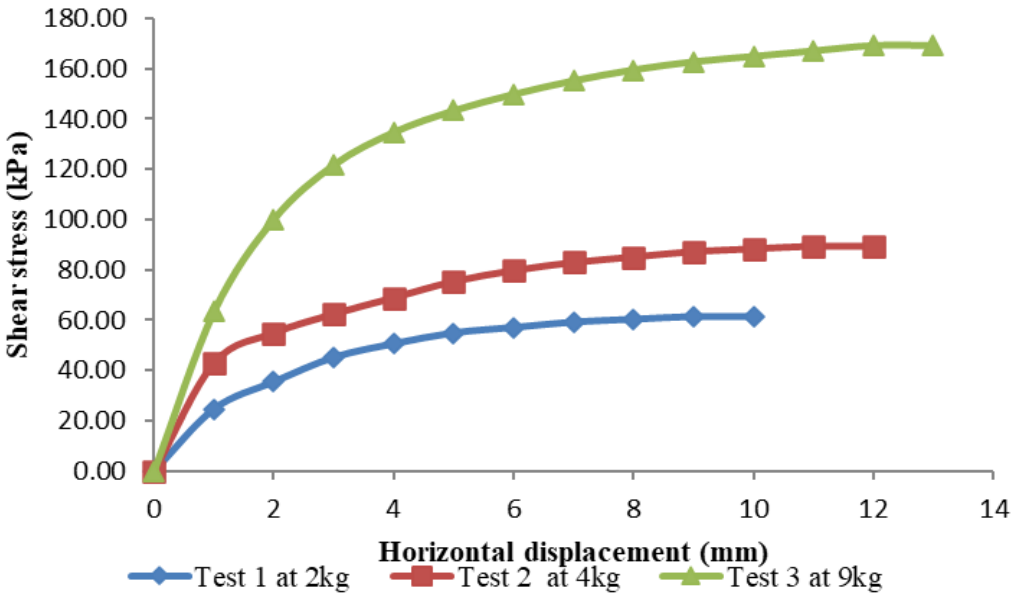

Figure 4.11: Stress-strain curve for 9.0 x10^8 *B. megaterium* suspension densities

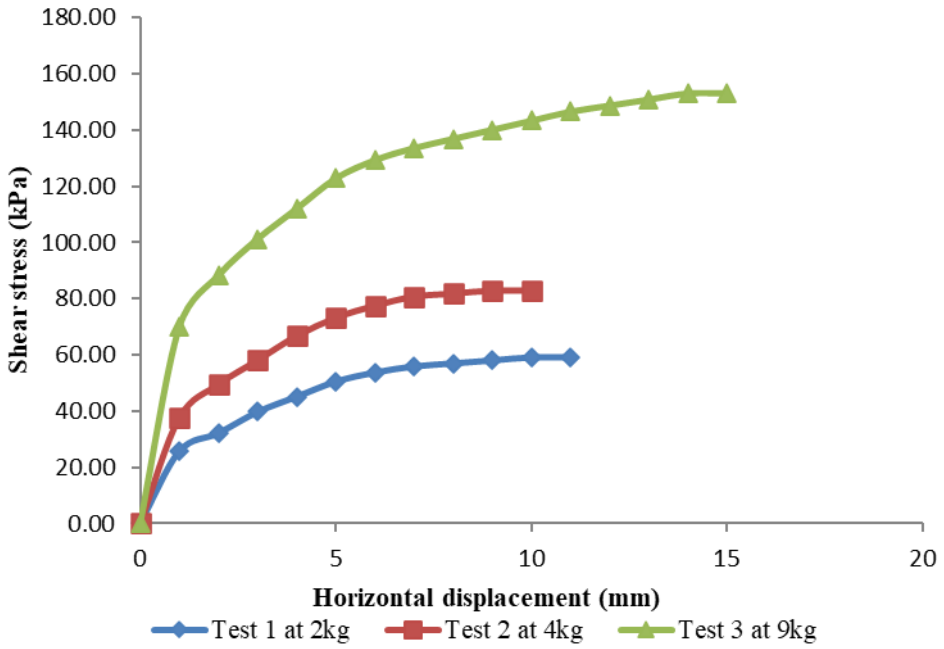

Figure 4.12: Stress-strain curve 18.0 x10^8 *B. megaterium* suspension densities

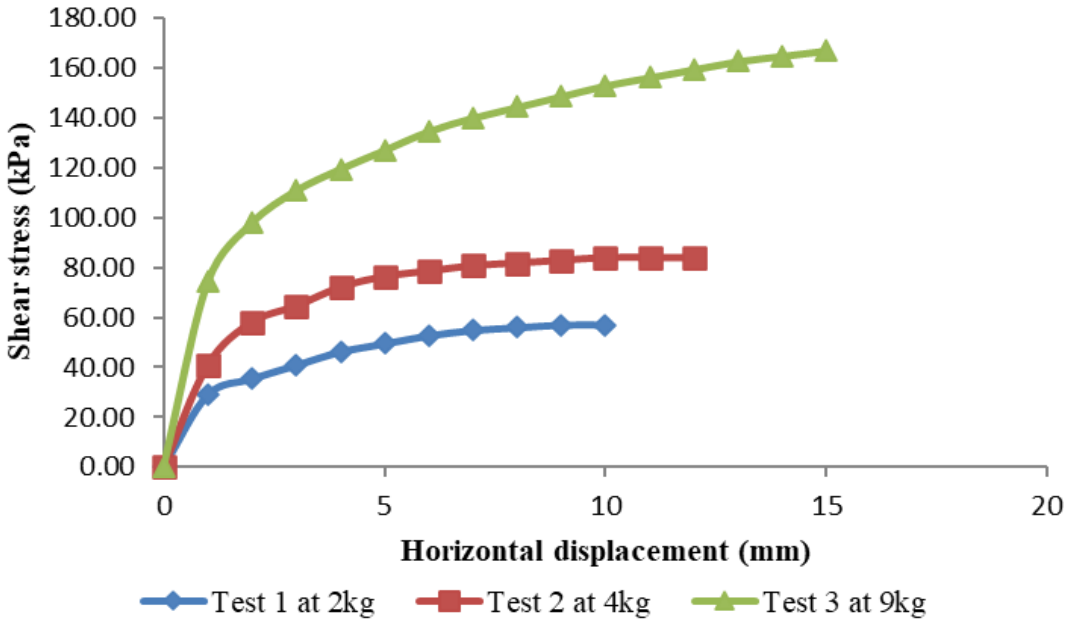

Figure 4.13: Stress-strain curve for 27.0 x10^8 *B. megaterium* suspension densities

4.5.2 Linear graph equation for C- ϕ of the soil samples

Linear graph equation (obtained from Appendix H-M) for cohesion (C in kPa) and angle of shearing resistance (ϕ in°) for natural and treated soil is given by equation 4.1.

$$y = mx + c \tag{4.1}$$

where,

c is cohesion,

m is slope (used in calculating the angle of shearing resistance)

1. For natural soil, y = 0.4966x + 44.076

   ϕ° = $\tan^{-1}(0.4966)$ = 0.460924 (in radians)

   $= \frac{0.460924x180}{3.142}$ = 26.41°

2. For 1.5 *B. megaterium* suspension density

   y = 0.6108x + 23.856

$\phi° = \tan^{-1}(0.6108) = 0.548322$ (in radians)

$= \frac{0.548322x180}{3.142} = 31.42°$

3. For 3.0 *B. megaterium* suspension density

$y = 0.6657x + 20.338$

$\phi° = \tan^{-1}(0.6657) = 0.587333$ (in radians)

$= \frac{0.587333x180}{3.142} = 33.65°$

4. For 9.0 *B. megaterium* suspension density

$y = 0.5697x + 29.088$

$\phi° = \tan^{-1}(0.5697) = 0.517842$ (in radians)

$= \frac{0.517842x180}{3.142} = 29.67°$

5. For 18.0 *B. megaterium* suspension density

$y=0.4966x + 30.768$

$\phi° = \tan^{-1}(0.4966) = 0.460924$ (in radians)

$= \frac{0.460924x180}{3.142} = 26.41°$

6. For 27.0 *B. megaterium* suspension density

$y=0.5834 + 23.270$

$\phi° = \tan^{-1}(0.5834) = 0.528124$ (in radians)

$= \frac{0.528124x180}{3.142} = 30.26°$

4.5.3 Effect of *B. megaterium* on shear strength parameters

The variation of cohesion with *B. megaterium* cells/ml is shown in Figure 4.14. Cohesion values for the treated soil samples were all lower than the natural soil. This could be as a result of soil composition, compaction and calcite formation. Cohesion for the treated soil varies between 20.30 to 30.8kPa for different *B. megaterium* cell concentrations**.** Similar trend was reported by Abdelmagied (2019); Sani *et al.,* (2020a) and Sani *et al.,* (2020b).

The variation of angle of shearing resistance with *B. megaterium* cells/ml is shown in Figure 4.15. The angle of shearing resistance increased with increasing *B. megaterium* cell/ml up to $18.0x10^8$ *B. megaterium* cell/ml were the highest value was recorded. It increased from 26.4° to 33.7°. The higher angle of shearing resistance recorded is due to the calcite formed during the MICP process. The calcite formed is responsible for the bond between the soil particles resulting in soil movement restriction thereby improving the angle of shearing resistance (Harkes *et al.,* 2010; Mujah *et al.,* 2017; Osinubi *et al.,* 2019d; Sani *et al.,* 2020a and Sani *et al.,* 2020b).

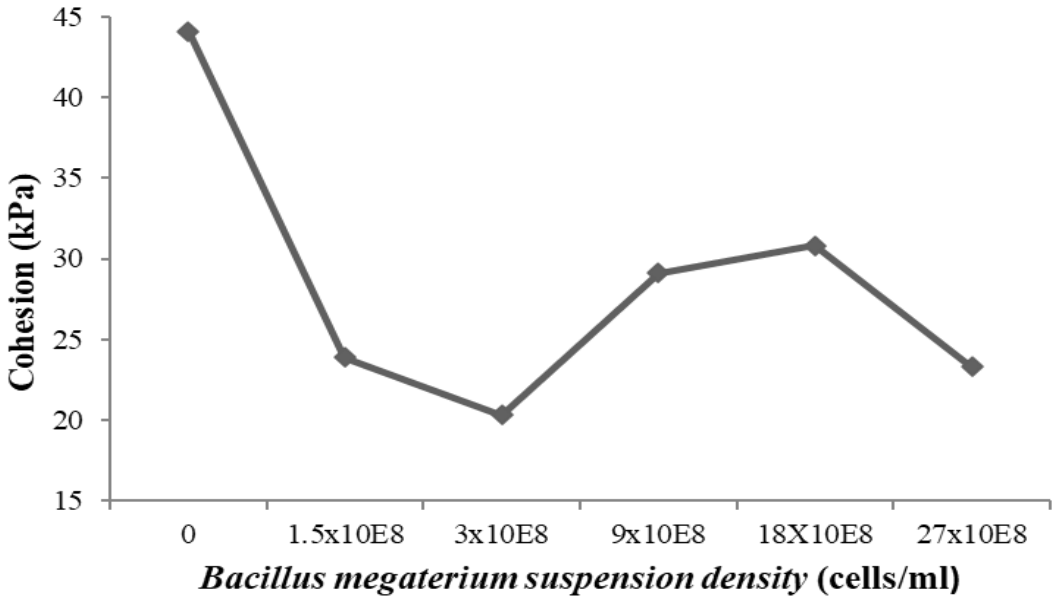

Figure 4.14: Variation of cohesion with *B. megaterium* suspension densities

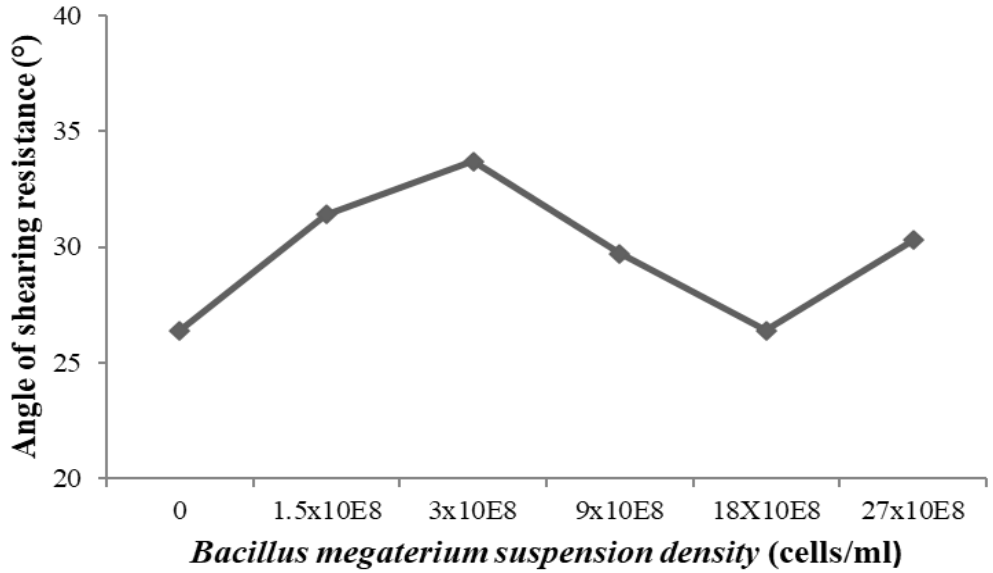

Figure 4.15: Variation of shearing resistance with *B. megaterium* suspension densities

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

From the investigation of effect of microbial induced calcite precipitation on strength properties of weak clayey soil, the following conclusions were drawn:

The index properties result revealed that the soil has a liquid limit of 41.90%, plastic limit of 28.60%, plasticity index of 13.30%, specific gravity of 2.55 and natural moisture content of 25.70%. The soil falls under clayey sand (SC) according to Unified Soil Classification System (USCS) and A-7-6(2) according to American Association of State Highway and Transportation Officials. The soil is greyish in colour and had a percentage passing of sieve no 200 as 38.74%.

The compaction characteristic shows that the maximum dry density (MDD) increased slightly with the introduction of *B. megaterium* at $1.5x10^8$ concentrations (cell/ml). At $3.0x10^8$ *B. megaterium* (cell/ml), a decrease in MDD was observed. The highest MDD was recorded at $1.5x10^8$ *B. megaterium* suspension densities. The optimum moisture content (OMC) increased with increase in *B. megaterium* suspension densities. Highest OMC of 14.00% was achieved at $18.0x10^8$ and $27.0x10^8$ *B. megaterium* suspension densities.

An inversely proportional relationship is shown between cohesion and angle of shearing resistance. The cohesion was decrease by 53.97% while angle shearing resistance was increased by 36.99% at $3.0x10^8$ *B. megaterium* suspension densities. This result shows a great improvement in the soil since the clayey nature of the natural soil was reduced from 44.10kN/m^2 to 20.30kN/m^2 whereas the angle of shearing resistance was increased from 26.4° to 33.7°. Normal stress increases with increase in shear stress.

5.2 Recommendations

From the study carried out, the following recommendations are given:

i. The *B. megaterium* suspension density of $3.0x10^8$ cells/ml can be used to improve the engineering properties of soil.

ii. Microbial induced calcite precipitation has shown its viability and suitability in improving the engineering properties of soil.

iii. The microstructure of MICP treated soil should be studied as it could shed more light on the effect of pore throat sizes on MICP process.

5.3 Contribution to Knowledge

This research showed the feasibility of using microbial induced calcite precipitation in soil improvement. Cohesion and angle of shearing resistance (angle of internal friction) are the two key factors considered during this research. Previous studies includes the effect microbial induced calcite precipitate has directly on strength and bearing capacity of soil used in construction. This study showed a great improvement of the soil since the clayey nature of the soil was reduced by 53.97% and angle of shearing resistance increased by 36.99%.

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