

STUDY ON EFFECT OF MICP USING BACILLUS CLAUSII

A dissertation submitted in partial fulfilment of the requirement for the award
of degree of

Master of Technology

In

Geotechnical Engineering

By

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Certificate

This is to certify that major project-II entitled — **Study on effect of MICP using bacillus clausii** is bona fide record of work carried out by Jaiprakash Kumawat (Roll No. 2K13/GTE/07) under the guidance and supervision, during session 2015 in partial fulfilment of the degree of Master of Technology (Geotechnical Engineering) from Delhi Technological University New Delhi.

The work in this major project- II has not submitted for the award of any other degree to the best of my knowledge.

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Candidate's Declaration

I do hereby certify that the work presented is the report entitled **Study on effect of MICP using bacillus clausii** in the partial fulfillment of the requirements for the award of the degree of “Master of Technology” in geotechnical engineering submitted in the Department of Civil Engineering, Delhi Technological University, is an authentic record of our own work carried out from December 2014 to July 2015 under the supervision of Prof. A.Trivedi (Professor), Department of Civil Engineering.

I have not submitted the matter embodied in the report for the award of any other degree or diploma.

Date: July 2015

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Abstract

Some soils have very low strength so it's quite difficult to construct civil engineering structures but due to a great space requirements we used to construct these structures on those locations. Microbially induced calcite precipitation is a phenomena by which soil properties are enhanced. In this study a silty sand sample is modified using MICP, Microorganism named *Bacillus clausii* is used in this study and its ureolytic effects with cementation agent containing urea are examined for various time periods namely 8 hours, 72 hours (3days) and 168 hours (7days) and laboratory tests are performed on soil to determine CBR, unconfined compressive strength, and shear parameters. The results of UCS and tri-axial tests showed a significant impact of MICP on the strength properties of the soil and the effects of curing duration on the strength properties of the soil. The presence of Calcite precipitates resulted by microbial activities identified by the scanning electron microscope. The three days soaked CBR increased by 49%, unsoaked CBR increased by 65% compared to results of untreated samples, which shows increase in stiffness of soil. UCS results were highly promising as 168 hours UCS increased 4 times as compared to untreated soil specimen. Selected microorganisms and MICP is likely to play a very important role in geotechnical engineering properties involving soil improvement techniques.

Keywords: MICP; Soil stabilization; Urea hydrolysis; *Bacillus clausii*; Shear parameters; UCS; CBR; Scanning electron microscope.

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Chapter 1

Introduction

1.1 Soil Stabilization

1.1.1 Definition

The use of chemical or mechanical treatment of a soil mass to improve its stability or maintain it or enhance its engineering properties.

Stabilization of soil is the process of maximizing the suitability of soil for given construction purpose.

1.1.2 Need for Soil Stabilization

A land – based structure of any type can be as strong as its foundation. Hence, soil is critically important element of any structure and is very important aspect for success of any construction project. Understanding the engineering properties of soil is of very crucial to obtain strength and for economic permanence.

Site feasibility studies for construction projects are very important and beneficial before a project can take off. Site survey is carried out at the place before the final design process starts, for getting an idea of the characteristics of soil on which the project location is to be decided. The following design criteria of geotechnical characteristics have to be determined during site selection.

- Design load and function of the structure.
- Type of foundation to be used.
- Bearing capacity of subsoil.

In earlier times, many of sites for construction were abandoned or rejected because of undesirable soil properties. This led to land scarcity and increased use of natural resources and it worsened with growing population.

But with advancements in technological developments this problem was tackled. The undesirable soil properties of soil were modified, so that the rejected construction site can be used.

1.1.3 Soil Stabilization Methods

The simplest stabilization processes are:

- Compaction – This mainly refers to reduction of void spaces or removal of air from soil in order to increase unit weight of soil which in turn increases the bearing capacity of soil.
- Drainage – This refers to removal of water from soil. Removal of water reduces the water content of soil and hence increases the unit weight of soil which in turn increases the bearing capacity of soil.
- In the other process particle size gradation is improved and following kind of enhancements can be gained by addition of binders with the weak soils. Stabilisation of soil can be achieved by many methods.

Stabilization of soil can be broadly classified as:

- Mechanical Stabilization
Under this category, stabilization of soil can be accomplished through physical process i.e. by modifying the physical characteristics of in-situ soil particles either by vibration induction or compaction or by introducing other physical properties like nailing and barriers.
- Chemical Stabilization
Under this category, reactions between Stabilizer (cementation agent) and soil minerals (pozzolanic) is the main factor on which the desired effects of soil stabilisation is depended.

1.1.4 Applications

Soil stabilization results in increase in the bearing capacity of the soil used in foundation of the structure and its water tightness, strength, washout resistance, and some more properties are enhanced.

It is mainly used in -

- the construction of industrial and residential buildings on loose soils;

- for preventing landslides where ground is susceptible to it and water saturated ground;
- where shafts are sunk and in formation of barriers for the foundations of hydraulic structures which are filtration-proof i.e. filtration-proof barriers;
- protection from aggressive industrial effluents of concrete structures (foundations);
- Enhancing the bearing capacity of piles and of large-diameter supports.

1.2 MICROBIALLY INDUCED CALCITE PRECIPITATION (MICP)

1.2.1 What is MICP?

Microbially induced calcite precipitation is a comparatively sustainable and modern soil improvement method. This method comprises of micro bacterial activity to form calcite precipitate, and to modify engineering properties of soil strata using depletion of layer or coating and formation of bonds between soil grains (Soon 2013).

A new ground improvement method that involves some micro-bially process, which is technically identified as Microbially induced calcite precipitation. It takes parts from various interdisciplinary researches based on geotechnical engineering, geochemistry, and microbiology to find out a natural way of treatment for ground improvement.

Microbial induced calcite precipitate is a microbial process that is naturally occurring. It is implemented using a severe amount of microorganisms which have capability to precipitate calcite and by using a cementation media mixed with soil, due to all this microbial and chemical activity a cementing compound is precipitated named CaCO_3 to enhance the properties of the soil useful in geotechnical engineering. The behaviour and properties of the calcite forming microorganisms are environment friendly and will cause no harmful effects to the environment, soil, and human health.

1.2.2 Mechanism of MICP

In general, Microbial induced calcite precipitate can be attained by hydrolysis of urea, aerobic oxidation, denitrification, sulphate reduction, etc. van Paassen et al. (2010) suggested that urea hydrolysis possesses the highest calcite conversion rate compared to other studied processes. Urea hydrolysis refers to a chemical reaction where urea ($\text{CO}(\text{NH}_2)_2$) is decomposed by urease enzyme that may be supplied either by some outside source (Nemati and Voordouw, 2003) or can be manufactured in situ by microorganism which have capability to produce urease

(DeJong et al., 2006; Whiffinet al., 2007; Martinez et al., 2011). The latter process requires urease positive type bacteria, i.e. genera Bacillus, Sporosarcina.

Suitable Microorganisms

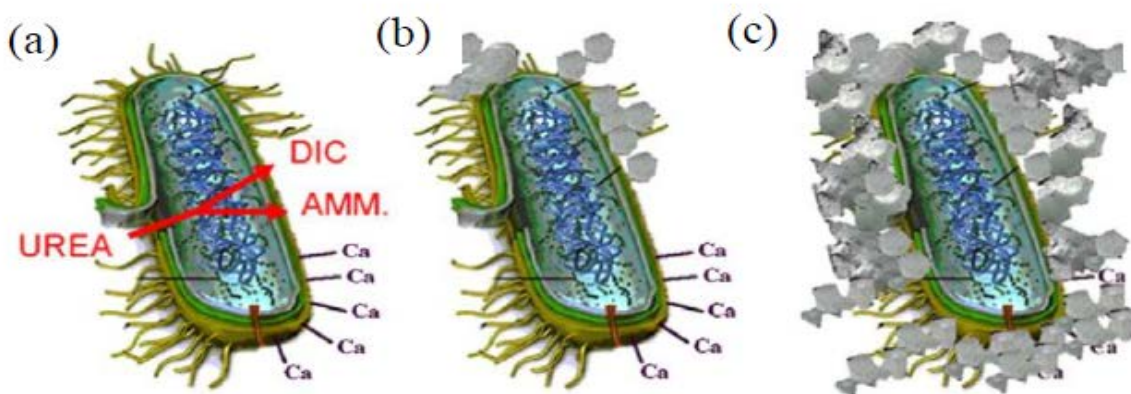
- Facultative anaerobic bacteria
- Micro-aerophilic bacteria
- Anaerobic fermenting bacteria
- Anaerobic respiring bacteria
- Obligate aerobic bacteria

Aerobic and anaerobic bacteria can be identified by growing them in liquid culture

Sporolactobacillus, *Clostridium* or *Desulfotomaculum* (Kucharski et al., 2008). This chemical reaction involves 1 mol of urea which decomposes into 2 mol of ammonium:



The release of ammonium (NH_4^+) increases the pH of system thus creates a favourable environment for precipitation of calcite with the presence of calcium ion (Ca^{2+}) from the supplied calcium chloride:



Source: <http://labmet.ugent.be/user/willem-de-muyneck>

Figure 1.1 Representation of the events occurring during MICP, (a) calcium ion attracted to cell wall; (b) calcite precipitated near cell wall; (c) calcite increased in quantity and encapsulate cell

The formation of calcite is responsible for increasing soil properties.

1.2.3 Soil Stabilization by MICP

The phenomenon of calcite precipitation by micro bacteria can be explained as positive ions of calcium in the solution are attracted towards opposite charges available on cell wall of microbes. Addition of urea leads to the release of dissolved inorganic carbon (DIC) and ammonium in the microenvironment of the microbes. The existence of calcium ions causes local supersaturation and thus heterogeneous calcite precipitation on microbial cell wall occurs. After certain period of time, the microbes become encapsulated by calcite, resulting in limited or no nutrient transfer and eventually exterminate the microbes. The calcite precipitates are gelatinous or gel like substances which bridge the gap between soil particles and act as binder. Most *Bacillus* strains can produce urease enzyme for urea hydrolysis (Hammes et al., 2003). Reported studies have mostly adopted *S. pasteurii* as the urease-producing microorganism. Studies on alternative bacilli are still very limited.

The calcite (CaCO_3) precipitated is responsible for improving inherent engineering properties of soil through biocementation and bioclogging. Biocementation is defined as an improvement of soil strength by formation of cementing materials through microbial activities, while in bioclogging permeability of soil or porous rock is reduced by pore-filling materials resulted from microbial processes (Ivanov and Chu, 2008).

1.2.4 Factors affecting MICP

- Nutrients

Nutrients are the energy sources for bacteria, and hence it is critical to provide proper and sufficient nutrient for calcite producing bacteria. This has been found from various previous reported that 3 g/l of nutrient broth into the treatment solution is sufficient to support the sustainable growth and viability of bacteria producing urease.

- Types of Bacteria

The type of bacteria which are suitable for MICP application should be able to catalyze the urea hydrolysis which are known as urease positive bacteria. The common type of bacteria used for soil improvement is *B. Pastuerri*.

- Geometric Compatibility of Bacteria

Bacteria are the most abundant and most sustaining microbes in soil. Due to shape and size of bacteria the small pore throat size would limit their free passage and also the soil grains geometry.

- Fixation and Distribution of Bacteria in Soil

The urease positive bacteria should be distributed evenly and fixed in place when they are injected into the soil. Fixation fluid in higher flow rate flushes bacteria cell over larger distances compare to lower flow rate.

- Temperature

The microbial activity and growth are less sensitive to the temperature within the range of 20 to 30 °C. The rate of urea hydrolysis is marginally higher in 30 °C, as compare to 20 °C.

- Reactant Concentration

The products from 1 mole of urea and 1 mole of calcium chloride would react to form calcite. A solution contains equimolar of both reactants would provide better conversion to calcite.

- pH

It was found out that the process is best exhibited when pH was found in range of 7.5 – 8

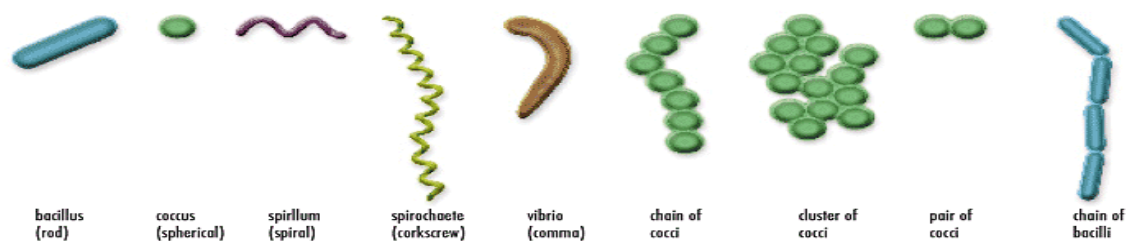
1.3 VARIOUS TYPES OF BACTERIA

Bacteria are single celled microbes and their cell structure is simpler than other microorganism because there is no nucleus and other membrane bound structures. Their control centre is contained in single loop of structure. In 1676 Anton van Leeuwenhoek first observed the bacteria through microscope and called them “animalcules”. Later German naturalist Christian Gottfried Ehrenberg called it bacteria meaning “little stick”.

1.3.1 Types of bacteria on basis of shape

Bacteria are classified in five groups according to their shape:-

- Spherical(cocci)
- Rod(bacilli)
- Spiral(spirilla)
- Comma(vibrios)
- Corkscrew(spirochaetes)



Source: http://www.microbiologyonline.org.uk/themed/sgm/img/slideshows/3.1.2_bacteria_1.png

Figure 1.2 Types and Shapes of various Bacteria

1.3.2 Habitats of bacteria

Bacteria found on every habitat of earth like soil, rock, oceans, snow and also in extreme condition where no animals can survive. Few bacteria live in or on plants and animals including humans. Large number of bacteria found on inner lining of digestive system. Bacteria play a very important role in the recycling of nutrients. Some bacteria causes food spoilage and crop damage but on the other hand they play a very important role in fermentation. Few bacteria are which causes disease in plants and animals.

1.3.3 Reproduction in bacteria

Bacteria reproduce by binary fission in which parent bacteria is divided into two daughter cells. DNA of bacteria is divided into two identical replicates and then cell elongates and divided into two daughter cells. In favourable condition and at appropriate temperature bacteria divide every 20 minutes and in 7 hours it become 2097152 and after one more hours bacteria will rise to 16777216. This is the reason we quickly get ill when bacteria comes in contact of our body.

1.3.4 Survival mechanism

Bacteria form spores which are dormant structure and are highly resistant to hostile physical and chemical conditions such as heat, UV radiation and disinfectants.

1.4 Urease Positive Bacteria

That bacterium which causes urea hydrolysis is called urease positive bacteria. Urease is an enzyme which catalyses hydrolysis of urea to form ammonia and carbonate. Urease activity increases the pH because it forms ammonia which is a basic molecule. Other examples of urease positive bacteria are *Proteus mirabilis*, *ureaplasma urealyticum*, *helicobacter jejuni*, *staphylococcus epidermitis*, etc.

1.5 Bacillus Clausii

Bacillus clausii is a rod shaped, Gram positive, motile and spore forming bacterium which is encountered in soil. It is a type of probiotic microorganism which holds a symbiotic relationship with the organism on which it lives. Presently it's a part of studies related to infections in respiratory system and some stomach disorders. It was discovered for *Bacillus clausii* that they produce some kind of antimicrobial substances which are active against gram positive bacteria and are also ureas positive in nature.

Scientific classification:-

Kingdom : - Bacteria
Phylum :- Firmicutes
Class :- Bacilli
Order :- Bacillales
Family :- Bacillaceae
Genus :- Bacillus
Species :- Bacillus Clausii

CHAPTER 2

LITERATURE REVIEW

Sung-Sik Park et al. [2014] conducted a study on “Effect of Microbially Induced Calcite Precipitation on Strength of Cemented Sand”.

- In this study weakly cemented soil is treated with a bacteria named *Sporosarcina pasteurii*.
- This study mainly focused on evaluation of unconfined compressive strength (UCS) and the amount of calcite precipitation within the cemented sand particles and analysing the effects of multiple time treating with cementifying solution over a span of 20 days at some regular interval.
- The results showed that the specimen treated one time with the bacteria shows a 5% increase in the unconfined compressive strength compared to the untreated specimen. Other observation was the specimen treated more than two times, its strength decreased gradually up to 50% compared to the untreated specimen.

Gomez et al. [2013] conducted study on “Field-scale bio-cementation tests to improve sands”

- In this paper, MICP is applied over the surface in field having loose sand deposit to improve reduce the erosion and to provide stabilisation of surface for dust control and future re-vegetation.
- In this study three test plots were treated with a bacterial media and nutrient media at different concentrations, and a fourth test plot used as a control.
- The improvement in sand deposits was analysed up to a depth of 40 cm by dynamic cone penetration (DCP) testing and measurement of calcite content precipitated as result of MICP and Water jet impingement erosion test was performed to analyse erosion resistance.
- From the observations, it is observed that the test plot treated with the least concentrations of urea and calcium chloride showed the most improvement and formed a stiff crust measuring 2.5 cm thick, which possesses improved erosion resistance.
- Results of DCP tests and calcite content measurements after 20 days treatment showed increase up to a depth of approximately 28 cm which is near the targeted depth of 30 cm.
- The loose sand deposit treated with MICP showed no significant signs of deterioration after 44 days of completion of the final treatment, and showed only moderate degradation occurred 298 days after the final treatment following a harsh winter.

Ivanov et al. [2008] conducted study on “Applications of microorganisms to geotechnical engineering for bioclogging and biocementation of soil in situ”

- In this paper, potential applications of MICP on soil are discussed as Biocementation and Bioclogging.
- If introduction of microorganism causes reduction in permeability of soil, it is termed as bioclogging.
- If introduction of micro-organism causes increment in compressive strength of soil, they termed it as Biocementation.
- They observed that MICP could be used in geotechnical engineering to enhance or alter the mechanical properties of soil like strength and hydraulic conductivity by biocementation and bioclogging. These methods can be the alternatives to energy demanding mechanical compaction methods or the expensive and environmentally unfriendly chemical grouting methods.

Paassen et al. [2010] conducted a study on “Quantifying Biomediated Ground Improvement by Ureolysis: Large-Scale Biogrout Experiment”.

- In this study large-scale in situ experiments [of 100m³ sized specimen] are conducted to test the possibility and usefulness of biomediated grouting as a method of ground improvement using similar methods as used in its potentials uses.
- The results showed that MICP significantly increases the stiffness of granular soils, which is varified by two types of experiments: time-lapse shear-wave transmission seismics and UCS test on excavated specimens.
- The increase in stiffness can be quantified as a function of volume of biogrouts injected and the distance from the points of injection.

Soon et al. [2012] conducted a study on “Improvements in Engineering Properties of Soils through Microbial-Induced Calcite Precipitation”.

- In this study two types of soil samples were considered i.e. Sandy soil and Residual soil (Sandy Silt).
- This paper mainly focused on effect of MICP on improving shear strength and reducing hydraulic conductivity.

- The results showed that for both sand and residual soil, effectively reduction in hydraulic conductivity and improved shear strength by use of MICP.
- The residual soil treated with MICP, showed improved shear strength ratios significantly higher (1.41-2.64) than that of the sand (1.14-1.25). On the other hand, sand resulted in better reduction in hydraulic conductivity ratios (0.09-0.15) than that of the residual soil (0.26-0.45).

Soon et al. [2012] conducted a study on “Optimum conditions for promoting improvements in engineering properties of tropical residual soil by Microbially-Induced Calcite Precipitation”.

- The main findings of this study were to evaluate the most favourable environment situations for utilising MICP in a typical tropical residual soil, observe the usefulness of the MICP technique in improving the soil properties which includes UCS, permeability, and compressibility.
- As per the results the most favourable treatment conditions were observed when using reagent flow head of 1.1 bars, treatment duration of 48 hours, reagent concentration of 0.5 M, and *B. Megaterium* concentration of 1×10^8 cfu/ml.
- As per these findings, the enhancement achieved recorded for the UCS, permeability, and recompression index were 69.1%, 90.4%, and 46.9 %, respectively.

Soon et al. [2013] conducted a study on “Factors Affecting Improvement in Engineering Properties of Residual Soil through Microbial-Induced Calcite Precipitation”

- This study focuses on using a different bacteria called bacillus megaterium with silty sand and effects of different pressure by which cementation fluid is injected and there effects.
- The biggest gain in shear strength and reduction in hydraulic conductivity obtained are 100 and 90%, respectively. The factors governing the improvement considered in this research consists *B. megaterium* conc., cementation reagent conc., flow pressure of the cementation reagent, and treatment duration.
- It was noticed that high cementation reagent flow pressure (2 bar) was leading to a continuous increment of pore-water pressure and distortion of soil structure, leading to an adverse impact on the soil improvement. On the other hand, very low flow pressure (0.2 bar) was leading to precipitate calcite near to the inlet to restrict the flow of reagent through the soil sample. A medium range flow pressure (1 bar) is suggested to maintain

a sufficient injection distance of the cementation reagent while minimising the possible development of excess pore-water pressure.

- It was observed that there was negligible modification in soil properties of the controlled specimens treated with cementation reagent only but when the bacteria was included in treatment process there was decrease in permeability by 26%. The possible reason for happening so was soil pores getting blocked with bacteria cells, which was a temporary effect.

Bing et al. [2011] conducted a study on “Geotechnical Properties of Biocement Treated Soils – a new approach”.

- In this study clayey soil (kaolinite) was considered and for evaluation of strength, Unconfined Compression Test was conducted and for Hydraulic conductivity, Soaking Test was conducted.
- It was found that Peak Strength increased from 28kPa to 73 kPa and Residual Strength also almost doubled.
- For hydraulic conductivity MICP was mixed in clay and balls of clay were made and were immersed in water. It was found that treated soil soaked relatively less amount of water.

Morgan et.al. [2011] conducted a study based on “Innovative Environmentally responsible techniques for Ground Improvement stimulating natural processes”.

- They studied feasibility of using two different urea hydrolysing bacteria for bio cementation of soil containing silt and kaolin clay.
- Bacteria used in this case was *Spor.pasturii*. Many samples were considered and they were left to cure at different conditions.
- Undrained shear strength of soil was evaluated at different moisture contents. The Results indicated positive response of bacteria used on soil cementation.

Lee et al. [2012] conducted a study based on “An Overview of the Factors Affecting Microbial-Induced Calcite Precipitation and its Potential Application in Soil Improvement”.

- In this study, residual soil with Sandy Silt composition was considered for studying the factors effecting the treatment of soil with MICP. The Bacteria considered in this study was *Bacteria Magneterium*.

- Factors affecting included Nutrients, type of bacteria used, bacteria cell concentration, pH, Temperature, Reactant Concentration.
- Finally it was concluded that MICP showed improvement in both hydraulic conductivity and shear strength of residual soil.
- The results implied that native urease-forming bacteria can be utilized in MICP soil improvement, with sufficient and appropriate nutrient provided.

Qabbany et al. [2010] conducted a study on “Microbial Carbonate Precipitation in soil”.

- In this study effect of MICP on soils was studied. Bacteria used was *Bacillus Pastuerri*.
- This study was aimed to controlling and optimizing the efficient use of *Bacillus pasteurii* and to increase the pH and microbial induced precipitation of CaCO_3 in sand specimen.
- UCS and hydraulic conductivity experiments were performed on sand specimen with treatment and the amount of CaCO_3 precipitation were correlated with improvement in strength and reduction in hydraulic conductivity.
- It resulted in strength increment, MICP was found highly promising (an average UCS of 1 MPa at 6 % cementation) but the increment was also related to the chemical concentration of the cementation reagents.

LU Wang Jie [2010] conducted a study on “Study on soil solidification based on microbiological precipitation of CaCO_3 ”

- In this study, compressive strength of soil treated with *Bacillus* bacteria is considered.
- This paper mainly deals with solidification of soil using micro-organism.
- Soil for the experiments was dried at 105°C and went through the sieve (5 mm).
- Compressive Strength Test were conducted on plain soil and soil treated with bacteria after 7 days.
- It was found that compressive strength of soil was increased.

Montoya et al. [2015] conducted a study on “Stress-Strain behaviour of sands cemented by Microbially Induced Calcite Precipitation”.

- In this study sand specimens are tested on triaxial test for drained and undrained shearing conditions.
- Sand treated using MICP is tested for undrained shearing at different cementation levels which are young, uncemented and highly cemented like sandstone type conditions.
- Changes in shear strength and volumetric behaviour are monitored for moderately cemented samples which are subjected to various stress path.
- Non-destructive monitoring is done using shear wave velocity to observe small strain stiffness changes during shearing and this can provide an indication that cementation degradation as a function of strain level, because due to level of cementation and effective mean stress change both influences shear wave velocity .

Montoya et al. [2013] conducted a study on “Bio-mediated soil improvement utilized to strengthen coastal deposits”.

- Four soil column specimens were prepared by dry pulverization
- The soil column specimens had 50.8 mm diameter and an aspect ratio of 2:1.
- *Bacillus Clausii* was used for treatment of soil.
- Unconfined compressive strength was calculated of natural soil and soil treated with MICP
- The applied cell density of the bacterial culture was the same for each plot, whereas the nutrient solution concentration was varied across the plots to evaluate the impact on treatment depth.
- The culture and nutrients were applied through a surficial spray application system over 20 days.
- Soil improvement was verified up to a depth of 30 cm using dynamic cone penetration (DCP) resistance, induced calcite content, and biological activity measurements.
- The measurements of DCP resistance and induced calcite content indicated modification up to a depth of approx 25 cm.

ChunXiang et.al. [2010] conducted a study on “Cementation of sand grains based on carbonate precipitation induced by microorganism”

- Quartz sand was used for this study.

- 3 types of bacteria were considered for this study, testing's were done and it was considered that Bacillus Bacteria S3 was best suited.
- Compressive Strength was calculated for the all three types of bacteria.
- The results concluded that, the highest strength was closely to 2 MPa, and the strength was of inhomogeneous distribution, which depended on the distribution of CaCO₃.

Whiffin et.al. [2007] conducted study on “Microbial Carbonate Precipitation as a Soil Improvement Technique”

- In this study, application of Microbial Calcite Precipitates on sandy soil was observed.
- A five meter sand column was treated with bacteria and reagents under conditions that were realistic for field applications.
- After treatment, the column was subjected to mechanical testing, which indicated a significant improvement of strength and stiffness over several meters.
- Improvement of the load bearing capacity of the soil without making the soil impermeable to fluids was shown with microbial carbonate precipitation

M. Rollins [2009] conducted a study on “Effect of Soil Treatment with Bio-Base on CBR%”

- Three tests specimen were considered in this study. The three specimens considered had fine, medium and coarse gradations respectively.
- Atterberg Limits and pH values of three soil specimens were calculated.
- CBR value was also calculated for untreated soil specimen.
- The soil specimens were then treated with Bio-Enzyme for 4 weeks.
- Again Atterberg Limits and CBR values were calculated.
- The CBR values for all gradations increased significantly with time
- The CBR values for the bio-base treated coarse and medium gradation specimens (15 and 24% fines, respectively) were less than those from fine gradation specimens (Appendix Figures 3, 4, 5). Improvement is projected from soil with higher percentages of clay size particles or a fine fraction with a higher plasticity index.
- Improvement with bio-base treatment may require up at least 4 weeks to fully develop.

Lee et al [2013] conducted a study on “Stress-deformation and compressibility responses of bio-mediated residual soils”

- In this study the stress-deformation and compressibility responses of bio-mediated soil was tested at laboratory scale.
- A residual soil was tested with MICP for different test durations, flow pressures and concentrations of cementation chemicals.
- The experiments resulted in significant improvement in the peak strength and stiffness of soil by the MICP treatment. A linear correlation was found between the amount of calcite precipitated with recompression index (Cr), reasonable correlations with peak strength (p) and total settlement (Sc), but a poor correlation with compression index (Cc).
- The compressibility responses of bio-mediated soils show certain similarities to typical aged clays that have undergone a long period of natural cementing process.

Table 2.1 Literature review: work and comparison between previous researches and present work

Sr.	Author	Journal	Scale of study	Type of soil	Bacteria used	Tests conducted	Results	Key Observation
1	Park et al. (2014)	ASCE	Small scale Lab testing	Weakly cemented sand	Sporosarcina pasteurii	UCS, SEM, XRD, TGA, XRF	Sample treated once showed 5% increase in UCS and samples treated more than twice shows gradual decrease in strength up to 50% compared to untreated sample	Calcite precipitation does not always increases the strength of cemented soils
2	Gomez et al. (2014)	ICE	Large scale Field and lab testing	Loose sand	Sporosarcina pasteurii	DCP, Calcite content, Water jet impingement erosion test	Stiff crust of 2.5 cm thick was formed and was verified by DCP, Water jet impingement erosion test verified increase in erosion resistance of sand	The test plot with least conc. of urea and calcium chloride showed the most improvement and formed a stiff crust measuring 2.5 cm thick
3	Lee et al. (2013)	Elsevier	Small scale Lab testing	Silty residual soil	Bacillus megaterium ATCC 14581	Consolidation, UCS, Gravimetric analysis	significant improvement in the peak strength and stiffness of soil,	linear correlation was found between the amount of calcite precipitated with

							compressibility responses of bio-mediated soils show certain similarities to typical aged clays that have undergone a long period of natural cementing process	recompression index (Cr), reasonable correlations with peak strength (p) and total settlement (Sc), but a poor correlation with compression index (Cc)
4	Paassen et al. (2010)	ASCE	Large scale Field and lab testing	Sand	Sporosarcina pasteurii	UCS, Shear-wave velocity	Increase in stiffness of soil is verified by in situ time lapse shear wave transmission seismic and UCS results	Stiffness of soil had significantly increased after one day of treatment
5	Soon et al. (2012)	Springer	Small scale Lab testing	Tropical residual soil and sand	B. Megaterium ATCC 14581	UCS, Hydraulic conductivity, Gravimetric acid washing, SEM	Improved shear strength ratios significantly higher (1.41-2.64) than that of the sand (1.14-1.25). On the other hand, sand resulted in better reduction in hydraulic conductivity ratios (0.09-0.15)	Effectively reduction in hydraulic conductivity and improved shear strength by use of MICP on both sand and residual soil

							than that of the residual soil (0.26-0.45)	
6	Soon et al. (2013)	ASCE	Small scale Lab testing	Silt	B. Megaterium	Shear strength test, Hydraulic conductivity Gravimetric analysis, SEM	Shear strength and reduction in hydraulic conductivity obtained are 100 and 90% respectively, Cementation reagent flow pressure 2 bar, 0.2 bar are not well suited for treatment, medium range flow pressure 1 bar is advisable.	The recommended treatment conditions for residual silty soil are B. megaterium concentration of 1×10^8 cfu/mL, cementation reagent concentration of 0.5 M, and flow pressure of 1.1 bar for a treatment duration of 2 days
7	Lee et al. (2012)	WASET	Small scale Lab testing	Residual soil	Bacillus Megaterium	UCS, Pemeability, SEM	MICP showed improvement in both hydraulic conductivity and shear strength of residual soil, native urease-forming bacteria can be utilized in MICP soil improvement, with sufficient	

							and appropriate nutrient provided	
8	Qabbany et al. (2012)	ASCE	Small scale Lab testing	Silica sand	Sporosarcina Pasteurii	Chemical analysis, Gravimetric acid washing, SEM	Below some input rate (0.042 mol/L/h) and on bacterial optical density (OD ₆₀₀) between 0.8 and 1.2 reaction efficiency observed high and amount of ppt has no effects of liquid medium concentration	At pore scale the pattern of precipitation was found to be affected by injection concentration.
9	Tsukamoto et al. (2012)	ISOPE	Small scale Lab testing	Toyoura sand	Sporosarcina Pasteurii (ATCC11859)	Tri-axial (CD), Permeability	Young's modulus and peak strength tend to increase as a function of the amount of precipitated calcium carbonate, with little change in the coefficient of permeability.	
10	ChunXiang et.al. (2010)	Springer	Small scale Lab testing	Quartz sand	Carbonate mineralization bacteria	UCS, Porosity, TGA, XRD, SEM	3 types of bacteria were considered and Bacillus Bacteria	The more the calcite was contained and the more

					Bacillus S3, Bacillus S4, Bacillus A4		S3 was best suited, The highest strength was closely to 2 MPa, and the strength was of inhomogeneous distribution, which depended on the distribution of CaCO ₃	homogeneous it was distributed, the higher the strength would be.
11	Montoya et al. (2015)	ASCE	Small scale Lab testing	Ottawa sand	Sporosarcina pasteurii (ATCC 11859)	Tri-axial, Gravimetric acid washing, Shear wave velocity	Peak stress ratio increased from 1.3 to 1.9 for cemented sand with shear wave velocity of 1400 m/s, the critical state stress ratio was not significantly affected by cementation	Shear wave velocity may be essential for verification of MICP cementation during the treatment phase and also used to monitor the level of cementation after loading has occurred (e.g., an earthquake).
12	Montoya et al. (2013)	ICSMGE	Small scale Lab testing	Ottawa sand	Sporosarcina pasteurii (ATCC 11859)	UCS, DCP, Gravimetric acid washing	culture and nutrients were applied through a surficial spray application system over 20 days, the lightly	Measurements of DCP resistance and induced calcite content indicated the modification up to a depth of approx 25 cm

							cemented sand had an increase in strength, as demonstrated with the unconfined compression tests, and increase in friction angle	
13	Morgan et al. (2011)	ICEST	Small scale Lab testing	Silt, Kaolin, Mixture of Silt and Kaolin	Sporosarcina pasteurii NCIMB8841 Sporosarcina ureae NCIMB9151	UCS, Cone penetration test	Sporosarcina pasteurii and Sporosarcina ureae was used many samples were considered and they were left to cure at different conditions, Undrained shear strength of soil was evaluated at different moisture contents and mixture of soils which indicated positive response of bacteria used on soil cementation	Sporosarcina pasteurii showed better results than Sporosarcina ureae.

14	Whiffin et al. (2007)	Taylor & Francis (GMJ)	Small scale Lab testing	Itterbeck sand	Sporosarcina pasteurii (DSMZ 33)	Triaxial test (CD), modified Nessler method, commercial cuvette test, constant head permeability test, acid gravimetric method	indicated a significant improvement of strength and stiffness over several meters, Improvement of the load bearing capacity of the soil without making the soil impermeable to fluids was shown with microbial carbonate precipitation	Strength increase is largely related to the supply of cementation reactants versus the bacterial activity in the column
15	Present Work (2015)	Desertation	Small scale Lab testing	Silty sand	Bacillus clausii	CBR, Tri- axial, Direct shear, UCS, SEM	Stiffness of soil increases with time verified by UCS and unsoaked CBR results but decreases with increase in soaking days, cohesion and angle of internal friction increases	Shear parameters increases with the time of curing

Table 2.2 Literature review: Methodology and reason for difference in Results of previous Researches and present work

Author	Bacteria	Method of treatment	Advantages	Disadvantages	Results
Soon et al. (2012)	B. Megaterium ATCC 14581	Special apparatus having steel mould connected to pressure controlling device to regulate flow (1.7×10^{-5} m/s) of cementing and bacterial reagent through soil specimen at a fixed rate at a interval of 6 hours for 48 hours	Continuous supply increases the chances of calcite precipitation because bacteria has more food and can cause urealalysis up to greater extent		UCS of residual soil increased from 50 to 140 kPa
Lee et al. (2013)	B. Megaterium ATCC 14581	A steel mould was prepared of specific dimension and connected to a pressure controlling device to regulate the flow of treatment reagent through specimen and consolidation was done at incremental loadings for 3 days	Specimen was prepared and putted in mould and reagents were supplied continuously which resulted in calcite precipitation	Consolidation was done and saturated sample after consolidation was trimmed to 100 mm length and then UCS test was performed	UCS increased from 38 to 70 kPa after 3 days treatment
Park et al. (2014)	Sporosarcina Pasteurii	3% Portland cement was mixed in sand and cured for 3 days after that microbe medium was injected continuously on some specimen for 10 days and 20 days for some others after which specimen was oven dried to make same water content in all samples and then tested	Continuous supply of microbe medium caused more calcite precipitation	Apart from continuous supply if microbe medium is injected different times reduces the strength of soil because pressure by which medium is injected can destroy the existing cementation	(UCS 683kPa) treated 10 days then oven dried and tested for UCS

Montoya et al. (2015)	Sporosarcina Pasteurii ATCC 11859	Triaxial specimens were prepared fixed in apprestus and using peristaltic pump cementation media was injected into the bottom pore line of triaxial instrument which initially consisted of bacterial media only thereafter cementing media and the process was repeated every 3-6 hrs	Sample was not disturbed at all as the treatment was done after fixing the specimen in apparatus, bacteria was supplied at a regular flow rate		UCS of cohesion less sand increased to 650 kpa and angle of internal friction increased from 39.04° to max 48.8°
Paassen et al. (2010)	Sporosarcina Pasteurii DSM33	A concrete container (100 m ³) (8.0*5.6*2.5m) was filled with 25 cm layer of sand and 48 geophones were planted then tank was filled with sand up to 2.25 m. 96 m ³ reagent was injected in 10 batches of 10 m ³ and the flow was divided equally over three wells and treated for a period of 16 days, before UCS test whole chamber was washed with water to wash out free sand	By such a large scale setup shear wave velocity test was easily performed and soil was treated like field situation and after treatment specimens were extracted and dried to perform UCS test	Uniformity of calcite precipitation was not there, therefor specimens were extracted from different parts and then tested which showed variation in strength	For different calcite content different UCS values were obtained which were ranging from 700 kPa to 12400 kPa for calcite content of 12.6% to 25% of dry weight respectively
Present Work (2015)	Bacillus clausii	Samples were prepared by mixing Bacterial and cementing reagent at fixed amount equal to OMC of soil. Specimens for desired tests were prepared and were packed in air tight bags and daily 10 ml of solution was poured freely on specimen and again packed and was repeated for 7 days. Stored at room temp. (30° - 40°).	Bacterial and cementing reagent were thoroughly mixed with soil and some amount of food and cementing reagent was supplied daily.	Continuous flow of reagents was not there due to which bacteria had lesser opportunity for hydrolysis of urea resulted in lesser cementation, distribution of cementation media was not uniform.	UCS of soil increased from 18.75 kPa to 76 kPa and angle of internal friction was increased from 25.8° to 35°.

Chapter III

Methodology

For this study, the soil will be treated with bio – organic based material. That is:

- Microbial Induced Calcite Precipitates

3.1 Microbial Induced Calcite Precipitation

In this method, Calcite Precipitates will be induced with help of an aerobic urease producing bacteria, i.e. *Bacillus Clausii*. The soil materials will be directly mixed with the prepared solutions of nutrient broth and *Bacillus Clausii*.

Concentration of *Bacillus Clausii* will be 1×10^{12} cfu/ml. This concentration is selected based on study performed by Lee M.L. et al. [2012] on similar type of soil.

Concentration of Nutrient Broth that will be added will be 3 gm /litre. Nutrient Broth is needed to be added to soil because it is necessary for survival of Bacteria in soil. Concentration of Nutrient Broth is selected based on earlier studies done. 3 gm /litre were found to be most viable amount.

Nutrient Broth composition:-

It is a food of bacteria and its composition is

- Peptic digest of animal tissue 5.0g/l
- sodium chloride 5.0g/l
- beef extract/yeast extract 3.0g/l
- Temperature maintained is around 25 degree.
- pH will be around 7.4-7.6.

Composition of chemicals used in bacterial reagent and cementation reagent per litre (Dejong *et al.*, 2006; Qabany *et al.*, 2011; Stocks-Fischer *et al.*, 1999; Stoner *et al.*, 2005)

- Nutrient broth 3g/l

- Urea (NH₂-CO-NH₂) 20g/l
- Ammonium chloride (NH₄Cl) 10 g/l
- Sodium bi-carbonate (NaHCO₃) 2.12 g/l

For Bacterial reagent 100ml per kg of soil

- 1*10¹² cells/ml Bacillus Clausii
- 2 ml Calcium Chloride solution (140g/l)

For Cementation reagent 72ml per kg of soil

- 1.44 ml Calcium Chloride solution (140g/l)

Bacteria and cementation reagents are mixed as per OMC of soil. After addition of Bacteria, soil will be compacted to Maximum Dry Density and to this cementation reagent will be added.

The soil will be placed in a mould and cementation reagent will be supplied from relatively higher level.

The cementation reagent for the MICP treatment will consist of urea and calcium chloride. The urea and calcium chloride serve as important ingredients for promoting calcite precipitation. Concentration of cementation reagent will be 1 M. This concentration is selected based on study performed by Lee M.L. *et al.* [2012] on similar type of soil.

The cementation reagent will be added from separate container and will be added from top and cementation reagent will be flowed into the soil. Effect of calcite precipitation will be studied on compressive strength, shear parameters and CBR value of soil.

Amount of cementation reagent will be varied, which in turn will vary the amount of calcite precipitated.

The soil specimen will be taken out of mould and will be tested for compressive strength, shear parameters and CBR Values.

Table 3.1 Experimental Program

Test	Direct shear		Triaxial		CBR		UCS	
	Virgin soil	Treated soil	Virgin soil	Treated soil	Virgin soil	Treated soil	Virgin soil	Treated soil
8 hrs (0 days)			3	3	2	2	3	3
1 day	1	1						
3 days				3	2	2	3	3
7 days				3	2	2	3	3

Chapter IV

Experimental Programme

4.1 Soil Properties

The tests on natural soil samples were carried out in the laboratory to determine the basic properties of the soil. So far, in this phase of the project, the tests are carried out on Soil Sample. The samples obtained were disturbed samples.

4.1.1 Experimental Investigation

Table 4.1 Test results

Area	DTU Campus, Delhi
Depth	0.5m
Grain Size Analysis	
Gravel	3.54%
Sand	80.2%
Fines(silt + clay)	16.26%
Index Properties	
Liquid limit %	24.20%
Plastic limit%	20.64%
Plasticity Index%	3.56%
Specific Gravity	2.579
Engineering Properties	
Optimum Moisture Content(OMC)	17.274%
Maximum Dry Density(MDD)	1.7275 g/c ³
CBR Unsoaked Virgin soil	7.12

CBR Unsoaked MICP treated soil	11.89
CBR (3days) Soaked Virgin soil	3.22
CBR (3days) Soaked MICP treated soil	4.72
CBR (7days) Soaked Virgin soil	1.75
CBR (7days) Soaked MICP treated soil	1.93

4.1.2 Sieve Analysis

Sieve analysis is done to know about the various sizes of particles present in soil. After doing sieve analysis on both virgin and bacterial treated soil we find that there is not much change on the particles of soil.

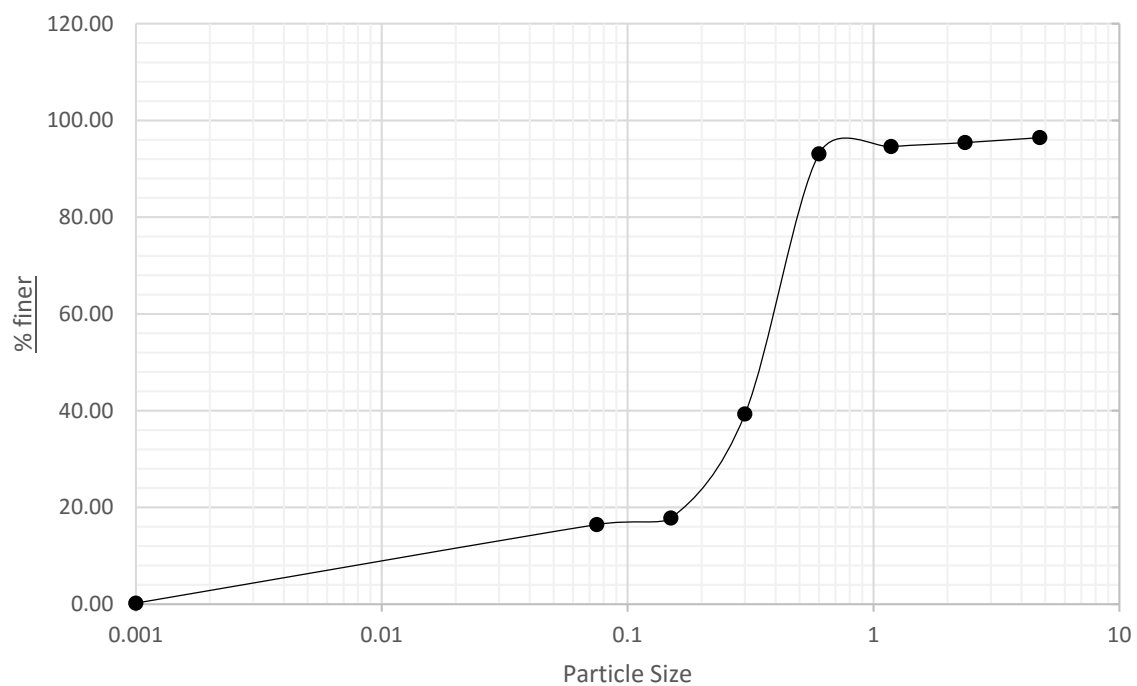


Figure 4.1: Grain size analysis

Table No.4.2 Grain size analysis

S. no.	Sieve size (mm)	Mass of soil retained (gm.)	Percentage on each sieve Retained Mass of soil/Wt. *100	Cumulative % retained	% finer, 100-cumulative retained
1	4.75	35.42	3.54	3.54	96.46
2	2.36	10.24	1.02	4.57	95.43
3	1.18	8.42	0.84	5.41	94.59
4	0.6	15.2	1.52	6.93	93.07
5	0.3	537.75	53.78	60.70	39.30
6	0.15	214.98	21.50	82.20	17.80
7	0.075	13.45	1.35	83.55	16.45
8	0.001	162.58	16.26	99.80	0.20

4.1.3 Liquid Limit

Table 4.3.: Liquid limit determination

No. of blows	Water content
14	60.26
19	39.42
24	27.12
30	18.1

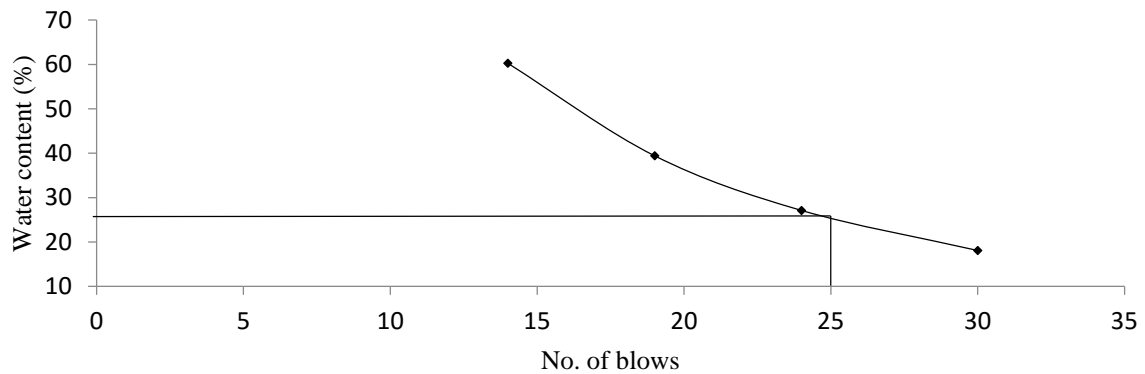


Figure 4.2: Liquid limit

The liquid limit for given soil sample is 24.20 percent.

4.1.4 Plastic Limit

Weight of empty pan = 13.84 gm

Weight of pan + weight of soil = 29.56 gm

Weight of pan + dried sample = 26.87 gm

The plastic limit of the adopted sample is 20.64 percent

4.1.5 Standard Proctor's Tests

Standard proctor test is done to know about the optimal moisture content of soil. It is that value at which we get the maximum dry density of soil. Bacteria is added according to the optimal moisture content of soil which we get in this test.

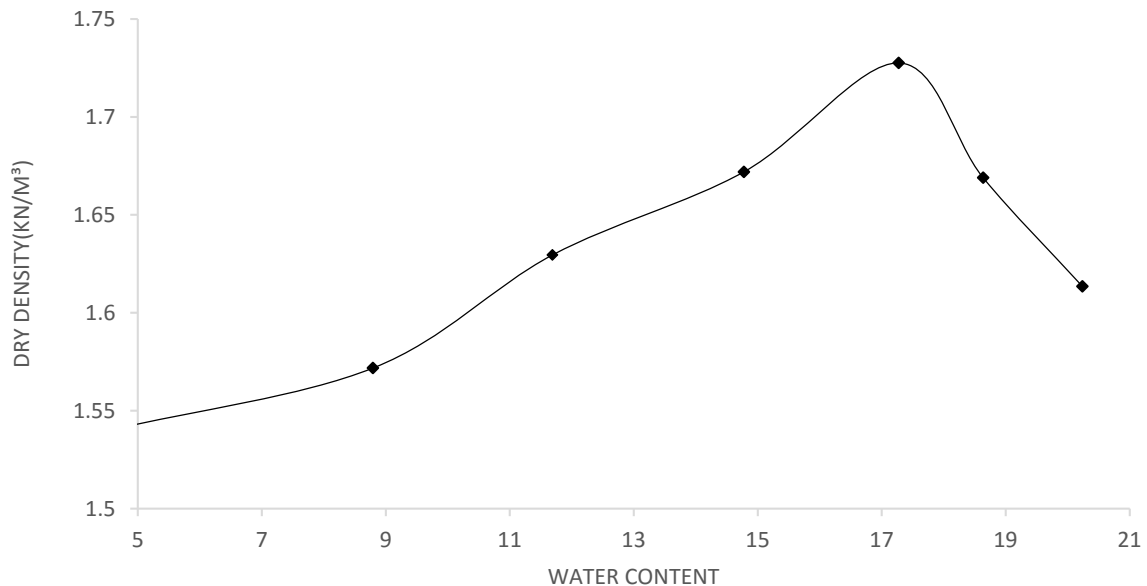


Figure 4.3: Proctor compaction

4.1.6 Unconfined Compressive Strength (UCS)

This test is case of Tri-axial test in which no confining pressure is applied, therefore this test is used to determine undrained unconfined compressive strength of soil. This test cannot be performed with cohesion less soils because such soils are not able to stand freely without any confinement.

Length of sample = 7.6 cm

Diameter of sample = 3.8 cm

Area of sample = 11.341 cm²

Results were performed in triplets and average values are shown here because results obtained were so consistent.

Table No. 4.4 Results of UCS test

Soil	Present Study (OMC) (kN/m ²)	Lee et al (2013) (Saturated) (kN/m ²)	Park et al (2014) (Oven Dried) (kN/m ²)	Soon et al (2014) (Saturated) (kN/m ²)	Passen et al (2010) Dry (kN/m ²)
Virgin soil	18.75	38	650	76	0
8 hours MICP	31.24	56 (24 hours)	-	-	700 (24 hours)
3 Days MICP	59.88	65	-	152	-
7 Days MICP	76.04	70	683 (10 days)	-	12400 (16 days)

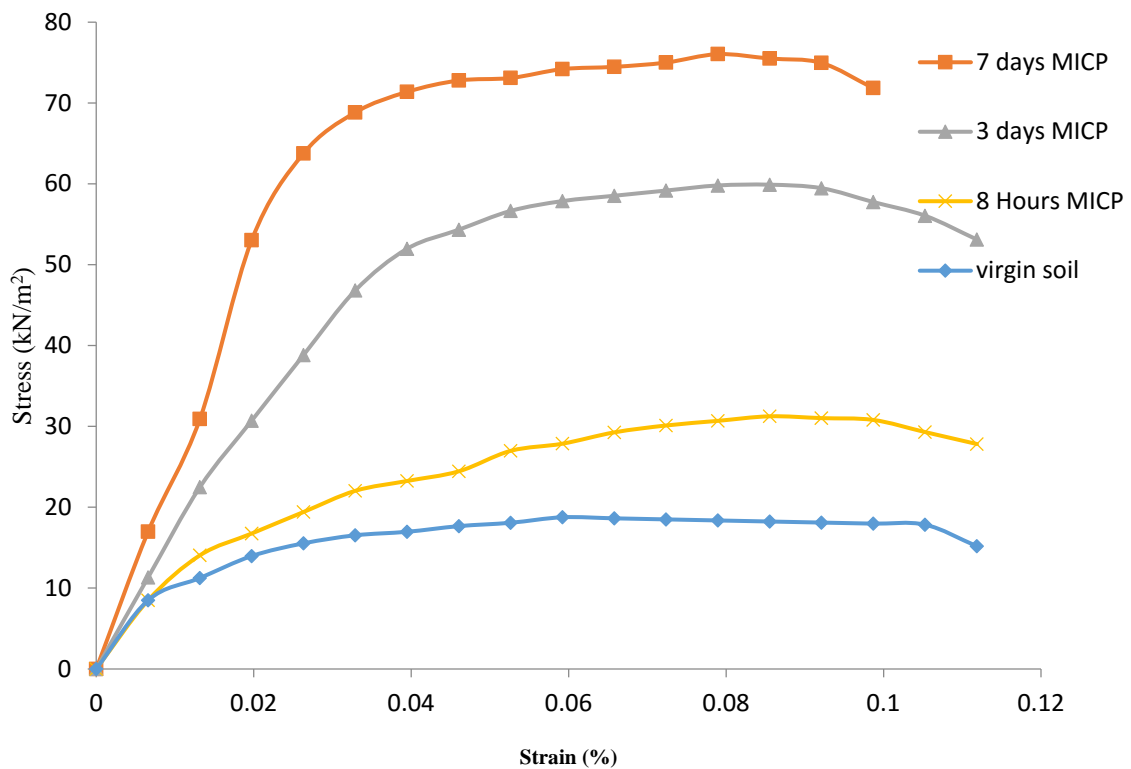


Figure 4.4 Unconfined compression test



Figure 4.5: Unconfined compression test failed sample

4.1.7 California Bearing Ratio (CBR)

CBR value is the percentage of force per unit area required to penetrate a soil mass with circular plunger of 50mm diameter at the rate of 1.25mm/min to that required for corresponding penetration in a standard material. In which loads are measured corresponding to 2.5mm and 5mm penetration. CBR test is used for the evaluation of penetration resistance and this test is generally used for road pavement and it is very significant for the geotechnical engineering.



Figure 4.6 CBR test setup and tested sample

Table 4.5 CBR un-soaked virgin soil (sample 1) (CS2)

S.NO.	Penetration of Rod (mm)	Load taken by sample (kg)	Standard load (kg)	CBR
1	2.5	96.20	1370	7.02%
2	5.0	141.30	2055	6.87%

Table 4.6 CBR un-soaked virgin soil (sample 2) (CS1)

S.NO.	Penetration of Rod (mm)	Load taken by sample (kg)	Standard load (kg)	CBR
1	2.5	99.10	1370	7.23%
2	5.0	144.30	2055	7.02%

Table 4.7 CBR un-soaked MICP (sample 1) (DS2)

S.NO.	Penetration of Rod (mm)	Load taken by sample (kg)	Standard load (kg)	CBR
1	2.5	159.00	1370	11.60%
2	5.0	237.50	2055	11.55%

Table 4.8 CBR un-soaked MICP (sample 2) (DS1)

S.NO.	Penetration of Rod (mm)	Load taken by sample (kg)	Standard load (kg)	CBR
1	2.5	162.90	1370	11.89%
2	5.0	242.40	2055	11.79%

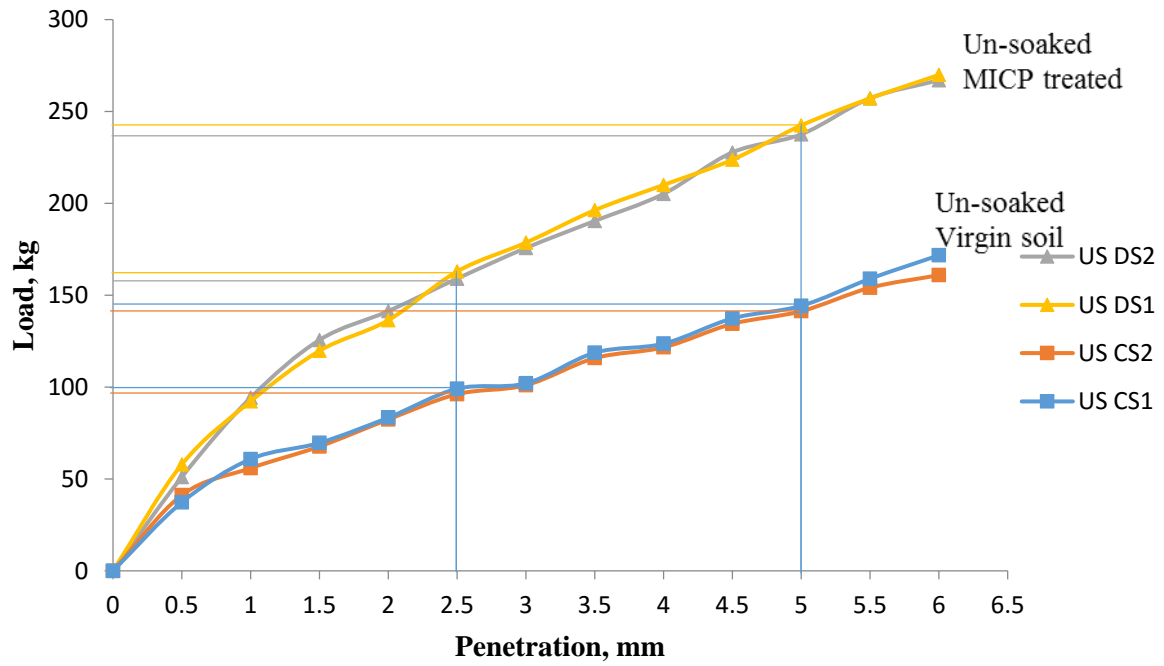


Figure 4.7: CBR un-soaked virgin soil and MICP treated Soil

Table 4.9 CBR 3 days virgin soil (sample 1) (BS1)

S.No.	Penetration of Rod (mm)	Load taken by sample (kg)	Standard load (kg)	CBR
1	2.5	43.20	1370	3.15%
2	5.0	60.80	2055	2.96%

Table 4.10 CBR 3 days virgin soil (sample 2) (BS2)

S.No.	Penetration of Rod (mm)	Load taken by sample (kg)	Standard load (kg)	CBR
1	2.5	45.10	1370	3.29%
2	5.0	62.80	2055	3.05%

Table 4.11 CBR 3 days MICP (sample 1) (AS1)

S.No.	Penetration of Rod (mm)	Load taken by sample (kg)	Standard load (kg)	CBR
1	2.5	66.70	1370	4.87%
2	5.0	96.20	2055	4.68%

Table 4.12 CBR 3 days MICP (sample 2) (AS2)

S.No.	Penetration of Rod (mm)	Load taken by sample (kg)	Standard load (kg)	CBR
1	2.5	64.70	1370	4.72%
2	5.0	94.20	2055	4.58%

Table 4.13 CBR 7 days virgin soil (sample 1) (SS2)

S.No.	Penetration of Rod (mm)	Load taken by sample (kg)	Standard load (kg)	CBR
1	2.5	23.50	1370	1.72%
2	5.0	33.40	2055	1.62%

Table 4.14 CBR 7 days virgin soil (sample 2) (SS1)

S.No.	Penetration of Rod (mm)	Load taken by sample (kg)	Standard load (kg)	CBR
1	2.5	24.50	1370	1.79%
2	5.0	34.30	2055	1.67%

Table 4.15 CBR 7 days MICP (sample 1) (MS2)

S.No.	Penetration of Rod (mm)	Load taken by sample (kg)	Standard load (kg)	CBR
1	2.5	27.50	1370	2.00%
2	5.0	40.20	2055	1.95%

Table 4.16 CBR 7 days MICP (sample 2) (MS1)

S.No.	Penetration of Rod (mm)	Load taken by sample (kg)	Standard load (kg)	CBR
1	2.5	26.50	1370	1.93%
2	5.0	39.30	2055	1.91%

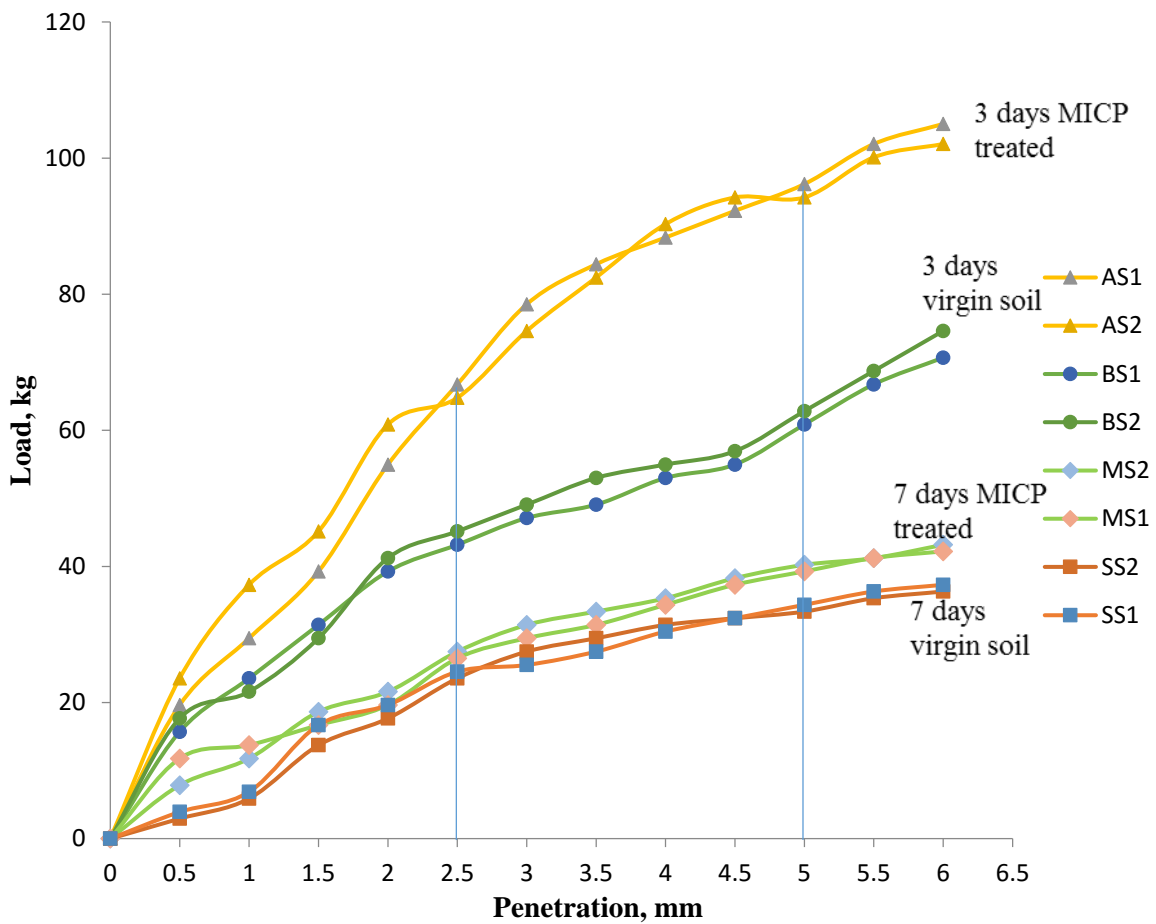


Figure4.8: CBR soaked virgin soil and MICP treated soil

4.1.8 Direct Shear Test

This test will us the shear strength of soil and shear strength is depends upon the cohesion and internal friction angle of soil. Direct shear strength of soil increases due to addition of urease positive bacteria because it will increases the cohesion and internal friction of soil.



Figure 4.9: Failed sample from direct shear test

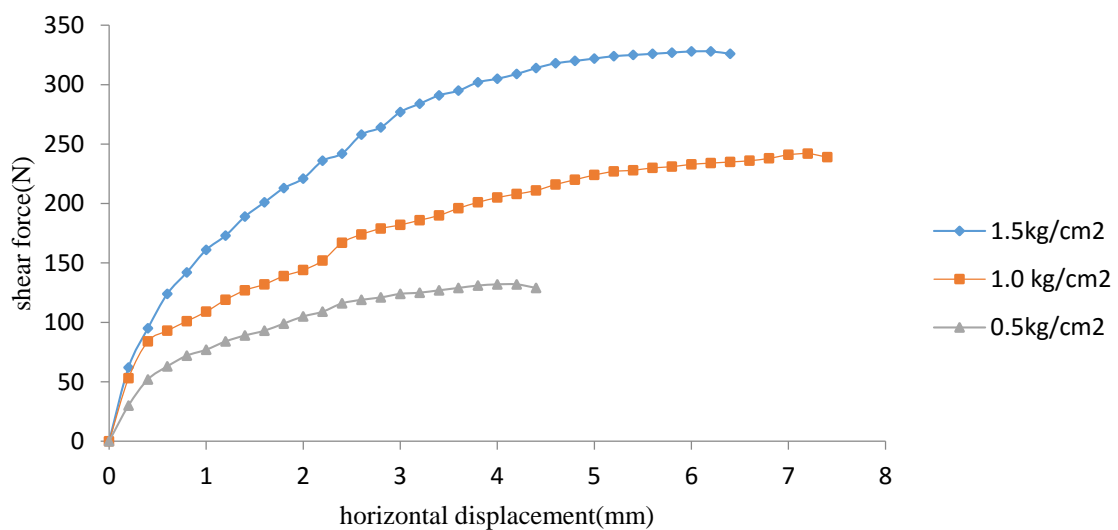


Figure 4.10: Load vs Displacement diagram without bacteria

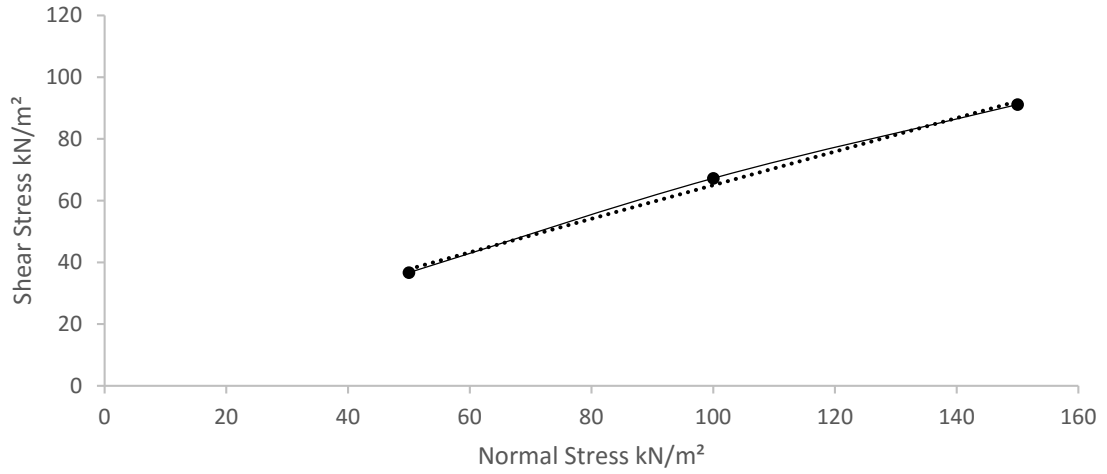


Figure 4.11: Normal stress vs Shear stress diagram without bacteria

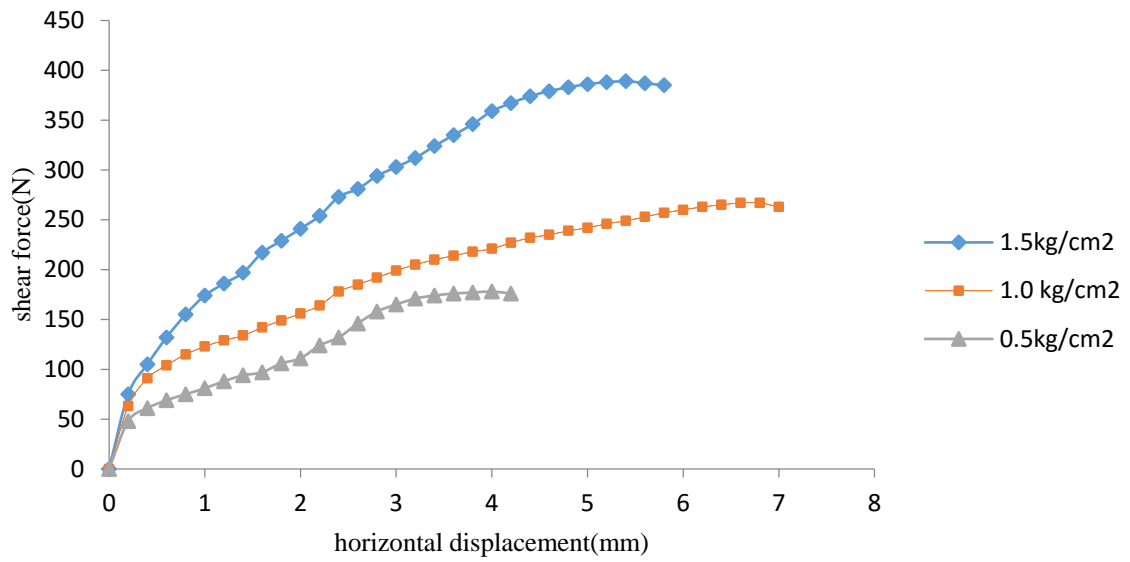


Figure 4.12: Load vs Displacement diagram with bacteria

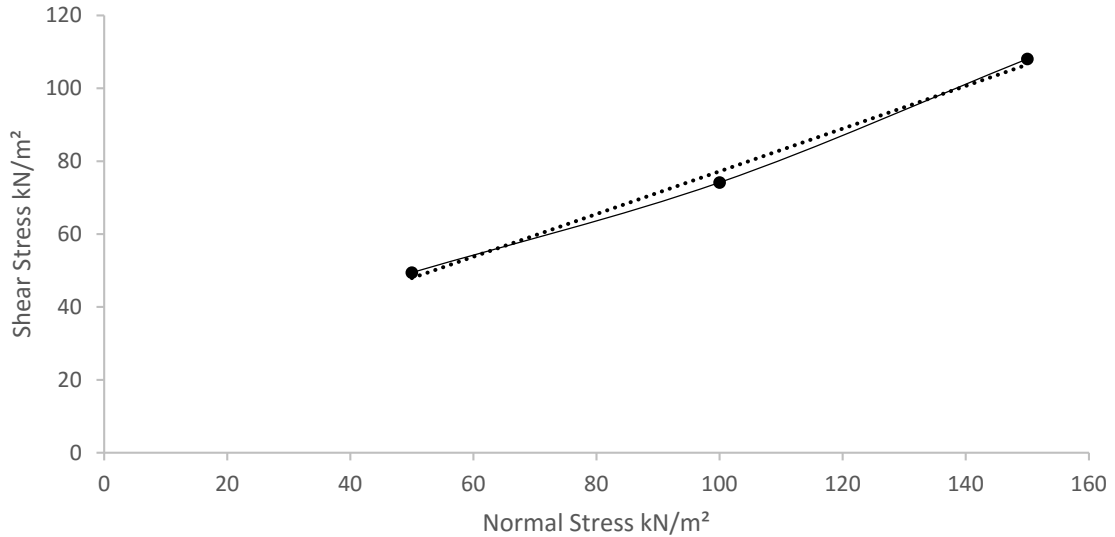


Figure 4.13: Normal stress vs Shear stress diagram with bacteria

Table 4.17 Shear parameters of soil by Direct shear test

S. No.	DTU SOIL	ANGLE OF FRICTION(°)	COHESSION(kN/m ²)
1	Virgin Soil	28.546	10.56
2	MICP Treated Soil 1day treated	30.379	18.607

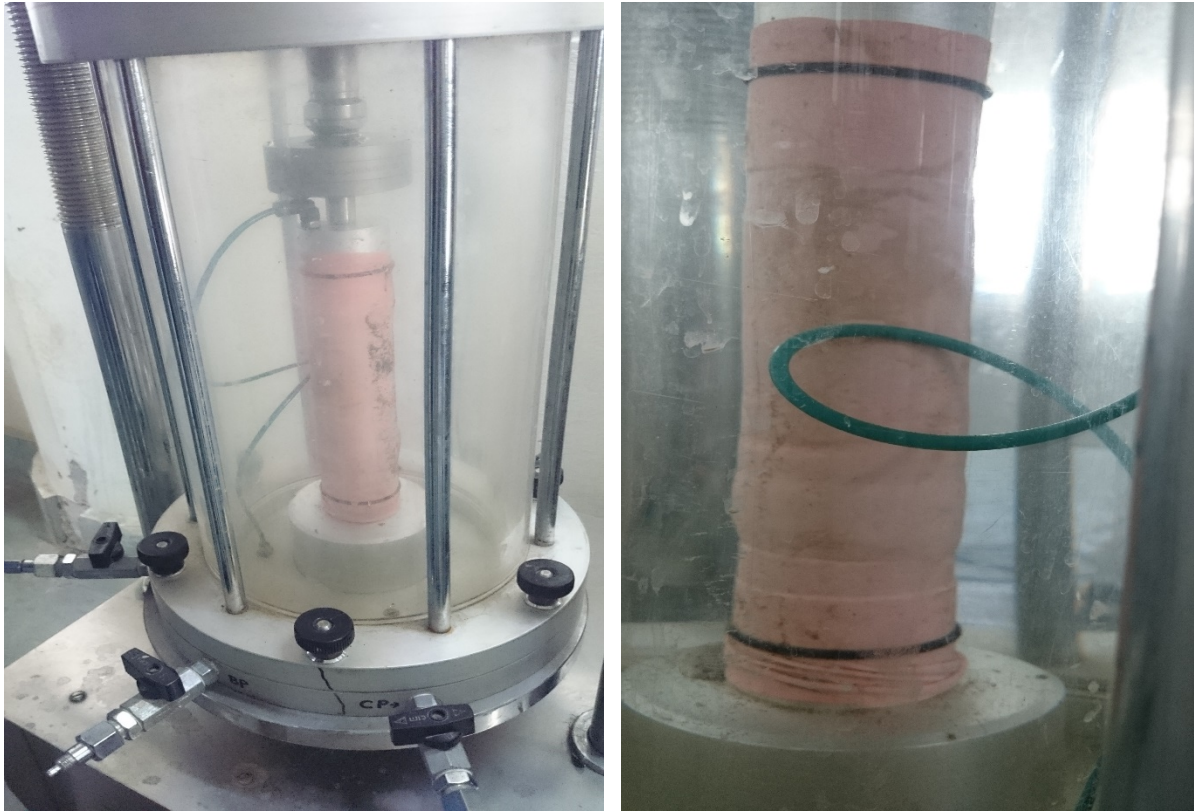
4.1.9 Tri-axial test

Tri-axial test is used to find the mechanical properties of soil and it will give us shear parameters of soil. Stress is applied along the one axis of the sample not on its perpendicular direction, in perpendicular direction stress is applied by the fluid which is water. Finally we will make mohr circle in graph between shear stress and normal stress. We will make three more circle using three different cell pressure and finally a line tangential to all three circles give us the value of shear parameter.

Length of sample = 7.6 cm

Diameter of sample = 3.8 cm

Area of sample = 11.341 cm²



(a)

(b)

Figure 4.14: Triaxial test (a) Initially fitted sample (b) Bulged sample

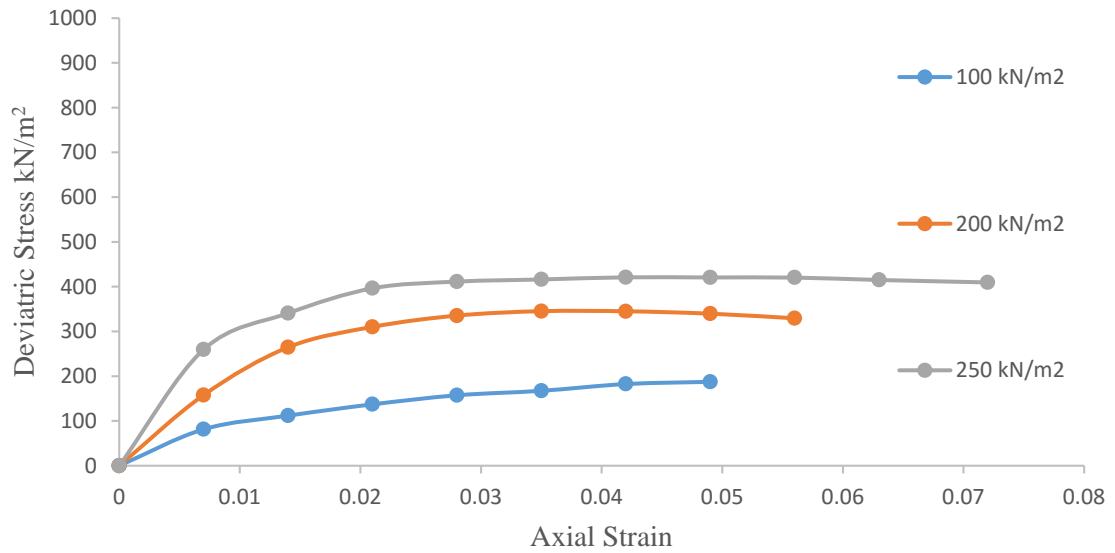


Figure 4.15: Stress vs Strain diagram of virgin soil by tri-axial test

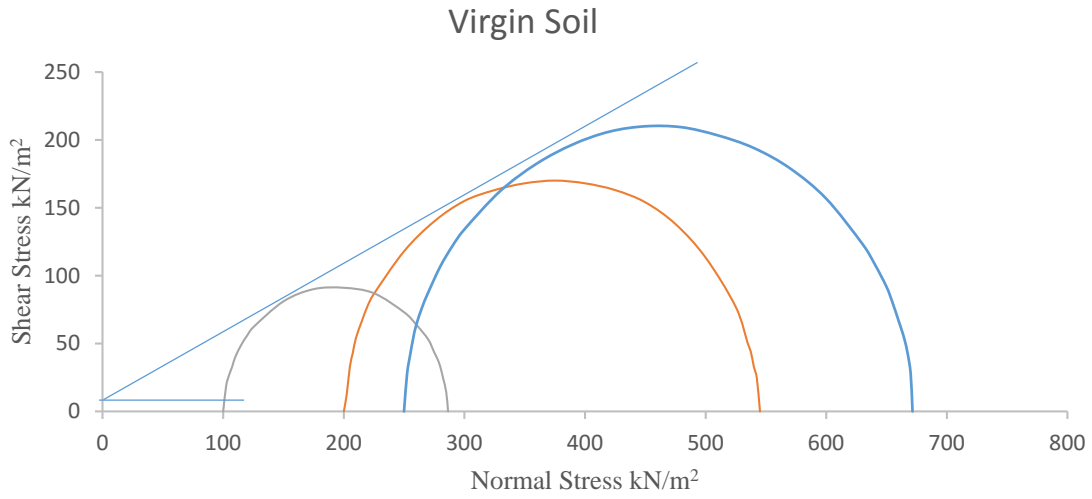


Figure 4.16: Mohr circle diagram of virgin soil by tri-axial test

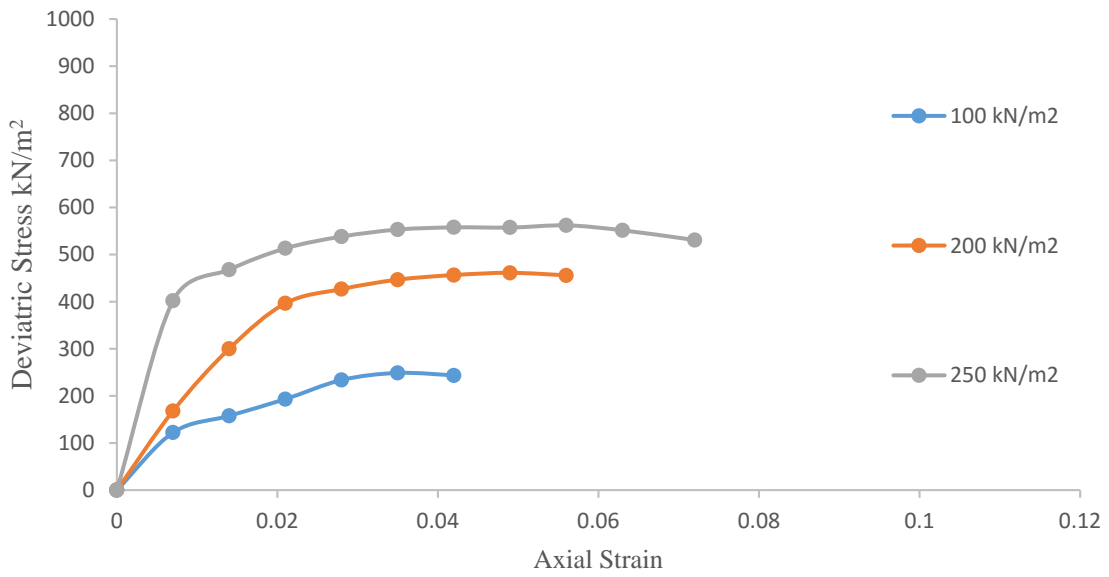


Figure 4.17: Stress vs Strain diagram of MICP treated soil by tri-axial test

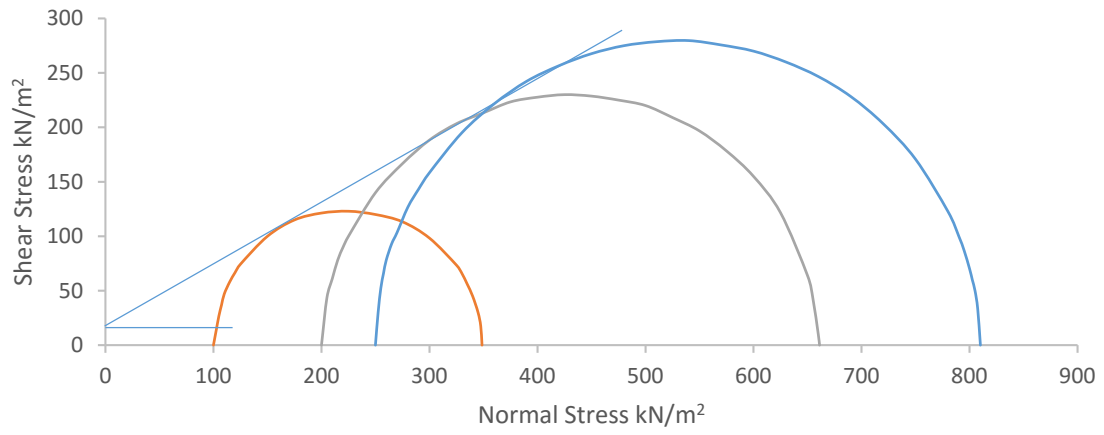


Figure 4.18: Mohr circle diagram of 8 Hours MICP treated soil by tri-axial test

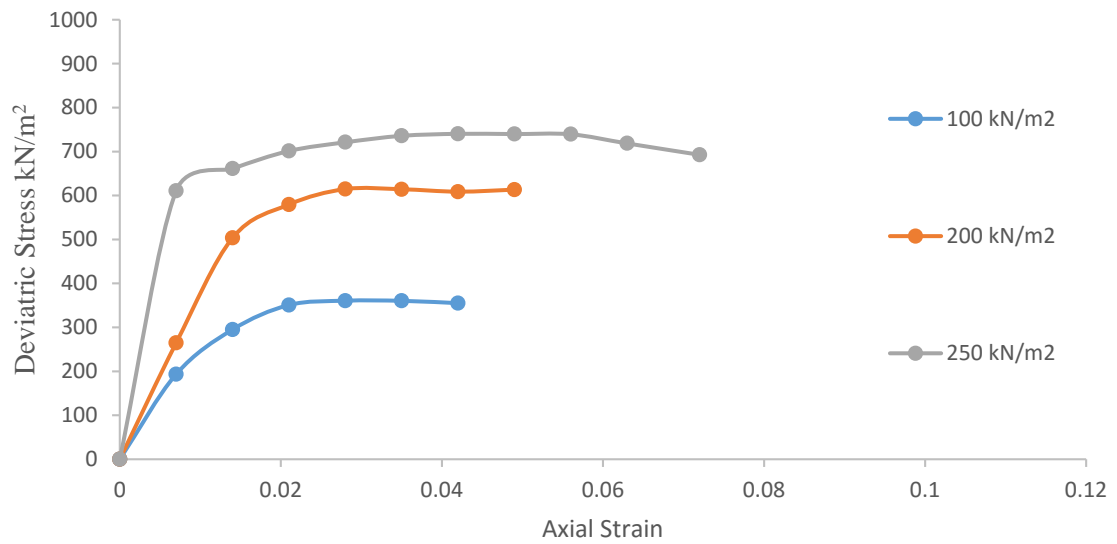


Figure 4.19: Stress vs Strain diagram of 3 days MICP treated soil by tri-axial test

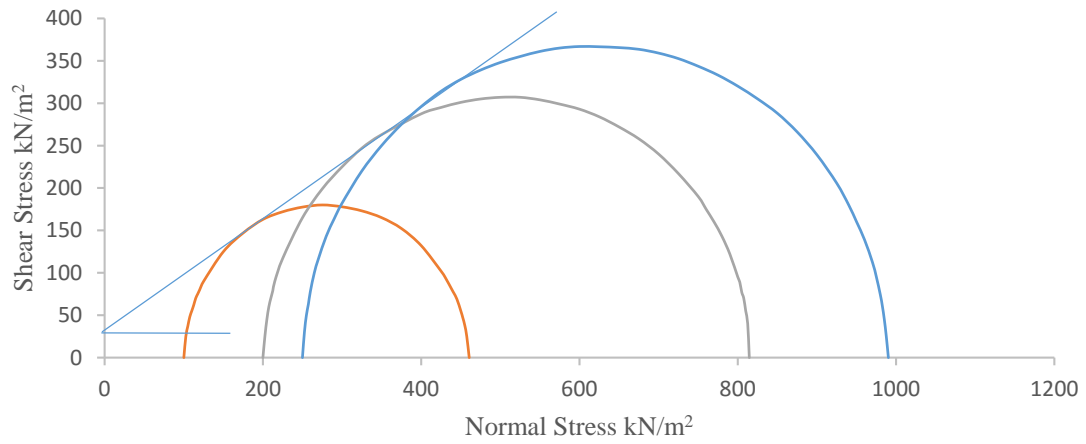


Figure 4.20: Mohr circle diagram of 3 days MICP treated soil by tri-axial test

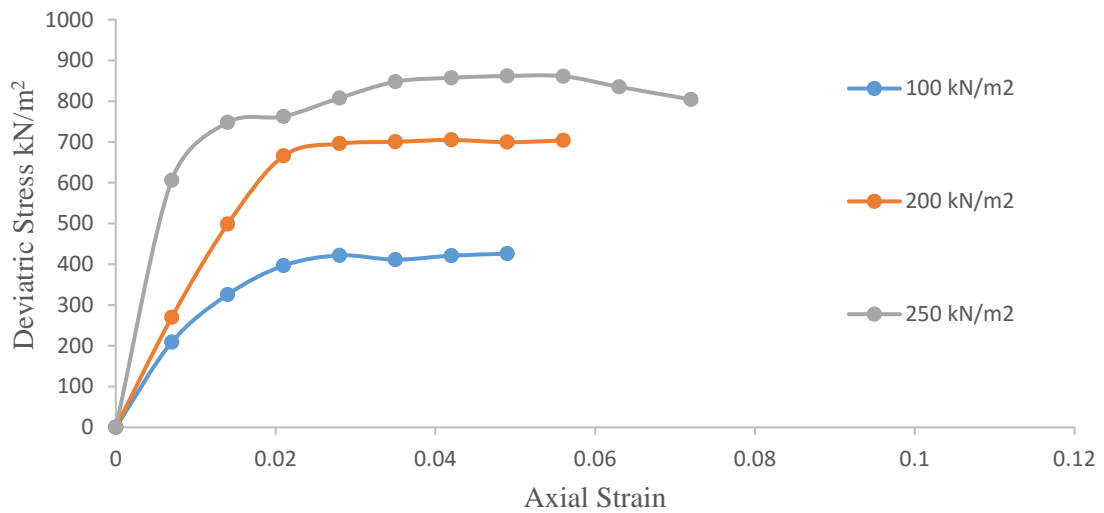


Figure 4.21: Stress vs Strain diagram of 7 days MICP treated soil by tri-axial test

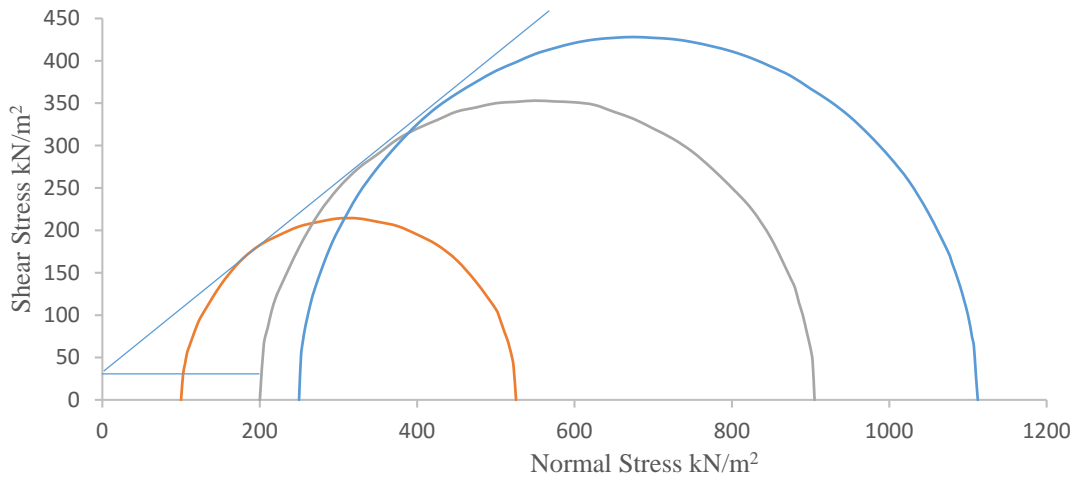


Figure 4.22: Mohr circle diagram of 7 days MICP treated soil by tri-axial test

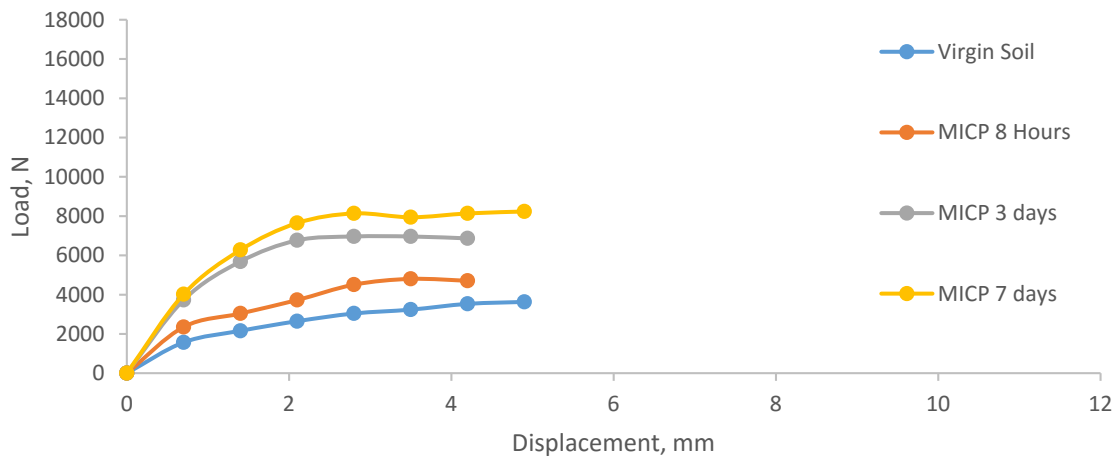


Figure 4.23: Load displacement curve of tri-axial test at 100 kN/m² cell pressure

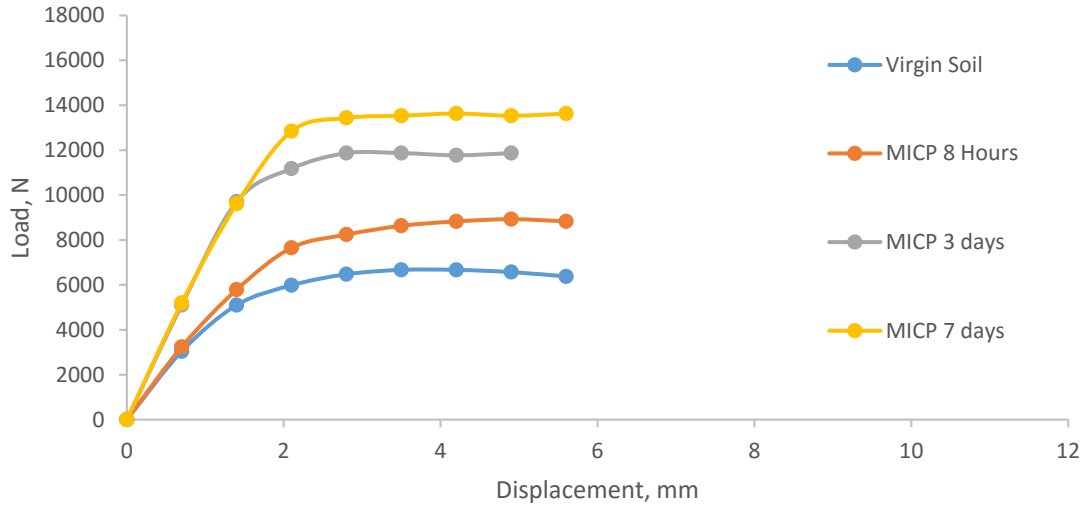


Figure 4.24: Load displacement curve of tri-axial test at 200 kN/m² cell pressure

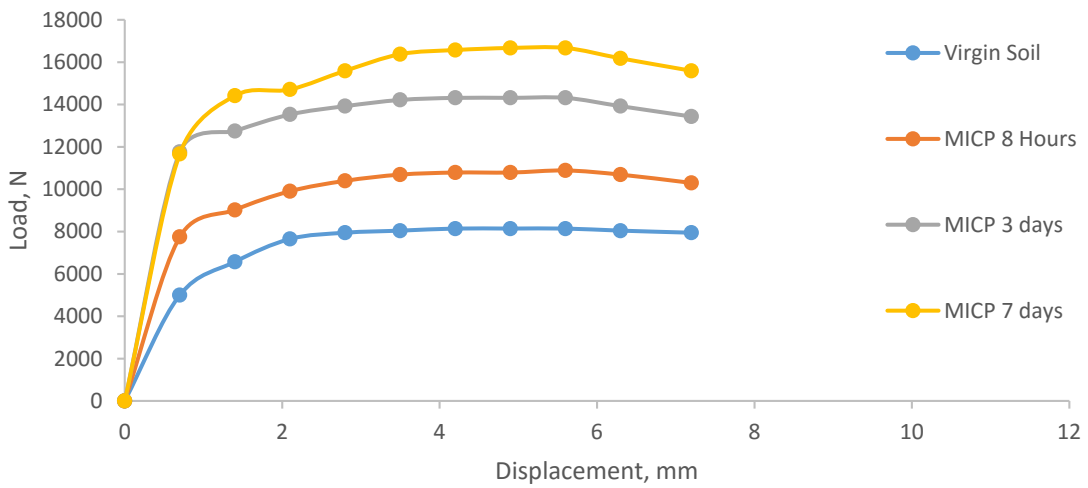


Figure 4.25: Load displacement curve of tri-axial test at 250 kN/m² cell pressure

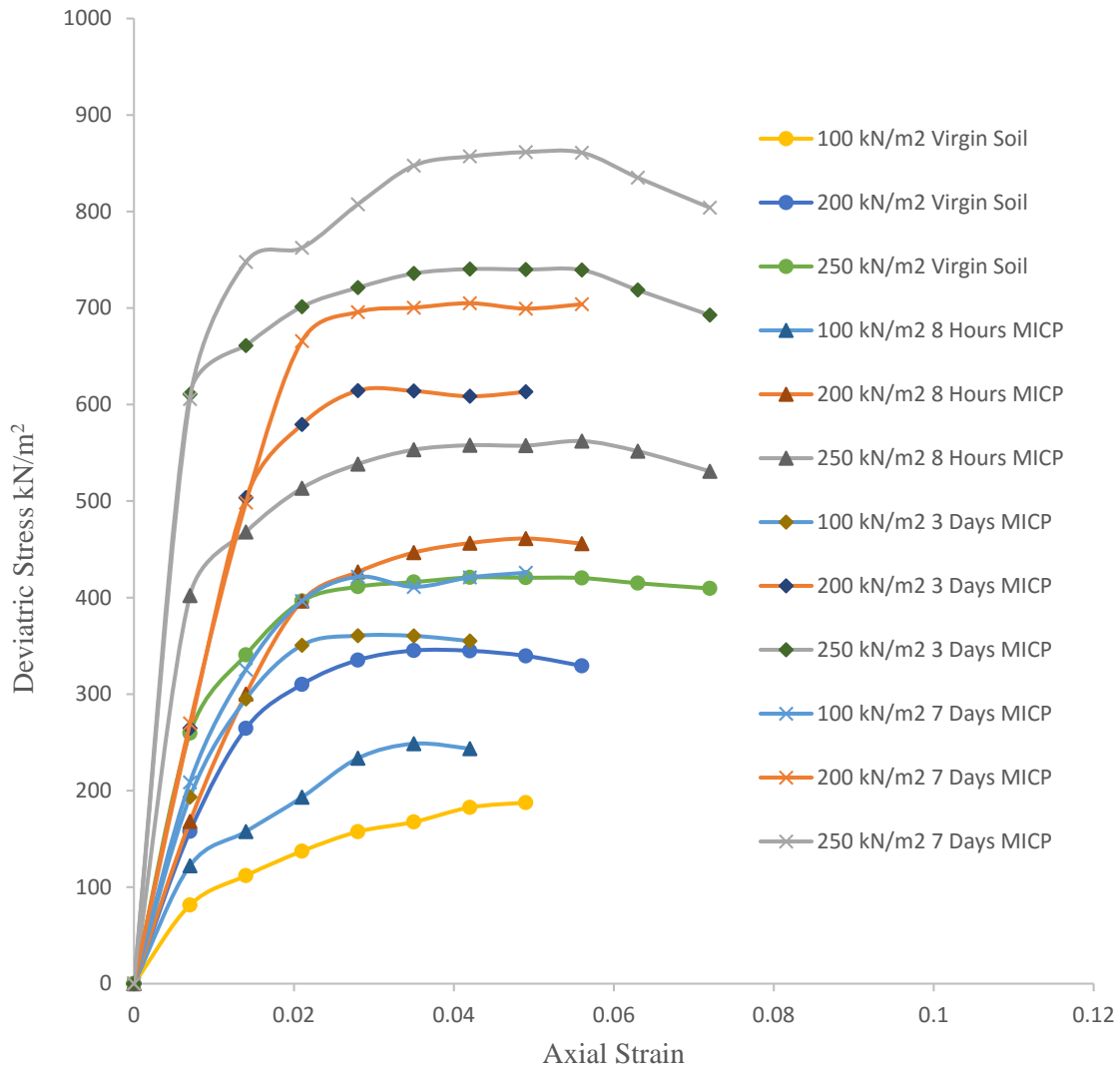


Figure 4.26: Deviatoric stress vs axial strain curve

Table 4.18 Shear Parameters of Soil by Tri-axial Test

S. No.	DTU Soil	Angle of Friction (°)	Cohesion(kN/m ²)
1	Virgin Soil	25.8	9.33
2	MICP Treated Soil 8 hours	30	15
3	MICP Treated Soil 3 days	33.5	30
4	MICP Treated Soil 7 days	35	37.5

4.1.10 Results of SEM Analysis

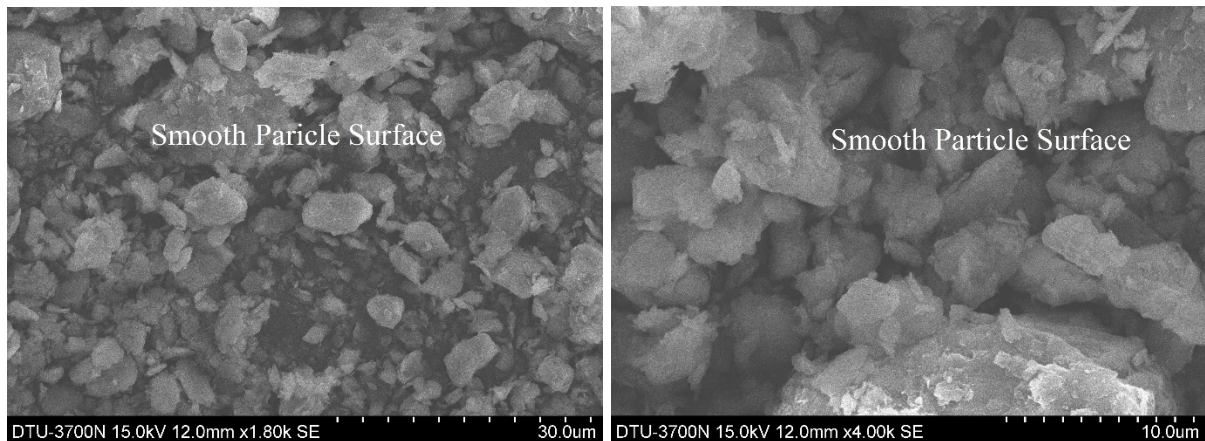


Figure 4.27 SEM results of virgin soil

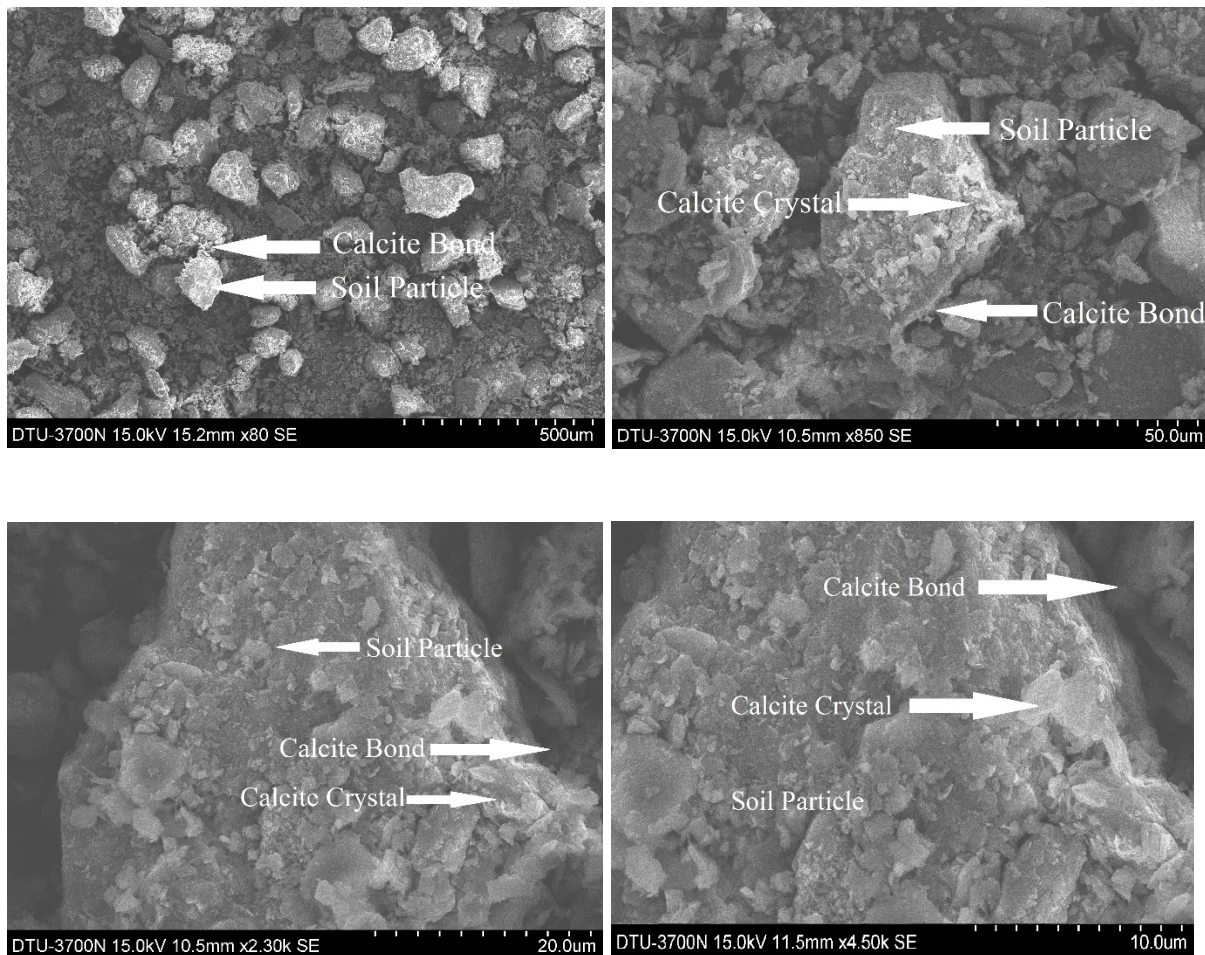


Figure 4.28 SEM results of MICP treated soil at different resolution

All the images were taken by SEM instrument in Advance instrumentation laboratory, Science block, DTU, Delhi. MICP treated images were taken after 7 days treatment and are of same sample at different resolution.

Chapter V

Result and Discussion

All the tests were carried out in accordance to respective Indian Standard Codes mentioned for respective experiments. All the tests were performed on Silty Sand. From the tests performed, following points were noted:

- Liquid Limit of soil is more than 20% and Plasticity Index was less than 4, hence the soil was classified as SM i.e. Silty Sand.
- The presence of cementation caused by calcite precipitation was verified by images of MICP treated soil from Scanning Electron Microscope.
- Unconfined compressive strength of soil was 18.75 kN/m², which was increased to 31.24 kN/m² after 8 hours, here a significant increase was observed because due to high microbial activity calcite precipitation rate was high. After 3 days UCS value increased to 59.88 kN/m² as a result of precipitation of calcite. Cementation agent was provided till 7 days which resulted in a UCS value of 76.04 kN/m².

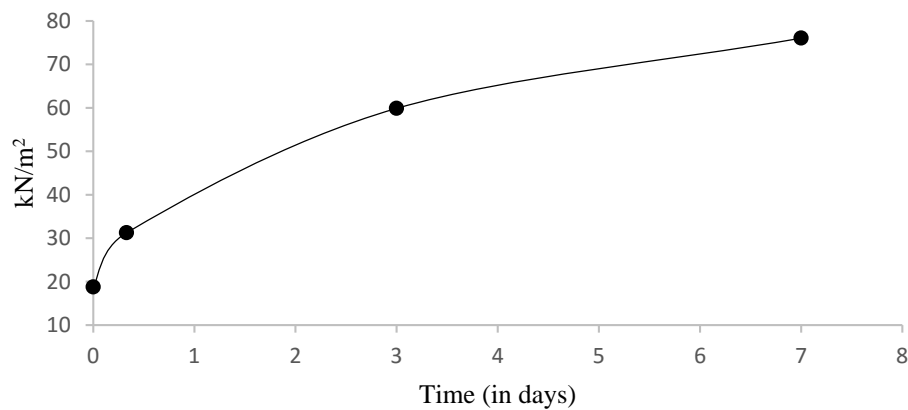


Figure 5.1 Variation in UCS with time

- CBR tests were performed on soil specimens in which unsoaked CBR value was observed as 7.125 and was improved up to 11.745 as a result of MICP and similar tests were performed on 3 days soaked specimen in which MICP treated specimen resulted in increase in CBR value 3.22 to 4.795 which is a result of cementation caused by calcite

precipitate. The 7 days soaking resulted in slight decrease in penetration resistance of soil but a comparatively increase with the sample treated microbially.

- Direct shear test results showed increase in cohesion of soil from 10.56 kN/m² to 18.607 kN/m² and also increase in angle of internal friction.
- Tri-axial tests also resulted significantly showed increase in shear parameters of soil because the cementation changes the surface textures thus increases angle of internal friction and enhances Undrained cohesion, in which an increment of 60% in Cohesion of soil was observed which was further increased to 200% after 3 days treatment and subsequently to 300% after 7 days, which was also verified by UCS results. Angle of internal friction increased from 25.8° to 30°, 33.5° and 35° for 8 hours, 3 days and 7 days duration respectively.

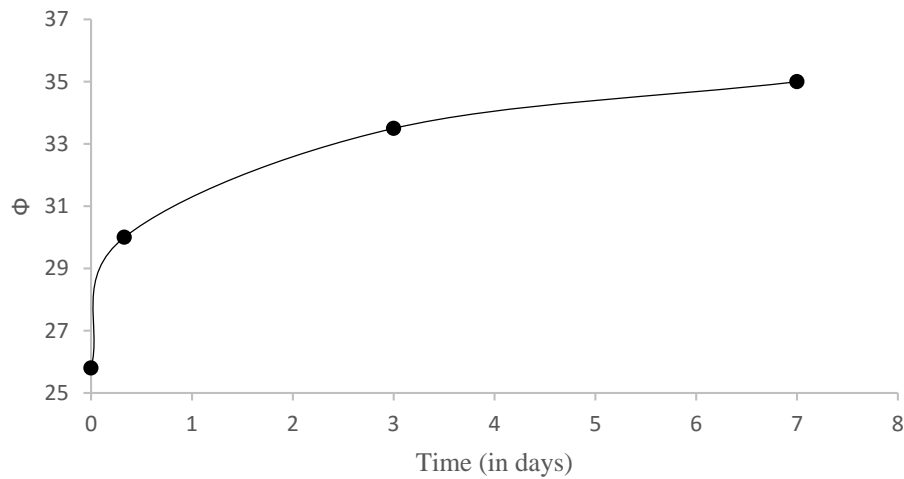


Figure 5.2 Variation in angle of internal friction with time

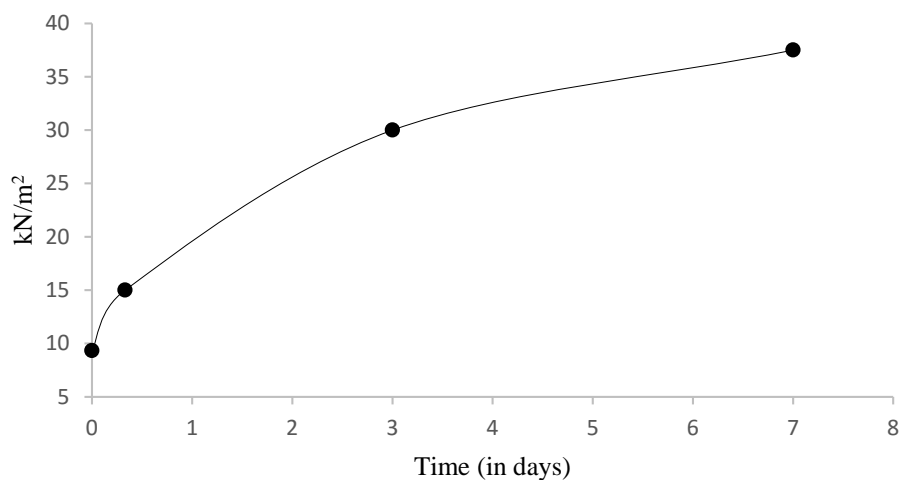


Figure 5.3 Variation in cohesion in soil with time

Chapter VI

Conclusion

- SEM results gives us exact idea of calcite precipitation between soil particles and this calcite precipitation is exact cause of improvement of soil precipitation. Initially soil grain surface was slight smooth but after treatment high amount of roughness is visible in SEM images which shows change in texture results in increase in angle of internal friction.
- Unconfined compressive strength of soil increases due to the action of urease positive bacteria and there is 0.65 times increase in strength of soil in 8 hours. After three days strength increases 2.2 times and after seven days strength increases 3 times.
- Similarly there is improvement in CBR value of soil, it become 1.6 times for unsoaked condition after treatment with bacteria. Then after three days soaking CBR value becomes 1.5 times of untreated 3 days soaked soil. Marginal improvement of CBR value also seen on after one week soaking but was slight decreased compared with 3 days soaked CBR value.
- We also see some improvement in the shear parameter value in direct shear also.
- Tri-axial tests give us very fine result and we will see increase in cohesion value of soil similar to the variation observed in UCS specimens. Instantly after 8 hours cohesion becomes 1.6 times and thereafter it becomes 3 times after three days and 4 times after seven days.

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Appendix 1

Triaxial Test Observation Table

1.1: Virgin soil 100 cell pressure

L (mm)	ΔL , mm	Load, P, kg	$\epsilon = \Delta L/L$	$A = A_0/1-\epsilon$	$\sigma = P/A$	Sig1 (kg/sq.cm)	Sig1 + Sig3 (kg/sq.cm)
76	0	0	0	1134.11	0	100.00	200
75.3	0.7	95	0.009211	1144.66	82.99	182.99	282.9942
74.6	1.4	130	0.018421	1155.40	112.52	212.52	312.5153
73.9	2.1	160	0.027632	1166.34	137.18	237.18	337.1809
73.2	2.8	185	0.036842	1177.50	157.11	257.11	357.113
72.5	3.5	200	0.046053	1188.87	168.23	268.23	368.2276
71.8	4.2	220	0.055263	1200.46	183.26	283.26	383.2637
71.1	4.9	230	0.064474	1212.27	189.73	289.73	389.726
70.4	5.6	225	0.073684	1224.33			
69.7	6.3		0.082895	1236.62			
68.8	7.2		0.094737	1252.80			

1.2: Virgin soil 200 cell pressure

L (mm)	ΔL , mm	Load, P, kg	$\epsilon = \Delta L/L$	$A = A_0/1-\epsilon$	$\sigma = P/A$	Sig1 (kg/sq.cm)	Sig1 + Sig3 (kg/sq.cm)
76	0	0	0	1134.11	0	200.00	400.00
75.3	0.7	180	0.009211	1144.66	157.25	357.25	557.25
74.6	1.4	305	0.018421	1155.40	263.98	463.98	663.98
73.9	2.1	360	0.027632	1166.34	308.66	508.66	708.66
73.2	2.8	395	0.036842	1177.50	335.46	535.46	735.46
72.5	3.5	410	0.046053	1188.87	344.87	544.87	744.87
71.8	4.2	415	0.055263	1200.46	345.70	545.70	745.70
71.1	4.9	410	0.064474	1212.27	338.21	538.21	738.21
70.4	5.6	405	0.073684	1224.33	330.79	530.79	730.79
69.7	6.3		0.082895	1236.62			
68.8	7.2		0.094737	1252.80			

1.3: Virgin Soil 250 Cell Pressure

L (mm)	ΔL, mm	Load, P, kg	ε = ΔL/L	A = A₀/1-ε	σ = P/A	Sig1 (kg/sq.cm)	Sig1 + Sig3 (kg/sq.cm)
76	0	0	0	1134.11	0	250.00	500.00
75.3	0.7	300	0.009211	1144.66	262.09	512.09	762.09
74.6	1.4	395	0.018421	1155.40	341.87	591.87	841.87
73.9	2.1	460	0.027632	1166.34	394.40	644.40	894.40
73.2	2.8	485	0.036842	1177.50	411.89	661.89	911.89
72.5	3.5	495	0.046053	1188.87	416.36	666.36	916.36
71.8	4.2	505	0.055263	1200.46	420.67	670.67	920.67
71.1	4.9	510	0.064474	1212.27	420.70	670.70	920.70
70.4	5.6	515	0.073684	1224.33	420.64	670.64	920.64
69.7	6.3	515	0.082895	1236.62	416.46	666.46	916.46
68.8	7.2	515	0.094737	1252.80	411.08	661.08	911.08

1.4: MICP 0 days 100 cell pressure

L (mm)	ΔL, mm	Load, P, kg	ε = ΔL/L	A = A₀/1-ε	σ = P/A	Sig1 (kg/sq.cm)	Sig1 + Sig3 (kg/sq.cm)
76	0	0	0	1134.11	0	100.00	200
75.3	0.7	140	0.009211	1144.66	122.31	222.31	322.3073
74.6	1.4	180	0.018421	1155.40	155.79	255.79	355.7904
73.9	2.1	225	0.027632	1166.34	192.91	292.91	392.9107
73.2	2.8	275	0.036842	1177.50	233.55	333.55	433.5464
72.5	3.5	295	0.046053	1188.87	248.14	348.14	448.1358
71.8	4.2	290	0.055263	1200.46	241.57	341.57	441.5749
71.1	4.9		0.064474	1212.27			
70.4	5.6		0.073684	1224.33			
69.7	6.3		0.082895	1236.62			
68.8	7.2		0.094737	1252.80			

1.5: MICP 0 days 200 cell pressure

L (mm)	ΔL, mm	Load, P, kg	$\epsilon =$ $\Delta L/L$	A = $A_0/1-\epsilon$	$\sigma = P/A$	Sig1 (kg/ sq.cm)	Sig1 + Sig3 (kg/ sq.cm)
76	0	0	0	1134.11	0	200.00	400.00
75.3	0.7	190	0.009211	1144.66	165.99	365.99	565.99
74.6	1.4	345	0.018421	1155.40	298.60	498.60	698.60
73.9	2.1	460	0.027632	1166.34	394.40	594.40	794.40
73.2	2.8	500	0.036842	1177.50	424.63	624.63	824.63
72.5	3.5	530	0.046053	1188.87	445.80	645.80	845.80
71.8	4.2	550	0.055263	1200.46	458.16	658.16	858.16
71.1	4.9	560	0.064474	1212.27	461.94	661.94	861.94
70.4	5.6	555	0.073684	1224.33	453.31	653.31	853.31
69.7	6.3		0.082895	1236.62			
68.8	7.2		0.094737	1252.80			

1.6: MICP 0 days 250 cell pressure

L (mm)	ΔL, mm	Load, P, kg	$\epsilon =$ $\Delta L/L$	A = $A_0/1-\epsilon$	$\sigma = P/A$	Sig1 (kg/ sq.cm)	Sig1 + Sig3 (kg/ sq.cm)
76	0	0	0	1134.11	0	250.00	500.00
75.3	0.7	460	0.009211	1144.66	401.87	651.87	901.87
74.6	1.4	540	0.018421	1155.40	467.37	717.37	967.37
73.9	2.1	600	0.027632	1166.34	514.43	764.43	1014.43
73.2	2.8	635	0.036842	1177.50	539.28	789.28	1039.28
72.5	3.5	655	0.046053	1188.87	550.95	800.95	1050.95
71.8	4.2	670	0.055263	1200.46	558.12	808.12	1058.12
71.1	4.9	675	0.064474	1212.27	556.80	806.80	1056.80
70.4	5.6	690	0.073684	1224.33	563.57	813.57	1063.57
69.7	6.3	680	0.082895	1236.62	549.88	799.88	1049.88
68.8	7.2	665	0.094737	1252.80	530.81	780.81	1030.81

1.7: MICP 3 days 100 cell pressure

L (mm)	ΔL, mm	Load, P, kg	ε = ΔL/L	A = A₀/1-ε	σ = P/A	Sig1 (kg/sq.cm)	Sig1 + Sig3 (kg/sq.cm)
76	0	0	0	1134.11	0	100.00	200
75.3	0.7	220	0.009211	1144.66	192.20	292.20	392.1972
74.6	1.4	340	0.018421	1155.40	294.27	394.27	494.2707
73.9	2.1	410	0.027632	1166.34	351.53	451.53	551.5261
73.2	2.8	425	0.036842	1177.50	360.94	460.94	560.9353
72.5	3.5	430	0.046053	1188.87	361.69	461.69	561.6894
71.8	4.2	425	0.055263	1200.46	354.03	454.03	554.0322
71.1	4.9		0.064474	1212.27			
70.4	5.6		0.073684	1224.33			
69.7	6.3		0.082895	1236.62			
68.8	7.2		0.094737	1252.80			

1.8: MICP 3 days 200 cell pressure

L (mm)	ΔL, mm	Load, P, kg	ε = ΔL/L	A = A₀/1-ε	σ = P/A	Sig1 (kg/sq.cm)	Sig1 + Sig3 (kg/sq.cm)
76	0	0	0	1134.11	0	200.00	400.00
75.3	0.7	300	0.009211	1144.66	262.09	462.09	662.09
74.6	1.4	580	0.018421	1155.40	501.99	701.99	901.99
73.9	2.1	675	0.027632	1166.34	578.73	778.73	978.73
73.2	2.8	725	0.036842	1177.50	615.71	815.71	1015.71
72.5	3.5	730	0.046053	1188.87	614.03	814.03	1014.03
71.8	4.2	730	0.055263	1200.46	608.10	808.10	1008.10
71.1	4.9	745	0.064474	1212.27	614.55	814.55	1014.55
70.4	5.6		0.073684	1224.33			
69.7	6.3		0.082895	1236.62			
68.8	7.2		0.094737	1252.80			

1.9: MICP 3 days 250 cell pressure

L (mm)	ΔL, mm	Load, P, kg	$\xi =$ $\Delta L/L$	A = $A_0/1-\xi$	$\sigma = P/A$	Sig1 (kg/ sq.cm)	Sig1 + Sig3 (kg/ sq.cm)
76	0	0	0	1134.11	0	250.00	500.00
75.3	0.7	700	0.009211	1144.66	611.54	861.54	1111.54
74.6	1.4	765	0.018421	1155.40	662.11	912.11	1162.11
73.9	2.1	820	0.027632	1166.34	703.05	953.05	1203.05
73.2	2.8	850	0.036842	1177.50	721.87	971.87	1221.87
72.5	3.5	875	0.046053	1188.87	736.00	986.00	1236.00
71.8	4.2	890	0.055263	1200.46	741.38	991.38	1241.38
71.1	4.9	895	0.064474	1212.27	738.28	988.28	1238.28
70.4	5.6	905	0.073684	1224.33	739.18	989.18	1239.18
69.7	6.3	890	0.082895	1236.62	719.70	969.70	1219.70
68.8	7.2	865	0.094737	1252.80	690.45	940.45	1190.45

1.10: MICP 7 days 100 cell pressure

L (mm)	ΔL, mm	Load, P, kg	$\xi =$ $\Delta L/L$	A = $A_0/1-\xi$	$\sigma = P/A$	Sig1 (kg/ sq.cm)	Sig1 + Sig3 (kg/ sq.cm)
76	0	0	0	1134.11	0	100.00	200
75.3	0.7	240	0.009211	1144.66	209.67	309.67	409.6696
74.6	1.4	375	0.018421	1155.40	324.56	424.56	524.5633
73.9	2.1	460	0.027632	1166.34	394.40	494.40	594.3952
73.2	2.8	495	0.036842	1177.50	420.38	520.38	620.3835
72.5	3.5	490	0.046053	1188.87	412.16	512.16	612.1577
71.8	4.2	505	0.055263	1200.46	420.67	520.67	620.6735
71.1	4.9	515	0.064474	1212.27	424.82	524.82	624.8212
70.4	5.6		0.073684	1224.33			
69.7	6.3		0.082895	1236.62			
68.8	7.2		0.094737	1252.80			

1.11: MICP 7 days 200 cell pressure

L (mm)	ΔL, mm	Load, P, kg	$\epsilon =$ $\Delta L/L$	A = $A_0/1-\epsilon$	$\sigma = P/A$	Sig1 (kg/ sq.cm)	Sig1 + Sig3 (kg/ sq.cm)
76	0	0	0	1134.11	0	19.61	39.23
75.3	0.7	310	0.009211	1144.66	270.82	46.17	65.79
74.6	1.4	575	0.018421	1155.40	497.66	68.42	88.03
73.9	2.1	775	0.027632	1166.34	664.47	84.78	104.39
73.2	2.8	820	0.036842	1177.50	696.39	87.91	107.52
72.5	3.5	830	0.046053	1188.87	698.14	88.08	107.69
71.8	4.2	845	0.055263	1200.46	703.90	88.64	108.26
71.1	4.9	850	0.064474	1212.27	701.16	88.37	107.99
70.4	5.6	860	0.073684	1224.33	702.43	88.50	108.11
69.7	6.3		0.082895	1236.62			
68.8	7.2		0.094737	1252.80			

1.12: MICP 7 days 250 cell pressure

L (mm)	ΔL, mm	Load, P, kg	$\epsilon =$ $\Delta L/L$	A = $A_0/1-\epsilon$	$\sigma = P/A$	Sig1 (kg/ sq.cm)	Sig1 + Sig3 (kg/ sq.cm)
76	0	0	0	1134.11	0	250.00	500.00
75.3	0.7	695	0.009211	1144.66	607.17	857.17	1107.17
74.6	1.4	865	0.018421	1155.40	748.66	998.66	1248.66
73.9	2.1	890	0.027632	1166.34	763.07	1013.07	1263.07
73.2	2.8	950	0.036842	1177.50	806.80	1056.80	1306.80
72.5	3.5	1010	0.046053	1188.87	849.55	1099.55	1349.55
71.8	4.2	1030	0.055263	1200.46	858.01	1108.01	1358.01
71.1	4.9	1045	0.064474	1212.27	862.02	1112.02	1362.02
70.4	5.6	1055	0.073684	1224.33	861.70	1111.70	1361.70
69.7	6.3	1030	0.082895	1236.62	832.91	1082.91	1332.91
68.8	7.2	1010	0.094737	1252.80	806.19	1056.19	1306.19

Appendix 2

UCS Test Observation Table

2.1 Virgin soil

ΔL , mm	Dial gauge reading	Load, P, kg	$\epsilon = \Delta L/L$	$A = A_0/1-\epsilon$	$\sigma = P/A$ kg/sq.cm	σ kN/sq.m
0	0	0	0	0	0	0
0.5	0.3	0.98776758	0.00657895	11.4162564	0.0865229	8.48495446
1	0.4	1.31702345	0.01315789	11.4923648	0.11459986	11.2383503
1.5	0.5	1.64627931	0.01973684	11.5694948	0.14229483	13.9542849
2	0.6	1.84383282	0.02631579	11.647667	0.15830061	15.5239078
2.5	0.6	1.97553517	0.03289474	11.7269029	0.1684618	16.5203749
3	0.6	2.04138634	0.03947368	11.8072241	0.172893	16.9549245
3.5	0.7	2.1401631	0.04605263	11.8886532	0.18001729	17.6535752
4	0.7	2.20601427	0.05263158	11.9712133	0.18427658	18.0712672
4.5	0.7	2.30479103	0.05921053	12.0549281	0.19119077	18.7493144
5	0.7	2.30479103	0.06578947	12.139822	0.18985377	18.6182003
5.5	0.7	2.30479103	0.07236842	12.22592	0.18851678	18.4870862
6	0.7	2.30479103	0.07894737	12.313248	0.18717978	18.3559721
6.5	0.7	2.30479103	0.08552632	12.4018325	0.18584278	18.224858
7	0.7	2.30479103	0.09210526	12.4917009	0.18450578	18.0937439
7.5	0.7	2.30479103	0.09868421	12.5828812	0.18316878	17.9626299
8	0.7	2.30479103	0.10526316	12.6754024	0.18183178	17.8315158
8.5	0.6	1.97553517	0.11184211	12.7692942	0.15470982	15.1717729

2.2 MICP 0 days

ΔL , mm	Dial gauge reading	Load, P, kg	$\epsilon = \Delta L/L$	$A = A_0/1-\epsilon$	$\sigma = P/A$	Column1
0	0	0	0	0	0	0
0.5	0.3	0.98776758	0.00657895	11.4162564	0.0865229	8.48495446
1	0.5	1.64627931	0.01315789	11.4923648	0.14324983	14.0479378
1.5	0.6	1.97553517	0.01973684	11.5694948	0.1707538	16.7451419
2	0.7	2.30479103	0.02631579	11.647667	0.19787577	19.4048848
2.5	0.8	2.63404689	0.03289474	11.7269029	0.22461573	22.0271665
3	0.8	2.79867482	0.03947368	11.8072241	0.23703072	23.2446545
3.5	0.9	2.96330275	0.04605263	11.8886532	0.2492547	24.4434119
4	1	3.29255861	0.05263158	11.9712133	0.27503967	26.9720407
4.5	1	3.42426096	0.05921053	12.0549281	0.28405486	27.8561242
5	1.1	3.62181448	0.06578947	12.139822	0.29834165	29.2571719
5.5	1.1	3.75351682	0.07236842	12.22592	0.30701304	30.1075404
6	1.1	3.85229358	0.07894737	12.313248	0.31285763	30.6806963
6.5	1.2	3.95107034	0.08552632	12.4018325	0.31858762	31.2426138
7	1.2	3.95107034	0.09210526	12.4917009	0.31629563	31.0178468
7.5	1.2	3.95107034	0.09868421	12.5828812	0.31400363	30.7930798
8	1.1	3.78644241	0.10526316	12.6754024	0.29872365	29.2946331
8.5	1.1	3.62181448	0.11184211	12.7692942	0.28363466	27.8149169

2.3 MICP 3 days

ΔL , mm	Dial gauge reading	Load, P, kg	$\epsilon = \Delta L/L$	$A = A_0/1-\epsilon$	$\sigma = P/A$	Column1
0	0	0	0	0	0	0
0.5	0.4	1.31702345	0.00657895	11.4162564	0.11536386	11.3132726
1	0.8	2.63404689	0.01315789	11.4923648	0.22919973	22.4767006
1.5	1.1	3.62181448	0.01973684	11.5694948	0.31304863	30.6994268
2	1.4	4.60958206	0.02631579	11.647667	0.39575153	38.8097696
2.5	1.7	5.59734964	0.03289474	11.7269029	0.47730843	46.8077289
3	1.9	6.25586137	0.03947368	11.8072241	0.52983337	51.9586394
3.5	2	6.58511723	0.04605263	11.8886532	0.55389934	54.318693
4	2.1	6.91437309	0.05263158	11.9712133	0.57758332	56.6412854
4.5	2.1	7.11192661	0.05921053	12.0549281	0.5899601	57.8550272
5	2.2	7.24362895	0.06578947	12.139822	0.59668329	58.5143438
5.5	2.2	7.37533129	0.07236842	12.22592	0.60325368	59.1586759
6	2.3	7.50703364	0.07894737	12.313248	0.60967128	59.7880235
6.5	2.3	7.57288481	0.08552632	12.4018325	0.61062628	59.8816764
7	2.3	7.57288481	0.09210526	12.4917009	0.60623328	59.450873
7.5	2.2	7.40825688	0.09868421	12.5828812	0.5887568	57.7370245
8	2.2	7.24362895	0.10526316	12.6754024	0.57147132	56.0419067
8.5	2.1	6.91437309	0.11184211	12.7692942	0.54148436	53.1012051

2.4 MICP 7 days

ΔL , mm	Dial gauge reading	Load, P, kg	$\epsilon = \Delta L/L$	$A = A_0/1-\epsilon$	$\sigma = P/A$ kg/cm ²	σ kN/m ²
0	0	0	0	0	0	0
0.5	0.6	1.975535	0.006579	11.41626	0.173046	16.96991
1	1.1	3.621814	0.013158	11.49236	0.31515	30.90546
1.5	1.9	6.255861	0.019737	11.56949	0.54072	53.02628
2	2.3	7.572885	0.026316	11.64767	0.650163	63.75891
2.5	2.5	8.231397	0.032895	11.7269	0.701924	68.8349
3	2.6	8.593578	0.039474	11.80722	0.727824	71.37476
3.5	2.6	8.824057	0.046053	11.88865	0.742225	72.78705
4	2.7	8.922834	0.052632	11.97121	0.745358	73.09423
4.5	2.7	9.120387	0.059211	12.05493	0.756569	74.19372
5	2.8	9.219164	0.065789	12.13982	0.759415	74.4728
5.5	2.8	9.350866	0.072368	12.22592	0.764839	75.00475
6	2.9	9.54842	0.078947	12.31325	0.775459	76.04617
6.5	2.9	9.54842	0.085526	12.40183	0.76992	75.50298
7	2.9	9.54842	0.092105	12.4917	0.764381	74.9598
7.5	2.8	9.219164	0.098684	12.58288	0.732675	71.85052