Effect of wetting and drying on the mechanical properties of MICP treated clay

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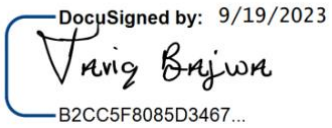

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DEDICATED TO

My parents , sisters , teachers and friends

This is the result of all your support and encouragement.

ACKNOWLEDGEMENT

I would like to begin by expressing my gratitude to the Almighty Allah for His blessings and guidance throughout my academic journey.

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Sincerely,

Engr. Abbas Khan

Abstract

Microbial-induced calcium carbonate (MICP) mineral precipitation is a fairly new and innovative technique in soil stabilization. MICP was originally applied to coarse grained soil with success. The use of MICP in the fine-grained soil is rare, so far. MICP process involves using bacterial strains that can secrete urease enzymes, called urease positive bacteria (UPB) and chemical which include urea and calcium carbonate. The bacterial strains used in this research are Bacillus subtilis and Priestia megaterium. An expansive soil obtained from Nandipur; Pakistan was used in the study. The unconfined compressive strength (UCS) and Atterberg limit tests were performed to examine the mechanical behaviour of MICP treated soil for different wet-dry cycles. Microstructural analysis, such as X-ray diffraction (XRD), and scanning electron microscopy (SEM) was conducted to observe the surface and internal structures. The result showed that the treated soil gave 4 times higher UCS than untreated soil, which is due to change in its composition. Furthermore, the MICP treated soil demonstrated insignificant decrease in strength after 7th wet-dry cycle. The results infer that that MICP is an efficient technique to stabilize the swelling soil.

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Introduction

1.1 Overview

Expansive soil are characterized as problematic soil when encountered in the field. They are dangerous for construction of residential buildings and roads. The reason for this is that expansive soil undergo large volume alterations when its moisture changes due to the presence of Monmorrilonite mineral. Therefore, they are stabilized and treated before construction of roads or buildings. The common stabilization techniques are either mechanical or chemical. Mechanical methods are not that effective in the field and mostly chemical techniques are preferred. These chemical techniques create a lot of ground pollution and contaminate body therefore they are considered a threat to the environment.

A new environmentally friendly technique called Microbial Induced Calcite Precipitation is introduced in the recent past to stabilize soil mostly coarse grained soil. This technique has been found effective for coarse grained soil and now it has been also researched in fine grained soil. Microbial Induced Calcite Precipitation uses bacterial strain that can secrete Urease in either dried or solution form. These bacteria in combination with Calcium Chloride produce a mineral called Calcite which can increase the strength of the soil. This technique is considered effective, environmental friendly and cost effective for the stabilization of soil.

In this research the MICP technique is investigated in the expansive soil. Two bacterial strains *Bacillus subtilis* and *Priestia megaterium* are used which are urease producing bacteria and both are considered as useful bacteria. The Calcium Chloride solution is used in three different molarities and its effect is also studied.

1.2 Need for research

As Expansive soil is found in a lot of area in Pakistan and around the world. Therefore, a cost effective and environmentally friendly technique to stabilize the soil is required. The use of locally identified bacterial strains in stabilizing soil is very important as a lot of area is covered with swelling soil. The damages caused by expansive soil to light weight residential structures and road

is more than any other disaster like flood and earthquake. Therefore research is required for checking the applicability of MICP in expansive soil.

1.3 Objective

The objective of this research are following

- Improve the mechanical properties of expansive soil through MICP modification.
- Improve the durability of soil against Wetting and Drying process

1.4 Scope and Methodology

The research will investigate the mechanical properties of MICP treated soil and untreated soil. The methodology flow chart is below.

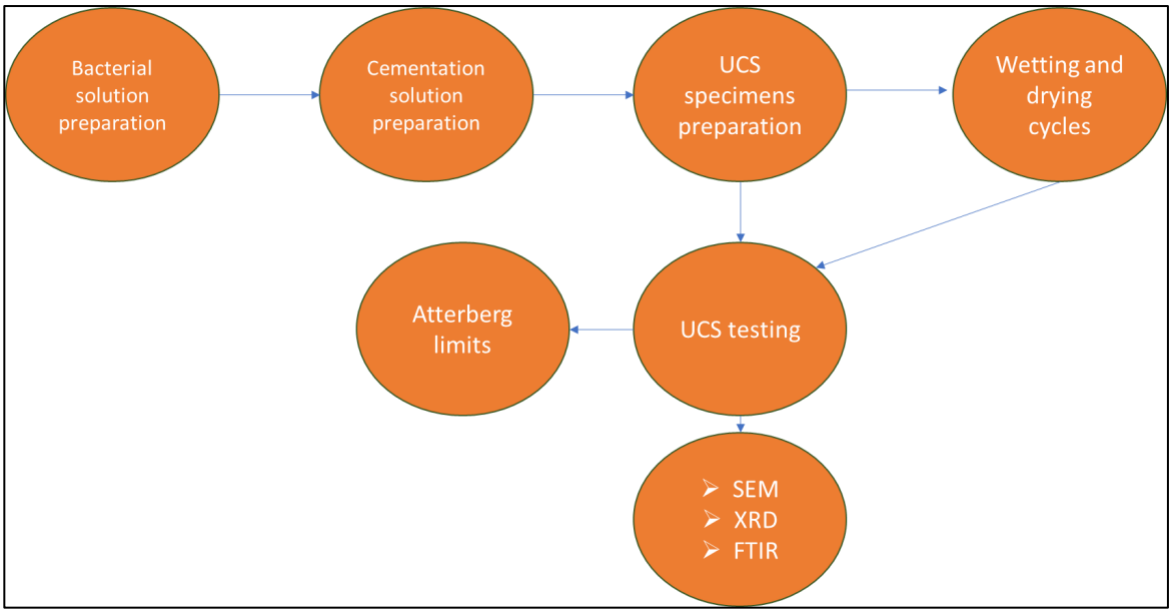

Fig 1.1 Methodology flow chart

2 Chapter

Literature Review

2.1 Background

Expansive soils, alternatively referred to as swelling soils, shrink-swell soils, or heavable soils, are a recognized geological phenomenon. These types of soils demonstrate substantial alterations in volume in response to variations in soil moisture levels. When water is absorbed by soils of this nature, there is an increase in their volume and they acquire a sticky consistency. As water is absorbed, there is a corresponding rise in volume. In addition, they undergo a reduction in size as the level of moisture diminishes, resulting in the formation of extensive surface fissures or a swollen look. These fissures enable the infiltration of water to greater depths during periods of moisture. The phenomenon described leads to a recurring pattern of contraction and expansion, resulting in cyclic loads exerted on constructions situated on soils exhibiting these characteristics (Chen, 1988).

Swelling soils are present throughout all continents and exhibit a particularly extensive distribution in regions characterised by wet and dry tropical climates. Therefore, it is crucial to prioritise the identification of swelling soils throughout the design phase of any project. This will enable us to make informed decisions that ensure the safety and cost-effectiveness of the construction (Al-Rawas et al., 2002). The prompt detection of soil swelling can aid in mitigating extensive structural damage resulting from the swelling phenomenon.

2.2 Damages caused by Expansive soil.

The possible adverse consequences to structures arise from the change in volume of both the underlying and surrounding soils, particularly when these soils are classified as moderate to highly expansive.

According to Chen (1988), it has been demonstrated that the hazards posed by expanding soils are more detrimental to construction projects compared to those generated by earthquakes, hurricanes, and floods. The growing recognition of the structural impairments linked to expansive soils has resulted in heightened scrutiny of several facets pertaining to their properties and behavior. The

estimation of anticipated heave is a important parameter in the decision of treatment alternatives and the design of foundations for structures on expansive soils (Shi et.al., 2002; Steinberg, 1998).

Expansive soils provide numerous challenges, particularly in relation to the structural integrity of lightweight constructions. The primary concerns associated with these soils are encountered in the following scenarios.

- Residential Buildings (Single and double story)
- Boundary Walls
- Flexible and Rigid Pavements
- Railways Embankments
- Floors
- Concrete Lining of Channels and Reservoirs
- Sewerage Linings

The damages caused by Expansive soil to civil engineering structures are shown in Fig 2.1 to Fig 2.8.

Fig 2.1 Damage to road due to the uplifting of underneath expansive soil

Figure 2.2 Cracks in walls of residential buildings

Figure 2.3 Damage to a flexible pavement due to swelling of clay

Figure 2.4 Cracks in walls of single storey building

Figure 2.5 45 degree crack in a wall due to uplift pressure

Fig 2.6 Crack in boundary wall due to the Swelling clay.

Fig 2.7 Crack in wall due to the Uplift pressure of the clay.

Fig 2.8 Heaving in the Lining of the channel

2.3 Expansive clay Minerology

According to Chen (1988), clay minerals possess the ability to adsorb water on their external surfaces, resulting in a degree of swelling that is associated with the expansion of capillary films. Clay minerals can be classified into distinct categories according to their crystalline structures. Some of the clay minerals are explained below.

2.3.1 Kaolinite

The Kaolinite group comprises many clays, with Kaolinite being the primary constituent. The mineral known as Kaolinite is comprised of two distinct sheets, with one sheet built of silica tetrahedrons and the other sheet composed of alumina octahedrons. The cohesion between these sheets is remarkably robust. Kaolinite exhibits high stability and demonstrates minimal susceptibility to volumetric alterations upon exposure to water or dry conditions. The absence of interlayer water in kaolinite can be attributed to the manner in which its sheets are arranged. Nevertheless, it possesses the capacity to absorb an ample amount of water in order to acquire

plasticity. The kaolinite minerals consist of repetitive arrangements of a tetrahedral (silica) sheet and an octahedral (alumina or gibbsite) sheet, hence falling into the category of 1:1 minerals. The cohesion between the two layers is facilitated by bonding of hydrogen, namely between ions of hydroxyl located on the octahedral sheets and atoms of oxygen situated on the tetrahedral sheets. The formation of a single layer is the result of this interaction. The presence of strong hydrogen bonding in the system provides resistance against swelling pressures induced by water, while simultaneously facilitating the formation of expansive crystal formations. The mineral layers possess an approximate thickness of 0.72 nm (7.2 Å) and exhibit indeterminate lateral extension in both directions. The structure of kaolinite crystals consists of regularly recurring layers of a mineral layer with a thickness of 0.72 nm. According to Holtz and Kovacs (1981), it is frequently seen that Kaolinite crystals can have a thickness ranging from 70 to 100 layers. The Cation Exchange Capacity of Kaolinite is characterized by a notably low range, typically ranging from 3 to 15 milliequivalents per 100 grammes. This limited capacity can be attributed to the minimal occurrence of substitution processes within the mineral sheets.

Kaolinite is a mineral with various practical applications across different industries. Some of the main uses of kaolinite include:

Ceramics: Kaolinite is a key ingredient in the production of ceramics, including porcelain, pottery, and tiles. Its fine particle size, white colour, and high plasticity make it an ideal material for shaping and forming various ceramic objects.

Paper Industry: Kaolinite is used as a filler and coating material in the paper industry. It enhances the smoothness, opacity, and printability of paper products, reducing ink absorption and improving the overall quality of the paper.

Paints and Coatings: Kaolinite is used as an extender in paints, providing improved durability, opacity, and whiteness. It also helps control the rheology of paint formulations, ensuring proper viscosity and flow characteristics.

These are just few examples of diverse use of Kaolinite in the industry. The unique propertiesof kaolinite like its fine structure, adsorption

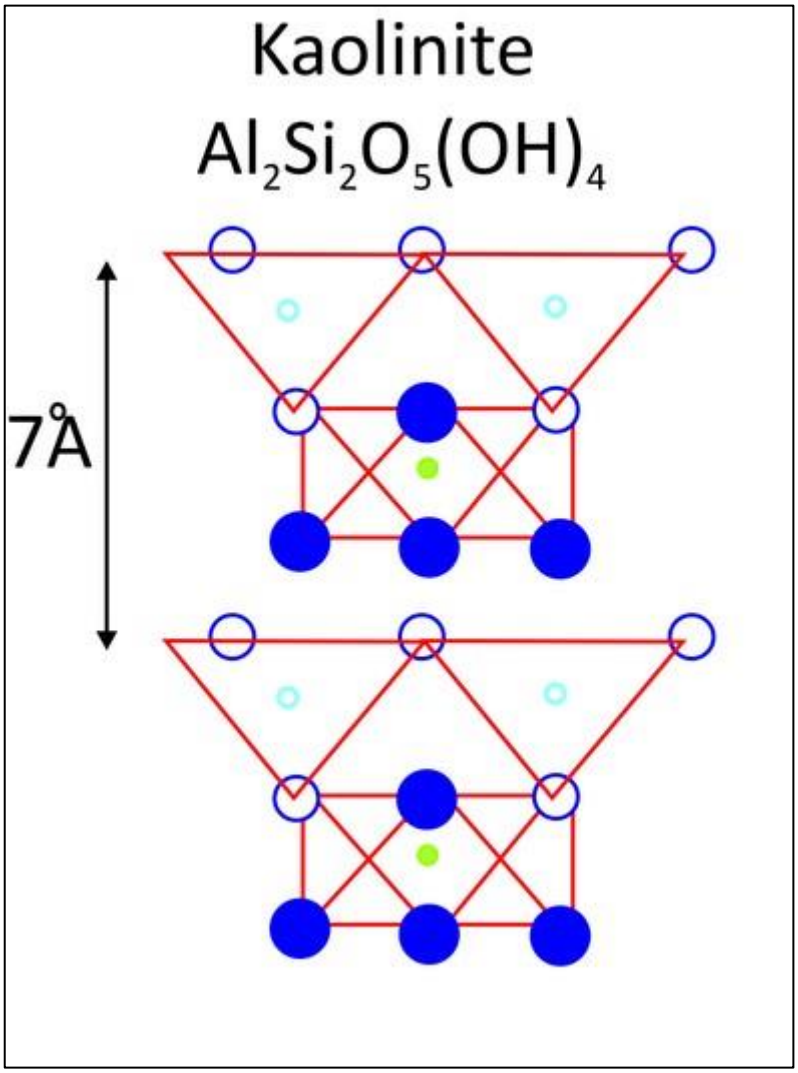

Fig 2.9 Structure of Kaolinite (Bauluz, 2015)

2.3.2 Illite

Illite is a type of 2:1 mineral characterized by the presence of a potassium atom that forms bonds within the interlayer. The mineral Illite is composed of a single octahedral sheet that is situated between two silica tetrahedral sheets. The Illite mineral has a structural arrangement wherein the potassium atom is accommodated within the hexagonal voids formed by the silica tetrahedron sheets, hence facilitating the bonding of adjacent mineral layers. The resultant charge is partially counterbalanced by the presence of potassium atoms within the hexagonal interlayer gap. The strong bonding of potassium renders it predominantly non-exchangeable. Ionic substitutions commonly take place within the Illite mineral, predominantly within the layers composed of silica tetrahedra. The strong interlayer bonding effectively limits expansion by inhibiting water infiltration, thereby maintaining the layers at a relatively constant state (Mitchell, 1993). Illite has a Cation Exchange Capacity (CEC) of between 10 and 40 milliequivalents per 100 grams

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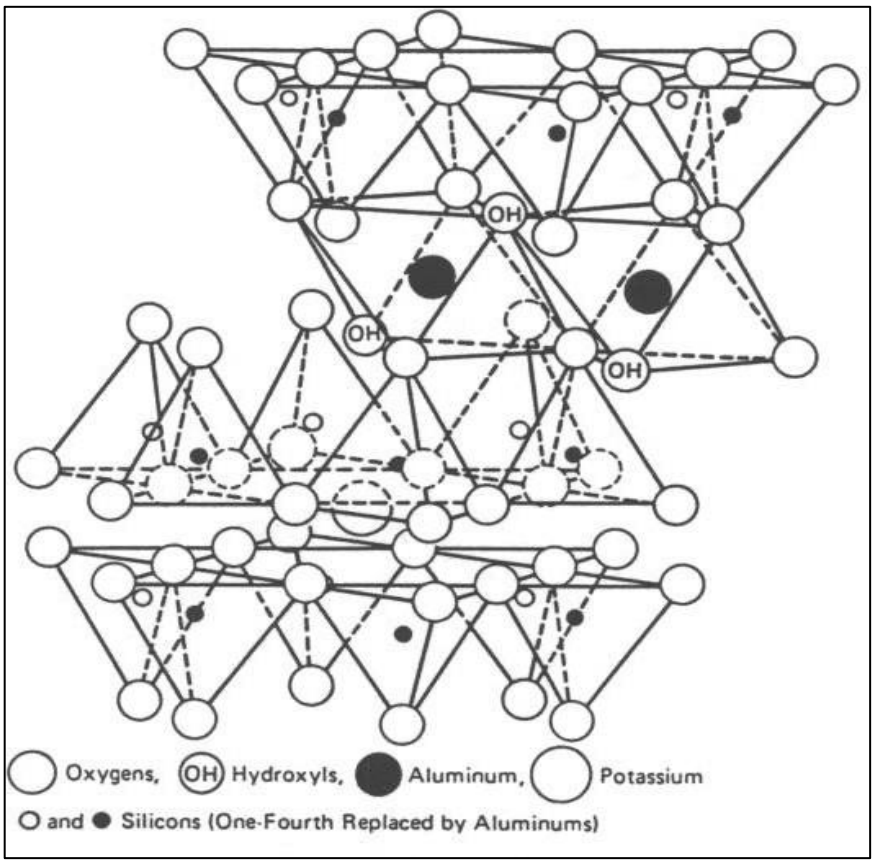


Fig 2.10 Structure of Illite (Mitchel 1993)

2.3.3 Smectite

The term "Montmorillonite" refers to a collective designation minerals characterized by their expansive structures. Additionally, it is used specifically to denote the primary mineral within this group. The presence of an alumina sheet sandwiched between two silica sheets in the composition of montmorillonite causes a loosely linked Tri layer structure to emerge. The observed variations in properties of this material can be attributed to the interchanging of components within each sheet. Materials such as iron or aluminum have the potential to serve as substitutes for aluminum in the alumina sheet, whereas aluminum can potentially replace certain silicones in the silica sheet.

This mineral demonstrates the unfavorable property of experiencing significant volumetric alterations when the soil mass is subjected to the addition or removal of moisture. This particular attribute has the potential to give rise to significant issues pertaining to heaving or settlement.

The mineral known as Montmorillonite, also referred to as Smectite, is composed of a 2:1 structure. This structure consists of two sheets of silica tetrahedrons and one sheet of alumina (gibbsite) octahedrons. The octahedral sheet is situated in the interlayer region, sandwiched between two silica tetrahedral sheets, so constituting a monolayer structure. The bonding between the tips of the silica tetrahedra and the hydroxyl groups of the alumina octahedra is facilitated by Vander Waals' forces. The intermolecular interactions responsible for bonding inside the layers are rather weak, hence facilitating the ingress of water molecules and exchangeable ions. The average thickness of a mineral layer with a 2:1 ratio is estimated to be around 0.96 nm (9.6 Å), as reported by Holtz and Kovacs in 1981. It is important to note that this layer extends indefinitely in both lateral directions. Montmorillonite exhibits significant expansiveness based on its initial and final moisture contents, owing to its minute dimensions and strong water affinity. Smectites have a cation exchange capacity (CEC) that ranges from 80 to 150 milliequivalents per 100 grams.

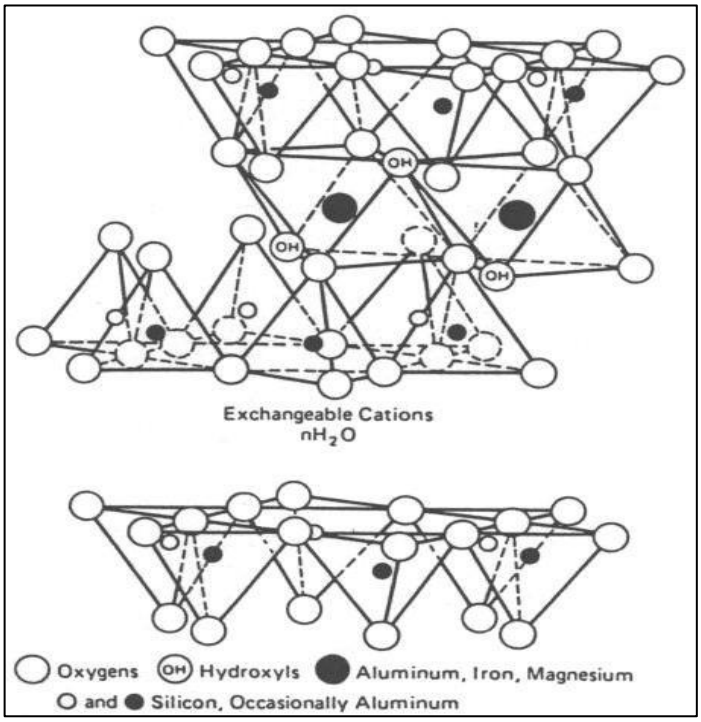


Fig 2.11 Structure of Smectite (Mitchel 1993)

2.4 Soil stabilization

When utilizing local land, projects often face challenges related to limited land availability and insufficient financial resources. Numerous places have negative soil characteristics that have undesirable engineering attributes, such as low bearing capacity, high shrink and swell potential,

and high moisture susceptibility, according to soil quality assessments. (Sharma et al., 2012; Harichane as al., 2011a, Harichane et al., 2011b).

The use of poor soil as a sub-grade layer in pavement is one example demonstrating the consequences of using subpar soil in construction.

According to Sharma et al. (2012), the pavement experiences rapid deterioration, leading to early pavement failure. Nevertheless, the geotechnical engineers tasked with the responsibility of pavement construction have the challenge of working within the constraints of limited financial resources, which are contingent upon the economic condition of their respective countries. Additionally, they must also navigate the scarcity of sufficient resources necessary for the task at hand. Hence, there are instances where a trade-off between quality and durability becomes necessary. Currently, researchers are actively working on the development of cost-effective procedures and enhanced stabilizing methods.

Due to the aforementioned reasons, soil stabilization has been used for many years as a very effective, affordable, and environmentally responsible method of treating soil. The approach described in the literature (Harichane et al., 2011a, Harichane et al., 2011b, Castro-Fresno et al., 2011, Ramadas et al., 2011, Kolias et al., 2005, Amiralian et al., 2012e, Amiralian et al., 2012a) can be customized to fulfill the specific engineering requirements of various projects.

2.5 Mechanical stabilization

According to Patel and Patel (2012), the utilization of soil stabilisation techniques serves to reduce the expenses associated with earthworks while also offering a means of treating soil to maintain, alter, or enhance its performance. The soil stabilisation method can be implemented through two approaches: mechanical means, which involves altering the soil's gradation, and chemical means, which involves inducing changes in the soil chemical characteristics (Amiralian et al., 2012e).

The process of stabilization through mechanical means involves the blending of multiple varieties of natural soil in order to attain a soil mixture that exhibits superior quality and gradation when compared to its individual constituents. To achieve the desired composition and establish the requisite requirements, the ratio of fine and coarse particles in the mixture is modified through the addition or removal of individual soil constituents. Ultimately, the optimal combination of materials is accurately positioned and compressed. According to Ramadas et al. (2011), the introduction of

the newly created soil combination is expected to enhance the soil's strength characteristics by effectively managing cohesion of the soil as well as internal friction. Additionally, the load bearing capacity of the soil is anticipated to increase due to its improved stability as a composite material.

2.6 Chemical stabilization

Furthermore, the implementation of a stabilizer enhances the soil engineering characteristics of weak soil, hence rendering it suitable for various applications such as construction, road construction, and subgrade stabilization in areas with soft soil. Cohesion has a significant role in enhancing the strength qualities of materials, hence reinforcing structures like embankments. According to Patel and Patel (2012), the use of this technique is expected to ultimately result in a decrease in building expenses. Several studies have documented the successful utilization of several additives, including chemicals such as silica fume, cement, gypsum, for the chemical stabilization of soft soils (Amiralian et al., 2012e, Amiralian et al., 2012a).

It is widely recognized that the utilization of resources from nature and industry, such as lime and fly ash, has a great impact on enhancing soil properties (Amiralian et al., 2012a; Castro-Fresno et al., 2011; Harichane et al., 2011a; Kavak and Akyarl, 2007; Kavak and Baykal, 2012; Seco et al., 2011; Degirmenci et al., 2007; McCarthy et al., 2012; Tu, 2009; Kim and Prezzi, 2008). However, the choice of stabilizers depends on the state of the selected construction site as well as on economic issues. In certain nations, like Nigeria, the predominant use of lime as a chemical stabilizer in research on account of financial limitations has been seen (Castro-Fresno et al., 2011).

2.7 Environmental impacts of Chemical stabilizers

Chemical stabilization is effective in stabilizing soft soil and expansive soil. These chemicals such as gypsum, cement, Fly ash, Lime and some other chemicals have been successful in increasing the cohesion of the soil due to which the mechanical parameters of clay has reported to enhance. Therefore, these chemicals are usually used in the field. The problem with these chemicals is that they are harmful to the environment. When these chemical leach in the ground they contaminate the ground water. They also contaminate the surrounding water bodies.

Therefore, the use of these chemicals must be reduced in order to protect the environment. Different new approaches are being adopted to stabilize the soil. Environmentally friendly techniques to stabilize soft soils are being studied by researchers. Microbial Induced Calcite Precipitation is one

of the processes to gain a lot of interest from Geotechnical Engineers due to its environmental friendly pros.

2.8 Introduction to MICP

The method of Microbially Induced Calcium Carbonate Precipitation (MICP) involves the Urea hydrolysis, followed by the precipitation of Calcite mineral (Stocks-Fischer et al., 1999; Hammes and Verstraete, 2002).During MICP, bacteria are responsible for the hydrolysis of one mole of urea ($CO(NH2)2$) into 1 mole of ammonia and 1 mole of carbamic acid, as depicted in Equation 1. The decomposition reaction of carbamic acid can be represented by Equation 2, whereby ammonia and carbonic acid are the resulting products. The hydrolysis of ammonia results in the formation of ammonium ions, leading to an increase in systems pH (as shown in Equation 3). This is followed by the dissociation of carbonic acid into dissolved inorganic carbonate (as depicted in Equation 4). The introduction of $Ca2+$ ions into the medium leads to the precipitation of calcium carbonate crystals on the cell wall, as demonstrated by Equations 5 and 6 (Burne and Chen, 2000).

$$CO(NH_2)_2 + H_2O \rightarrow NH_2COOH + NH_3 \quad (1)$$

$$NH_2COOH + H_2O \rightarrow NH_3 + H_2CO_3 \quad (2)$$

$$NH_3 + H_2O \rightarrow NH_4^+ + OH^- \quad (3)$$

$$H_2CO_3 \rightarrow HCO_3^- + H^+ \quad (4)$$

$$HCO_3^- + H^+ + 2OH^- \rightarrow CO_3^{2-} + 2H_2O \quad (5)$$

$$CO_3^{2-} + Ca^{2+} \rightarrow CaCO_3 \quad (6)$$

The concentration of calcium ions, the concentration of dissolved inorganic carbon (DIC), the pH level, and the existence of nucleation sites are the four parameters that have the biggest effects on the MICP process. (Hammes and Verstraete, 2002). Furthermore, the capacity to metabolize, develop, and reproduce has a significant impact on the viability of microorganisms (Rebata-Landa, 2006). The variables might alternatively be referred to as "limiting growth factors."

The shift in pH has been found to have an impact on cell surface charge, microbial growth, and Metabolic activity (Rebata-Landa, 2006). The increase in pH of the medium can cause due to the

ammonia generated by the process of urea hydrolysis. According to Stocks-Fischer et al. (1999), there was a notable increase in urease activity primarily within the pH range of 6.0 to 8.0. The Urease producing capability of Sporosarcina Pasteurii exhibited its peak at pH 8.0, and thereafter declined as the pH increased. However, a certain activity of Urease production was still seen at 9 pH. Nevertheless, when a enough amount of chemical reagent is present, the hydrolysis of urea rate has a positive correlation with the number of bacterial cells. An increased quantity of bacteria results in a higher production rate of urease per unit volume, hence initiating the process of urea hydrolysis.

In study, Stockfischer et al. (1999) made the observation that the bacterial cell has the potential to function as a site of nucleation for the precipitation of calcite mineral. According to Lian et al. (2006), the process of calcite nucleation occurs at the cell walls of bacteria, as shown by the use of Scanning Electron Microscopy (SEM) pictures. The presence of elevated salinity levels has been seen to result in the inhibition and cessation of activity of microbes (Rivadeneyra et al., 1998). The basic nature of the cementation liquid exhibits a dependence on calcium chloride. The presence of elevated salinity levels has the potential to impede microbial activity, hence restricting the generation of urease by Urease producing bacteria (Nemati et al., 2005).

2.9 Previous Work done on MICP

Numerous experimental research have extensively documented a substantial alteration in soil strength (DeJong et al., 2006; Whiffin et al., 2007; Van Paassen et al., 2009; Chu et al., 2011, 2013; Al Qabany and Soga, 2013). Moreover, numerous experimental data provide evidence for the stiffening effect. The literature suggests that there is a potential for a significant increase in stiffness, with studies indicating a magnitude increase of up to three orders (DeJong et al., 2006; Van Paassen, 2011; Esnault-Filet et al., 2012). Nevertheless, an undesired consequence of the precipitation of calcite is the decrease in hydraulic conductivity, as studies by Al Qabany (2011), Rusu et al. (2011), and Martinez et al. (2013) have reported a loss of more than two orders of magnitude. While this outcome may not necessarily lead to advancements in conventional load-bearing geotechnical applications, it can offer significant advantages in other geo-environmental contexts, including hydraulic barriers and geological waste disposal. Another effect that is more complex in nature is the increase in dilative tendency, as discussed by Chou et al. (2011),

Mortensen and DeJong (2011), and Tagliaferri et al. (2011), which carries significant implications for liquefaction processes.

2.10 Factors affecting MICP

2.10.1 Urease Enzyme availability

The MICP process has two crucial elements, namely efficient urea hydrolysis and calcite precipitation. In order to facilitate the hydrolysis of urea at a satisfactory pace, it is important to make sure the presence of an adequate quantity of urease enzymes capable of catalyzing the process. The presence of bacteria that secretes the enzyme Urease in the system directly impacts the efficiency of the process. The presence of a substantial quantity of bacteria within the soil has been found to contribute to a enhanced performance of calcite precipitation, whereas a deficiency or scarcity of microorganisms is often identified as the primary cause for a shortage of precipitated calcite (Harkes et al., 2010; Al Qabany et al., 2012; Lauchnor et al., 2013; Martinez et al., 2013). Therefore, it is of utmost need to cultivate the bacterial strains, safeguard their viability, and extend their functionality to attain adequate precipitates of calcite minerals. The comprehension of the intricate biotechnological mechanisms behind enzyme activation remains enigmatic.

2.10.2 Concentration of cementation solution

The primary constituents of a cementation media consist of Urea and CaCl2. According to the principles of stoichiometry, it is expected that higher molarity of Urea and CaCl2 will result in a relatively greater production of calcite compared to lower amounts. The veracity of this statement was demonstrated in batch tests (Al Qabany et al., 2013). However, instances have arisen where lower molarity of cementation chemicals yielded stronger treated samples compared to higher concentrations when conducting microbial-induced calcium carbonate precipitation (MICP) in a porous media (Al Qabany et al., 2013).

Gomez et al. (2015) conducted field trials on MICP and reported a similar pattern. In their study, a loosely packed soil ground was divided into parts and subjected to varying amounts of different chemicals. Upon the conclusion of the therapy, the plot that underwent the aforementioned treatment exhibited certain characteristics.

Plots treated with lower amounts of chemicals had superior performance and enhanced levels of calcite quantity compared to parts modified with more concentrations of chemicals. Despite the absence of empirical data to elucidate this phenomenon, Al. Qabany et al. (2013) posited that the observed characteristics are more plausibly attributed to production of a less premium variant of mineral (vaterite) under conditions of elevated concentration of cementation chemicals. According to existing knowledge, it has been suggested that vaterite is eliminated from the body through the process of precipitation following injections. According to Gomez et al. (2015), it was hypothesized that elevated levels of calcium lead to the encasement of bacterial cells in calcium carbonate, ultimately causing their demise prior to the completion of the reaction involving subsequently administered chemicals.

2.10.3 Grain size

In order to facilitate unrestricted movement through the soil matrix, it is important for bacteria to possess dimensions that are smaller than the throat of pores present in the clay. Simultaneously, the presence of wide holes may impede the effective retention of bacteria in the vicinity of soil grains, as these bacteria can be quickly flushed out. The diameter of the majority of bacterial cells typically falls in the range from 0.5 to 3 μm (Madigan and Martinko 1997). Consequently, in order to facilitate the ingress and mobility of bacteria within the soil, the throat of pore size of the porous soil must be marginally larger than 0.5 μm (DeJong et al. 2006). According to DeJong and Fritzges (2006), it has been proposed that soils with larger pore throats, specifically sands and silts, are more conducive to bacterial migrations. The suggested threshold for pore throat size is 4 μm or larger. The compatibility between Sporosarcina Pasteurii and Ottawa 50-70 sand (D50= 0.12 mm) was confirmed by Mitchell and Santamarina (2005).

Soils characterized by smaller throats of pores, such as clayey soil, may not be conducive to the MICP process due to their characteristic to impede the movement of cells within the system. Additional research conducted by Rebata-Landa and Santamarina (2006), DeJong et al. (2010), and Phadnis and Santamarina (2012) has similarly demonstrated that the movement and ability of bacteria to persist are influenced by their shape compatibility with the throats of pore inside the soil.

2.10.4 Methodology of mixing

The remediation of contaminated soils using the Mechanically Induced Chemical Precipitation (MICP) method necessitates the injection of biological and cementation solutions or amendments

into the soil. In order to optimize the adherence of bacteria to soil particles and achieve a homogeneous dispersion within the system, numerous aspects must be taken into consideration. According to the findings of Martinez et al. (2013), the key determinants for the successful dispersion of microorganisms inside soil are the initial concentration and the duration of retention. The latter is typically influenced by the injection mechanism employed for fluid delivery into the system (Van Paassen, 2009).

In MICP research, two typically employed techniques for introducing liquids into the soil are the continuous injection method and the Stopped-Flow injection method. The former method involves the continuous and uninterrupted pumping of solution into the system for the whole duration of the treatment. In contrast, the other method uses the injection runs being interrupted by breaks. Different research groups have implemented this approach, but the duration of the intervals between injection runs differs considerably due to the consideration of various factors such as sample size and concentration of cementation solution, which may not be consistent across these studies (Whiffin et al., 2007; Van Paassen, 2009; Harkes et al., 2010; Al Qabany et al., 2012; Montoya et al., 2012; Gomez et al., 2015).

In general, up to this point, the implementation of the stopped-flow technique has facilitated a more homogeneous process of cementation than before. The continuous flow injection method has been previously described by Martinez et al. (2013) and DeJong et al. (2013). The continuous injection approach has been seen to provide clogging at the injection point, as well as significantly cemented in non-uniform manner in comparison to the stopped flow injection method. One obvious reason for this is that, in contrast to non-stop injection, the cessation of injection provides a sufficient duration for cementation chemicals to undergo reactive processes inside the soil prior to being purged.

2.10.5 In situ studies on MICP

Only a limited number of MICP trials have been conducted in the field thus far. A field study using MICP was performed by Van Paassen (2011) in the southern region of the Netherlands. The trial involved treating a soil volume of 1000 m3 using the MICP method, which was implemented at depths ranging from 3 to 20 meters (see Fig 2.11). The treatment process consisted of the administration of around 200 cubic meters of bacterial liquid, continued by the injection of 300 to 600 cubic meters of urea and salt (Calcium chloride). The purpose of this investigation was to

enhance the structural integrity of a gravel-based borehole in preparation for the underground installation of massive pipes by horizontal directional drilling (HDD) techniques.

The monitoring of the Moisture Induced Calcite Precipitation (MICP) phenomenon involved the utilization of various techniques, including electrical resistance, sampling of groundwater, and clay sample, to quantify the calcite. Upon the conclusion of the trial, it was observed that the measures of CPT and SPT did not exhibit a statistically significant disparity between pre-treatment and post-treatment conditions. Nevertheless, the examination of the undisturbed and excavated samples yielded conclusive data about the impacts of microbial-induced calcium carbonate precipitation (MICP), as shown by calcium carbonate levels reaching up to 6% of the overall dry weight. In general, the borehole was effectively stabilized using microbial-induced calcium carbonate precipitation (MICP) treatment, and the installation of pipes was accomplished satisfactorily, with only a modest enhancement in strength being necessary.

Gomez et al. (2015) conducted an additional field trial on the use of the Mechanically Induced Consolidation Pressure (MICP) technique for ground improvement. This trial took place in loose, eroded sands at a mining site in Canada (see Fig 2.12 (a)). The experimental area was partitioned into four equitably sized plots denoted as TP1 to TP4. Each plot had dimensions of 2.4 m x 4.9 m and was assigned distinct treatment solutions with varying concentrations of urea and $CaCl_2$. This process was done to find out the effect urea and calcium chloride on the MICP process in the field. This is important as this will prove the field application of MICP.

In recent geotechnical research, a study conducted by Li et al. (2020) investigated the application of Microbially Induced Calcium Carbonate Precipitation (MICP) for enhancing the engineering properties of clay soils. The researchers explored the potential of using the bacterium *Sporosarcina pasteurii* to induce the precipitation of calcium carbonate within the clay matrix. The study aimed to evaluate the effectiveness of MICP in improving soil strength, reducing permeability, and enhancing overall geotechnical characteristics.

The findings of this study underscored the potential of MICP as a sustainable and eco-friendly method for enhancing the geotechnical properties of clay soils.

Following a treatment period of 20 days, TP4, which was subjected to the lowest quantities of chemicals, demonstrated the most significant improvement compared to other plots. This improvement was observed in terms of an increased depth of roughly 28 cm and a quantifiable calcite quantity of 2.1 %. The remaining parts exhibited no substantial enhancement.

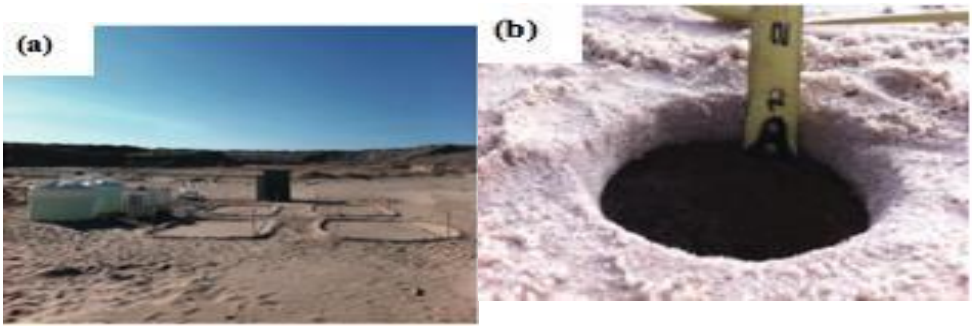


Fig 2.12 MICP conducted in field

The TP4 plots shown a superior level of improvement in comparison to the TP2 and TP3 plots. However, the unexpected nature of this improvement remained unexplained, since no solid rationale was presented to account for the unpredicted results. One obvious explanation was hypothesized to be the entrapment of bacterial bodies within the calcite that was precipitated around it. During the process of calcite crystal formation and precipitation, bacterial cells become engulfed, so impeding their performance. Hence, in the initial stages of fluid injection, the presence of higher chemical concentrations leads to a relatively greater production of calcite compared to lower concentrations. The substantial quantity of calcite generated during the initial stages of MICP will effectively encapsulate a significant quantity of bacterial cells, hence impeding their ability to hydrolyze urea.

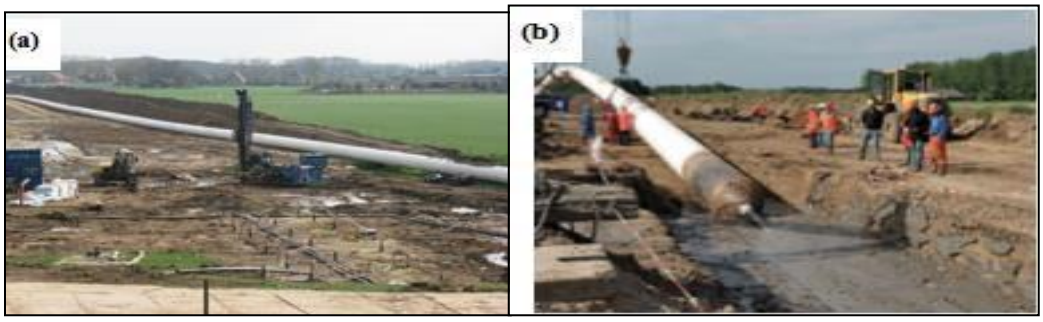


Fig 2.13 Another MICP study conducted in field

2.11 Major challenges in MICP

Predictably, the challenges in reproducing these achievements in the domain are likely to be associated with a lack of comprehensive comprehension of complex mechanisms. There are two main considerations when it comes to scaling up the use of microbial-induced carbonate precipitation (MICP). The first is the need to effectively treat a substantial area of soil in a consistent manner. The second is to ensure the long-term stability and durability of the modified soil (DeJong et al., 2013). Additional considerations and constraints encompass various aspects such as shortening the duration of treatment, the potential recirculation of injected substances, the promotion of existing microbial communities, optimizing the distance over which nutrients are transported, and minimizing the accumulation of undesirable byproducts resulting from microbial metabolism, specifically ammonium. This section will provide a detailed discussion on the key issues and requirements for enhancing the comprehension of (MICP) processes.

2.11.1 Bacterial activity distribution

Martinez et al. (2013) suggest spread of microorganisms plays a crucial role in facilitating a homogeneous precipitation of calcium carbonate. Significant obstacles in the context of bacterial cell distribution within soil pertains to the insufficiency of knowledge and control about their mobility within the soil matrix. According to a investigation conducted by DeJong et al. (2013), it has been documented that microorganisms present in the soil exhibit movement by means of either propulsion or diffusion through the pore mouths located between particles. The optimal scenario entails the bacterial cells achieving a uniform distribution throughout the system, so facilitating the even precipitation of calcite in the soils. This, in turn, leads to the formation of homogeneously cemented soils. In the context of microbial-induced Calcite mineral precipitation (MICP), the movement of bacteria growth is affected by mass diffusion and fluid convection processes. These processes are highly dependent on various variables and cannot be precisely regulated, often resulting in an uneven distribution of bacteria.

In addition to the spatial distribution pattern, there is also a notable worry regarding the vertical extent of microbial penetration inside the soil. In several prior investigations (Whiffin et al., 2007; Van Paassen et al., 2009; Martinez et al., 2013), it was consistently shown that a higher number of bacteria persisted in the vicinity of the injection point. This spatial distribution pattern corresponded to an increased presence of calcite in the proximal regions and a decreased presence of calcite in the

distal regions relative to the injection source. A further point of consideration pertains to the potential duration of survival once reaching a specific depth. The unanswered matters pertaining to the activity, motions, and dispersion of introduced bacteria during the process of Microbially Induced Calcium Carbonate Precipitation (MICP) provide a significant obstacle for the use of MICP on site in the real world (Van Passen et al., 2009).

2.11.2 Distribution of calcium carbonate

An uneven distribution of bacterial strains cells in the soil often consequents in an unequal distribution of precipitated Calite mineral in the soil. The observed outcome is deemed undesirable due to its propensity to result in an unevenly treated sample, wherein certain sections see a more pronounced alteration in soil qualities compared to others. Several research (Van Paassen et al., 2007; Whiffin et al., 2007; Martinez et al., 2013; Akimana et al., 2016) have documented the presence of non-uniform precipitation patterns in treated samples. These studies have observed an accumulation of calcite in regions proximal to the injection point, whereas locations near the exit have shown a deficiency of calcite. The treatment results in a variation in the soil parameters, specifically (porosity and stiffness), creating a gradient. However, this outcome is considered undesirable.

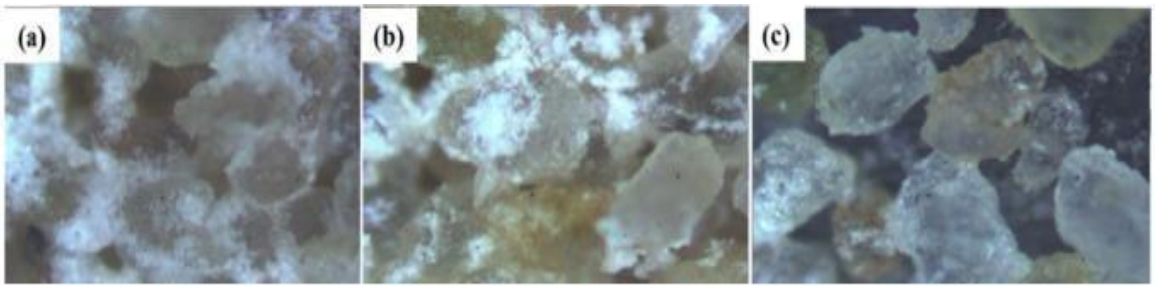


Fig 2.14 SEM Image of calcite (a) entrance of sample (b) middle of sample (c) wnd of sample

2.11.3 Bacteria as nucleation site for calcite

According to Cuthbert et al. (2012), the process of chemical reactions and calcite precipitation might result in the entrapment of bacterial cells within calcite crystals, hence impeding their ability to carry out urea hydrolysis, which is their primary purpose. The phenomenon of bacterial encapsulation has been hypothesized as a potential explanation for the atypical precipitation results observed in cases when less concentrated cementation chemicals lead to greater calcite formation

and improved soil cementation, as previously discussed. This phenomenon may be attributed to the rapid nucleation caused by highly concentrated solutions, resulting in the formation of substantial quantities of calcite during the initial phases of the reaction. Therefore, a significant number of bacterial cells are encapsulated by the calcite, impeding their ability to catalyze urea hydrolysis.

2.11.4 Cost

This process encompasses a diverse array of chemicals and operational tools to ensure its effective execution. Hence, the expenses associated with field size applications can be substantial. In a study conducted by Ivanov and Chu (2008), the authors provided an estimation of the cost of materials required for Microbially Induced Calcium Carbonate Precipitation (MICP) treatment. The anticipated price range for MICP raw materials, including urea, CaCl2, and microorganisms, was reported to be between US$4.0-9.0 per cubic meter. On the other hand, DeJong et al. (2013) proposed a broader cost range for MICP treatment in saturated soils, encompassing expenses related to materials, equipment, and installation. According to their findings, the cost of MICP treatment in such conditions may vary from $25-75 per cubic meter. While the cost of this approach may exceed that of traditional ground improvement methods, Cheng and Cord-Ruwisch (2012) have suggested that there is potential for cost reduction through improved cementation processes. In addition, the development of efficient processes for Microbially Induced Calcium Carbonate Precipitation (MICP) is of significant importance.

The objective is to attain consistent and manageable results in the practical application of a particular subject, moving away from the reliance on experimental investigations characterized by a trial-and-error approach.

2.11.5 Durability

DeJong et al. (2009) have articulated their apprehensions over the long-term stability of precipitated calcite inside soil matrices. Based on their assertions, the stability of recently formed calcite may be disrupted by the re-emergence of geochemical circumstances before to treatment and alterations in the overall composition of calcite subsequent to the conclusion of treatment. In the MICP field trial conducted in Saskatchewan, Gomez et al. (2015) observed a modest degradation of improved sands 298 days following the completion of the last treatment. The decline in condition was ascribed to an inclement winter that occurred in the Canadian area shortly following the MICP experiments.

The complex process of MICP requires a particular strain of bacteria which has the following properties and two bacterial strains were used which are explained in the following section.

2.11.6 Bacterial strains

The process of MICP requires two solutions which include the bacterial solution and the cementation solution. The Bacterial solution contains bacteria in suspended form. This bacteria has to be of certain qualities to be used in the process of MICP. Therefore, selection of bacterial strain was the most important part of the research. The qualifications of the bacterial strain are following

- Locally identified strain in Pakistan
- Urease producing bacterial strain
- Easy to grow in laboratory
- Resistant to Environmental changes
- Spore producing bacterial strain
- Should not have harmful impact on human health

The bacterial strains with the above mentioned qualities were searched in Pakistan and two bacterial strains were selected which are *Bacillus subtilis* and *Priestia megaterium.*

2.11.6.1 Bacillus subtilis (NCCP-3003)

The *Bacillus subtilis* was obtained from National Agriculture Research Centre (NARC).

Bacillus subtilis is a Gram-positive bacterium with a distinctive rod-like shape, renowned for its significance in various scientific and industrial domains. Its remarkable versatility, ecological relevance, and diverse applications have rendered it a subject of extensive study. Initially discovered and characterized by Christian Gottfried Ehrenberg in 1835, Bacillus subtilis has evolved into a model organism for investigating fundamental aspects of bacterial genetics, physiology, and cellular mechanisms.

2.11.6.1.1 Genomic Attributes and Taxonomy

Positioned within the Bacillaceae family and Bacillus genus, Bacillus subtilis has undergone taxonomic refinements driven by advances in molecular methodologies. The comprehensive sequencing of its genome in 1997 (Kunst et al., 1997) uncovered its intricate genetic makeup and shed light on its adaptive strategies to various environments.

2.11.6.1.2 Physiological Phenomena and Metabolism

Bacillus subtilis stands out for its robustness and adaptability across diverse conditions. It can effectively transition between distinct metabolic states, including biofilm development, sporulation, and exponential growth. Its metabolic flexibility has spurred extensive investigations into aspects like gene expression regulation, nutrient utilization, and stress response mechanisms (Molle et al., 2003).

2.11.6.1.3 Cellular Specialization and Sporulation

A captivating feature of Bacillus subtilis is its capacity for sporulation—a complex developmental process culminating in the formation of resilient endospores. This process involves intricate genetic and morphological alterations, rendering it an intriguing model for exploring cellular differentiation and intercellular signaling pathways (Errington, 2003).

2.11.6.1.4 Genetic Competency and Lateral Gene Transfer

Bacillus subtilis exhibits a natural competency, enabling it to uptake external DNA and integrate it into its genetic material. This inherent capability has facilitated investigations into horizontal gene transfer mechanisms and DNA assimilation processes (Dubnau, 1999), contributing to insights into bacterial evolution and adaptive mechanisms.

2.11.6.1.5 Applications

Beyond its role as a model organism, Bacillus subtilis finds practical utility in multiple industries. It is harnessed for enzyme, antibiotic, and biofuel production. Additionally, its utilization as a probiotic in animal feed has garnered attention due to its potential to enhance animal health and growth.

In summation, Bacillus subtilis emerges as a captivating bacterium with a rich legacy of scientific exploration and pragmatic applications. Its genetic manipulability, metabolic adaptability, and intricate developmental dynamics continue to fuel interdisciplinary research endeavors.

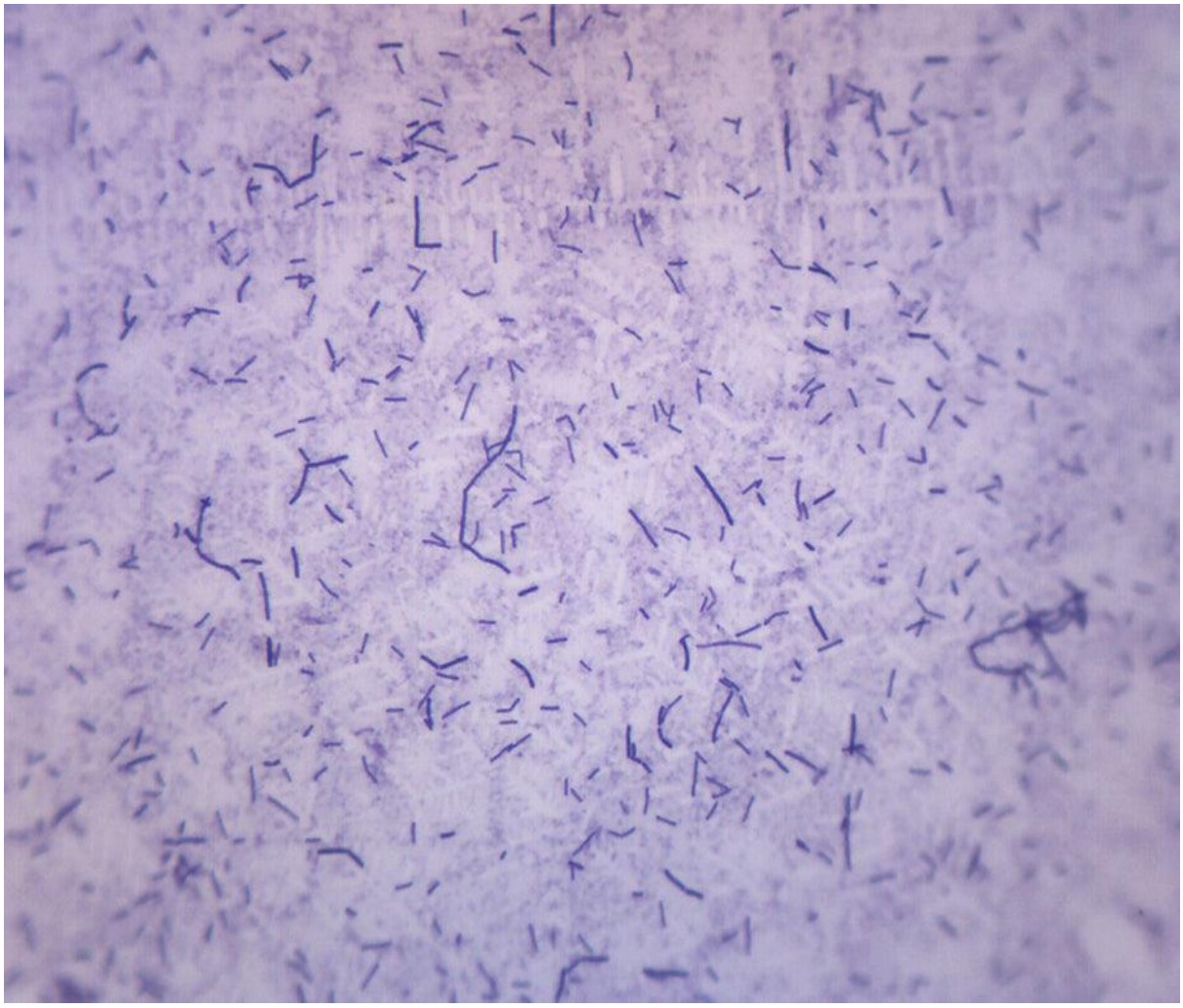


Fig 2.15 Image of *Bacillus subtilis* under a microscope

2.11.6.2 Priestia megaterium (NCCP-2993)

Bacillus megaterium, a Gram-positive bacterium belonging to the Bacillus genus, is recognized for its distinctive larger size relative to many other bacterial species, earning it the appellation "megaterium," signifying its "large organism" nature (Rey et al., 2019). Its habitat encompasses diverse environments such as soil, water, and other ecological niches. Bacillus megaterium stands

out due to its varied metabolic capabilities, rendering it particularly suitable for numerous biotechnological applications.

2.11.6.2.1 Morphology

Bacillus megaterium assumes a typical rod-shaped morphology, often capable of forming highly resilient endospores which facilitate survival in challenging conditions (Rey et al., 2019).

2.11.6.2.2 Metabolic Diversity

This bacterium exhibits a remarkable range of metabolic activities, allowing it to exploit diverse carbon and energy sources. It demonstrates proficiency in aerobic and facultative anaerobic respiration, sugar fermentation, and processing of an array of organic compounds (Rey et al., 2019).

2.11.6.2.3 Biotechnological Significance

Bacillus megaterium has garnered attention in the biotechnology domain for its aptitude in producing valuable enzymes, bioactive compounds, and other industrially pertinent products. Notably, it finds employment in the large-scale synthesis of enzymes like amylases, proteases, lipases, and exopolysaccharides, which hold relevance across food, pharmaceutical, and industrial sectors (Chandel et al., 2020).

2.11.6.2.4 Genomic Insights

Comprehensive genomic investigations have shed light on Bacillus megaterium's metabolic pathways and its potential for generating diverse metabolites. Genetic engineering techniques have been harnessed to tailor the bacterium for augmented yield of specific compounds (Rey et al., 2019).

2.11.6.2.5 Ecological Role

Bacillus megaterium plays an integral part in nutrient cycling and decomposition of organic matter within various ecosystems. Its prevalence in soil and water environments contributes to the breakdown of organic substances and the recycling of essential nutrients (Holt et al., 2020).

2.11.6.2.6 Research Utility

Its relatively larger size and ease of cultivation render Bacillus megaterium an ideal model organism for diverse research undertakings. It has been extensively employed to investigate cellular processes, metabolic pathways, and inter-microbial interactions (Rey et al., 2019).

To encapsulate, Bacillus megaterium emerges as a versatile bacterium, offering substantial potential for a spectrum of biotechnological applications. Its diverse metabolic repertoire, capacity to generate valuable enzymes, and adaptability to distinct habitats position it as a promising candidate for numerous industrial and scientific pursuits.

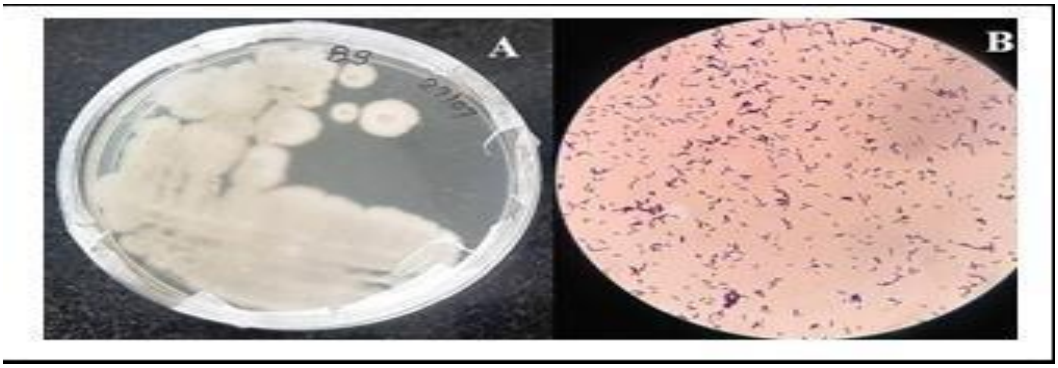


Fig 2.16 (a) *Bacillus megaterium* in Petri dish (b) Microscopic image of *Bacillus megaterium*

2.12 Soil properties

2.12.1 Sieve analysis

The methodology of sieve analysis, as outlined in ASTM E11, involves a precise and standardized approach to determine the particle size distribution of granular materials. This method ensures consistency and comparability of results across different laboratories and studies.

Soil gradation refers to the arrangement and distribution of different-sized particles within a soil sample. There are several types of soil gradation:

1. Well-Graded Soil: Well-graded soil contains a wide range of particle sizes, from large to small. This type of gradation provides good stability and excellent drainage properties. It typically includes a mixture of sand, silt, and clay.

2. Poorly-Graded Soil: Poorly-graded soil consists mostly of particles of similar size. This type of gradation lacks diversity in particle sizes, which can lead to limited drainage and reduced stability. It is often dominated by one particle size, such as sand or clay.

3. Uniformly-Graded Soil: Uniformly-graded soil contains particles that are primarily of one size. This type of gradation can have predictable engineering properties but may suffer from poor drainage due to the absence of diverse particle sizes.

4. Gap-Graded Soil: Gap-graded soil has an uneven distribution of particle sizes, with some sizes missing or sparsely represented. This type of gradation can lead to erratic engineering behavior, making it less desirable for certain construction purposes.

5. Well-graded gravel, well-graded sand, and well-graded clay: These subcategories of well-graded soil describe the specific particle types dominant in the gradation, indicating whether gravel, sand, or clay particles are prevalent.

Understanding the gradation of soil is crucial in various engineering and construction applications, as it affects the soil's mechanical properties, permeability, and suitability for specific projects. Different gradations are chosen based on the desired characteristics for a particular application.

2.12.2 Pycnometer test

Specific gravity of soil is a measure that tells us how dense or heavy a particular soil sample is compared to the density of water. It indicates the ratio of the weight of a given volume of soil to the weight of an equal volume of water. In simpler terms, it helps us understand how much heavier or lighter soil is compared to water.

To find the specific gravity of soil, we typically compare the weight of a known volume of dry soil to the weight of an equal volume of water. The formula for specific gravity is:

Specific Gravity (Gs) = (Weight of dry soil) / (Weight of an equal volume of water)

Specific gravity values are important in geotechnical engineering because they provide information about the soil's composition and its ability to support structures or retain water. Different types of soil, such as sand, silt, and clay, have different specific gravity values, and these values can influence factors like soil compaction and buoyancy forces on underground structures. By knowing the specific gravity of soil, engineers can make informed decisions about the suitability of soil for construction and other applications.

The pycnometer test is a widely used method to determine the specific gravity of solid materials, providing valuable insights into their density and composition. This test involves utilizing a pycnometer, a specialized glass container with a known volume, to measure the ratio of the density of the material to the density of a reference substance, typically water.

This test's accuracy relies on factors such as eliminating air bubbles, ensuring precise weight measurements, and using an appropriate reference liquid with a known density. Additionally, temperature plays a role, and it's important to account for thermal expansion effects.

2.12.3 Unconfined compressive strength

Unconfined compressive strength is a measure of a material's ability to withstand axial loads or pressure without any external lateral confinement or support. In simpler terms, it assesses how much force can be applied to a sample of material, such as soil or rock, in a compressive (squeezing) manner before it fails or breaks.

The test to determine unconfined compressive strength typically involves subjecting a cylindrical specimen of the material to a steadily increasing axial load until it ruptures. This measurement is significant in fields like geotechnical engineering and construction because it helps engineers understand the load-bearing capacity of soils, rocks, or other materials that may be used in various structures, foundations, or excavations. It provides critical information for designing and assessing the stability of structures and ensuring they can withstand the pressures and forces they may encounter during their lifespan.

In summary, unconfined compressive strength is a fundamental property used in engineering and geosciences to assess the load-bearing capacity of materials like soil and rock. It helps engineers make informed decisions when designing structures and evaluating the stability and safety of construction projects.

2.12.4 Standard proctor test

The Standard Proctor Test, outlined in ASTM D698, is a crucial method for determining the maximum dry density and optimum moisture content of a soil for compaction purposes. This procedure involves a series of controlled compaction efforts on soil samples at varying moisture contents to establish the optimal moisture-density relationship.

The process commences with obtaining a representative soil sample, which is then air-dried and broken down into smaller particles. The sample is then thoroughly mixed to ensure uniformity.

The dry density of clay can vary significantly depending on several factors, including the type of clay, its mineral composition, compaction effort, and moisture content. Typically, the dry density of clay falls within a range of 1.1 to 1.9 grams per cubic centimeter (g/cm^3). Here's a breakdown of this range:

1. Low Dry Density

Dry Density Range: Approximately 1.1 to 1.5 g/cm^3

This range is typical for expansive clays and highly organic soils. These soils tend to have a lower particle density and are more prone to shrinkage and swelling with changes in moisture content.

2. Moderate Dry Density

Dry Density Range: Approximately 1.5 to 1.7 g/cm^3

Many types of clay, including silty clays and some plastic clays, fall within this range. They have moderate dry densities and are often used in various construction applications.

3. High Dry Density

Dry Density Range: Approximately 1.7 to 1.9 g/cm^3

Certain types of clay soils, particularly well-compacted, dense clay soils with low porosity, can exhibit higher dry densities. These soils are generally stable and have good load-bearing characteristics.

It's important to note that the dry density of clay can also be influenced by the compaction effort during construction. The Standard Proctor Test, as mentioned in a previous response, is used to determine the maximum dry density and optimum moisture content for a specific clay soil under standardized compaction conditions. Engineers use this information to achieve the desired compaction and density when using clay soils in construction projects.

Additionally, soil classification systems, such as the Unified Soil Classification System (USCS) or the AASHTO Soil Classification System, may provide further insights into the specific type of clay

based on particle size distribution and plasticity characteristics. These classifications can help engineers better understand the engineering properties of the clay and its expected dry density range.

2.12.5 X-Ray Diffraction

X-ray diffraction (XRD) is a fundamental analytical technique widely used in soil science and geology to ascertain the mineral composition of soil samples. Its principle relies on the diffraction of X-rays by crystalline materials, with each mineral exhibiting a distinct diffraction pattern. To perform XRD on soil, samples are first prepared by drying and grinding them into a fine powder, ensuring random orientation of mineral crystals. These powdered samples are then exposed to X-rays generated by an X-ray source, and the resulting diffraction pattern is captured and analyzed. By comparing this pattern to a database of known minerals, XRD allows for precise identification of the minerals present in the soil. This mineralogical insight aids in soil classification, understanding its engineering properties, characterizing its behavior, and even assessing its interactions with contaminants in environmental studies. In essence, XRD is an indispensable tool for unraveling the mineralogical composition and properties of soil, thereby contributing to various fields such as agriculture, geotechnical engineering, and environmental science.

2.12.6 Fourier-transform Infrared (FTIR)

Fourier-transform infrared spectroscopy (FTIR) is a powerful analytical technique widely employed in various scientific fields, including soil science, chemistry, and material science. FTIR works on the principle that molecules absorb infrared radiation at characteristic frequencies, allowing for the identification of chemical bonds and functional groups present in a sample. In the context of soil analysis, FTIR enables researchers and scientists to gain insights into the organic and mineral components of soils. To perform FTIR on soil samples, a finely ground and homogenized soil specimen is typically mixed with a potassium bromide (KBr) matrix and compressed into a transparent pellet. This pellet is then exposed to a broad spectrum of infrared light, and the resulting absorption spectrum is measured and analyzed. The peaks and patterns in the spectrum provide valuable information about the soil's organic matter content, mineral composition, and various chemical properties. FTIR is particularly useful for studying soil organic matter, assessing soil quality, and understanding the impact of environmental factors on soil chemistry. Its versatility and

precision make it an essential tool for researchers and soil scientists seeking a deeper understanding of soil composition and its role in ecosystems and agricultural practices.

2.12.7 Scanning electron microscopy

Scanning Electron Microscopy (SEM) is an advanced imaging technique employed in various scientific disciplines, including material science, geology, biology, and nanotechnology. SEM enables researchers to visualize and analyze the microstructure and surface morphology of samples with exceptional detail. In SEM, a focused electron beam scans across the sample's surface, and interactions between the electrons and atoms on the sample's surface result in various signals, such as secondary electrons and backscattered electrons. These signals are detected and translated into high-resolution images, providing insights into the topography, texture, and composition of the sample at a nanoscale level. SEM has a wide range of applications in soil science, where it is used to examine soil particles, minerals, microorganisms, and aggregates. It helps researchers understand soil structure, particle size distribution, and pore networks, contributing to our knowledge of soil behavior, fertility, and environmental processes. SEM's ability to reveal intricate details at the micro- and nano-levels makes it an invaluable tool for investigating complex soil systems and advancing our understanding of soils' role in agriculture, geology, and environmental science.

3 Chapter

Materials and Methodology

3.1 Materials

3.1.1 Soil Used

The soil utilized in this study was obtained from Nandipur, located in the province of Punjab. The soil can be categorized as a high plastic clay, characterized by a liquid limit that surpasses 50. Table 1 provides a concise summary of the physical parameters exhibited by the Nandipur clay. The classification of clay as swelling clay (CL) is depicted in the figure.

Table 1 : Engineering properties of natural soil

Engineering properties of soil	
liquid limit	51
specific gravity	2.74
optimum moisture content	21.3
maximum dry density	1.69 g/cm3
unconfined compressive strength	177.5 kpa
PI	26

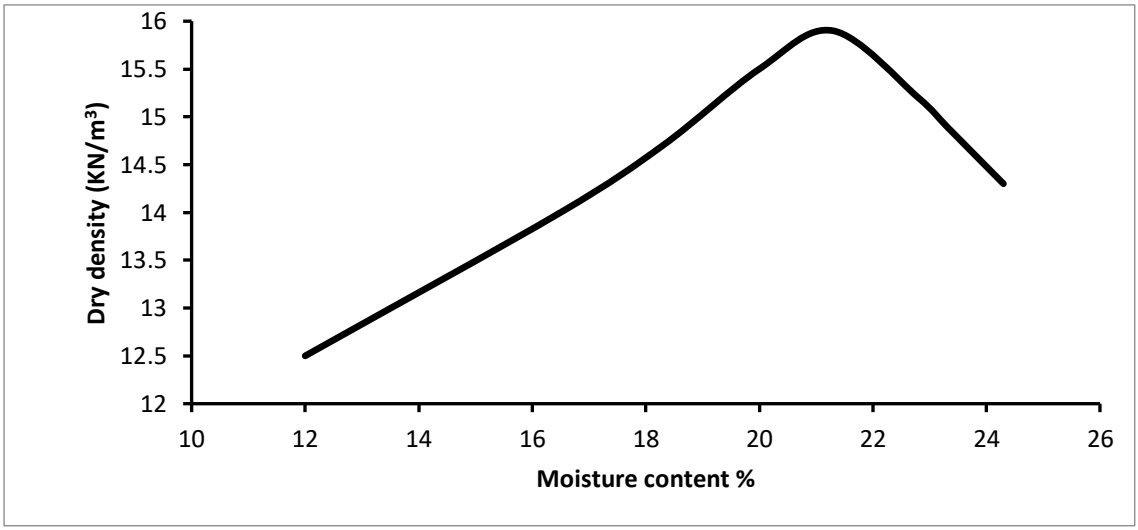


Fig 3.1 compaction curve of natural soil

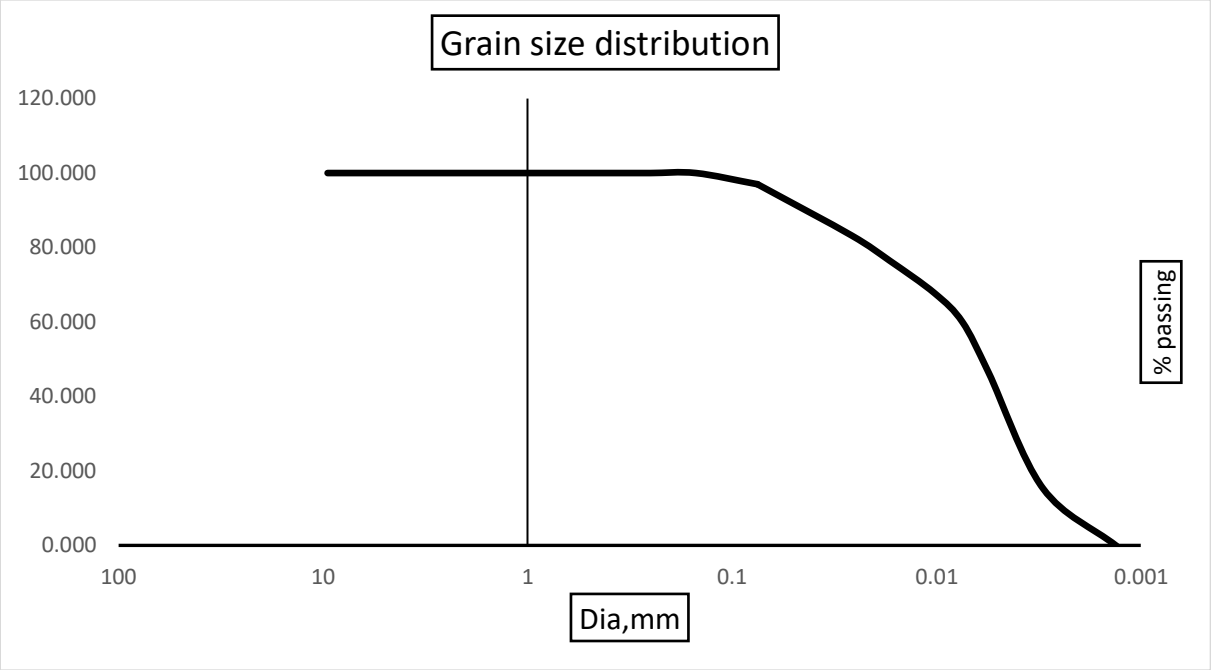


Fig 3.2 Grain size distribution of the soil

3.2 Methodology for transferring bacterial strains to fresh Petri dishes

3.2.1 Acquisition of Bacterial Strains

Bacillus subtilis and Priestia megaterium bacterial strains were procured from the National Agriculture Research Center (NARC), a recognized source of microbial cultures and research materials.

3.2.2 Transferring to Fresh Petri Dishes

To ensure the continued availability and viability of the bacterial strains, a procedure was undertaken to transfer them from their original sealed plastic Petri dishes to new sterile ones. This process is a fundamental aspect of maintaining long-term bacterial cultures.

3.2.3 Agar Media Preparation and Sterilization

Agar media, a nutrient-rich gelatinous substance serving as a growth medium for bacteria, was meticulously prepared. It was then subjected to sterilization through autoclaving at a high temperature of 121°C. This autoclaving process effectively eliminates any existing microorganisms, preventing contamination of the cultures.

3.2.4 Pouring Agar into Petri Dishes

The freshly autoclaved and now sterile agar media was poured into pre-sterilized glass Petri dishes. This step provides a solid substrate upon which the bacterial strains can thrive and propagate.

3.2.5 Incubation for Contamination Check

Following the agar media solidification, the Petri dishes were transferred to a controlled environment – an incubator – set to an appropriate temperature. Over a 24-hour period, the cultures were closely monitored for any signs of contamination, such as the emergence of unintended microorganisms.

3.2.6 Aseptic Streaking Technique in Laminar Flow Hood

Petri dishes that exhibited no observable contamination after the incubation period were meticulously opened within a specialized laminar flow hood. This sterile workspace minimizes the risk of introducing external contaminants. Using an aseptic streaking technique, bacterial strains were carefully streaked onto the agar surface in a distinctive zig-zag pattern. The primary purpose of this technique is to isolate individual bacterial colonies from the mixed culture, allowing for the propagation of pure strains.

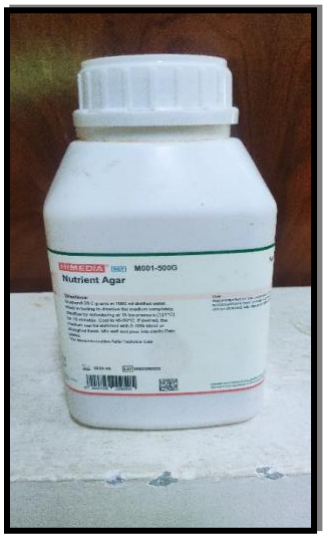
3.2.7 Incubation to Foster Growth

Once the streaking procedure was completed, the Petri dishes were promptly closed and securely sealed. These sealed dishes were then returned to the incubator, where conditions were optimized for the growth of the isolated bacterial colonies. The chosen temperature of 37°C is conducive to the rapid proliferation of many bacterial species.

3.2.8 Transfer and Preservation in Freezer

Following a subsequent 24-hour incubation period, the Petri dishes, now adorned with well-defined bacterial colonies, were transferred to a freezer for preservation. To prevent degradation and contamination, the Petri dishes were carefully enclosed within sealed plastic bags. The temperature within the freezer was set to -4°C, a temperature suitable for short-term storage of bacterial cultures.

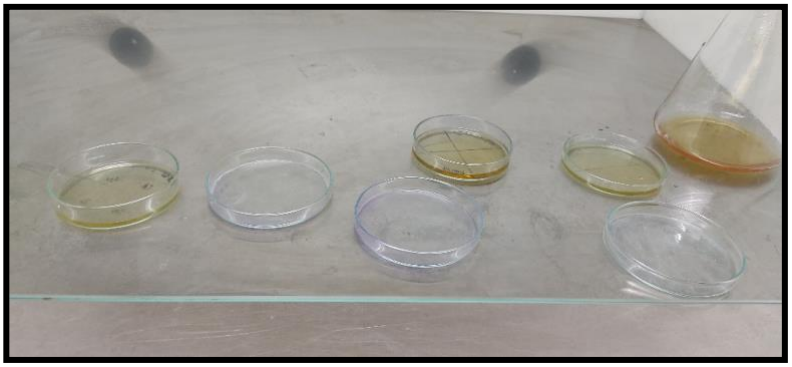
Throughout each phase of this meticulously orchestrated process, stringent aseptic techniques were employed to safeguard the purity and integrity of the bacterial strains. The attention to detail, sterile conditions, and controlled incubation parameters collectively contribute to the successful growth.



1. Nutrient Agar mixed with distilled water.

2. Nutrient Agar autoclaved.

3. Nutrient agar after autoclaving



4. The agar is poured in the Petri dishes.

5. The plates are kept in the incubator for 24 hours to check for any contamination, if there is no contamination the plates.

Fig 3.5 Growing bacterial strains in Petri dishes

3.3 Methodology for Inoculation

3.3.1 Preparation of Nutrient Broth (Tryptone Soya Broth, TSB)

Tryptone Soya Broth (TSB) serves as a rich and nutritious medium to support the growth of bacterial cultures. In a meticulously controlled procedure, a carefully measured amount of each ingredient is meticulously mixed to create the TSB. This includes incorporating 17 grams of pancreatic digest of casein, which provides a source of amino acids; 3 grams of papaic digest of soya bean meal, contributing essential nutrients; 5 grams of sodium chloride, maintaining osmotic balance; 2.5 grams of dipotassium hydrogen phosphate, supporting cellular functions; and 2.5 grams of dextrose/glucose, furnishing a carbon source. The resulting mixture, representing a carefully balanced nutritional composition, is dissolved into distilled water.

With an emphasis on maintaining sterility and preventing contamination, the solution is subjected to the autoclave process. At a high temperature of 121°C, pressure builds within the autoclave, effectively sterilizing the solution and rendering it devoid of any potentially harmful microorganisms. This crucial sterilization step ensures that the subsequent bacterial culture will exclusively comprise the desired strain, allowing for accurate and controlled experiments.

3.3.2 Inoculation and Incubation

Building upon the foundation of the meticulously prepared TSB, the process of bacterial culture initiation commences. Using a specialized tool known as an inoculating loop, bacterial colonies sourced from a Petri dish are carefully transferred into the autoclaved TSB broth. This meticulous transfer ensures that the bacterial culture remains uncontaminated and uncontested by unwanted microbes.

The next phase involves subjecting the inoculated TSB broth to a controlled environment that encourages bacterial growth. This environment is a shaking incubator, set at a precisely maintained temperature of 37°C. In addition to temperature control, the incubator's shaking mechanism ensures uniform distribution of nutrients and oxygen throughout the medium, facilitating optimal bacterial growth. The vigorous shaking, performed at a consistent rate of 120 revolutions per minute, fosters the development of bacterial populations over a period of 24 hours.

After this carefully monitored incubation period, a transformative change becomes evident. The formerly transparent TSB broth has become turbid and cloudy, a visual indicator of successful

bacterial proliferation. This transformation signifies the fulfillment of the culture's growth potential, serving as a crucial checkpoint in the process.

3.3.3 Bacterial Harvesting

With the bacterial culture now flourishing within the TSB broth, the focus shifts towards its careful collection for subsequent steps. To achieve this, a centrifugation process is employed. The bacterial culture in the TSB broth is transferred to specialized containers called falcon tubes, each capable of accommodating a specific volume.

Upon initiation of the centrifugation process, the falcon tubes are subjected to rapid rotation at a speed of 4000 revolutions per minute (rpm). This centrifugal force serves as a powerful tool for separation, causing the denser bacterial cells to sediment at the bottom of the tubes. Meanwhile, the liquid component of the culture remains suspended above.

The result of this process is the effective isolation and concentration of the bacterial population, as the sedimented cells form a pellet at the bottom of each tube. This concentrated biomass is a valuable resource for subsequent stages of the procedure.

3.3.4 Preparation of Fresh Media

With the concentrated bacterial biomass now harvested, the next step involves the replenishment of the culture medium. The TSB broth, having served as the nurturing environment for bacterial growth, is carefully removed from the falcon tubes. In its place, a fresh batch of autoclaved liquid media is introduced.

The introduction of this fresh media serves multiple purposes. Primarily, it provides an opportunity for the bacterial population to transition into a new environment, replete with the necessary nutrients and conducive to their continued growth. Additionally, this step ensures the sustainability of the bacterial culture, allowing for subsequent experimental iterations.

3.3.5 Optical Density (OD) Measurement and Adjustment

A key aspect of monitoring bacterial growth involves quantification of their population density. This is achieved through the measurement of optical density (OD) at a specific wavelength, often designated as OD600. To facilitate this measurement, a spectrophotometer is employed, capable of quantifying the amount of light absorbed by the bacterial suspension.

In this instance, OD600 readings of 1.5 and 1.7 are reported for megaterium and subtilis bacterial strains, respectively. These readings represent standardized measures of the bacterial culture's density, providing valuable insights into their proliferation and growth.

However, the procedure does not conclude with measurement alone. The data obtained from the OD600 readings is utilized to guide an essential adjustment. By supplementing the culture with additional fresh autoclaved liquid media, the OD600 is carefully adjusted to a uniform value of 1. This step ensures consistency in bacterial density and enables accurate experimentation.

1. Nutrient broth

2. Broth autoclaved for sterilization.

3. Broth after autoclaving

4. After inoculating the broth, the inoculum is kept in a shaking incubator for 48 hours

5. Inoculum turned cloudy due to bacterial growth.

Fig 3.6 Inoculation process for bacteria

3.4 Methodology for preparation of Cementation solution

3.4.1 Components of Cementation Solution

The cementation solution is meticulously composed of distinct chemical constituents, each playing a unique role in the overall process:

3.4.1.1 Ammonium Chloride (NH_4Cl)

This compound, consisting of ammonium and chloride ions, can influence the formation of specific compounds during the cementation process.

3.4.1.2 Sodium Sulfate (Na_2SO_4)

Sodium sulfate, a salt composed of sodium, sulfur, and oxygen, may contribute to the chemical reactions and solid formation occurring within the solution.

3.4.1.3 Calcium Chloride Anhydrous ($CaCl_2$)

Calcium chloride, an anhydrous form of the compound, is known for its hygroscopic properties, which might impact the water content and reactivity of the solution.

3.4.1.4 Urea

Urea, a diamide of carbonic acid, is often utilized in various chemical processes due to its ability to form complexes and engage in hydrogen bonding.

3.4.1.5 pH Adjustment

The pH of a solution is a fundamental parameter influencing the nature of chemical reactions. By carefully adding drops of sodium hydroxide (NaOH) solution to the cementation solution, the pH is raised to 8. This adjustment is significant as it can impact the solubility of compounds, the rate of reactions, and the overall stability of the solution.

3.4.1.6 Autoclaving and In-Situ Conditions

Autoclaving, a process involving elevated temperature and pressure, is employed to sterilize distilled water. This meticulous step ensures the removal of any potential contaminants that could interfere with the intended reactions. Notably, the decision not to autoclave the other components (Ammonium chloride, Sodium sulfate, Calcium chloride anhydrous, and urea) is deliberate. This choice is likely made to maintain a representation of real-world or specific experimental conditions where these components are exposed to the inherent environment, devoid of sterilization procedures.

3.5 Mixing of MICP solution with soil

The soil was dried out in an oven for an entire night, and then it was placed in sterile plastic bags to prevent any sort of bacterial contamination. In a laminar hood, the plastic bags were sanitized by being exposed to ultraviolet radiation. The bacterial solution and the cementation solution were both introduced to the soil at the same time in accordance with the optimal volumetric moisture content of the soil. The bacterial solution accounted for one half of the optimal moisture level, while the cementation solution covered the other half. According to Tiwari et al., 2021b, the soil was stirred thoroughly enough to allow the solution to permeate each every grain of dirt. According to ASTM D2166, the UCS sample had a width of 40 millimeters and a height of 80 millimeters. During the process of curing, the sample was kept in an open atmosphere in the laboratory. After 7 days of curing, testing was performed on the substance. In order to prevent any loss of moisture during the closed curing process, the samples were wrapped in food-grade plastic before being placed in the oven.

3.6 Methodology for wetting and drying

UCS samples were cured for a full week in an atmosphere including fresh air. Following the conclusion of the seven-day period allotted for the curing process, the samples were appropriately bandaged, as indicated in figure 6. The samples were prepared by being submerged in a dish of water. The container received an amount of water sufficient to submerge the sample to its midpoint. The samples were allowed to soak in the tray until they were completely absorbed by the liquid. At every 12-hour interval, the samples' saturation levels were analyzed. The link between the moisture content of the samples, the specific gravity of the clay, and the void ratio was used to determine whether or not the samples were saturated. The difference in mass between the samples before and after they were soaked in the water was used to derive the percentage of moisture present in each sample.

$S = WGs / e$

After being completely saturated, the samples were stored in an oven at a temperature of 40 degrees Celsius. The samples' levels of saturation were evaluated using the same methodology as those for drying. The experimental test for strength was carried out on an automatic direct shear apparatus for a total of one, three, five, seven, nine, and eleven cycles of wetting and drying.

3.7 Methodology of laboratory tests performed

The following test were performed on untreated and treated soil to confirm the applicability of MICP on the soil.

- Sieve analysis
- Specific gravity test
- Standard proctor test
- Unconfined compression test (UCS)
- X-Ray Diffraction test
- Fourier transform infrared (FTIR)
- Scanning Electron Microscopy (SEM)
- Spectroscopy

3.7.1 Sieve analysis

Soil was excavated from a depth of 3 feet from the ground and then a representative sample was taken for the process of soil gradation. The soil was oven dried before the gradation.

Next, a set of sieves with known mesh sizes is arranged in a stack, starting with the finest sieve at the bottom and progressively coarser sieves stacked on top. The material sample is placed on the top sieve, and the entire stack is placed in a mechanical sieve shaker.

The shaker is then operated for a predefined duration and at a specified amplitude and frequency. This mechanical action causes smaller particles to pass through the openings in the sieves, while larger particles are retained on the corresponding sieves. It's worth noting that the selection of shaker parameters is crucial to ensure consistency and accuracy.

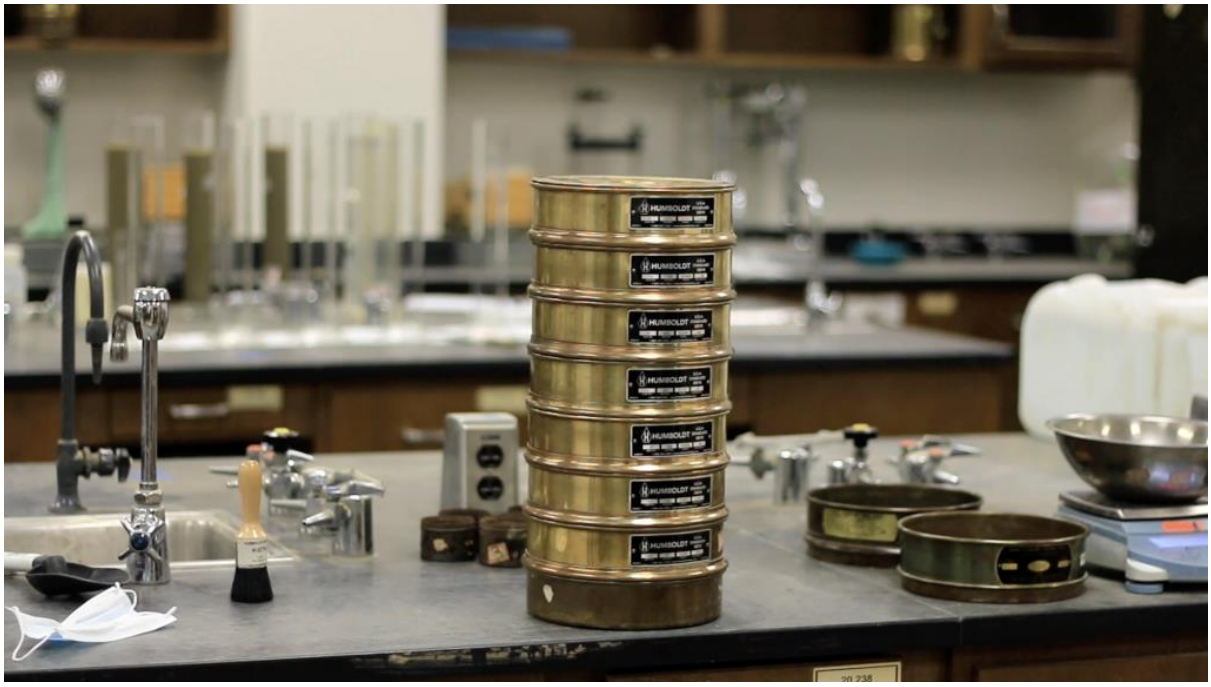


Fig 3.7 Stack of sieves

3.7.2 Specific gravity test

The process begins by thoroughly cleaning and drying the pycnometer to ensure accurate measurements. A known weight of the dry, solid material is then carefully placed into the pycnometer. The pycnometer is weighed again to determine the weight of the material.

The pycnometer is then filled with a reference liquid, usually water, up to a marked level. Any air bubbles are removed to prevent inaccuracies. The pycnometer is again weighed, this time with the liquid and the material inside.

From the collected data, the volume of the solid material within the pycnometer was calculated by considering the initial and final weights of the pycnometer filled with both the material and the liquid. The specific gravity of the solid material was determined by dividing the weight of the material by the difference in weight between the pycnometer with the material and the liquid, and then multiplying by the specific gravity of the reference liquid.

3.7.3 Unconfined compressive test (ASTM D2166 / D2166M):

To begin, a cylindrical specimen of the material is carefully prepared, either by remolding undisturbed soil or compacting loose soil into the desired shape. The specimen's height-to-diameter ratio is typically around 2:1 to 2.5:1.

The prepared specimen is placed on the lower platen of a universal testing machine. The lower platen is fixed, while the upper platen moves at a constant rate. Before testing, the initial height and diameter of the specimen are measured.

The test starts by applying a vertical load to the specimen at a constant rate of strain, commonly 1.25 mm/min (0.05 in/min). The load is increased continuously until the specimen fails or undergoes significant deformation. Throughout the test, axial load and deformation are continuously monitored and recorded.

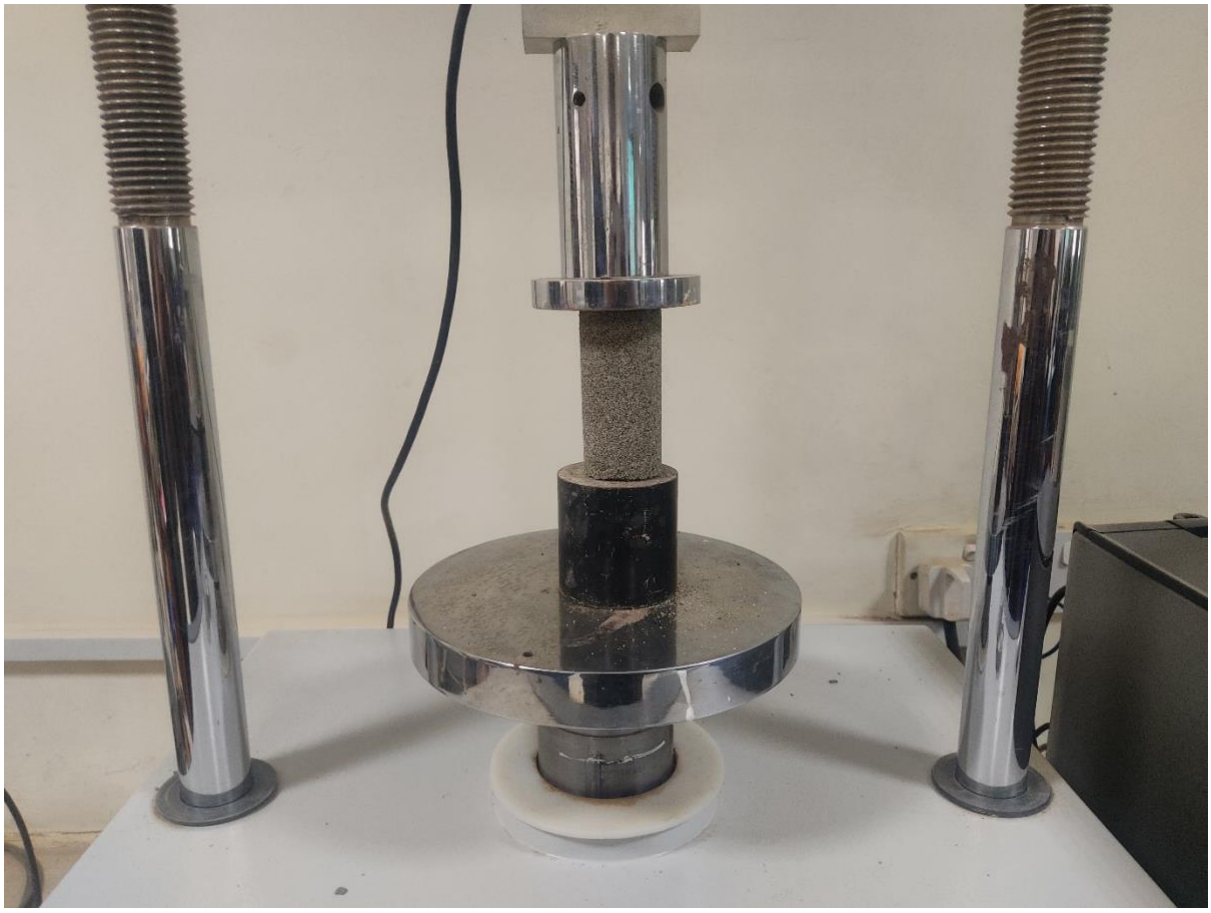


Fig 3.8 Unconfined compressive strength machine

3.7.4 Standard proctor test

Several identical compaction molds, each with a volume of 1/30 ft^3 (944 cm^3), are prepared for the test. A sample of the soil is placed into the mold in several layers, with each layer being compacted using a specified number of blows from a standard compaction hammer, typically 25 blows. The weight of the hammer is around 5.5 lbs (2.49 kg), and it drops from a height of 12 inches (305 mm).

The compaction process is repeated for various moisture contents, ranging from relatively dry to slightly wet. For each moisture content, the compaction effort is controlled to ensure consistency in the compaction energy applied to the soil.

After compaction, the soil's volume and weight are determined, allowing for the calculation of the dry density for each moisture content level. The moisture content corresponding to the maximum dry density is identified as the optimum moisture content for compaction.The results are plotted on a moisture-density curve, known as the Proctor curve.

3.7.5 X-Ray Diffraction

X-ray Diffraction (XRD) is a crucial technique revealing the atomic arrangement in materials. By analyzing X-ray patterns, it identifies crystallographic properties, phases, and composition. With broad applications, XRD informs fields like materials science, geology, and chemistry. It determines lattice parameters, unit cells, and phase ratios, aiding in phase analysis and complex structure characterization. Advanced methods like Rietveld refinement enhance accuracy.

The samples for XRD were extracted from the UCS specimens. The samples were taken from top, middle and bottom of the specimen to reperesent the entire specimen and also the effect of MICP at different height of the specimens.

3.7.6 Fourier transform Infrared (FTIR)

Fourier Transform Infrared (FTIR) spectroscopy is a fundamental technique that reveals molecular composition by analyzing infrared light interactions. It's applied in diverse fields like chemistry and biology. FTIR identifies molecular vibrations, aiding in understanding structures and functional groups. It's invaluable for qualitative and quantitative analysis of complex mixtures, enabling substance identification and reaction monitoring. Recent advancements enhance sensitivity and speed.

The samples for FTIR were extracted the same way as XRD.

3.7.7 Scanning Electron Microscopy (SEM)

Scanning Electron Microscopy (SEM) is a vital nanoscale imaging method. It utilizes an electron beam to visualize surface structures, offering insights into topography, morphology, and composition. SEM finds applications in fields like materials science and biology, providing high-resolution images and compositional data through energy-dispersive X-ray spectroscopy (EDS).

The samples were extracted the same way as XRD.

3.7.8 Spectrophotometry

Spectrophotometry is a fundamental technique that measures how much light is absorbed or transmitted by a substance at different wavelengths. It's used to analyze concentrations and properties of substances in various fields like chemistry and biology. By examining light-matter interactions, spectrophotometry helps identify compounds, study reactions, and offers both qualitative and quantitative insights.

The bacterial solution from the culture bottle was extracted with the help of pipette and poured into the glass container. Distilled water was kept as control.

Fig 3.9 Spectrophotometer for obtaining OD_{600}

4 Chapter

Results and Discussion

This chapter comprises results. The results are shown in graph. The results are divided into two parts. One are the laboratory test results and the other are microstructural results to confirm those laboratory results.

4.1 Effect of MICP on the type of soil

In order to evaluate the influence that MICP has on the Atterberg limits and the type of soil, a comparison was made between the Liquid limit and plasticity index of untreated samples and treated samples. Grinding UCS samples allowed to calculate the liquid limit and the plasticity index (Tiwari et al., 2021a). The soil characteristics have been transformed from CH highly plastic clay (expansive clay) to CL low plasticity clay (Normal clay) as a result of the precipitation of MICP. This transformation occurred in soil that had been treated with Bacillus subtilis and Priestia megaterium and then given 0.25 cementation solution. Because of the influence of MICP, the expansive soil has undergone biocementation, which has led to a reduction in the liquid limit. The precipitates of mineral Calcium carbonate that form in the pores of the soil as well as on the surface of the grains of soil help to strengthen the cohesion and, as a result, the bonding of attraction between the grains of the soil. The coating of the grains by the calcium carbonate crystals lowers its affinity for water, and as a consequence, the Atterberg limits of the soil are both lowered.

The changing of type of soil from high plastic clay to low plasticity clay is a great achievement for the MICP process as it proves the effectiveness of the process in the fine grained soil. This result shows the production of calcite has a very huge impact on the soil properties and it in the right quantity to conclude for us that the MICP process can be used in the field for stabilization of expansive soil. From figure 4.1 we can see that the liquid limit of the soil changed from almost 60 to 35 percent and the plasticity index changed from almost 30 to 19. This shows a huge impact of production of calcite on the water affinity and water holding ability of the expansive soil grains. The affinity of the water of the soil grains decreases due to which the liquid limit and plasticity of the soil changes.

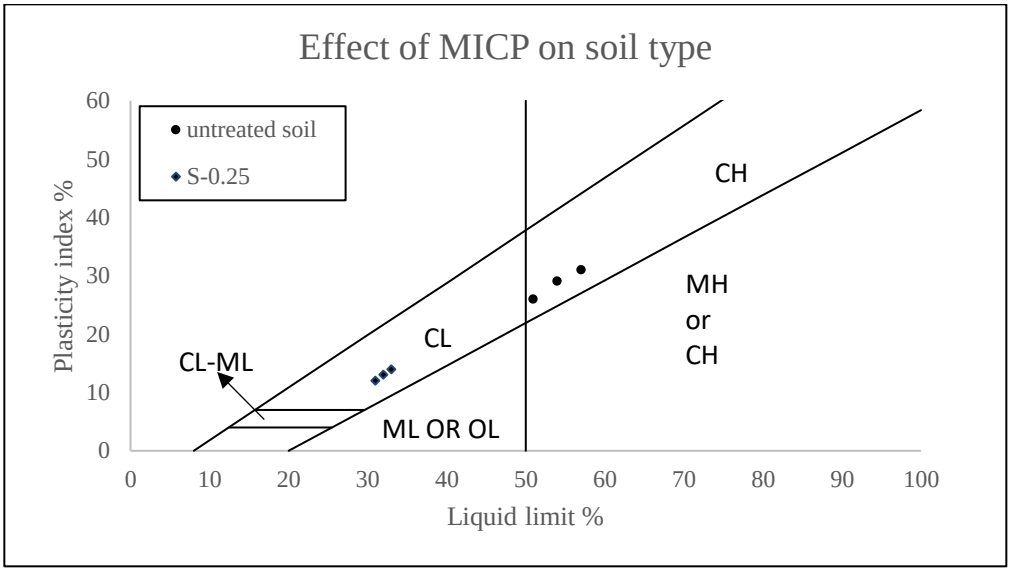


Fig 4.1 Effect of MICP on the liquid limit and plasticity of the soil

4.2 Effect on the UCS of the clay

The procedures used in the tests were those outlined in the ASTM D2166 [35]. The samples were examined under a variety of different circumstances. The sample was cured in a food-safe plastic, and the findings for that sample are depicted in figures 4.2 and 4.3. The findings of the samples that were left out in the open air for seven days are depicted in figures 4.4 and 4.5, respectively. This was done so that the effect of in-situ conditions on the effectiveness of MICP process could be evaluated.

In comparison to the samples that were not subjected to any treatment, the strength of the samples that were treated was much higher, as seen in figures 4.2 and 4.3. The specimens that were treated with a bacterial solution containing bacillus subtilis and a cementation solution that included ¼ molar calcium chloride and ¼ molar urea solution (S-0.25) showed the greatest improvement in unconfined strength. This was the case because these solutions produced the best cementation. The 0.25 M cementation produces a greater gain in strength than the other variations. The microbial activity that occurs in the soil is thought to be the cause of this phenomena. Calcium chloride is capable of inhibiting the activity of microbes, and a high molarity of calcium chloride salt results in suppression of growth of bacteria. This result can also be seen with naked eye which depicts the appearance of a protective coating of calcium carbonates on the surface of the specimens after they have been cured for 48 hours. In the samples that were treated with 1/2 M and 1 M cementation

solution, there was no evidence of this coating on the outer layer of the calcium chloride. Because of the molarity of the cementation liquid, the naturally occurring microbial activity in the soil has been significantly reduced. Crystals of calcium carbonate, also known as calcite, are thought to be responsible for the increased cohesiveness of the clay, which in turn contributes to the clay's increased strength. According to Liu et al. 2021 and Rahman et al. 2020, the increase in strength can be related to the precipitation of calcium carbonate between the pores of the clay particles. This increases the link between the clay particles, which in turn raises the strength and cohesion of the specimens. The unconfined compressive strength of the clay specimens is increased as a result of the bonding activity that the calcium carbonate precipitates have.

Both Figure 4.4 and Figure 4.5 demonstrate that the effect that the samples being kept in an open environment has on the microbial activity is the same. An increase in strength of up to 92% is seen in comparison to the untreated specimens in the case of the M-0.25 specimens. It is clear from looking at figure 4.4 that the specimens with the code S-025 have the highest strength.

According to the findings, the optimal performance in terms of strength was accomplished with a cementation solution that had a low molarity. Both bacterial strains delivered their highest performance at a molarity of 0.25 M, which was the lowest of those tested. The molarity of the Calcium chloride solution was the primary factor that determined how the treated specimens turned out in terms of their strength.

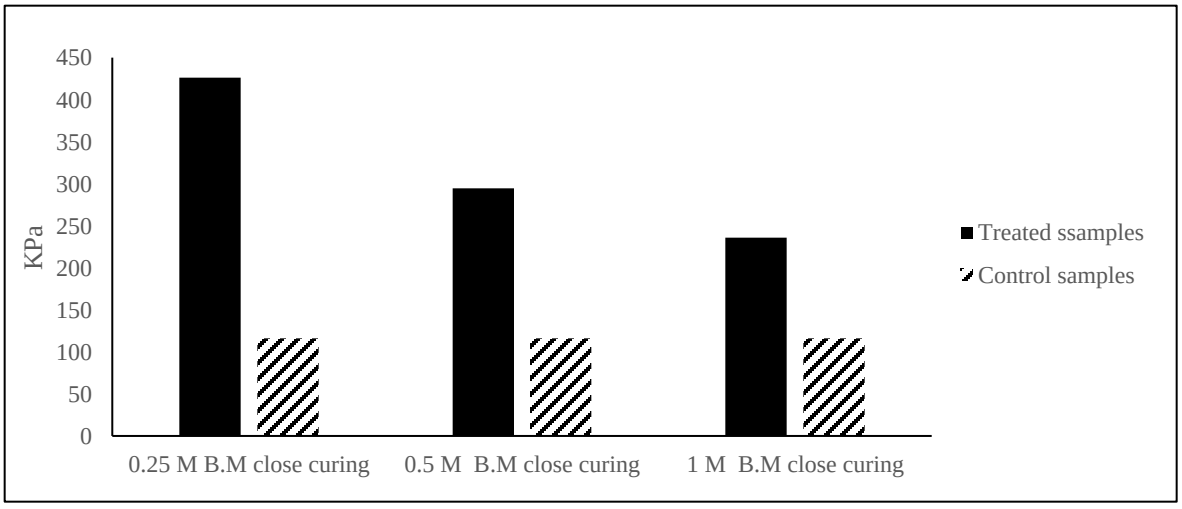


Fig 4.2 UCS of sample treated with *Priestia megaterium*

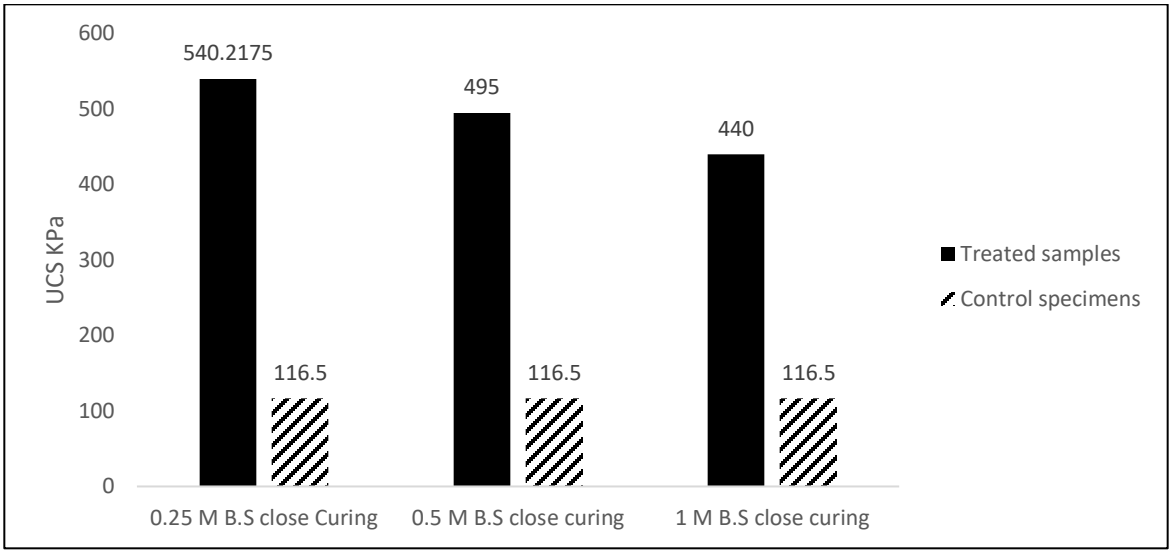


Fig 4.3 UCS of sample treated with *Bacillus subtilis*

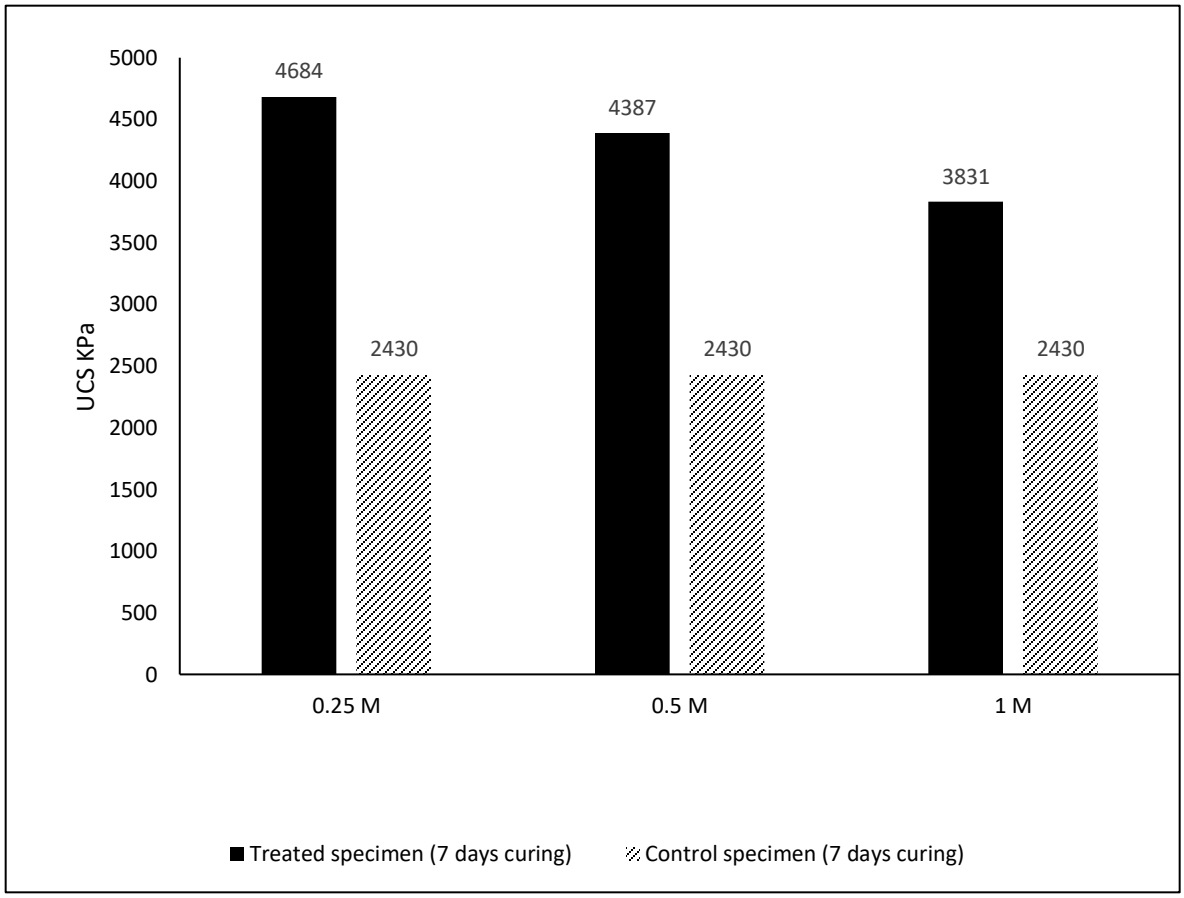


Fig 4.4 UCS of sample treated with *Bacillus subtilis and kept in open environment*

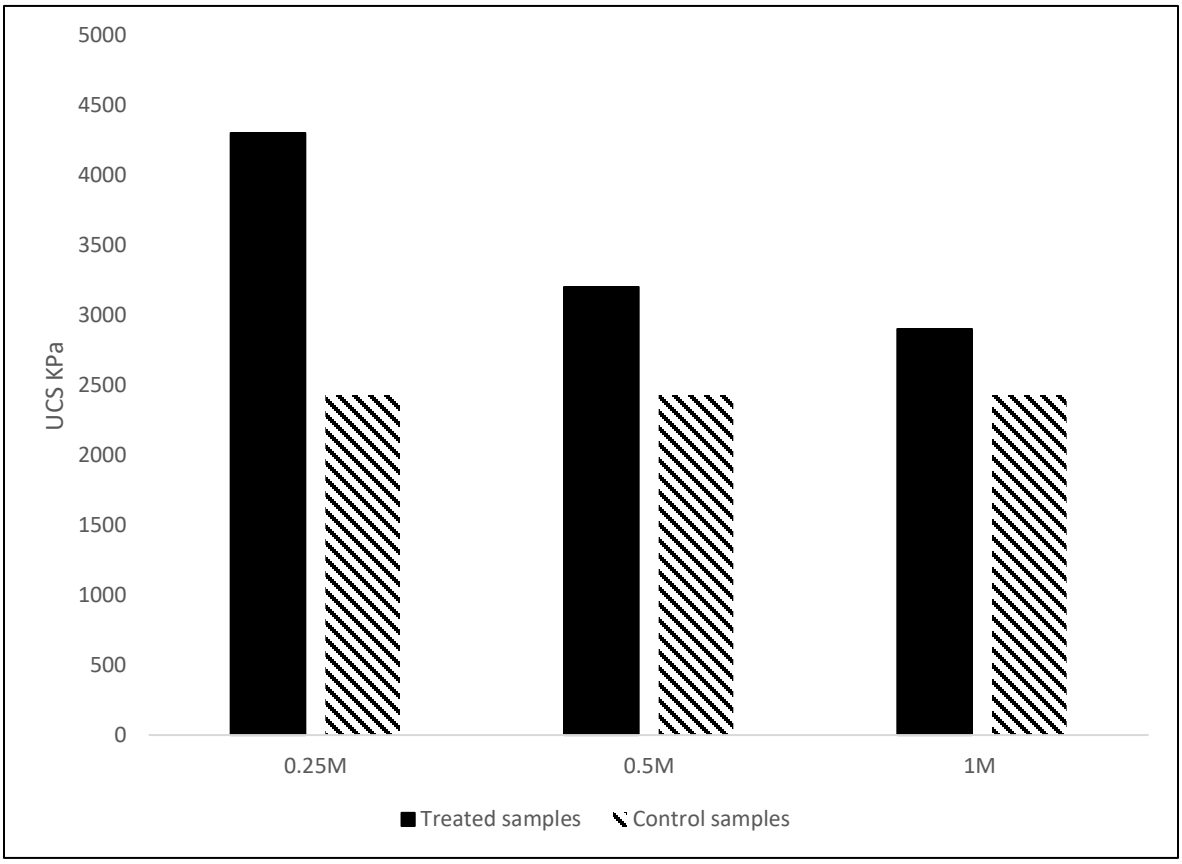


Fig 4.5 UCS of sample treated with *Priestia megaterium*

4.3 Effect of Wetting and Drying cycles on MICP treated soil

Samples that performed very well in the UCS test were put through a series of Drying after Wetting the soil. As a result, samples that had been treated with a bacterial solution of Bacillus subtilis and a cementation solution of 0.25 M (S-0.25) were put through a procedure that involved both wetting and drying. In accordance with ASTM D2166 [35], the specimens were put through their paces on the compression machine. After a certain Drying and Wetting cycles, the specimens were unwrapped, and then after drying, they were put through a series of tests. Testing the samples after they had been dried was much more practical. Due to the fact that it had a tendency to fall apart in the middle of the test, the specimens that were examined at the completion of the wetting cycle were not practically handy.

After being exposed to wetting and drying cycles, the unconfined compressive strength of the MICP-treated sample resulted to be more than that of the natural specimens. The plots show the UCS for the first, third, fifth, seventh, ninth, and eleventh wetting and drying cycles for both a 0.25 M cementation solution and a 0.50 M cementation solution. The percentages 188%, 183%, 211%,

218%, 244%, and 275% are the increase in the UCS between MICP treated specimens and untreated specimens treated with 0.25 M cementation solution and exposed to the first, third, fifth, seventh, ninth, and eleventh cycles respectively. In comparison, the samples that were modified with 0.50 M cementation solution and then subjected to the first, third, fifth, seventh, ninth, and eleventh cycles of wetting and drying had respective percentages of 176%, 166%, 200%,188%, 211%, and 230%.From the graph, the treated samples' strengths stopped declining after the seventh cycle, and this was true for both the S-0.25 and the M-0.25 specimens. During the wetting cycles, the calcium carbonate minerals were removed from the specimen, which resulted in a decrease in the specimen's strength. This phenomenon was observed for treated specimens up to the seventh cycle. The majority of the calcium carbonate that has been washed away can be seen on the specimens' external surfaces. This buildup of Calcite on the outer surface of the specimens cut down on the amount of additional mineral that washed away from the soil, and it also cut down on the amount of soil that was eroded away from the specimens. The bonding of the particles of clay due to the production of calcium carbonate is connected with the rise in the strength of the treated specimens after different cycles (Osinubi et al., 2019; Proto et al., 2016). This is the reason for the increase in the specimens' strength. A film is formed by the particles of calcium carbonate on top of the particles of the clay. There is an increase in unconfined strength as a result of the bonding effect (Oyediran and Ayeni, 2020; Sharma and R., 2016b; Tiwari et al., 2021a). The creation of a calcium carbonate layer on the surface of the specimen, which can be seen with the naked eye is connected with the rise in the specimen's strength after being subjected to a combination of several moist and dry particles.

On the specimen that was modified with 0.25 M cementation solution and bacterial solution of bacillus subtilis while the layer was being treated, the crystal layer of calcium carbonate is quite conspicuous. When the inside of the sample was viewed with the naked eye, however, there were no calcium carbonate crystals of any significant size or shape visible. It's possible that this is because the bacterial liquid and the cementation liquid are unable to fully permeate the soil due to the extremely low permeability of the high plastic clay. If this is the case, then this is the reason for the observed phenomenon. This layer has two purposes: first, it prevents the specimen from being eroded, and second, it creates a protective shell around the specimen. When compared to the first two cycles of soaking and drying, the UCS of the substance decreases more after the third cycle

than it does after the first two cycles. This is due to the periodic wetness and drying that causes the protective layer to be washed away in large part.

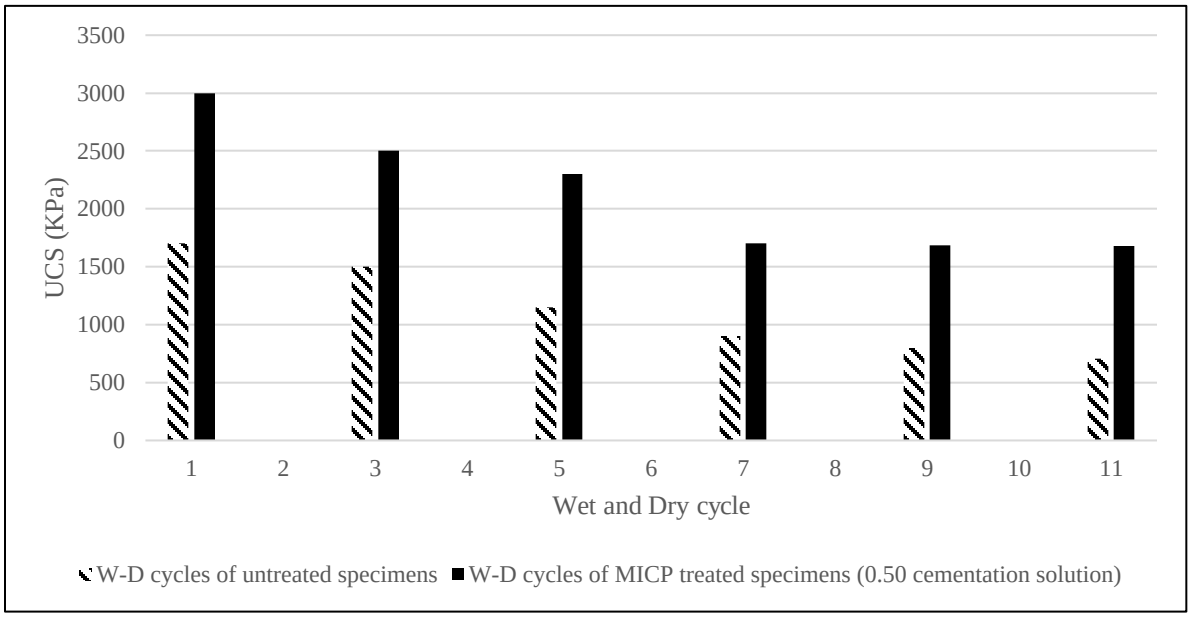


Fig 4.6 Results of UCS subjected to Wetting and Drying (0.50 M)

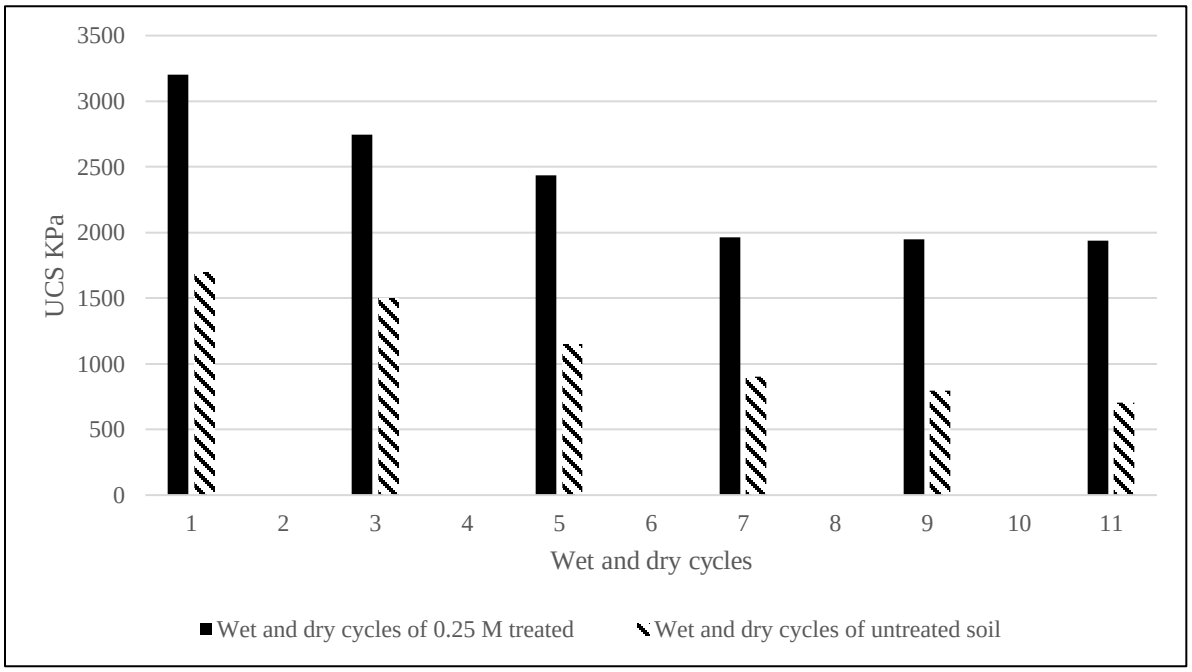


Fig 4.7 Results of specimens subjected to Wetting and Drying cycle (0.25M)

4.4 X-Ray Diffraction results

Through the use of X-ray diffraction (XRD), a comparison was made between the mineralogical change that occurred in the modified soil and the change that occurred in the natural soil. The XRD pattern shows evidence of calcite having precipitated out of solution. All of the samples were found to have a distinct peak, which was discovered to indicate quartz, with the untreated sample having the peak that was the most intense. The intensity of the quartz has been diminished in the samples that have been treated, with the greatest diminution occurring in the samples that have been treated with Bacillus subtilis and 0.25 M cementation solution. At a temperature of 29.5 degrees, the production of the calcium carbonate mineral has been seen. The fact that the peaks are distinguishable in the treated samples demonstrates that mineral calcite precipitates as a result of the application of MICP. The absence of a peak at a 2 theta value of 29.5 degrees in the samples that were not subjected to any treatment demonstrates that the samples did not contain any Calcium Carbonate. It has been noticed that, along with the creation of calcium carbonate, the mineral known as tobermorite is formed. The enhancement in the expansive clay strength and durability may be traced back to the production of Calcite mineral, which is responsible for the production of the mineral.

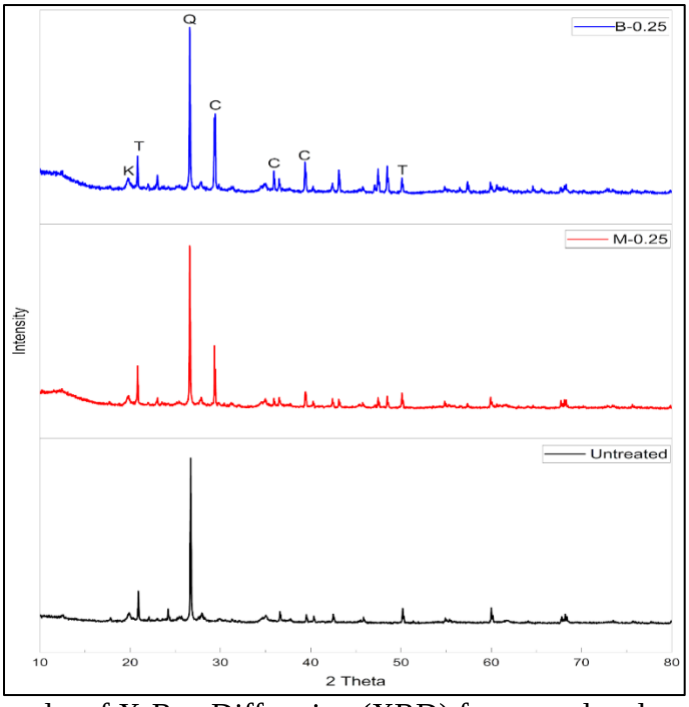


Fig 4.8 Results of X-Ray Diffraction (XRD) for natural and modified specimens

4.5 FTIR results

The FTIR analysis was performed on both modified and natural soil, using MICP as the treatment. The untreated soil specimens were compared to the soil specimens that had been treated with MICP. Powdered samples were used for the IR analysis that was conducted. The treated specimens' soil was removed by scratching, and the resulting powder was used. The findings are depicted in the image below. We chose samples that had been treated with Bacillus subtilis and Priestia megaterium and 0.25 M cementation solution for the treated specimens. These samples were given the names S-0.25 and M-0.25 in accordance with the treatment they had received. The value for the wave number was chosen from a range of 500 to 4000 cm-1. Minerals were detected in the range of 500 to 1200 cm-1, organic compounds were detected between 1200 and 3000 cm-1, and clay minerals were detected between 3500 and 4000 cm-1. According to the graph, the range of wavelengths 3092–3717 cm-1 is where clay minerals such as kaolinite and illite can be found. The production of Calcite minerals in the S-0.25 and M-0.25 specimens is indicated by the existence of a broad peak.

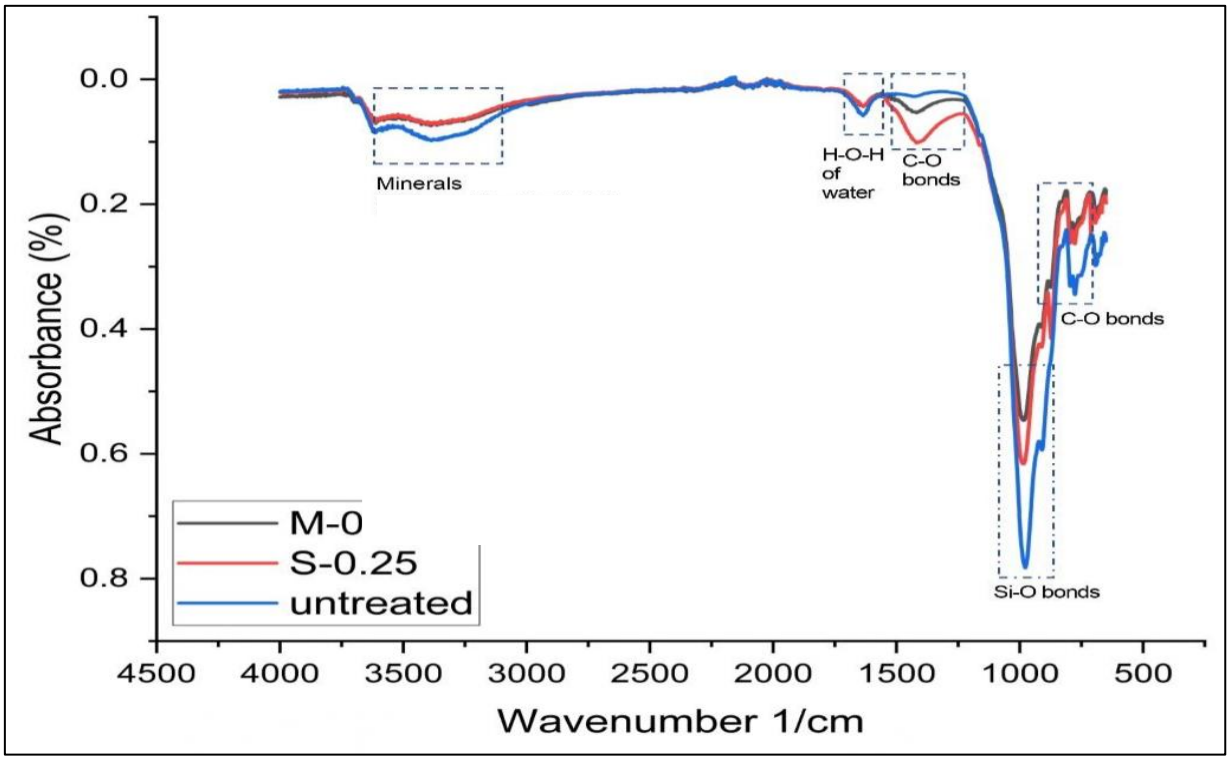


Fig 4.9 Fourier-Transform Infrared spectroscopy (FTIR) for treated and untreated soil

with an IR value of 3454 cm-1 in the same general area. The peak is accentuated with the value of S-0.25. More calcium carbonate minerals are present if the intensity of the peak for S-0.25 is higher. Within the frequency range of 874 to 1468 cm-1, a carbon-oxygen bond, often known as a C-O bond, was detected. Quartz (SiO) was detected in the infrared spectrum at wavenumbers 798 cm-1 and 782 cm-1 respectively. At a wavenumber of 3623 cm-1, the fundamental characteristic of the expanding soil mineral known as montmorillonite was identified. Comparing to the untreated samples that were treated with MICP, the untreated samples have a peak that is significantly higher. This suggests that the MICP treatment causes a change in the expanding soil's mineralogical composition.

4.6 Scanning Electron Microscopy

It can be seen quite plainly that there are pores in between each of the grains of soil. In the succeeding images that were taken from the treated samples, these spaces are seen to be filled by precipitates of calcium carbonate. The coating effect, which can be shown to be greater in the samples treated with Priestia megaterium and 0.25 M cementation solution, can be seen in the pictures produced by the SEM. The photos also make clear that the spread of calcite mineral is not uniform in any way. This is something that can be seen. This is because the expansive clay does not contain any pores that are related to one another. The photos of the treated samples show what appears to be a bridge of linkages between the individual grains of soil that are generated by calcium carbonate.

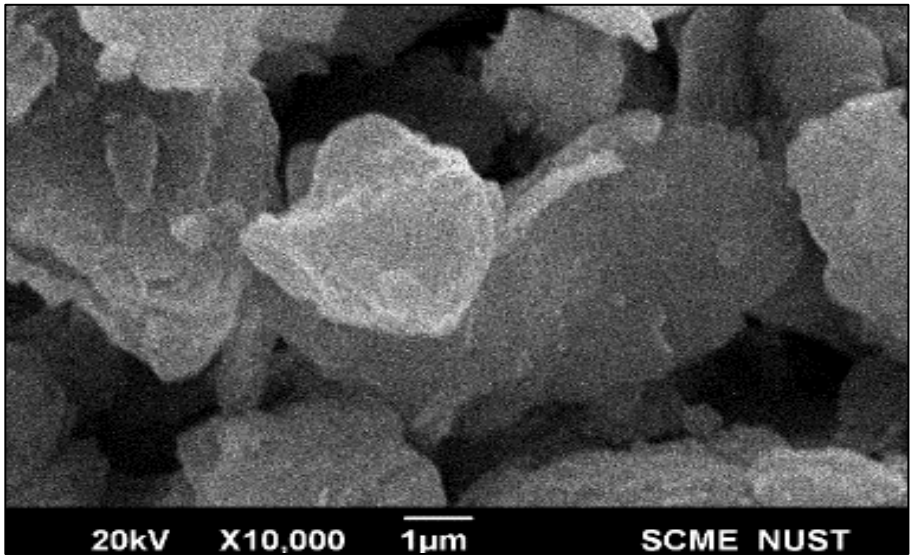


Fig 4.10 Scanning Electron Microscopy Images of Natural soil

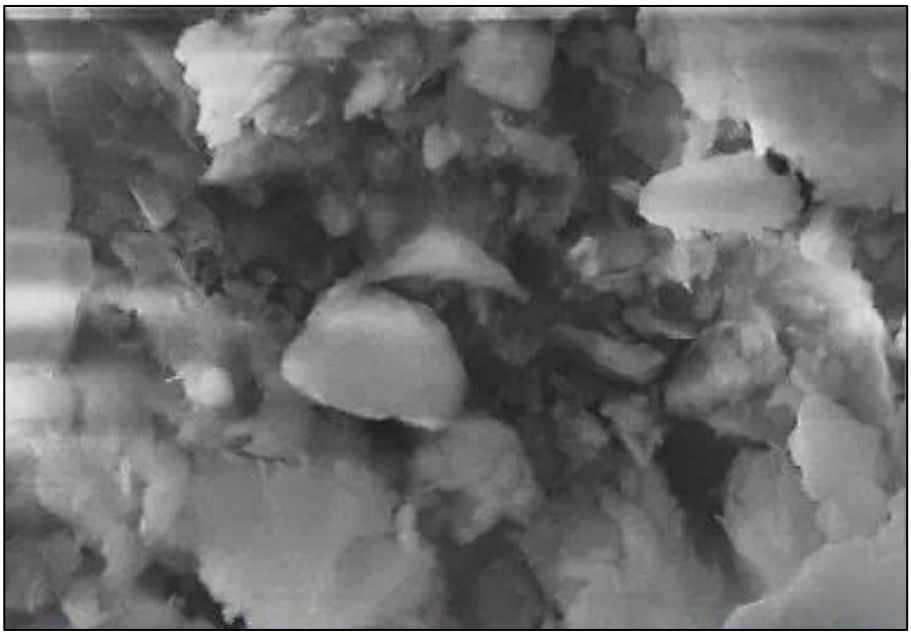


Fig 4.11 Scanning Electron Microscopy images of treated soil

5 Chapter

5.1 Conclusions

The research investigated the use of bacterial induced calcification of expansive soil. The expansive soil was then exposed to wetting and drying cycles to determine the durability of the calcification. The soil was obtained from Nandipur and initially tested for its engineering properties. The Atterberg limits of the soil were tested and the liquid limit of the soil turned out to be 51 % which is on the higher side. The soil was classified as a expansive soil. The bacterial strains for the process of MICP were selected and grown in a sterile environment in the lab. The cementation solution was also prepared in a very sterile environment to avoid any contamination from the surroundings. The soil was mixed with bacterial solution and cementation solution in a way any other shallow stabilization technique is done. The sample were tested on a UCS machine for checking the strength of the MICP treated soil. The Atterberg limits were tested after the calcification. The modified UCS samples were subjected to wetting and drying cycles which confirmed to increase the durability of MICP treated soil.

The results of this research are summarized in the following conclusions. The test performed on the treated and untreated soil resulted in the following conclusions.

1. The type of soil modified from highly plastic soil to low plasticity soil due to modification from MICP.
2. The Compressive Strength of the soil increased for treated soil. The best performance was found for samples treated with *Bacllius subtilis* and 0.25M cementation solution.
3. The compressive strength of the modified soil decreased with addition in the molarity of the cementation solution.
4. The resistance of the modified soil increased with subsequent cycle of Wetting and Drying as the strength of the soil become constant after 7^{th} cycle of Wetting and Drying.
5. SEM, FTIR and XRD confirmed the production of calcite in the soil. This calcite was produced due to the process of MICP which enhanced the mechanical properties of the soil.

5.2 Recommendation

The recommendations regarding the use of MICP in soil are given below.

1. Use of different bacterial strains for MICP. The use of locally identified bacterial strains should be preferred.
2. Use of different calcium source in the cementation solution to investigate its effect on the bacteria and mechanical properties of MICP treated soil.
3. Performing wetting and drying cycles with a unique and different method.
4. Use of MICP in the field to stabilize the soil to check the applicability of MICP in the field for expansive soil.

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