

MICROBIOLOGY IN GROUND IMPROVEMENT



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MICROBIOLOGY IN GROUND IMPROVEMENT

ABSTRACT

Different techniques are used for improving the properties of soil (reducing the permeability and strengthening the soil) such as deep dynamic compaction, Vibro-flotation and Vibro-compaction, chemical grouting, soil nailing and piling etc. Along with advantages of all the above listed techniques there are disadvantages too such as high cost, high energy consumption and their adverse effects on environment restrict their applications.

Microbial Geo-technology have three major applications including Bio-clogging, Bio-cementation and Biogas. Bio-clogging refers to a process in which pore-filling materials are produced through microbial activities so that the porosity and hydraulic conductivity of soil can be reduced. Bio-cementation refers to a process in which particle-binding materials are generated through microbial activities so that the shear strength of soil can be increased. Biogas refers to generation of gas (N_2) to increase the liquefaction resistance of soil.

We can also use bio-grouts to reduce the migration of heavy metals and organic pollutants, control ground water flow, and prevent piping of earth dams and dikes. Bio-cementation is used to prevent soil avalanching, reduce the swelling potential of clayey soil, to mitigate the liquefaction potential of sand, to enhance stability of slopes and dams, and compact soil on reclaimed land sites.

Our research is based on using bio-grouts to improve engineering properties of soil which is based on microbial induced calcite precipitation, we are majorly focused on bio-cementation.

MICROBIOLOGY IN GROUND IMPROVEMENT

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DEDICATION

We dedicate our work to our beloved parents and teachers who enabled us to achieve education and meet our objectives with such dignity and respect.

MICROBIOLOGY IN GROUND IMPROVEMENT

ACKNOWLEDGMENT

I am deeply obliged to acknowledge and thank those people who put their ever best contribution into our thesis. First of all thanks to Almighty Allah for blessing us with everything that he has provided us.

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CHAPTER 1

INTRODUCTION

Man has been trying to stabilize the soil for millions of years using different techniques. These techniques had their advantages and disadvantages, with time there was a lot of modification and innovation in stabilization methods of soil. Different improvement techniques are being used for stabilization of soil which includes micro piling, Vibro-compaction and soil densification etc. but these techniques can be used only at undeveloped sites, for the sites having construction there is new emerging branch of Geotechnical engineering called Microbial Geo-technology. This technique can be used at developed as well as undeveloped sites.

Microbial Geo-technology have three major applications including Bio-clogging, Bio-Cementation and Biogas. In microbial Geo-technology calcite precipitation using bacillus pasteurii is a branch which is being progressed more comparatively.

Bio-cementation is a process which have all the possible modifications that can be made with microbiology application in soil. It helps to reduce permeability, increase shear strength and also helps to increase liquefaction resistance of soil. In MICP precipitation of CaCO_3 in the form of calcite may help to alter soil properties positively.

Microbial soil improvement is far better than the other techniques that are being used already in terms of environment, economy and applicability.

This technique is site specific according to type of soil, environment and requirement, method to be used for soil improvement will vary in terms of bacterial method that can be used.

To make our research site specific, we have taken loose sand from bank of Soan River in front of Naval Anchorage, Japani road, Islamabad, Pakistan. It was loose sand present at a bank river, a lot of permeability and shear strength issues were expected there.

From previous research it is suggested that microbial induced calcite precipitation can be effective to resolve the shear strength and permeability issues present at site. In this technique both exogenous and indigenous bacteria can be used but it is not obvious that

exogenous bacteria will survive at the site, so we have used indigenous bacteria (bacillus) for calcite precipitation. Along with bacteria different reagents were injected to carry out the process. After that laboratory tests were performed to confirm the changes occurred in soil properties.

LITERATURE REVIEW

Microbiology is derived from two Greek words; mikros meaning “small” and Bios meaning “life”.

As its name indicates, Microbiology is the study of microscopic organisms. These microorganisms may be:

- Unicellular (single cell)
- Multicellular (cell colony)
- Acellular (lacking cells)

2.1 Now what does “Microbiology in Ground Improvement” imply?

It is basically the application of different microbiological methods to the geological material in order to improve its properties so that it becomes more suitable for use. The properties to be improved may include:

- Shear strength
- Permeability
- Bearing capacity
- Void ratio etc.

2.2 Various Ground Improvement Techniques:

Listed below are some common techniques of Ground Improvement.

- Vibro Compaction
- Vacuum Consolidation
- Preloading of soil
- Vitrification
- Ground freezing
- Vibro-replacement stone columns
- Mechanically stabilized earth structures
- Soil nailing
- Micro-piles
- Grouting

Ground improvement through the above mentioned methods has been causing various environmental and geological issues like metal elements increase in soil, leachate percolation, contamination of ground water, disturbance in natural striatal alignment of soil etc. The mechanical techniques are usually expensive. Thus applying them to commercial scale on such a large volume sometimes renders the method uneconomical. Similarly, the techniques which are based on chemicals may cause different unwanted reactions during the process. Also, these methods are not very eco-friendly.

Thus, in order to overcome all the environmental, geological and economical problems, the idea of microbiology in ground improvement has taken its strong place. This method is environmental friendly, no hazardous chemical reactions are expected, and it would also be economical.

2.3 Bio Techniques:

There are three major applications of microbiology in ground improvement that include:

1. Bio-cementation
2. Bio-clogging
3. Bio-gas

2.3.1 Bio-Cementation:

Bio-cementation is a method to improve shear strength of soil through the production of soil particle-binding materials as a result of bacterial action on cementation reagents. A bacteria along with nutrients and reagents are injected into the soil according to its compatibility as a result a chemical reaction is carried out at some rate according to conditions and cementing reagents are yielded which bind the soil particles together to increase its shear strength and bearing capacity.

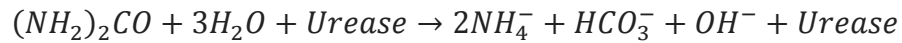
Bio-Cementation has many applications in construction field such as

- Wall and Building coating method
- Soil Strengthening
- Soil Stabilizing

- Sand Stabilizing in Earth Quake prone zones

The process of Bio-Cementation is mainly based on the MICP mechanism. MICP stands for Microbial Induced Carbonate Precipitation.

The facultative bacteria are able to precipitate calcite through the enzymatic hydrolysis of urea. The microbial urease enzyme hydrolyzes urea to produce dissolved ammonium, dissolved inorganic carbon and CO₂, and the ammonia released in the surroundings subsequently increases pH, leading to accumulation of insoluble CaCO₃ in a calcium rich environment, Hydrolysis of Urea by enzyme urease causes calcium carbonate precipitation and formation of a cemented product according to the following equation:



Different microbial processes which can lead to Bio-Cementation are as follows:

(a) **Urea Hydrolysis:**

Calcite precipitation in presence of Urea and salts by ammonifying bacteria.

(b) **Sulfate Reduction:**

Sulfide precipitation in the presence of salts and carbon by sulfate reducing bacteria.

(c) **Iron Reduction:**

Insoluble CO₃ or OH of Fe/Mn by iron reducing bacteria.

(d) **De-nitrification:**

Formation of nitrogen gas by nitrite and nitrate reduction through de-nitrifying bacteria.

The conditions in which de-nitrification occurs are anaerobic conditions.

2.3.2 Bio-Clogging:

“Bio-clogging is a process where soil voids are filled by the microbial activity or products.” The facultative aerobic or facultative anaerobic bacteria are injected only with nutrients and with time an organic biodegradable material is produced by the biological activity of these bacteria that fills the voids to decrease the permeability.

This method is used for treatment of such soils which only have permeability issues. No reagent solution is used in this method as in bio-cementation.

Different microbial processes which can lead to Bio-Clogging are as follows:

- Accumulation of bacterial Biomass:

Biomass is the amount of bacteria or the bacterial cell concentration present in the given volume of soil. Now as time progresses, bacterial reproduction occurs which increases the bacterial biomass. This biomass gets accumulated in the soil pores which reduces permeability.

- Insoluble bacterial slime:

Bacterial slime is basically the intact shell or saliva formed around the bacteria to protect themselves from outer environment such as pH change, temperature, Chemical reactions etc. This is inorganic in nature. This slime accumulates in the pores and decreases permeability.

- Bio-degradation of organic matter:

Organic matter such as straw and manure etc. are introduced into the soil as nutrients for bacterial growth and reproduction. These organic matters are bio degraded by bacterial activities to produce insoluble organic matter that fill the soil pores and reduces permeability.

2.3.3 Bio-Gas:

It is the production of gas by microorganism as a result of their microbial activity.

Biogas is used for the mitigation of liquefaction. Liquefaction is the major factor leading to major disasters such as landslide and subsidence. In this method denitrifying bacteria is injected to sand sample to produce nitrogen gas thus reducing liquefaction potential. Hence when sudden loading happens the impact is negated by gas and settlement occurs before failure.

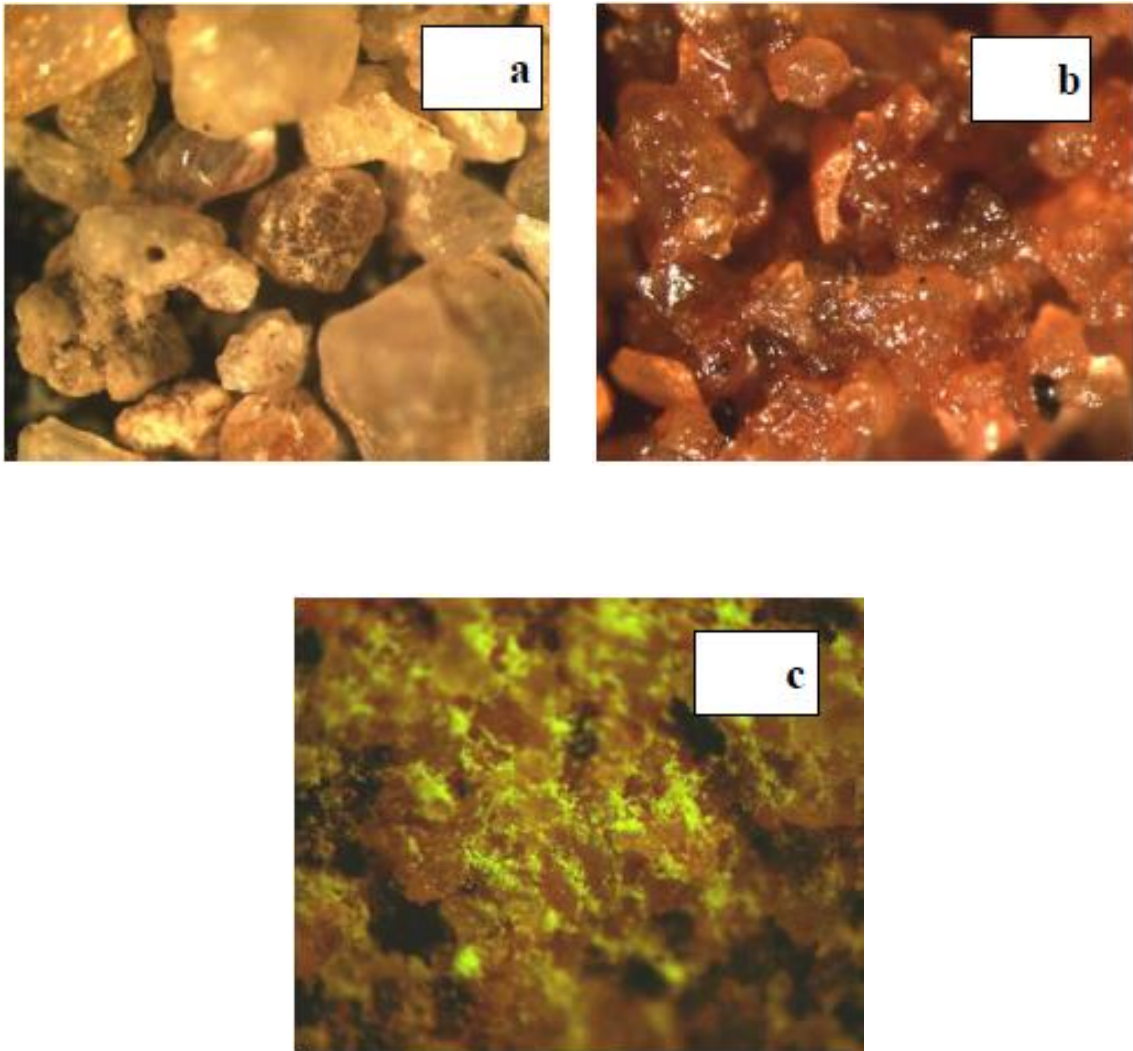


Fig 1: Micrographs of untreated and bio-treated sand samples
(a) Untreated sand (b) Sand treated with the iron-based bio-grout
(c) Sand treated with the calcium-based bio-grout

2.4 Types of Bacteria that can be used:

Based upon the source, there are two types of bacteria that can be used for ground improvement purposes.

1. Indigenous Bacteria
2. Exogenous Bacteria

2.4.1 Indigenous Bacteria:

As their name indicates, Indigenous bacteria are those bacteria which inhabit the soil which is under observation and testing. For indigenous bacteria, these are extracted from a sample first, cultured in a suitable media to increase its concentration and then injected back into the same sample along with reagents and nutrients.

2.4.2 Exogenous Bacteria:

Exo means external. Thus exogenous bacteria are those bacteria which are introduced into the soil which is under testing from the outside or external source. For them, they are extracted from an external source, cultured in a suitable media and injected into any desired sample along with nutrients and reagents to get the desired results.

Use of indigenous bacteria is preferred over exogenous bacteria because of the following factors:

- Temperature
- pH
- Competing bacteria
- Geometric compatibility of bacteria
- Fixation and distribution of bacteria

Since Indigenous bacteria are the ones already present in the soil, the environment for them is always feasible and they do not have to adapt to it. Conditions like pH, temperature, moisture content etc. are not a problem for them. The conditions would obviously be favorable.

On the other hand, exogenous bacteria since come from an external source into the new environment, the conditions may or may not be favorable for them.

Similarly, when exogenous bacteria are added into the soil, they have to compete with other already present bacteria for their nutrition, growth and survival. This renders the chances of their survival very low.

Lastly, the bacteria added to the soil should have such a geometry that they go into the soil voids forming geometrical links between the soil particles.

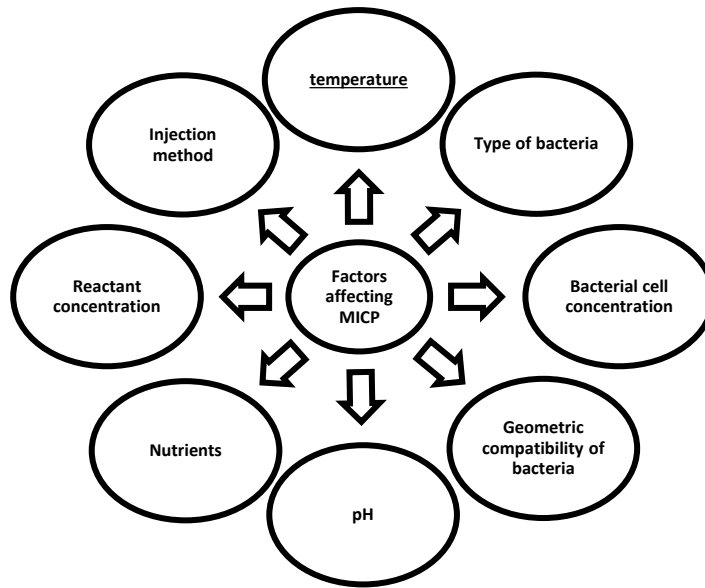


Fig 2: Factors Affecting MICP

2.5 Factors Affecting Bio-Grouting:

2.5.1 Nutrients:

Nutrients provide energy to bacteria and therefore it is essential to know that how much nutrients amount has to be provided for calcite production. Nutrients are provided along with the bio-mineralized solution in all injections. The constituents of nutrients that can be provided includes CO₂ , N, P, K, Mg, Ca, Fe etc. Organic compounds should also be present in nutrients otherwise it will limit the bacterial growth.

2.5.2 Type of Bacteria:

The bacterial selection should be according to the technique that is MICP. Such bacteria should be selected that must be urease positive and can do hydrolysis of urea in the given circumstances. The general urease positive bacteria are *Bacillus*, *Sporosarcina*, *Spolooactobacilus*, *Clostridium* and *Desulfotomaculum*. In MICP aerobic bacteria are preferred over anaerobic because they produce CO₂ that acts as a buffer and maintain required pH in the soil.

2.5.3 Geometric Compatibility of Bacteria:

The most abundant living organism in soil is microbes. Their sizes range from 0.5 to 3.0 μm . they moves between the soil particles under the action of two moving processes i-e self-propelled movement and by passive diffusion. As in MICP, free movement of bacteria in required within soil so it is critical for the treatment of soil. Pore throat size less than bacterial size will limit the efficient distribution of bacteria as well as nutrients and bio-mineralized solution. This limit in movement is due presence of silt and clay particles. So it is very necessary to take into considerations the type of soil, its pore throat size, and size of bacteria as these parameters govern the free movement within soil. A particle-organism size compatibility relationship that indicates the relative dimensional boundaries of compatibility is presented by Mitchell and Santamarina 2005, ASCE as shown in the figure below.

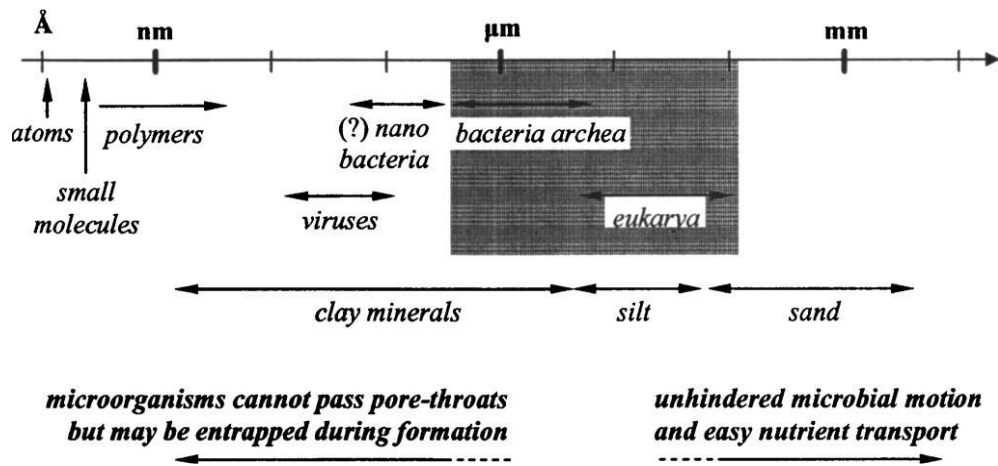


Fig 3: Particle-Organism Size Compatibility Relationship

2.5.4 Bacteria Cell Concentration:

Bacterial cell concentration has a direct relation with the production of CaCO_3 in MICP. With an increase of bacterial cell concentration, the rate of urea hydrolysis also increases. Hence for the area where results are required in very short time, the time of MICP can be reduced by increasing the bacteria cell concentration in soil.

2.5.5 Fixation and Distribution of Bacteria:

Fixation is directly related to geometric compatibility of bacteria. Bacteria size compatible to pore throat size of soil leads to efficient bacterial distribution. This will ensure equal production of cementing material. After the equal distribution, there should be proper fixation of bacteria in soil. High salinity solutions leads to flocculation but low salinity solution are required to avoid rapid flocculation in sands as solution has to cover large distances. Last but not least, fixation fluid in higher flow rate flushes bacteria cell over larger distances compare to lower flow rate.

2.5.6 Temperature:

The soil temperature varies with latitude, altitude, incident solar radiation, moisture content, conduction, type of soil, depth of soil and etc. Microbial activity is less sensitive to temperature. The rate of MICP has almost no effects in 20 to 30 °C but it increases from 30°C and gets its peak at 60°C. MICP shows almost constant rate after 60°C. But when temperature increases to 100°C and further it inhibits the process as bacteria can't survive in such severe conditions. But this optimum temperature for urease activity, however, is impractical to be applied for soil treatment either on site or in laboratory.

2.5.7 pH:

Like all other enzymes, urea also acts at certain range of temperature. The calcite production increases from pH 6 to 8. Production will be at maximum on pH 8. After pH 8 the rate of calcite precipitation starts decreasing. The pH of soil gradually increases with ammonia production but CO₂ is also produced which acts as a buffer and controls the pH ranges between 6 and 8.

2.6 General Hint about Selection of Bacteria:

General selection of bacteria according to site condition.

Table 1: The Periodic Table of Physiological Classification of Chemotrophic Prokaryotes for the Screening of the Physiological Groups Suitable for Soil Bioclogging and Biocementation

Ecology of origin	Relation to oxygen and type of energy generation			
	Anaerobic fermenting prokaryotes	Anaerobic respirating prokaryotes	Facultative anaerobic and microaerophilic prokaryotes	Aerobic respirating prokaryotes
Prokaryotes of aquatic origin	<i>Bacteroides</i>	<i>Desulfobacter</i>	<i>Escherichia</i>	<i>Pseudomonas</i>
	<i>Prevotella</i>	<i>Geobacter</i>	<i>Shewanella</i>	<i>Acinetobacter</i>
	<i>Ruminobacter</i>	<i>Wolinella</i>	<i>Beggiatoa</i>	<i>Nitrosomonas</i>
Prokaryotes of terrestrial origin	<i>Clostridium</i>	<i>Desulfotomaculum</i>	<i>Microthrix</i>	<i>Bacillus</i>
	<i>Peptococcus</i>	<i>Desulfobacterium</i>	<i>Nocardia</i>	<i>Arthrobacter</i>
	<i>Eubacterium</i>	<i>Bacillus infimus</i>	<i>Streptococcus</i>	<i>Streptomyces</i>
Prokaryotes originating from extreme environments (Archaea)	<i>Desulfurococcus</i>	<i>Methanobacterium</i>	<i>Metallosphaera</i>	<i>Picrophilus</i>
	<i>Thermosphaera</i>	<i>Thermococcus</i>	<i>Acidianus</i>	<i>Ferroplasma</i>
	<i>Pyrodictium</i>	<i>Haloarcula</i>	<i>Haloferax</i>	<i>Sulfolobus</i>

2.6.1 In Bioclogging:

Different processes can lead to bio-clogging, these includes production of slime, formation of impermeable layer, precipitation of non-degradable materials like sulphides of metals by sulphate reducing bacterial formation, precipitation of carbonate by ammonifying bacteria and precipitation of hydroxides of iron by iron reducing bacteria in the presence of iron ore. According to site conditions, we can use different types of bacteria like aerobic, anaerobic and oligotrophic etc. Efficiency of all processes is not ensured in laboratory or field.

The microbial processes that can possibly leads to bio-clogging are summarized in Table 2.

Table 2: Microbial Processes that can lead potentially to bio-clogging

Physiological group of microorganisms	Mechanism of bioclogging	Essential conditions for bioclogging	Potential geotechnical applications
Algae and cyanobacteria	Formation of impermeable layer of biomass	Light penetration and presence of nutrients	Reduce of water infiltration into slopes and control seepage
Aerobic and facultative anaerobic heterotrophic slime-producing bacteria	Production of slime in soil	Presence of oxygen and medium with ratio of C:N > 20	Avoids cover for soil erosion control and slope protection.
Oligotrophic microaerophilic bacteria	Production of slime in soil	Low concentration oxygen and medium with low concentration of carbon source	Reduce drain channel erosion and control seepage
Nitrifying bacteria	Production of slime in soil	Presence of ammonium and oxygen in soil	Reduce drain channel erosion
Sulphate-reducing bacteria	Production of undissolved sulphides of metals	Anaerobic conditions; presence of sulphate and carbon source in soil	Form grout curtains to reduce the migration of heavy metals and organic pollutants
Ammonifying bacteria	Formation of undissolved carbonates of metals in soil due to increase of pH and release of CO ₂	Presence of urea and dissolved metal salt	Prevent piping of earth dams and dikes
Iron-reducing bacteria	Production of ferrous solution and precipitation of undissolved ferrous and ferric salts and hydroxides in soil	Anaerobic conditions changed for aerobic conditions; presence of ferric minerals	Prevent piping of earth dams and dikes

2.6.2 In Bio-Cementation:

In Bio-cementation Non-degradable compounds are being formed that can leads to increase in binding of soil. Du increased binding strength of soil can also be increased. Physical structure and mechanical properties of sand will be changed.

The microbial processes that can possibly lead to bio-cementation are summarized in Table3.

Table 3: Possible Microbial processes that can lead potentially to bio-cementation

Physiological group of microorganisms	Mechanism of biocementation	Essential conditions for biocementation	Potential geotechnical applications
Sulphate-reducing bacteria	Production of undissolved sulphides of metals	Anaerobic conditions; presence of sulphate and carbon source in soil	Enhance stability for slopes and dams
Ammonifying bacteria	Formation of undissolved carbonates of metals in soil due to increase of pH and release of CO ₂	Presence of urea and dissolved metal salt	Mitigate liquefaction potential of sand Enhance stability for retaining walls, embankments, and dams; Increase bearing capacity of foundations
Iron-reducing bacteria	Production of ferrous solution and precipitation of undissolved ferrous and ferric salts and hydroxides in soil	Anaerobic conditions changed for aerobic conditions; presence of ferric minerals	Density soil on reclaimed land sites and prevent soil avalanching Reduce liquefaction potential of soil

These tables can be effectively used for selection of bacteria and in these tables all required restrictions are considered.

The selection of bacteria that can lead towards bio-cementation and bio-clogging can be indigenous or exogenous depending upon the presence of bacteria in soil and availability of bacteria in market.

The indigenous bacteria are those bacteria that will be already present in the soil and can lead to the bio-cementation and bio-clogging if proper nutrients are provided and other affecting factors are considered.

If required type of bacteria are not present in soil then exogenous bacteria can be used, these will be artificially injected to soil.

Indigenous bacteria will be more useful in comparison of exogenous bacteria because of their soil compatibility.

2.7 Positive Aspects of Bio-cementation and Bio-clogging:

2.7.1 Compressive strength is comparable to cement or chemical treated soil:

Sand specimens were prepared by depositing sand into a plastic cylinder. Bio-cement solutions, or *bio-grouts*, were poured on top of the specimens. Dry sand columns treated by three different types of bio-cement:

- (a) Microbial Polysaccharide xanthan
- (b) Cultivation of oligotrophic bacteria
- (c) Iron-reducing bacteria

It should be noted that dry sand cannot even stand as a column; obviously it is not able to sustain weight. Therefore, the effect of the microbial treatment is obvious. Under suitable conditions, the unconfined compressive strength of the bio-cement treated sand can be as high as 1500 kPa as shown in Figure 2. Such a compressive strength is comparable to cement or chemical treated soil.

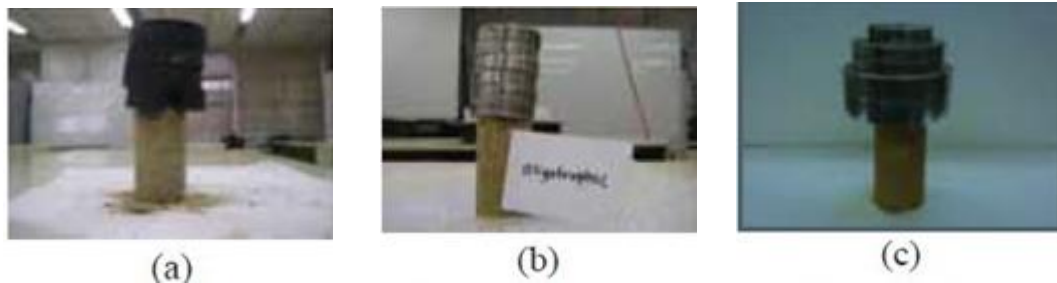


Fig 4: Sand Columns produced by sand treated by

(d) Microbial Polysaccharide Xanthan

(e) Ciltivation of Oligotrophic Bacteria

(f) Iron-Reducing Bacteria

2.7.2 Bio-cement Sustainable and energy saving material:

Bio-cement a new sustainable and energy saving material can be produced using naturally occurring microorganisms and used for soil improvement in a way similar to the use of cement. The production of bio-cement is more energy.

Saving and environmentally friendly. It is also sustainable as microorganisms are abundant in nature and can be reproduced easily at low cost. Harnessing this natural, unexhausted resource may result in an entirely new approach to geotechnical or environmental engineering problems and bring in enormous economic benefits to construction industries. The study made so far has shown that soil treated by bio-cement can have desirable engineering properties as that treated by ordinary cement.

2.7.3 Impermeable crust:

Bio-clogging and Bio-cementation can form impermeable crust which can further lead to decrease in permeability of soil.

Applying 0.6 g of Ca per cm² of sand surface, the permeability of the bio-cemented sand can be reduced from 10⁻⁴ m/s to 1.6 · 10⁻⁷ m/s (or 14 mm/day) due to formation of the crust on sand surface. The rupture modulus (maximum bending stress) of the crust was 35.9 MPa, which is comparable with that of limestone.

2.7.4 Bio Cement as a Construction Material:

In the construction process, a lot of materials used are from no-renewable resources and they add to the emission of carbon dioxide to the atmosphere thus polluting it.

Therefore, we must have some alternate construction materials that maintain the sustainability and reduce production of CO₂ emission.

Bio cement meets this purpose.

- Bio cement is environmental friendly since it has less CO₂ emission in the production process as compared to other construction materials like cement etc. and it also consumes less energy.

- It also requires lower temperature and shorter time for its production as compared to the ordinary cement which is usually produced at up to 1500 degree Centigrade.
- Bio cement increases mortar strength by around 38%.
- It has the ability to remediate in cracks.
- It increases the durability of the bricks by improving permeability and compressive strength factors.

2.8 MICP using Bacillus Pasteurii:

Bio-grout is a new soil improvement method based on microbiologically induced precipitation of calcium carbonate. Bacteria, which are able to convert urea into ammonium and carbonate, are injected in the soil, followed by a solution containing urea and calcium chloride and essential nutrients. The produced carbonate precipitates with calcium. The calcium carbonate crystals form bridges between the sand grains, which increases the strength of the sand mass.

2.8.1 Screening of microbes:

For effective microbially induced calcite precipitation (MICP) following factors should be considered. A microbe to be selected must have high capability of producing CO₂ as well as should increase PH in the surrounding environment to alkaline that will enhance the precipitation of calcium carbonate. Aerobic microorganisms capable of consuming urea as an energy source are particularly good candidates because they provide two sources of CO₂: respiration by the cell and decomposition of urea.

Mostly in literature Bacillus pasteruii was being used to carry out MICP. In this process bacterias were injected into solutuion after doing incubation in laboratory. Along with the Bacterial culture solution containing urea and CaCl₂ was also injected.Cementation treatments were done in different intervals to maximize the efficiency of bio-cementation. After that series of tests like consolidated undrained compression _CIUC_ triaxial tests. These tests indicate that shear capacity of treated sand was much more greater than untreated sand.

2.9 Applications of Bio-Cementation:

1. In slopes, Dams and Embankments:

These are the sites more prone to water bodies and have sulphide content greater than any other reagents. So production of undissolved sulphides can be obtained by anaerobic bacteria in presence of sulphate and carbon sources as cementing reagents.

2. Retaining walls and bearing capacity of foundations:

Formation of undissolved carbonates in the presence of ammonifying bacteria by the process of urea hydrolysis in a basic media maintained by the production of ammonia and carbon dioxide.

3. Liquifaction:

This problem can be negated by the action of nitrifying bacteria. Nitrifying bacteria leads to the production of nitrogen gas which get trapped into the pores and voids of soil particles. Hence when dynamic loading is done on fully saturated sand, these gas bubbles reduce the incompressibility of pore water.

4. Soil Avalanching:

Areas having high concentration of iron compounds can be mitigated by iron reduction bacteria. Production of undissolved ferrous and ferric salts will act as binding material which increases the density as well as the shear strength which ultimately increases the Factor of safety of any slope.

2.10 Applications of Bio-Clogging:

1. Water Infiltration:

In sites of low level penetration of water, Algae and Cyano-bacteria can be used along with nutrients, they form a thin biomass which reduces the infiltration of water.

2. Slope Protection:

Aerobic and facultative anaerobic heterotrophic slime producing bacteria are used along with their nutrients. They produce slime or saliva which adheres the soil particles together and control erosion.

3. Seepage and Drain channel Erosion:

Nitrifying and oligotrophic bacteria are used to control seepage. They also produce organic slime which fills the pores to reduce the permeability of soil. But this leads to saturation. To counter liquefaction in saturated sand, nitrifying bacteria are used. Oligotrophic bacteria have advantage over all other bacteria because they can survive in nutrient deficient conditions.

4. Grout Curtains:

Sulphate reducing bacteria are used for this purpose. In this technique an impermeable vertical wall is built by bacteria by the production of sulphate salts. This wall will cease the migration of metals and other contaminants which will ultimately contaminate the water body.

5. Piping:

The phenomena in which dissolved material is washed away with water leads to formation of clean and easy path for extensive seepage. Washing of dissolved strata can be reduced by bio-clogging with ammonifying bacteria and iron reducing bacteria.

2.11 Bio-Concrete:

Bio-concrete is a type of concrete with self-healing ability. It can regenerate itself when cracks occur in it. Two Dutch scientists from University of Delft- Eric Schlangen and Henk Jonkers, invented bio-concrete.

Bio-concrete can repair itself thus preventing structural degradation. It consists of Calcium lactate and bacteria. These bacteria have the ability to remain dormant for years inside the concrete. When a crack occurs, the bacteria come in contact with moisture and are thus activated. They start feeding on calcium lactate thereby producing calcite which is

accumulated in the crack to fill it up. Also the bacteria consumes oxygen which prevents internal corrosion of reinforced concrete.

Bacteria are encapsulated within two to four mm wide clay pellets and added to the cement mix with separate nitrogen, phosphorous and a nutrient agent. This innovative approach ensures that bacteria can remain dormant in the concrete for up to 200 years. Contact with nutrients occurs only if water penetrates into a crack - and not while mixing cement.

Bio concrete has many advantages over normal concrete such as it improves thermal comfort in buildings, it is environmental friendly and reduces Carbon dioxide levels in the atmosphere etc. It acts as an insulating material and a thermal regulator. It is also used for decorative purposes such as decorating the facades of buildings (vegetated facades).



Fig 5: Vegetated Facade

METHODOLOGY

3.1 Methodology for MICP:

- Addition of urea source.
 - Urea breaks into ammonia (NH₃) and carbon dioxide (CO₂) by bacterial cell, this ammonia will react with water to produce Hydroxyl ion (OH⁻) and NH₄⁺. (OH⁻) will react with CO₂ to form carbonic acid (HCO₃⁻) and NH₄⁺ will help to achieve the required pH of process.
- Addition of calcium salt.
 - Calcium ion from calcium salt will react with carbonic acid to precipitate Calcium Carbonate (CaCO₃).
- Chemical reaction by Urease producing bacteria.
 - These all chemical reactions will be carried out by Bacterial Cell.

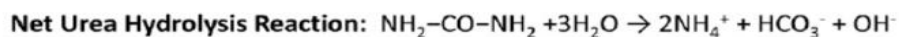
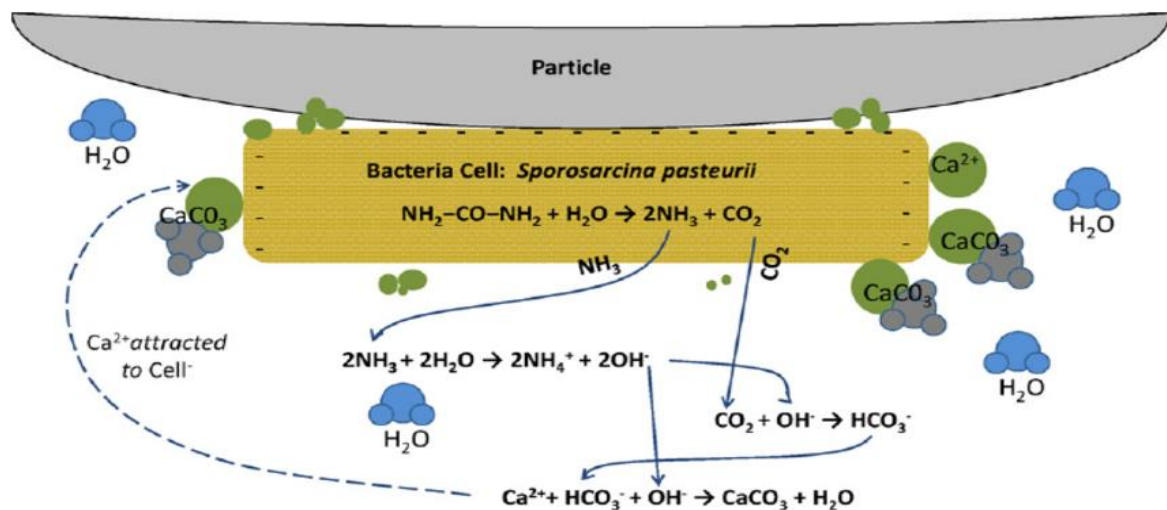


Fig 6: Microbial Induced Calcite Precipitation

3.2 Site Selection:

MICP is site specific technique with the change of sight type of bacteria, method leading to calcite precipitation, effecting factors and some other conditions will change. Before starting this project, selection of site was very important, we have to select the site where we can effectively apply this technique and can show the effective results of this technique. We have selected a site having low bearing capacity due to low shear strength and high permeability.

3.2.1 Our site Location:

Our site is located in front of naval anchorage Japani road near Soan River Rawalpindi Pakistan.

3.2.2 Why this site:

Our site consists of sand and according to Mitchell and Santamarina microbial and nutrients motion is free in sand. There are also different other reasons behind the selection of this particular site. Some of them are listed and explained below.

3.2.3 Loose Sand:

Loose sand has high permeability and low shear strength so we selected this site to increase the shear strength and to reduce permeability.

3.2.4 Gap Graded Sand:

Gap graded sand have permeability issues.

3.2.5 Near River:

Our site is near river. There will be high water table in the vicinity of river. Also Seepage and permeability issues will be there.



Fig 7: Site View

This is an ideal side to check the effect of MICP, this site is present at river bank so permeability issues are obvious and as sand is loose sand which leads to shear strength issues.

3.3 Field Testing:

In field different tests were performed to check the soil properties. For further proceeding it was very necessary to know the characteristics of natural soil.

3.3.1 Oven Dried Method:

This test is used to determine the water content of a materials by drying a sample to constant mass at a specified temperature. “The water content of a given soil is defined as the ratio, expressed as a percentage, of the mass of the pore water to the mass of the solid material (or "solids").”

We did not perform the speedy moisture test in field because speedy moisture test is not as accurate as oven dried method.

We took the sand sample in plastic bag for the measurement of field moisture content and fully covered the sample to not disturb the field moisture content and then in lab we

performed oven dried method to calculate the moisture content. Following table shows the results of oven dried method.

Dial reading(DR)	M.C= $DR \times 100 / (100 - DR)$ (%)
5.03	5.3%

5.3 % is relatively low moisture content which signifies high permeability of the soil sample.



Fig 8: Oven Dried Method

3.3.2 Sand Replacement Method:

The main objective of this test is to determine the in-situ density of the soil sample. We preferred using this method over core cutter method and rubber balloon method because it

is more accurate. Field Density was required for calculations in the later experimentation. Sand bottle, calibrating container, glass plate, base plate, pan, tools for excavating hole, clean dry sand passing from No.10 (2.0mm) sand retained on No.20 (850 μ m) sieve, speedy moisture tester apparatus were used for performing the test.



Fig 9: Sand Replacement Test at Site

Table 4: Sand Replacement Test

Calibration of unit weight of sand		1	2
1	Mass of Calibrating container, W1 (gm)	1376	1376
2	Mass of Calibrating container +sand, W2 (gm)	5038	5038
3	3 Mass of Sand W3= (W2-W1) (gm)	3662	3662

Table 4 (cont'd)

4	Volume of Container V (Cm3)	2649.4	2649.4
5	Dry unit Weight of sand, $\gamma_{\text{sand}} = W_3/V$ (gm)/ (Cm3)	1.3821	1.3821
Calibration of Cone			
6	Mass of bottle +Cone +sand (before use), W4 (gm)	6499	6499
7	Mass of bottle +Cone +sand (after use), W5 (gm)	4793	4793
8	Mass of sand to fill the cone $W_c = (W_4 - W_5)$ (gm)	1706	1706
Results from Field Tests			
9	Mass of bottle +Cone +sand (before use), W6 (gm)	8999	8790
10	Mass of bottle +Cone +sand (after use), W7 (gm)	3277	3064
11	Volume of hole , $V_h = (W_6 - W_7 - W_c) / \gamma_{\text{sand}}$ (gm) ³	2905.53	2908.43
12	Mass of excavated soil, W8 (gm)	4702	4782
13	Bulk density, $(\gamma_{\text{wet}}) = (W_8 / V_h) \gamma_{\text{sand}}$	1.62	1.64

Table 14 (cont'd)

14	Moisture content in the field (Mc %)	4.165	6.42
15	Dry unit weight in the field, $(\gamma_d) = (\gamma_{wet} \times 100) / (1 + mc \%)$	31.36	22.102

To simulate the field conditions we have performed these tests to calculate the moisture content and field density of soil, in further lab testing we performed all tests using these values.

3.4 Laboratory Testing:

To completely know the soil type, its specific gravity, relative density, permeability and shear strength different test were performed.

3.4.1 Sieve Analysis:

Sieve analysis is widely used in identification and classification of soil. It is also utilized in the part of the specification of soil for airfields, roads, earth dams and other soil embankment construction.

Advantages of the **sieve analysis** include easy handling, low investment costs, precise and reproducible results in a comparably short time and the possibility to separate the particle size fractions. Therefore, this method is an accepted alternative to **analysis** methods using laser light or image processing.

For performing sieve analysis, we used the following apparatus:

Set of ASTM sieves containing sieve # 4, 10, 20, 40, 60, 100, 200, lid and pan

1. Oven dried Soil sample
2. Balance sensitive to 0.1gm
3. Soil pulverizer
4. Sieve shaker
5. Soft brush for cleaning



Fig 10: Sieve Analysis

Following graph was plotted b/w %age passing and Sieve No. for further calculations.

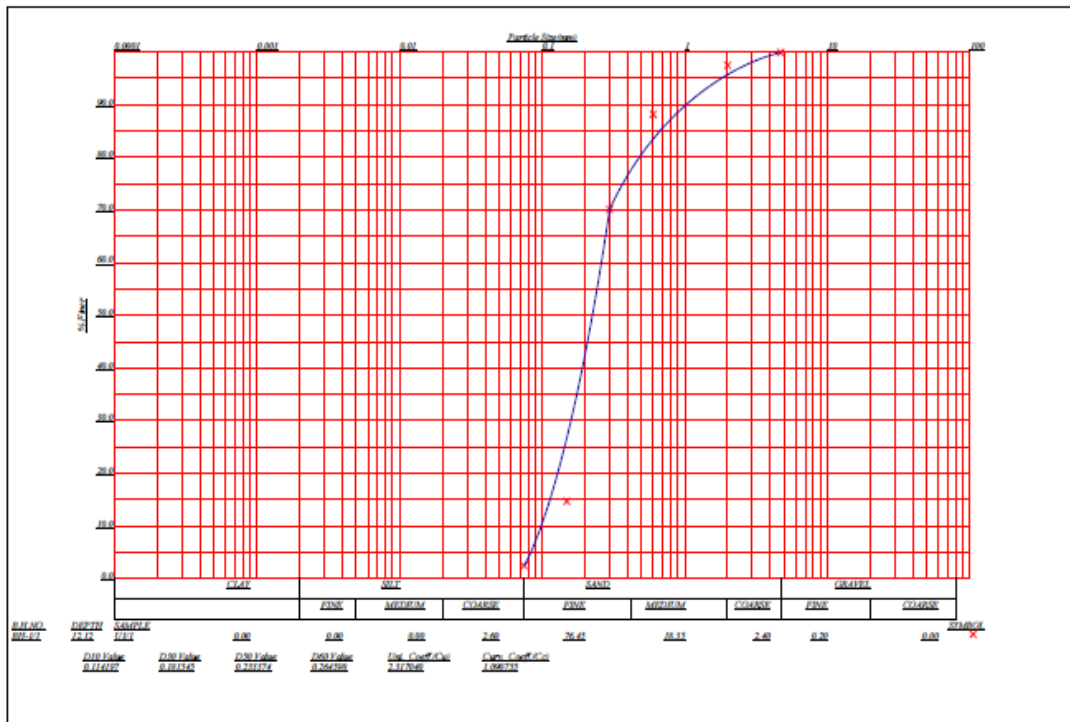


Fig 11: Graph between Percentage Passing and Sieve Number

Results from graph:

D10=0.13125

D60=0.2864

Cc=1.34

D30=0.225

Cu=2.218

From these results we have concluded that:

- Our sample is Gap graded soil(Gap in curve of sieve analysis) By (UCSC ASTM D 2487)
- According to (AASHTO Classification) our sample is A₂.

3.4.2 Specific Gravity:

Specific gravity of a soil is an important soil parameter that is used together with some soil parameters (such as void ratio, degree of saturation etc.) to compute other useful soil parameters. It is defined as “Ratio of the weight of a given volume of material to the weight of an equal volume of water.”



Fig 12: Specific Gravity Apparatus

Table 5: Finding Specific Gravity of Water

	Weights	Trail1	Trail2
Mass of flask	W1	85	89
Mass of flask +dry soil	W2	123	129
Mass of flask +soil+water	W3	357	363
Mass of flask+water	W4	333.5	338
Specific Gravity =(W2-W1)/[(W4-W1)-(W3-W2)*K]	Gs	2.63	2.66

Value of K =0.9974 at 30°C

Table 6: Values of Specific Gravity according to the soil type

Type of Soil	Specific Gravity
Sand	2.65-2.67
Silty sand	2.67-2.70
Inorganic clay	2.70-2.80
Soils with mica or iron	2.75-3.00
Organic soils	Variable but may be under 2.0

The value of specific gravity found out from the performed calculations lies within the range of Sand. Thus our sample is a sandy sample.

3.4.3 Constant Head:

This test is used basically to determine the coefficient of permeability for granular soil.

Apparatus used for the test included:

1. Constant head Permeameter device (including constant head filter tank and manometer tubes)
2. 500ml beaker
3. Balance
4. Stop watch
5. Thermometer.



Fig 13: Constant Head Test Performed in Geotech Lab, NICE

Following results were obtained.

Table 7: Constant Head Test

Test No	H1 (cm)	H2 (cm)	h=(h1-h2)	time (sec)	Q (cc/sec)	L (cm)	Area A(cm ²)	Temp (°C)	K (cm/sec)	K at 20°C(cm/sec)
1	270	155	115	22	3.31	20	78.54	21.8	7.33*10 ⁻³	7.02*10 ⁻³
2	270	175	95	22	2.96	20	78.54	22.6	7.93*10 ⁻³	7.48*10 ⁻³
3	270	169	101	22	2.88	20	78.54	23.0	7.26*10 ⁻³	6.92*10 ⁻³

Average K=7.14 ×10⁻⁵ m/sec

Table 8: Permeability and Drainage Characteristics of Soils

		Coefficient of Permeability k (m/s)											
		10 ⁰	10 ⁻¹	10 ⁻²	10 ⁻³	10 ⁻⁴	10 ⁻⁵	10 ⁻⁶	10 ⁻⁷	10 ⁻⁸	10 ⁻⁹	10 ⁻¹⁰	10 ⁻¹¹
Drainage		Good					Poor			Practically Impervious			
Soil types	Clean gravel	Clean sands, clean sand and gravel mixtures				Very fine sands, organic and inorganic silts, mixtures of sand silt and clay, glacial till, stratified clay deposits, etc.				"Impervious" soils, e.g., homogeneous clays below zone of weathering			
						"Impervious" soils modified by effects of vegetation and weathering							

The above table is from TPM which gives us range of permeability for different material. The permeability of our sample lies near 10⁻⁶ so we have a great range of reducing permeability to reduce permeability and seepage issues in our soil.

3.4.4 Direct Shear:

The direct shear test is used to determine the shear strength of the soil on a predetermined failure surface. This test is used to measure the friction angle and drained shear strength. It can be conducted on both coarse (sand) and fine (clays) soils. This method is very effective in case of sandy soils.



Fig 14: Direct Shear Apparatus

Following graphs were plotted between displacement and shear stress to show the behavior of soil at different loadings.

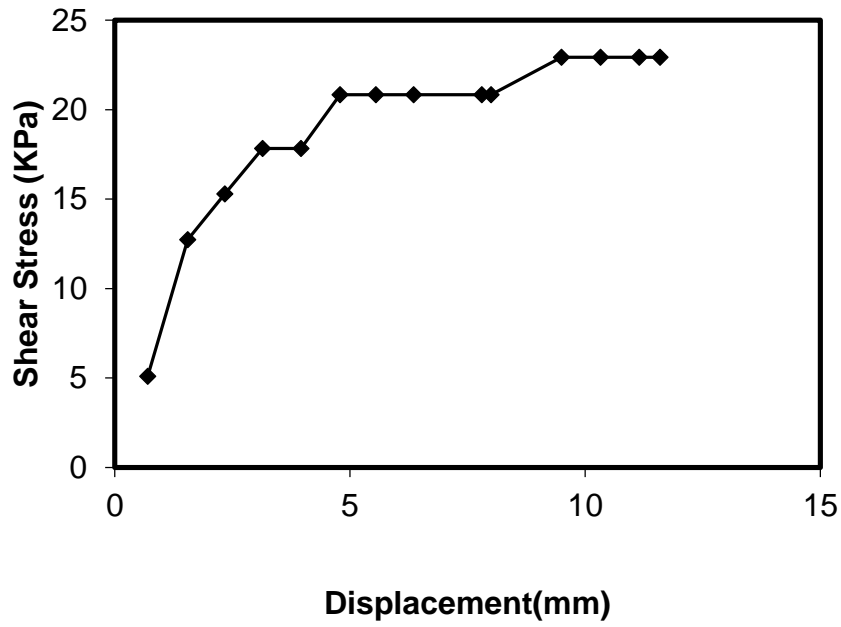


Fig 15: Displacement Vs Shear Stress at 50 kPa

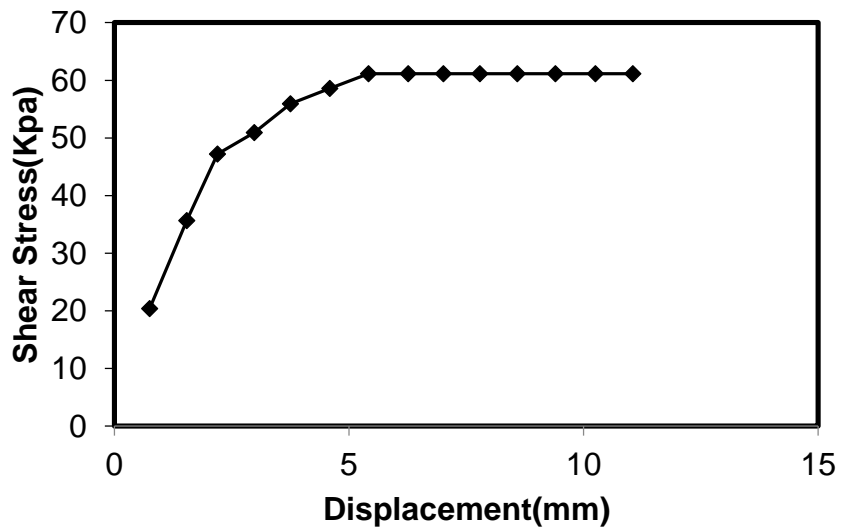


Fig 16: Displacement Vs Shear Stress at 100 kPa

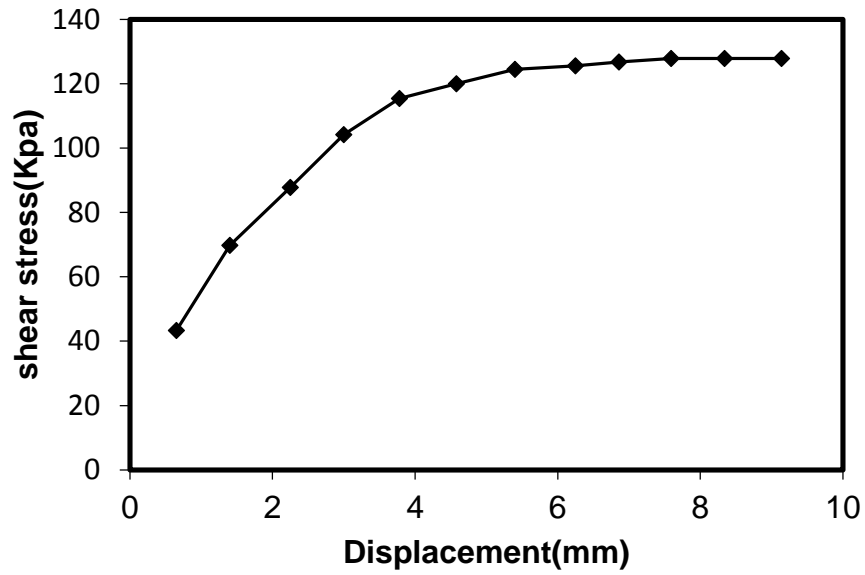


Fig 17: Displacement Vs Shear Stress at 200kPa

Shear Stress vs normal Stress:

C and Φ' values were extracted from this graph.

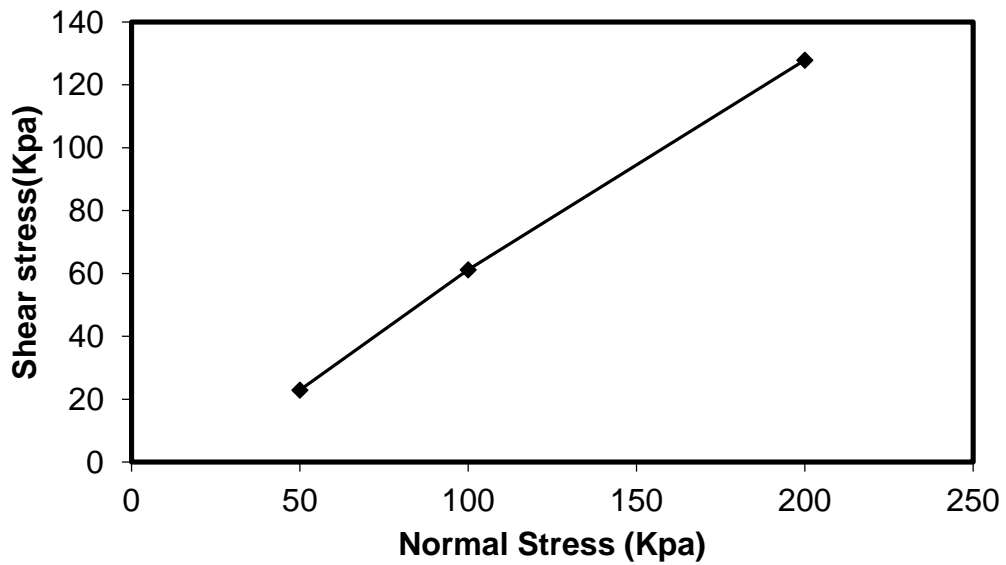


Fig 18: Shear Stress Vs Normal Stress

From the above graph we got $C'=0$

$$\Phi'=33^\circ$$

3.4.5 Relative Density:

Relative density is defined as “percentage difference of max void ratio and given void ratio divided by difference of max and min void ratio of soil.”

$$D_R = \frac{e_{max}-e}{e_{max}-e_{min}}$$

The value of Maximum Void Ratio (e_{max}) and Minimum Void Ratio (e_{min}) is obtained by using following equations.

$$e_{max} = \frac{G_s \rho_w}{\rho_{d_{min}}} - 1$$

$$e_{min} = \frac{G_s \rho_w}{\rho_{d_{max}}} - 1$$

Observation and Data Collection:

Table 9: Relative Density Test

Mass of Empty mould	3.779Kg
Dia of empty mould	15cm
Height of empty mould = L_o	15cm
Mass of mould + soil = M_1	
Mass of soil before vibration (M_{s1})	7.725Kg
Mass of soil after vibration (M_{s2})	7.725Kg
Average initial reading = R_i	7.555mm

Average final reading = R_f	27.79mm
Thickness t_p	13mm
X – Sectional area of mould	17671.458cm ²
$\Delta H = (R_f - R_i) + t_p$	33.235mm
Calibrated mould volume = V_c	2650718.8mm ³
Calibrated volume of soil = $V = V_c - (A_c \times H)$	2063407.871mm ³

Table 10: Relative Density Calculations

Sr.No	$\rho_{d_{min}} = \frac{M_{s1}}{V_c}$	$\rho_{d_{max}} = \frac{M_{s2}}{V}$	e_{max}	e_{min}	D_R
1	1.489	1.913	0.77	0.38	0.15



Fig 19: Relative Density Apparatus

3.5 Bacterial Selection:

As explained in literature review that we can either use indigenous bacteria or exogenous bacteria. But if urease producing bacteria are present in soil, they will be preferred. Indigenous bacteria will be preferred because of their compatibility with soil, less competition faced by indigenous bacteria because they were part of that environment and factors that effect this activity will be more compatible.

If bacteria compatible to run the process are not present we can use exogenous bacteria by considering all concerned factors.

3.6 Bacterial Detection:

To detect whether urease producing bacteria present we have followed this process:

- Tenfold dilutions of soil sample were made in distilled water.
- 0.5 ml of solution from each dilution was spread on nutrient agar plates. These plates were then kept in incubator overnight and bacteria were allowed to grow.
- Different bacterial colonies appeared as shown in picture. Mainly two types of colonies were observed i.e. small sized, circular, yellowish colonies and large, flat, sticky and white colonies.

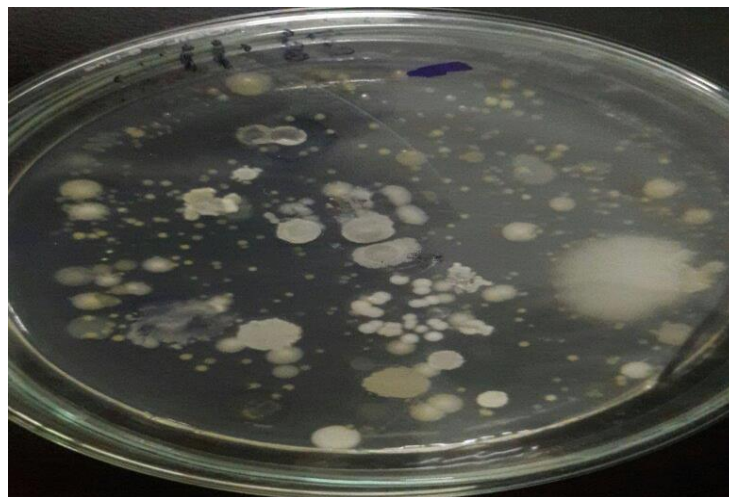


Fig 20: Bacterial Colonies

- These bacterial colonies were then picked from nutrient agar plates and streaked separately on Christensen`s agar media plates for identification of urease producing bacteria.
- Christensen`s agar medium is a selective medium for identification of urease producing microorganisms. Test organisms are cultured on this media. Decomposition of urea by urease enzyme produced by bacteria results in production of ammonia and CO₂. The medium becomes alkaline and color of medium changes from magenta to pink.
- While streaking different urea agar base plate (Christensen`s agar medium) with bacterial colonies of sample, salmonella typhi was used as control and a plate was streaked with salmonella typhi. As salmonella do not produce urease therefore there should be no change of color in its particular plate. Plates were then kept in incubator and bacterial growth was allowed. Control (salmonella typhi) did not show color change whereas a change in color was observed in other plate

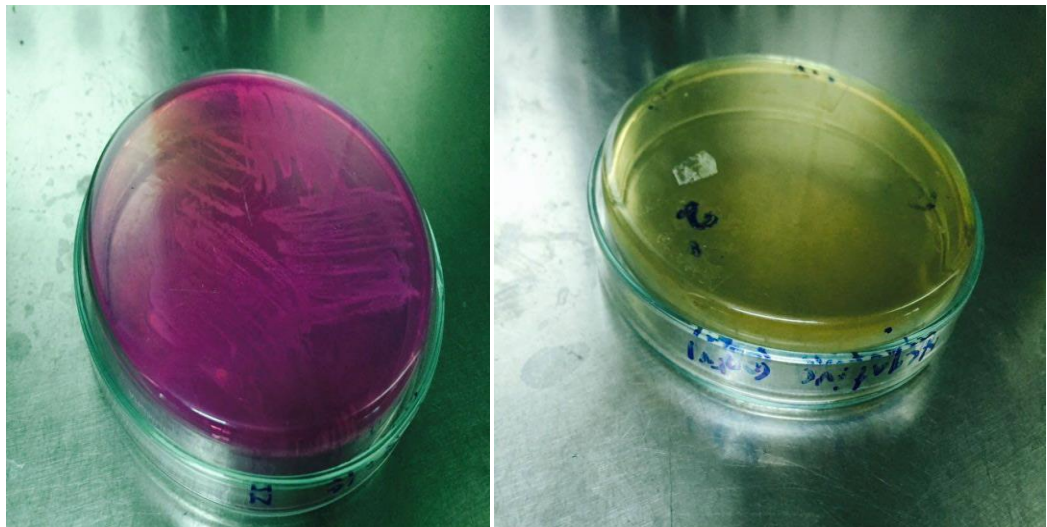


Fig 21: Control (Salmonella typhi), no color change (left side) whereas plate containing bacterial colonies from sample changed to pink which confirmed presence of urease producing bacteria in sample

- Bacterial culture on Christensen`s agar media was then further purified and streaking was performed on more urea agar base (Christensen`s agar medium)

plates. a single bacterial colony was then obtained which showed urease activity. This colony was then picked and transferred to liquid culture containing LB medium for growth. It was kept overnight in shaker incubator. This colony appeared rod shaped under microscope which shows that our isolated strain would most probably be bacillus.

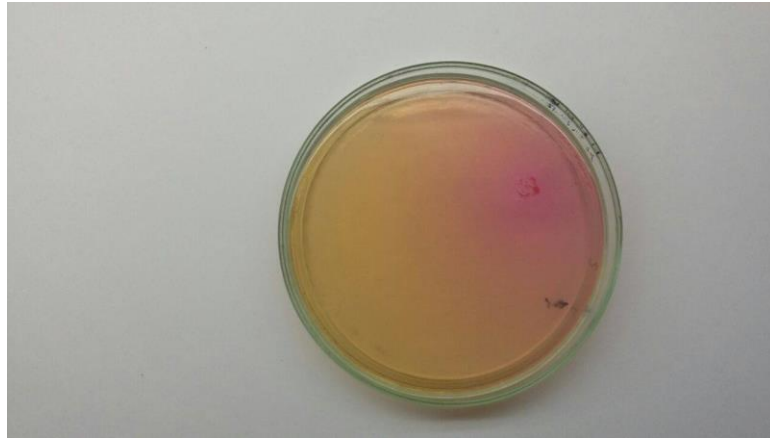


Figure 12: Urease producing Bacteria (Bacillus)

3.7 Bacterial Reproduction in lab:

In case of indigenous bacteria to stimulate the growth of bacteria in soil we can use enrichment solution, but at lab to ensure the bacterial growth we have extracted the bacteria from soil then place them in LB medium and kept them into incubator. After that we have injected these bacteria into our soil samples.

3.8 Soil Samples:

Soil samples were prepared to inject bacteria and enrichment solution and to observe the changing behavior of soil.

Test Moulds Instead of Soil Tank:

Different soil samples in respective moulds of Direct shear, UCS (unconfined compression Test) were prepared instead of using a tank of soil because if we will do the process of calcite precipitation in tank and then we will extract sample to perform tests, these samples

will be disturbed samples and calcite will not be in pores. These disturbed sample will give us the same results as untreated soil.

It is necessary to perform tests on undisturbed sample to ensure the efficiency of process.

Total sample prepared:

Table 11: Total Samples Required for Testing

TOTAL SAMPLES REQUIRED	
TESTS	NUMBER OF SAMPLES
UCS	5
Constant Head	5
Direct Shear	5*3=15



Fig 23: Soil Samples

3.9 Preparation of Bio-mineralized solution:

As explained in process followed we want to generate CaCO_3 in our soil sample, for this process along with bacteria we have to inject bio-mineralized solution. This solution contain urea and calcium salt which is required for MICP.

We have selected the following amounts to get maximum calcite preparation.

Table 12: Amounts of different Chemicals present in the prepared Bio-mineralized Solution

BIO-MINERALIZED SOLUTION			
TOTAL VOLUME	COMPOSITION	AMOUNT PER LITER	TOTAL VOLUME REQUIRED (33.2 L)
Urea	[$\text{CO}(\text{NH}_2)_2$; 46-0-0, 46% total nitrogen],	19.98 g	663.336 g
Calcium Chloride	($\text{CaCl}_2 \times 2\text{H}_2\text{O}$, .99% pure, EMD),	36.75 g	1220.1 g
Sodium Acetate	($\text{CH}_3\text{COONa} \times 3\text{H}_2\text{O}$, .99% pure,)	13.6 g	451.52 g

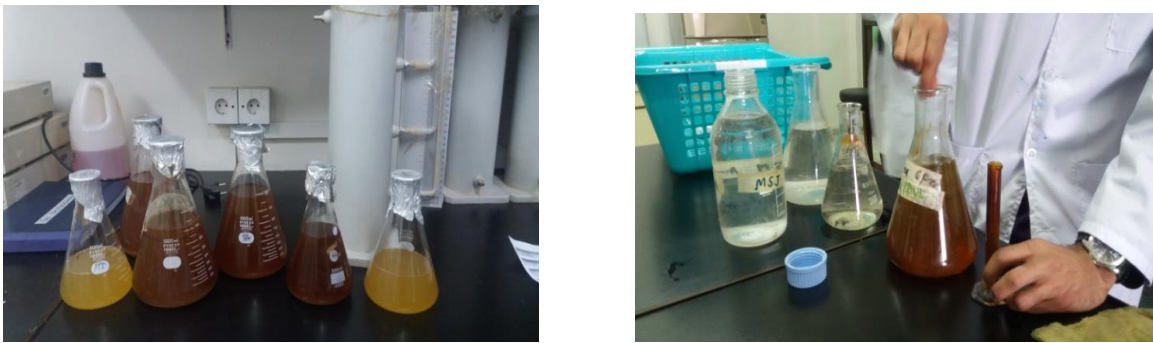


Fig 24: Solution Preparation for Injection

3.10 Injection of bacteria and bio-mineralized solution:

For uniform precipitation of calcite in soil injection of bacteria and bio-mineralized solution is very important.

3.10.1 Injection in Field:

Injection methods used in field for cement grouting can also be used for bio-grouting.

- Low pressure grouting:
Injection of low viscosity grout into soil to fill the voids. Grout will be injected at low pressure to avoid soil bulging.
- Jet grouting:
Mixing of soil and cement grout by injecting cement grout at high pressure.
- Stage-down method
To the full depth borehole was drilled and after that grout was injected as the drill was withdrawn.
- Stage-up method
Injection of grout was started from top and done to the desired depth.
- Grout port method:
In this method a slotted injection pipe and a double packer will be used to inject the grout at specific intervals.
- Vibrating beam method,
Beam will be vibrated and after that grout will be injected to desire depth.

Technologies for the microbial grouting could be similar to those used in chemical grouting. Depth of penetration depends on the size of used microorganisms.

The typical size of unicellular bacteria is from 1 to 3 μm , but the length of microbial cellular filaments can be up to 100 μm , which can be an obstacle in penetration of filamentous

microorganisms into soil. The specificity of microbial grouting is that such optimal for microbial activity conditions as optimal pH, salinity, oxidation-reduction potential, concentrations of nutrients, and content of water must be provided for.

A model proposed by us for Field injection:

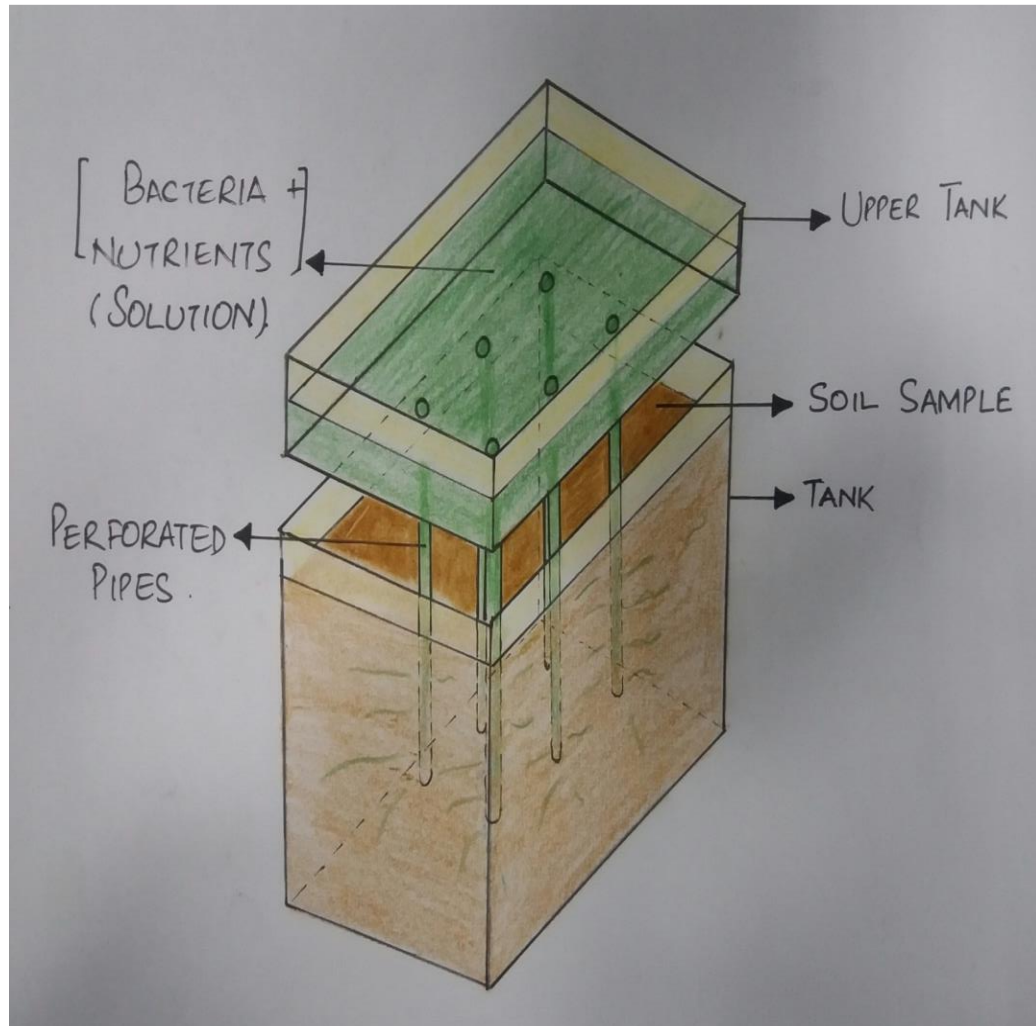


Figure 25: Model Proposed for Field injection

3.10.2 Injection in Lab:

To ensure the uniform injection in soil we used syringes to inject soil, for injection syringe opening and applied pressure must be considered. Syringe opening must be such that it

ensures the passage of bacterial cells. We made the grid at top of sample and then injected the solution at different points:

To make grid we must consider:

- Soil Permeability
- Viscosity of injected solution

3.10.2.1 Total Number of Injections:

We have made single injection of bacteria, and bio-mineralized solution was injected six times with difference of two days between each injection. In sample of UCS and direct shear constant amount was injected every week, but in constant head amount was decreased linearly because there was decrease in permeability with time.

Table 13: Total amount of material Injected into samples

TOTAL SAMPLES REQUIRED		Total injection=1	Total Injections=7
Tests	Number of Samples	Bacterial Injection/ sample	Bio-mineralized solution Injection per Sample
UCS	5	50 ml	30ml
Constant Head	5	150 ml	879.6ml, 780 ml, 680 ml, 580 ml, 580 ml, 480 ml (linearly Changed with time)
Direct Shear	5*3=15	30 ml	20 ml



Fig 26: Enrichment solution Injection

3.11 Optimum Injection:

After research we have made the optimum injection of bio-mineralized solution, in samples of direct shear and UCS we have made the injection with the help of syringes of length 3 cm and 6 cm. But in Constant head we have made injection by pooling method. In constant head injection was not possible with syringes because of

- Length of sample

- Intrusion of soil into syringe
- Limited length of syringe available in market.

To ensure the uniform solution distribution in constant head we have injected the optimum amount of solution, at end there was small opening and the outflow of solution from this opening ensure the uniform distribution. Height of mould was in the limits to ensure proper distribution by pooling. This technique can't be used at larger scale but at this level this technique was beneficial. For uniform distribution injection pump can be used but they will also have some restraints like soil surface disturbance etc.

3.12 Testing after Bacterial treatment:

After bacterial injection at different time period test were performed to check the change in soil properties like shear strength, permeability and unconfined compression strength. Afterward CaCO₃ precipitation was ensured by titrations test, XRF (X-ray Fluorescence: Used for elemental analysis), XRD (X-ray Diffraction: Used for compound analysis) and SEM (scanning electron microscopy: Imaging technique).

3.12.1 Test after 2 weeks:

Following tests were performed after 2 weeks of injection.

- Constant Head

Table 14: Constant Head Results after 2 weeks of Injection

Test No	H1 (cm)	H2 (cm)	h=(h1-h2)	Time (sec)	Q (cc/sec)	L (cm)	Area A(cm ²)	Temp (°C)	K (cm/sec)	K at 20°C(cm/sec)
1	270	170	100	20	3.15	20	78.54	25.1	8.02*10 ⁻³	7.12*10 ⁻³
2	270	132	138	20	3.45	20	78.54	21.2	6.36*10 ⁻³	6.18*10 ⁻³
3	270	142	128	20	3.20	20	78.54	22.0	6.37*10 ⁻³	6.07*10 ⁻³

Average k (at 20 °C) = 6.46*10⁻⁵ m/sec

- **Direct Shear:**

Following graph shows the results of direct shear test and increase in the shear strength of our sand sample.

First three graphs are between shear stress and displacement at 50, 100 and 200 kPa normal loads respectively.

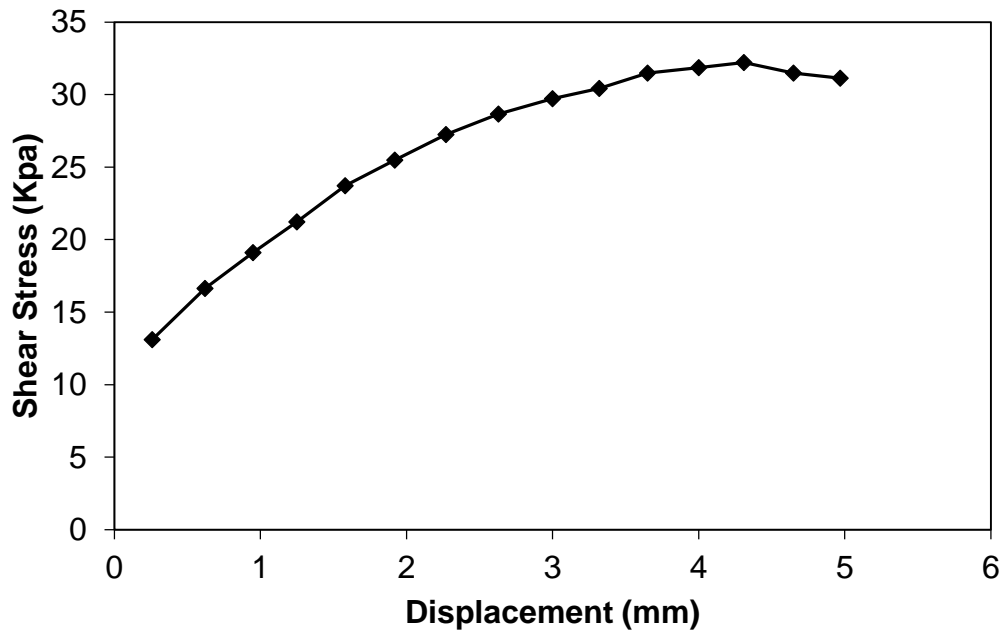


Fig 27: Displacement Vs Shear Stress at 50kPa

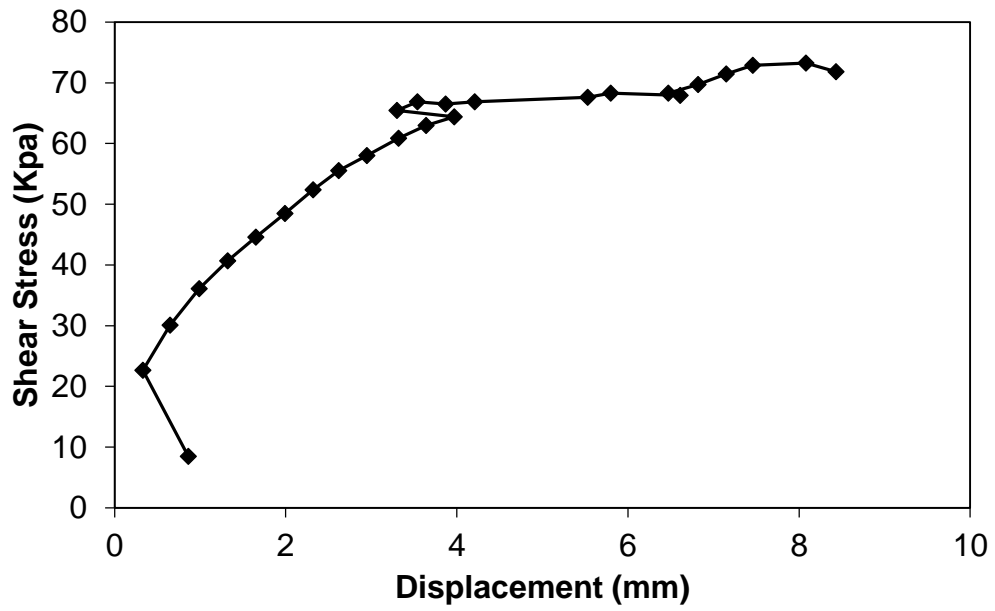


Fig 28: Displacement Vs Shear Stress at 100kPa

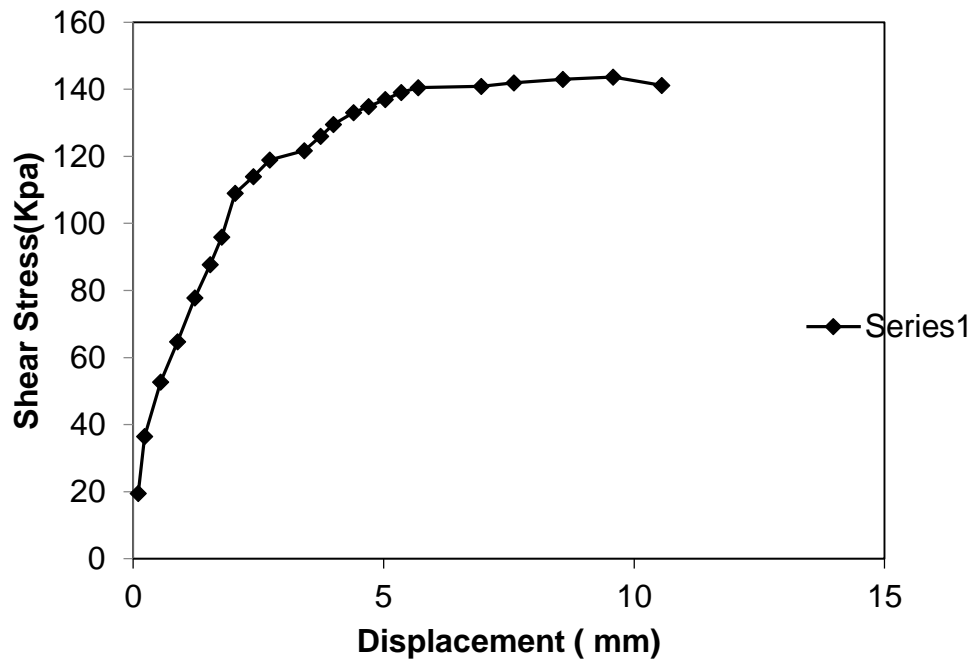


Fig 29: Displacement Vs Shear Stress at 200 kPa

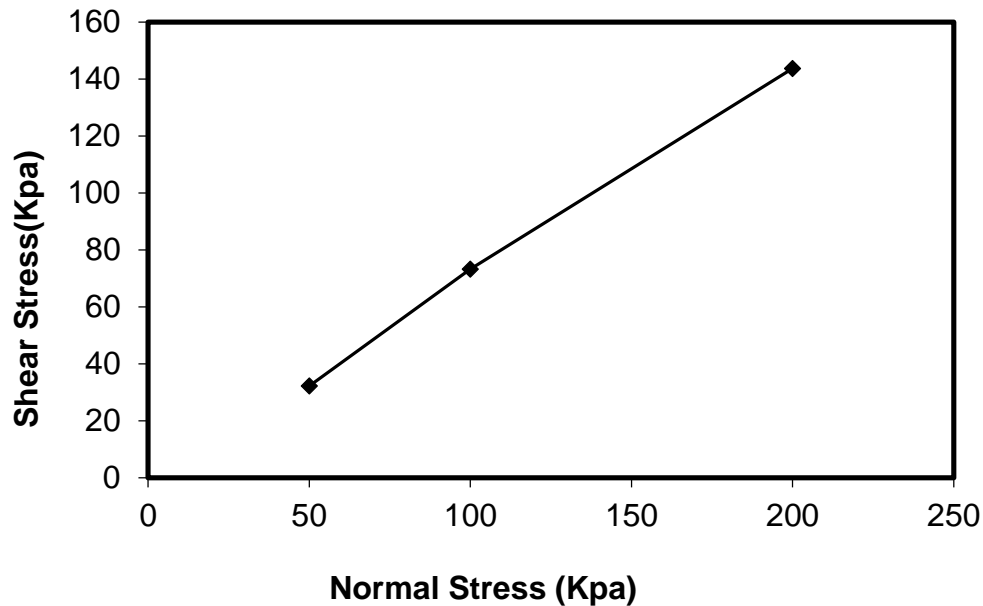


Fig 30: Shear Stress Vs Normal Stress

Comments:

Above graph shows the increase in shear strength of sand at different normal loads. The shear strength at 50Kpa normal load is increased from 22.92 to 32.2Kpa, at 100Kpa normal load from 61.146 to 73.246Kpa and finally at 200Kpa normal load from 127.88 to 143.665Kpa.

From graph it is clear that that there is small cohesion in sand sample. As cohesion increases the sand gets denser. Denser sand have less permeability as compared to loose sand and definitely have greater shear strength. Our sand sample got dense as originally it was loose and have increased shear strength.

- **Unconfined Compression Test:**

Our sample is sand and do not have any cohesion that is why we did not performed the unconfined compression test before injection of bacterial media but after injection of bacterial media we performed the unconfined compression test because the cohesion of our sample increased due to microbial activity.

Following are the results of UCS.

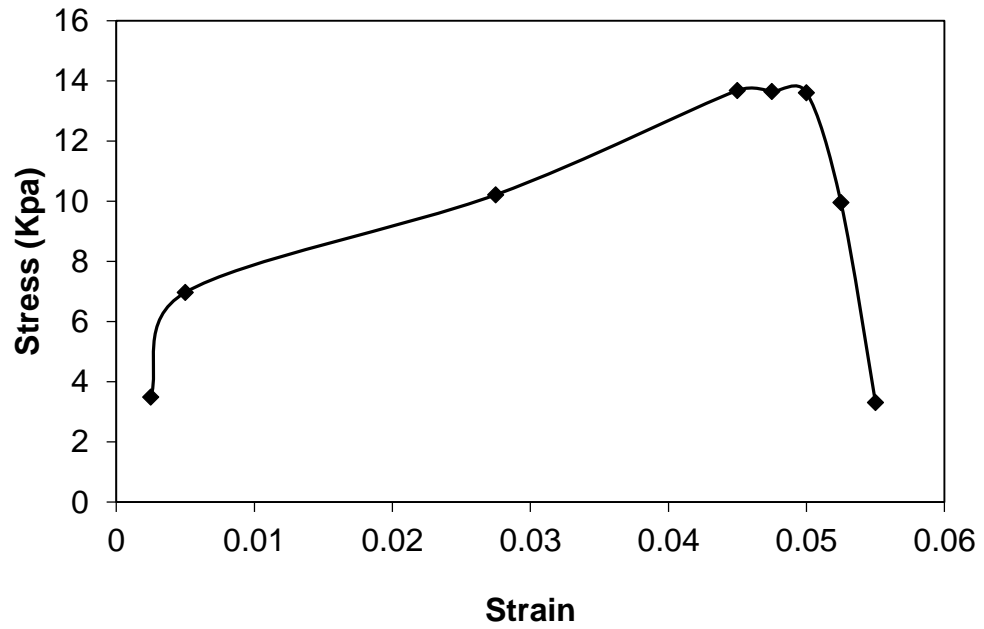


Fig 31: Graph between Stress and Strain

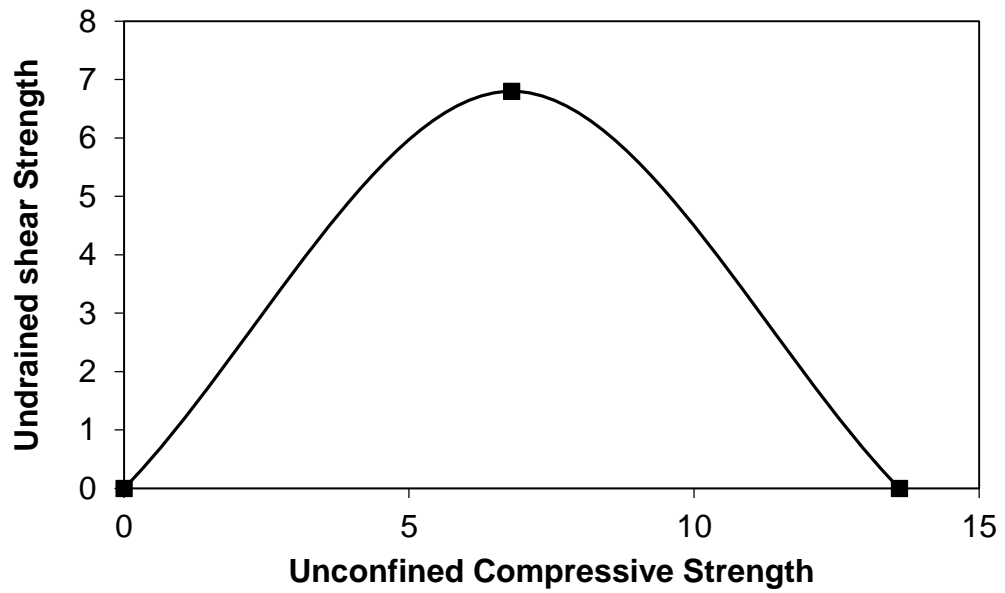


Fig 32: Undrained Shear Strength Vs Unconfined Compressive Strength

From graph:

$q_u = 13.67 \text{ kPa}$

$c = 6.835 \text{ kPa}$

- **Titration:**

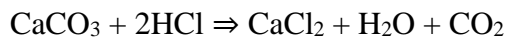
To check amount of soil we have used carbonate analysis by titration and XRF.

In Titration we have followed this method:

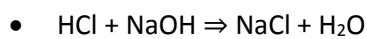
This method follows Rowell (1994).

This is a two-phase analysis:

1. The soil is mixed with a known amount of hydrochloric acid (HCl) causing the dissolution of the carbonate (CaCO_3) and creating Calcium chloride (CaCl_2), water and carbon dioxide.



2. The amount of acid left over is measured by titrating it with sodium hydroxide (NaOH) to produce sodium chloride (NaCl) and water. Adding phenolphthalein indicator to the solution causes it to turn pink when all the acid has reacted.



Percentage of CaCO_3	16.75%
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- **XRF:**

This is used for elemental analysis, moles of calcium and calcium carbonate will be same. So this can be used to know about CaCO_3 amount in sample.

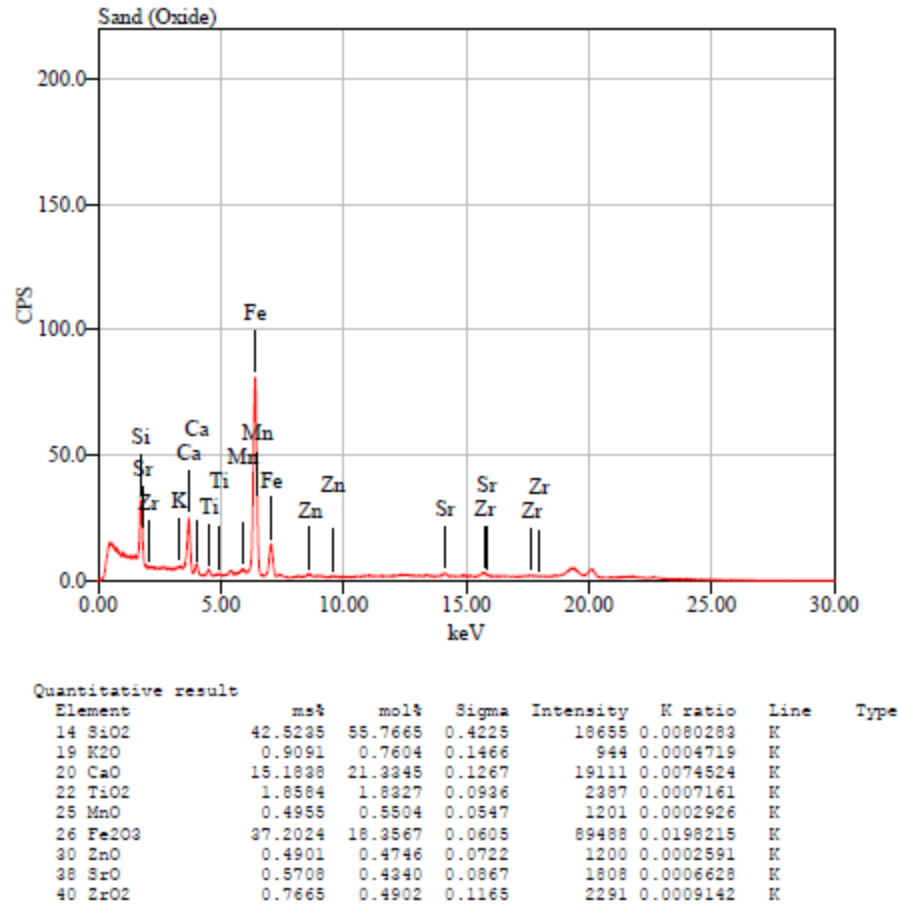


Fig 33: XRF Results (After 2 weeks)

- **SEM Results:**

After two weeks SEM was performed this test was performed to get images of sand and inside sand pores. We have performed this test at 10Kev and Mag 464X. To get our required results this magnification was perfect because we want to see CaCO₃ precipitation on sand, in these picture we can clearly see sand as well CaCO₃ precipitation. CaCO₃ is precipitated on soil grains and between soil pores.

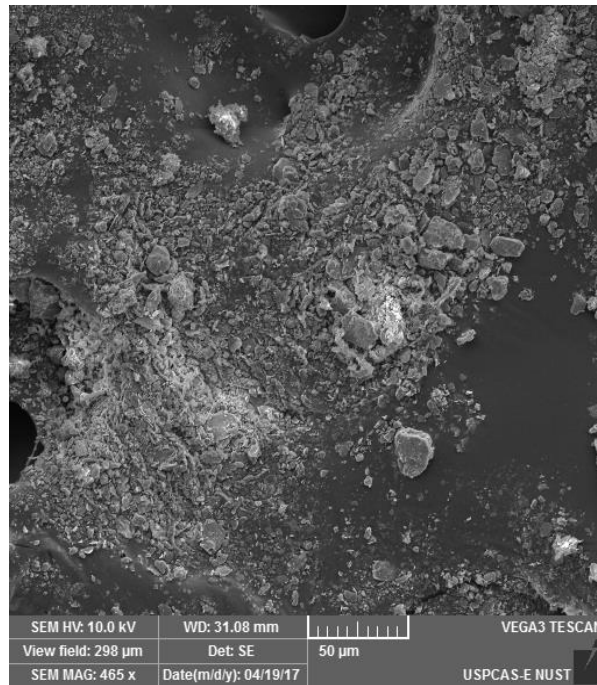
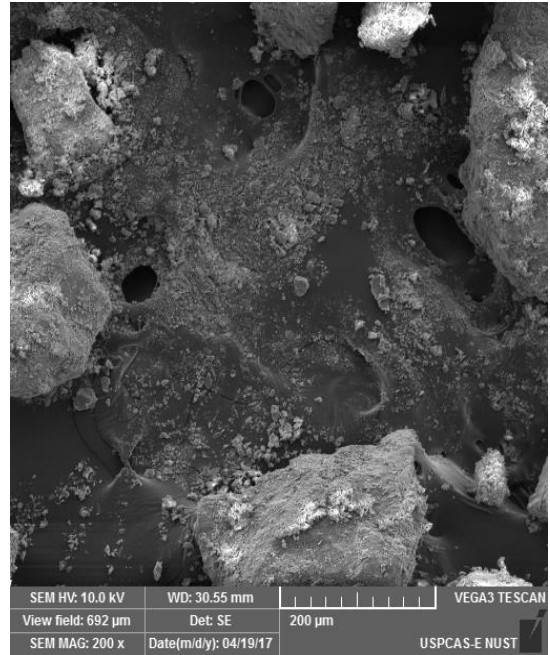
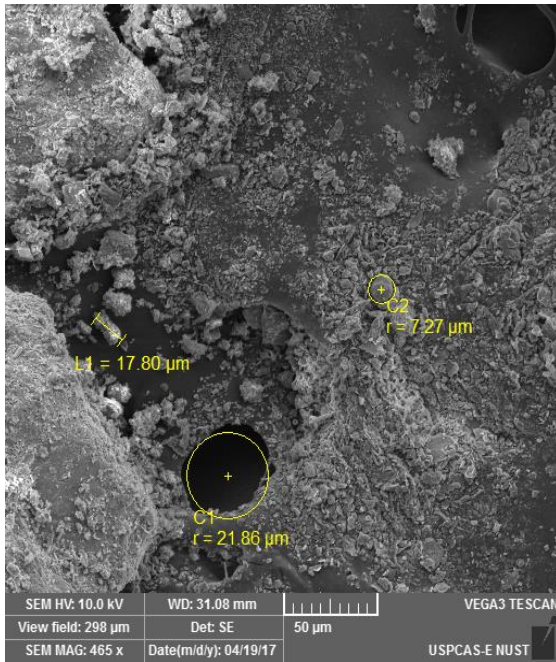


Fig 34: SEM Results (After 2 weeks)

3.12.2 Test after 4 weeks:

- **Constant head:**

Table 15: Constant Head Results after 4 weeks of Injection

Test No.	h1	h2	h=(h1-h2)	Temp. (°C)	Time (sec)	Q (cc/sec)	L (cm)	Area (cm ²)	k (cm/sec)	K at 20°C (cm/sec)
1	270	122	148	25.1	20	3.75	20	78.54	6.45*10 ⁻³	5.73*10 ⁻³
2	270	132	138	25.1	20	3.45	20	78.54	6.37*10 ⁻³	5.65*10 ⁻³
3	270	140	130	25.1	20	3.15	20	78.54	6.17*10 ⁻³	5.48*10 ⁻³

Average k (at 20 °C) = 5.62*10⁻⁵ m/sec

- **Direct Shear:**

Following graphs shows the results of direct shear test and increase in shear strength of sand sample due to microbial activity after four weeks of bacterial injection. First three graphs are between shear stress and displacement at 50, 100 and 200 kPa normal loads.

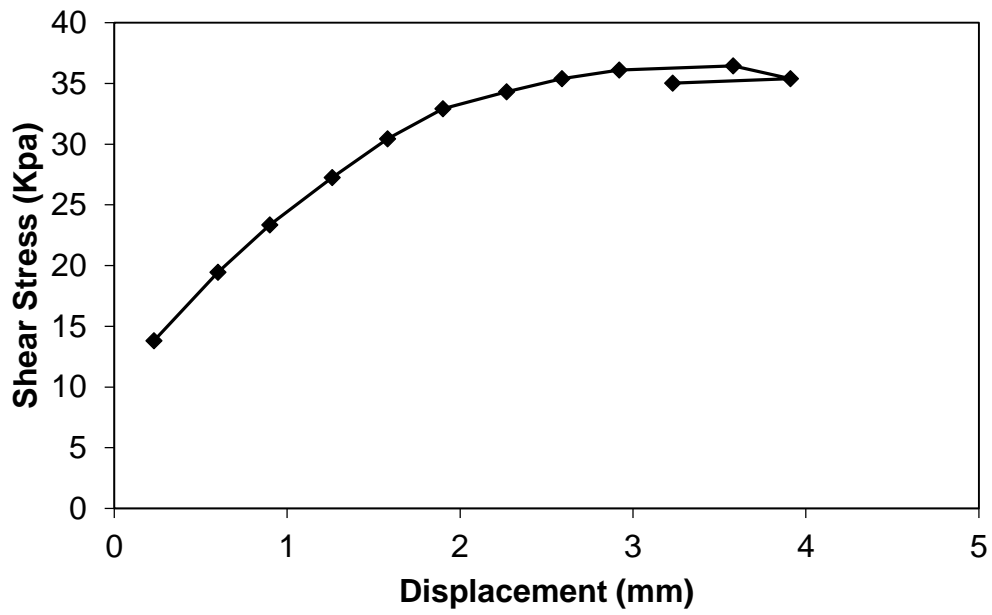


Fig 35: Shear Stress Vs Displacement at 50kPa

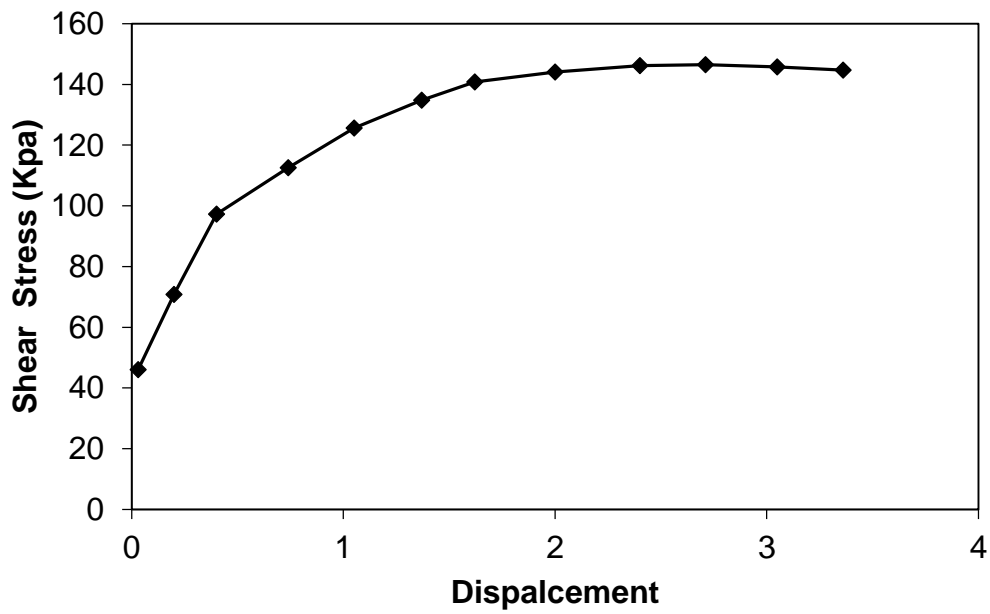


Fig 36: Displacement Vs Shear Stress at 100kPa

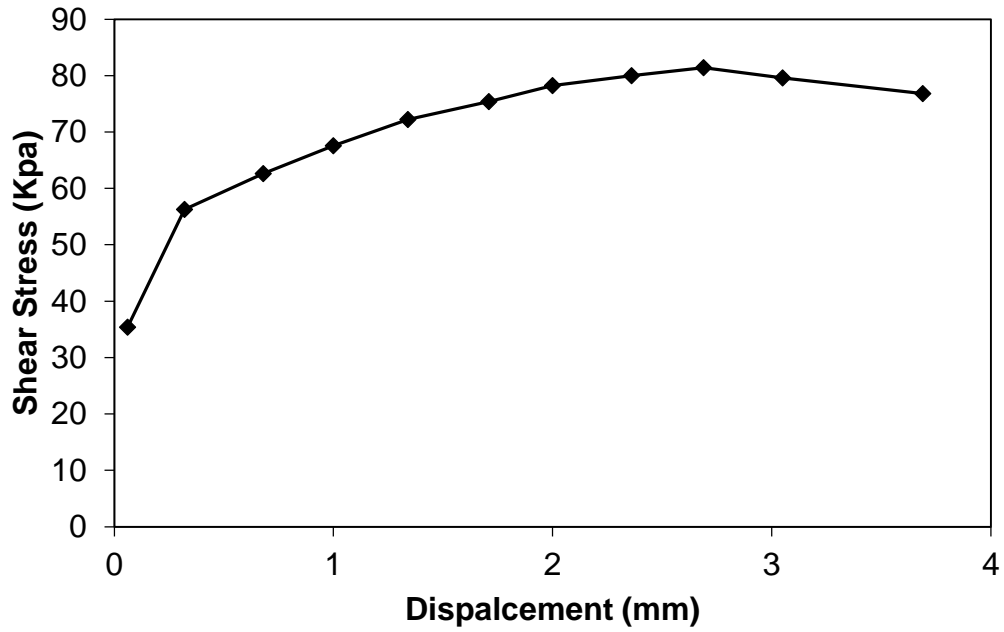


Fig 37: Displacement Vs Shear Stress at 200kPa

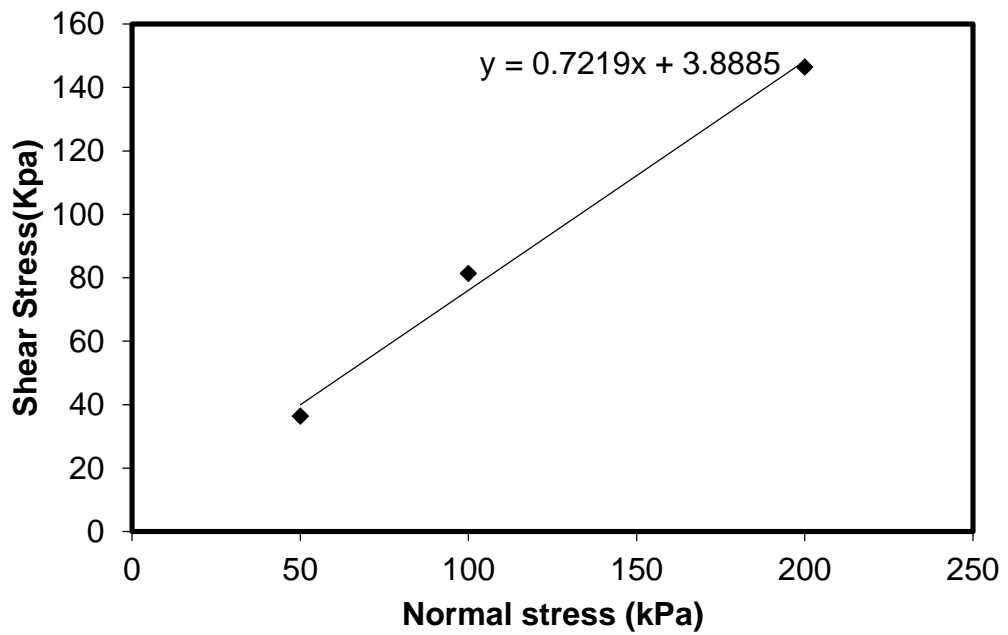


Fig 38: Shear Stress Vs Normal Stress

Comments:

After four weeks of bacterial injection the shear strength at 50Kpa normal load is increased from 22.92Kpa to 77.73Kpa, at 100Kpa normal load from 61.146Kpa to 114.41Kpa and at 200Kpa normal load from 127.88 to 187.75Kpa with $C=41.06$ and $\Phi=36.26$. As the cohesion of our sand is increased as compared to week 2 results and to sand without bacterial injection so our sample also got denser with respect to week 2 sand and sand without bacterial injection. Dense sand have less permeability and high shear strength.

- **Unconfined compression test:**

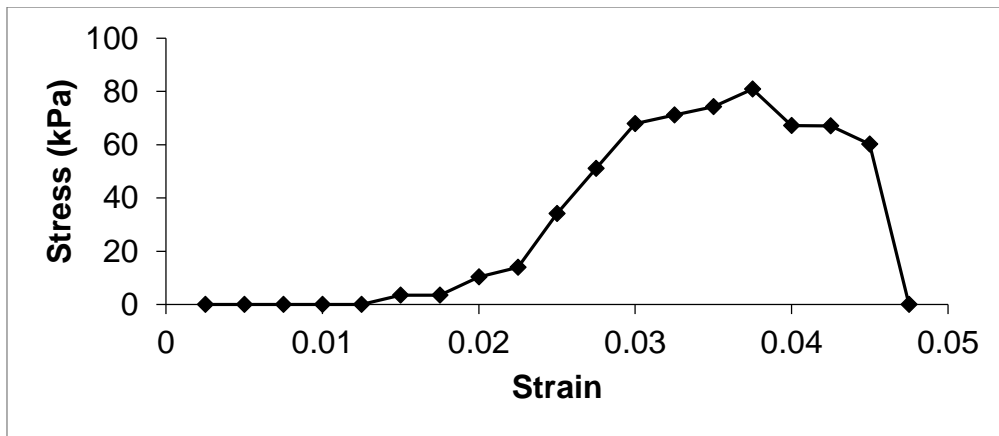


Fig 39: Graph between Stress and Strain

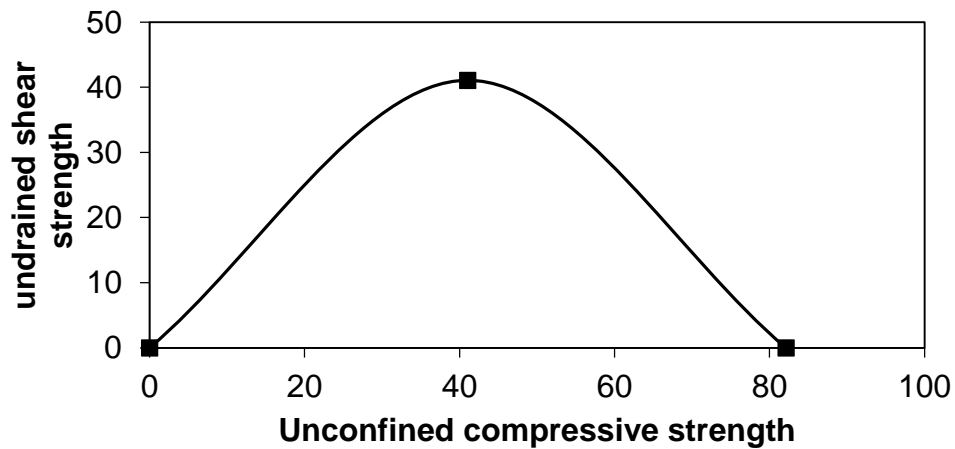


Fig 40: Undrained Shear Strength Vs Unconfined Compressive Strength

From graph:

$q_u=82$ kPa

$c=41.06$ kPa

- **Titration:**

Following are the results of titration after 4 weeks.

Percentage of CaCO_3	17.75%
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- **XRF:**

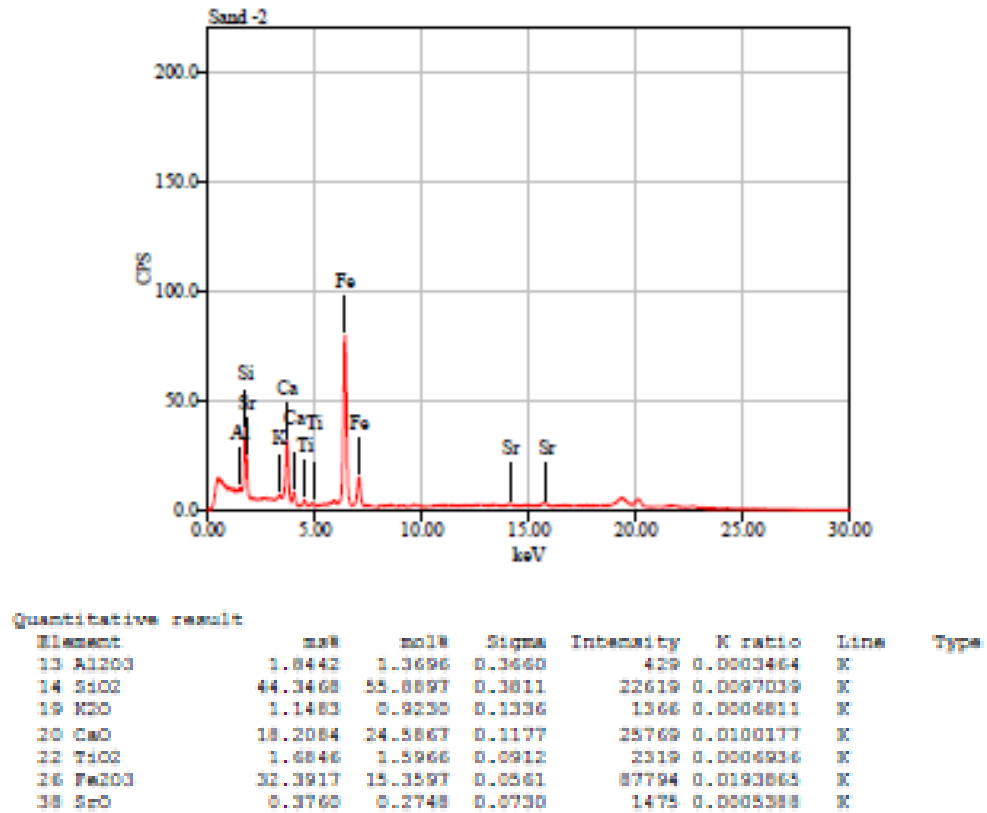


Fig 41: XRF Results (After 4 weeks)

3.12.3 Test after 6 weeks:

Test on Air dry sample:

- **Constant Head:**

Table 16: Constant Head Results after 6 weeks of Injection

Test No	H1 (cm)	H2 (cm)	h=(h1-h2)	Time (sec)	Q (cc/sec)	L (cm)	Area A(cm ²)	Temp (°C)	K (cm/sec)	K at 20°C (cm/sec)
1	270	120	150	20	3.00	20	78.54	27	5.09*10 ⁻³	4.33*10 ⁻³
2	270	110	160	20	3.15	20	78.54	27	5.01*10 ⁻³	4.26*10 ⁻³
3	270	118	152	20	2.70	20	78.54	27	4.52*10 ⁻³	3.84*10 ⁻³

Average k (at 20 °C) = 4.14*10⁻⁵ m/sec

- **Direct Shear:**

Following graphs shows the results of direct shear test and increase in shear strength of sand sample due to microbial activity after six weeks of bacterial injection. First three graphs are between shear stress and displacement at 50, 100 and 200 kPa normal loads.

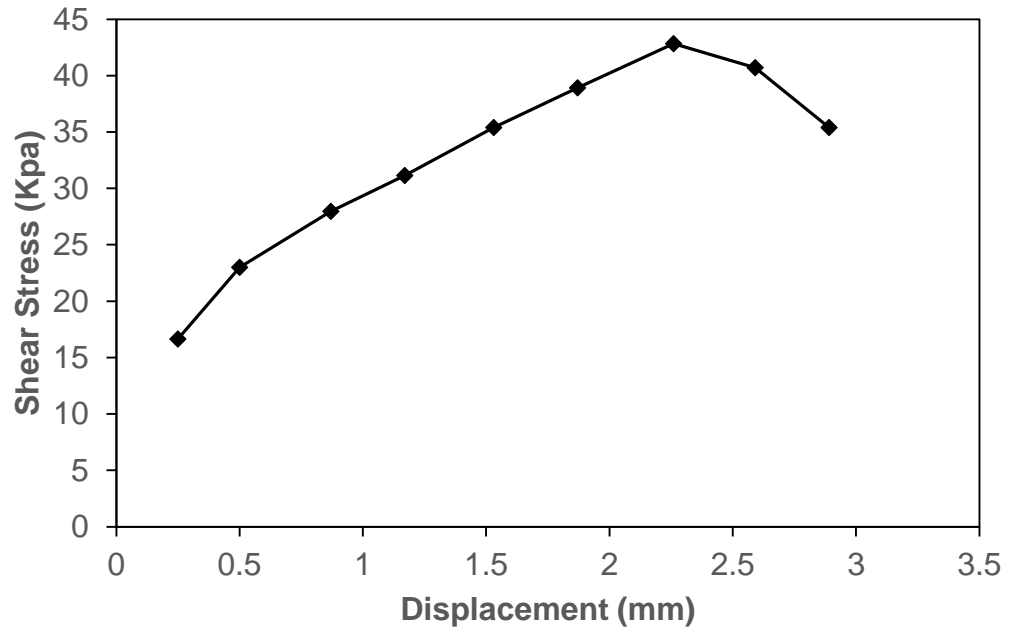


Fig 42: Shear Stress Vs Displacement at 50kPa

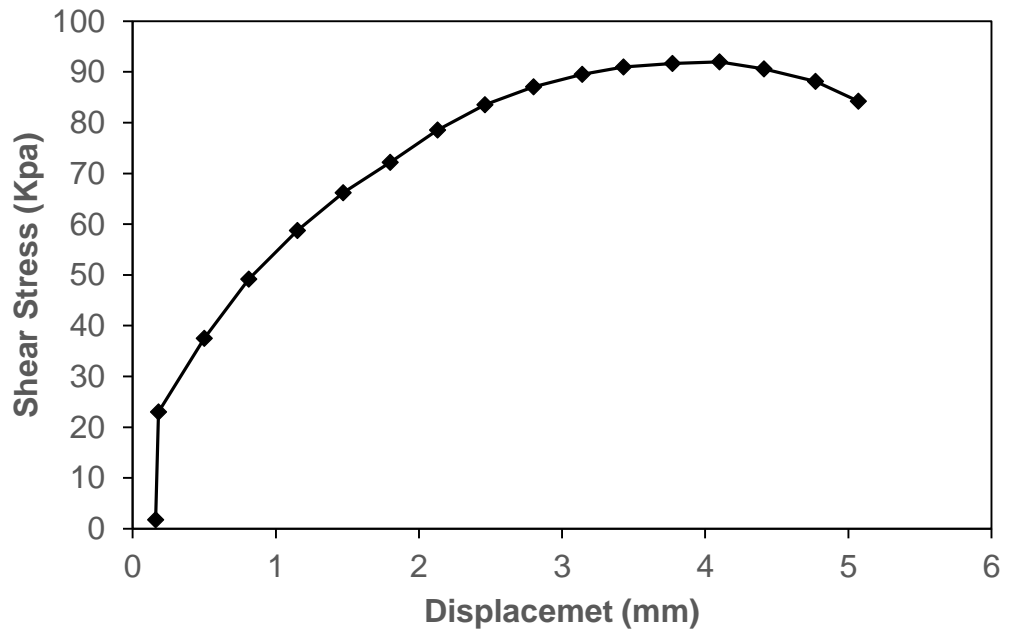


Fig 43: Shear Stress Vs Displacement at 100kPa

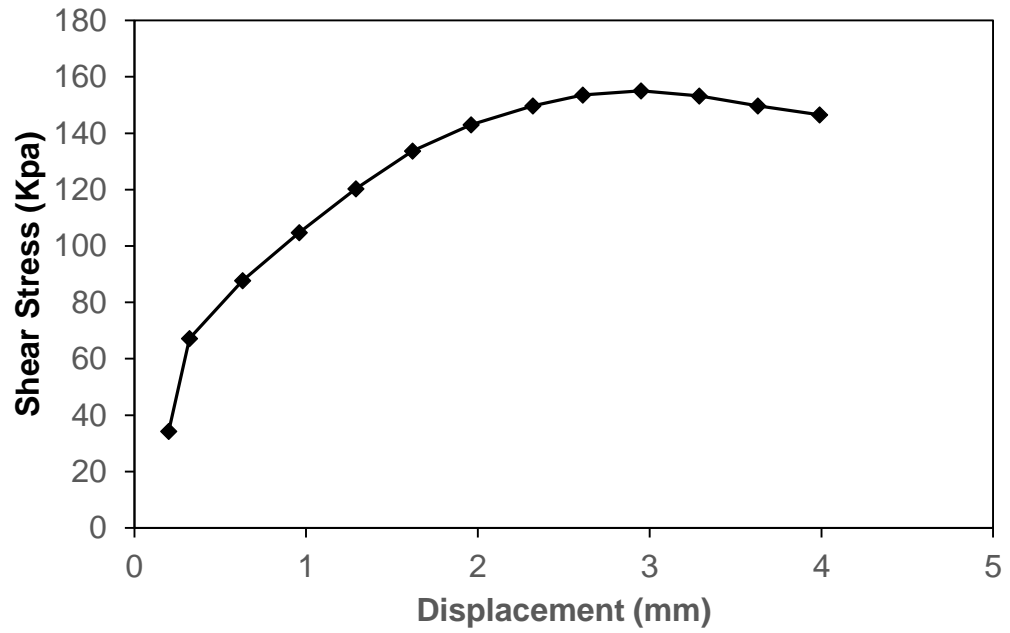


Fig 44: Shear Stress Vs Displacement at 200kPa

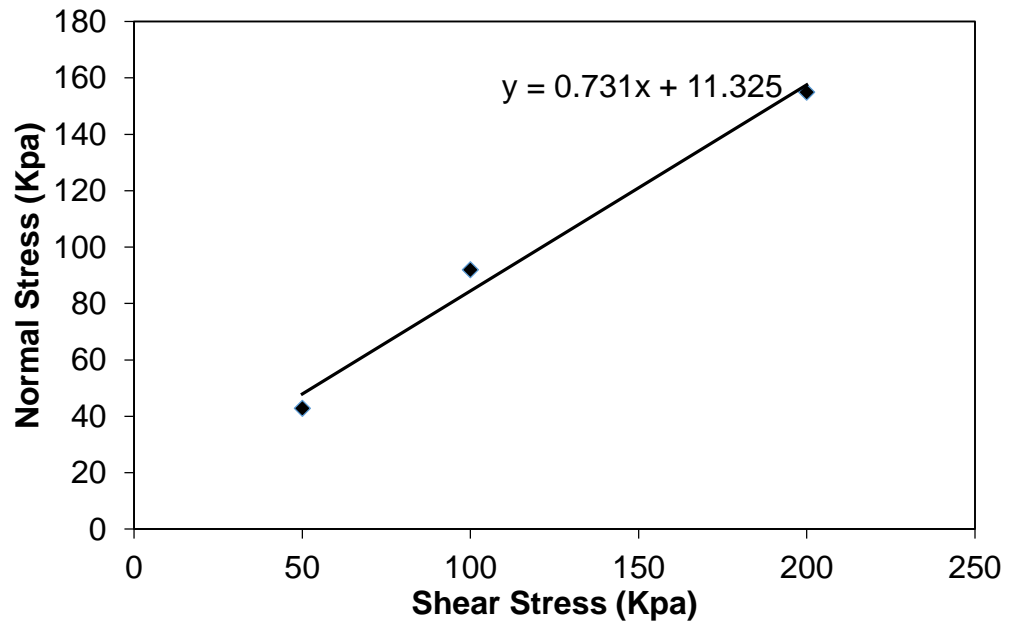


Fig 45: Shear Stress Vs Normal Stress

- **Unconfined Compression Test:**

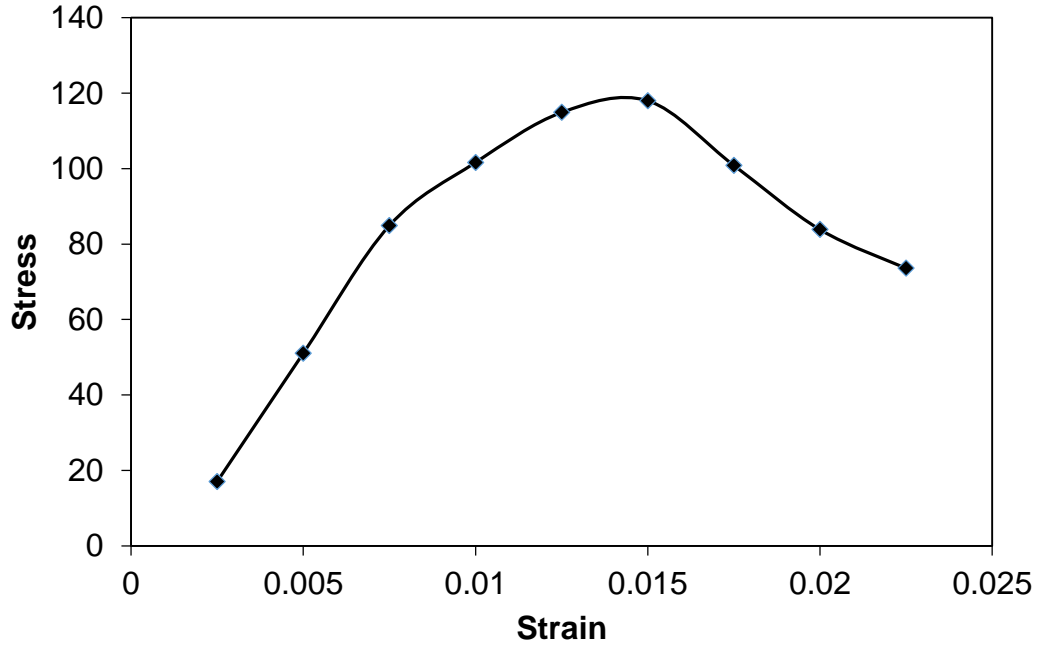


Fig 46: Graph between Stress and Strain

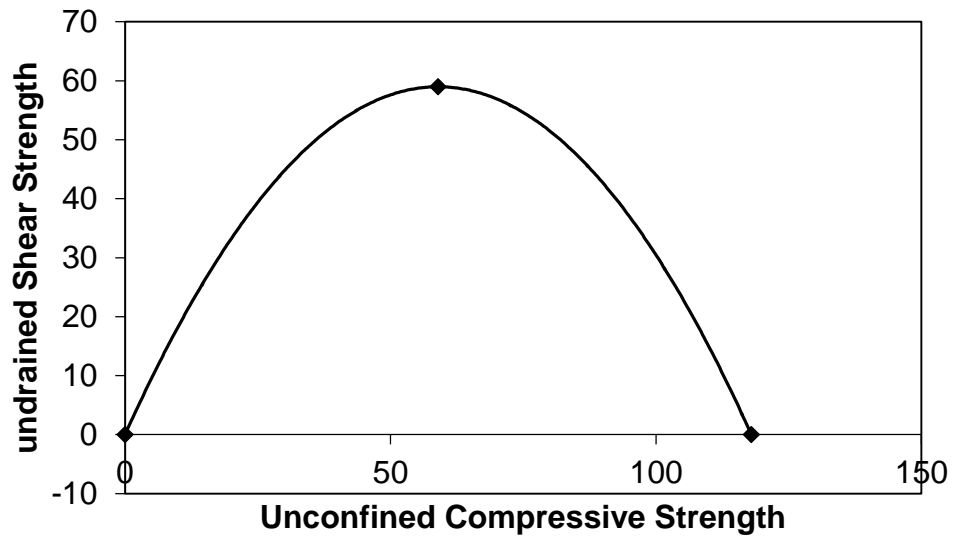


Fig 47: Undrained Shear Strength Vs Unconfined Compressive Strength

From graph:

$q_u=117.96$ kPa

$c=58.98$ kPa

- **Titration:**

Following are the results of titration after 6 weeks.

Percentage of CaCO_3	19.6%
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3.12.4 Test after 7 weeks:

Test on Air dry sample:

- **Unconfined Compression Test:**

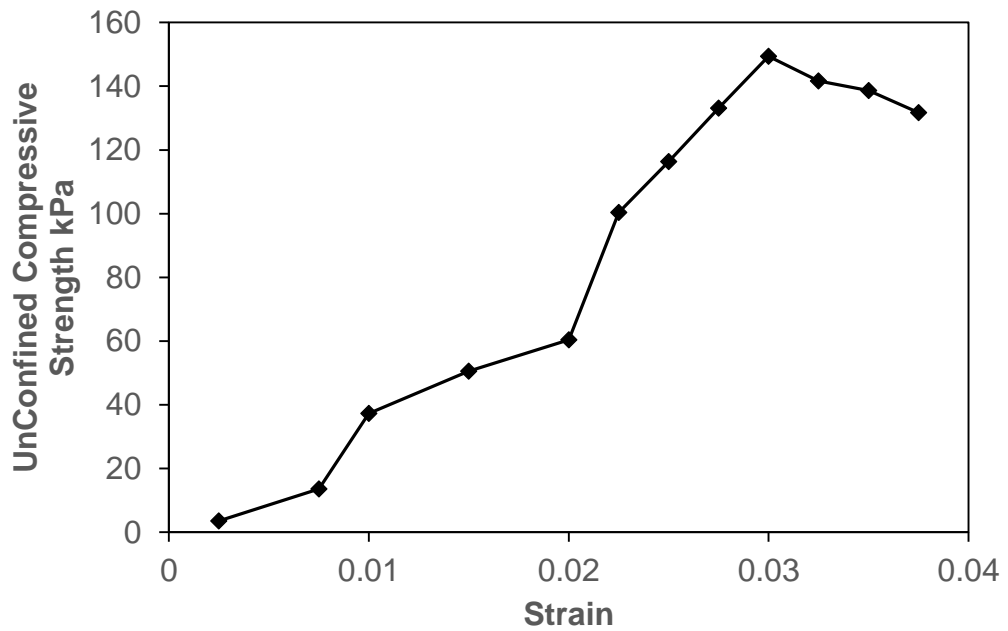


Fig 48: Graph between Stress and Strain

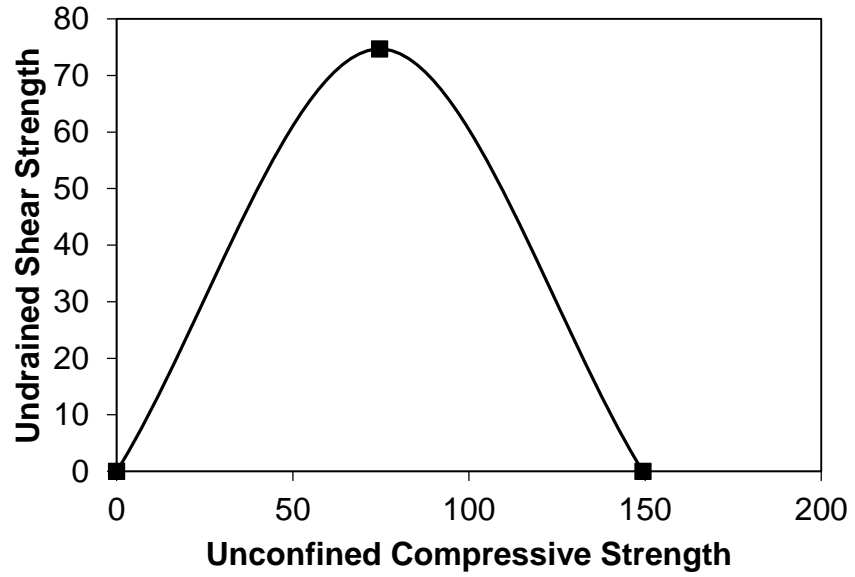


Fig 49: Undrained Shear Strength Vs Unconfined Compressive Strength

- **Titration:**

Following are the results of titration after 7 weeks.

Percentage of CaCO ₃	19.75%
---------------------------------	--------

CHAPTER 4

CEMENT GROUTING

Cement-based grout is made of mixed water and cement, which is sometimes also added with sand and admixtures. It is commonly used for soil improvement, such as dam curtain walls using jet grouting methods for masonry wall crack repairs, or for preplaced-aggregate concrete applications. In the application the grout is placed by using injection methods, with pressure or by its own weight only.

Therefore it is necessary for the grout to have adequate flowability so that the injection process can be easily carried out; additionally, it is also necessary for the grout to have adequate mechanical properties such as compressive and tensile strengths.

In Geotechnical engineering, the application of cement grouting is to get the shear strength and mainly to reduce the permeability of soil. When cement paste is injected, it moves under the action of gravity or it is pumped with certain pressure which the soil can hold in which cement is to be injected. Cement base grout mixes are commonly used for gravely layers or fissure rock treatment. But the suspension grain size may be too big to penetrate sand or silty-sand layers. In this case, chemical or organic grout mixes are also used. In recent years, the availability of ultrafine grout mixes has extended the performance of hydraulic base grout for soil treatment.

4.1 Classification of Grouting in Ground Improvement:

The Following flowchart shows the classification of grouting in ground Improvement.

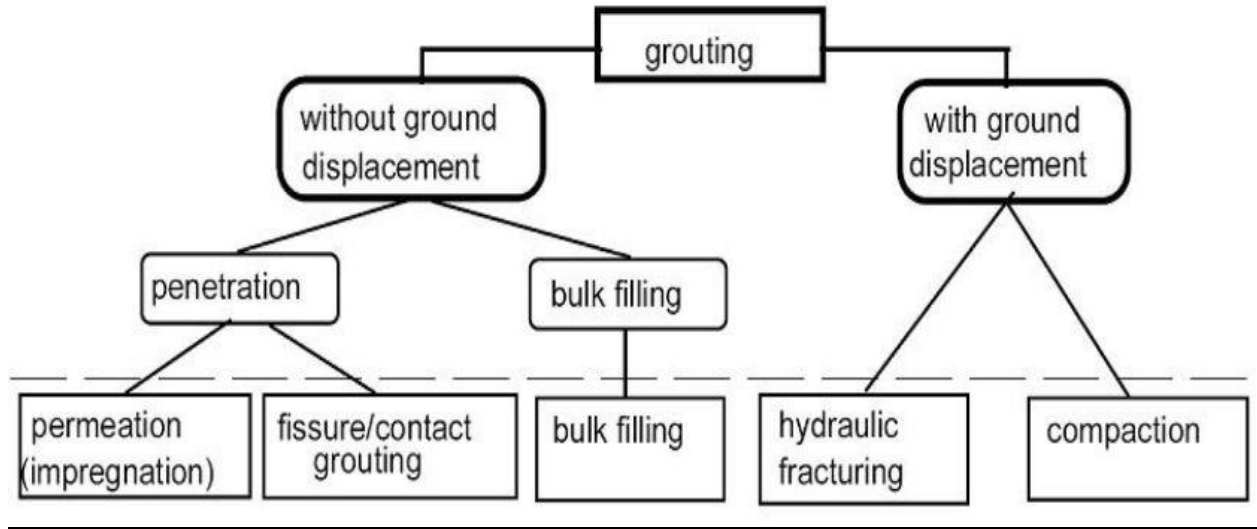


Fig 50: Classification of Grouting In Ground Improvement

4.2 Demerits of Cement Grouting:

1. The grouting methods are the most economical methods to improve soil properties like weak seams and unsound rocks as compared to barrier walls. But cement and chemical grouting are toxic and chemical used in chemical grouting are mostly soluble in water. They are not easy to handle as they contain some acidic compounds.
2. Another limitation of cement grouting is that it can only be injected into the medium soils and into the soils whose permeability is greater than or equal to 80 m/day. So for soils with small permeability micro fine cement has to be used. Hence for the treatment of soil with greater number of fines cannot be treated only with Portland cement paste. Moreover, a pumping or the lowering of water table is required before pumping of cement grout.
3. The limited tensile resistance, as well as the technique's strict quality control are needed in order to obtain an element in Jet grouting with the characteristics set out in draft. The risks of ground lifting, subsoil settlement and subsoil's chemical aggressiveness are the main constraints of this technique that should be avoided through a strict quality control.

4.3 Cost of Cement Grouting:

Cost of Cement Grouting includes Establishment Cost, Transportation Cost and Material Cost. Equipment used for the grouting are very expensive and are not available easily.

4.4 Comparison of Cement and bio grouting:

1. The cost of establishing the equipment for jet grouting is high.
2. Ground water level has to be lowered by pumping.
3. Special measures, such as piling and groundwater infiltration outside the construction site, may be needed to avoid unacceptable mechanical impact in adjacent areas and structures due to loss of stability and bearing capacity in the ground or excessive settlements.

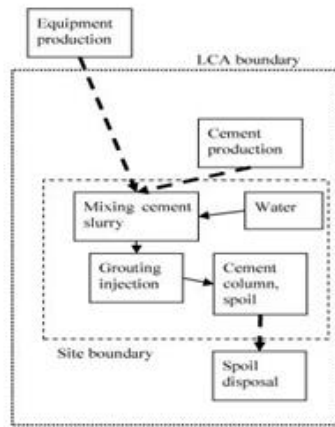


Fig 51: Material flows for jet grouting.
Bold, broken arrow indicates transport.

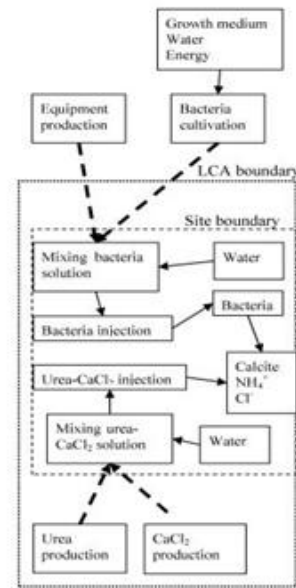


Fig 52: Material flows for bio-grouting.
Bold, broken arrow indicates transport.

4. Cement grouting has adverse effects on environment as compared to bio-grouting.

Table 17: Environmental comparison of jet grouting and bio grouting

	<i>Amount Jet grouting</i>	<i>Bio-grouting</i>		<i>Materials for production</i>	<i>Environmental impact from production</i>
Drinking water	13	3	m ³	Surface water Cleaning chemicals	Natural resources (fresh water), electricity related impact (pumping)
Cement	0.45	—	m ³	Limestone Sand Gypsum Fossil fuel Electricity	CO ₂ , SO ₂ , NOx emissions (global warming, acidification, eutrophication, photochemical smog and human health), natural resources (fuel, limestone), biodiversity (quarry)
Urea	—	200	kg	Ammonium Fossil fuel Electricity	Natural resources (fossil petrocarbons, fuel). Particles, NH ₃ , N _{tot} , NH ₃ , CO, CH ₄ . Positive for CO ₂ .
CaCl ₂	—	300	kg	Limestone Waste-HCl Thermal energy	Biofuel emissions, CO ₂ emission, natural resources (limestone), biodiversity (quarry and landfilled filter cake). Positive for wasteHCl.
Bacteria	—	?		Starting culture Growth media Water Heat and electricity	Natural resources (water), unknown impact from growth media, energy related impact.
Spoil	0.7	—	m ³	Landfill	Biodiversity (landfill)

5. Energy budget for jet grouting and bio-grouting for cement grouting of Stockholm road case and per m³ bio-grouting in Swedish kronor.

Table 18: Energy budget Comparison of Jet Grouting and Bio grouting

Description		Jet Grouting	Bio-Grouting	Jet Grouting	Bio-Grouting
Transport	Equipment	400	40	1700	160
	Material	1	5	3	23
Production	Materials	560	600	2300	2500
	Grouting	460	130	1900	540
TOTAL ENERGY REQUIRED		1400	800	6000	3200

6. Cost of grouting by jet grouting and bio-grouting. The table below shows the case study of cement grouting of Stockholm road case and per m³ bio-grouting in Swedish kronor.

Table 19: Cost Comparison of jet Grouting and cement Grouting

	Cost per m ³ Sealed soil (SEK)	
	Jet Grouting	Bio Grouting
Establishment	3500	330
Running Cost of Personnel and equipment	11900	8100
Materials and waste	3600	
Water	Included above	11
Bacteria	0	-
Urea	0	1200
CaCl ₂	0	860
TOTAL COST	19000	10500

4.5 Conclusions:

In summary, we found an economical benefit for bio-grouting compared to jet-grouting. This benefit will be even larger for smaller projects. Bio-grouting also seemed to have a lower environmental impact than cement grouting, but this could not be ascertained with certainty. For bio-grouting the contribution to environmental cost from production of raw materials is high, and the amount of raw materials could not be assessed with certainty. The environmental aspects that are affected are mostly those associated with the burning of fossil fuel: global warming, scarce natural resources, particle emission and human health, photochemical smog, etc. The case study involved long transport of equipment, since no jet grouting equipment is available in Sweden. There would be a great advantage to the environment if transport by train could be arranged.

RESULT ANALYSIS

5.1 Change in Calcium Carbonate Concentration with time:

The bacterial treatment done on the soil samples resulted in Calcium Carbonate Precipitation. This Calcium Carbonate filled up the pores between the soil particles thereby increasing its strength. Titration Tests were conducted at 2, 4, 6 and 7 weeks to find out the percentage of calcium carbonate present in the bio- treated samples. Following graph is obtained.

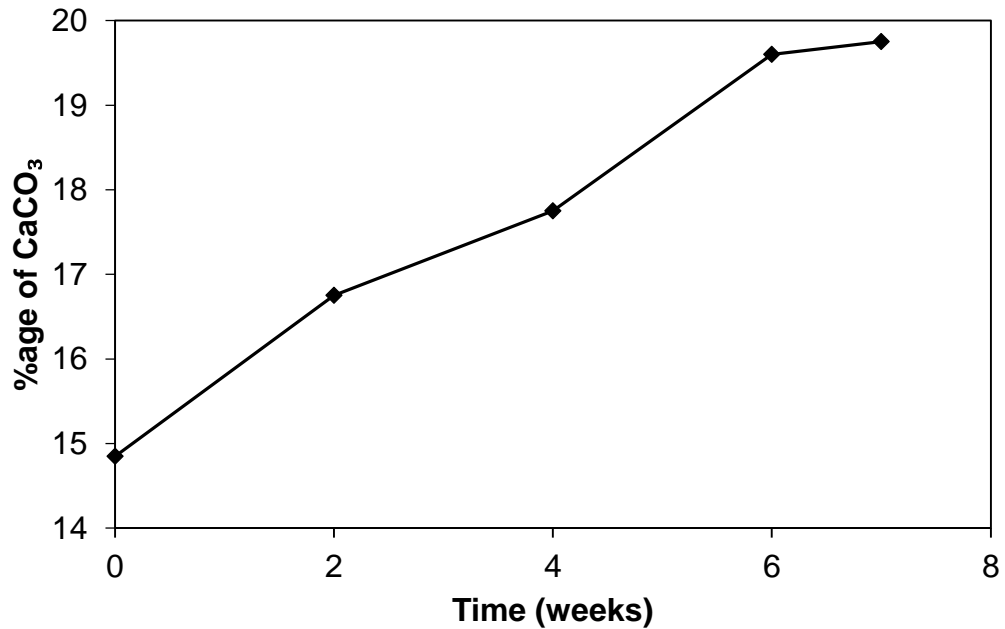


Fig 53: Percentage of CaCO₃ Vs Time

XRF tests conducted at the same samples showed the presence of Calcium Oxide in them. The percentage of Calcium Oxide obtained was equal to the percentage of Calcium Carbonate obtained through Titration. This suggests that the Calcium Oxide shown in the XRF results is basically Calcium Carbonate.

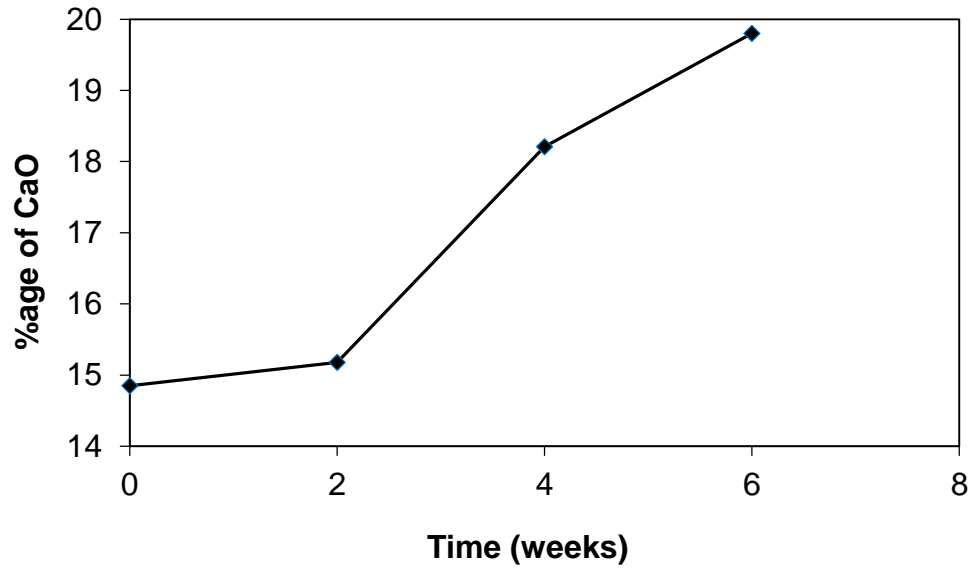


Fig 54: Percentage of CaO Vs Time

5.2 Change in Unconfined Compressive Strength with time:

This is the main property that has been changed under the process of bio-grouting. As the Undrained shear strength of sand is known to be zero but after the treatment by bio-grouting technique. The undrained shear strength has been changed form 0 kPa to 74.68 kPa.

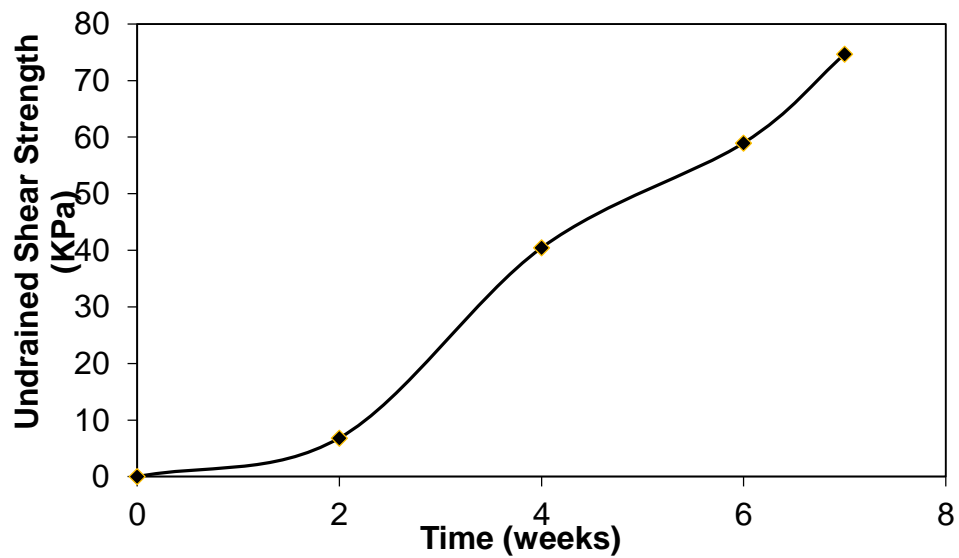


Fig 55: Undrained Shear Strength Vs Time

The un-drained compressive strength of sand is known to be zero because of no cohesive forces between the sand particles. But after the implementation of bio-grouting it has been increased to 149.36 kPa.

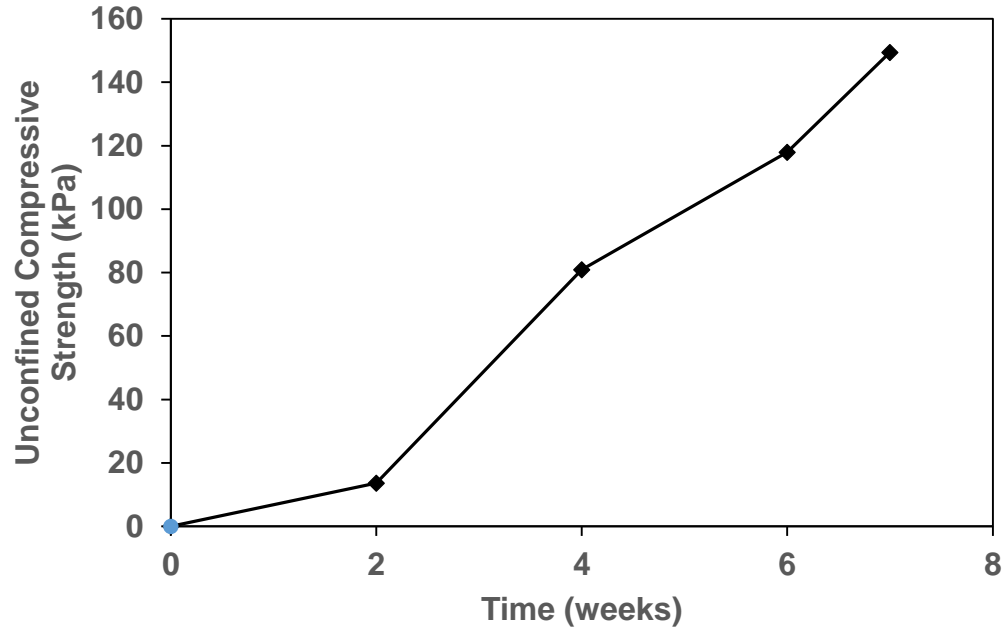


Fig 56: Unconfined Compressive Strength Vs Time

5.3 Change in Shear Strength of Soil with time:

Our sample had zero cohesion in the beginning since it is sandy soil. But after bio-treatment, the Calcium Carbonate precipitated started causing adherence between the soil particles. This increased the Cohesion values as shown by the following graph. The cohesion value achieved after 6 weeks is 11.32 KN per meter square.

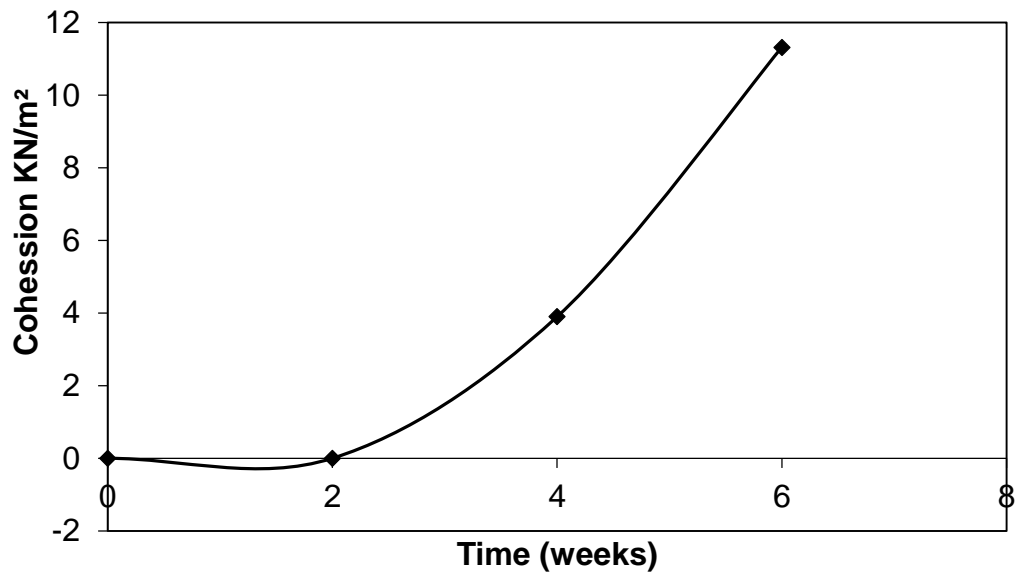


Fig 57: Cohesion Vs Time

Initially the angle of friction of our sand sample was 33 degrees. After the treatment by this technique, it has been increased from 33 degrees to 36 degrees. This increment shows the densification of sand sample with an increment in an amount of CaCO_3 .

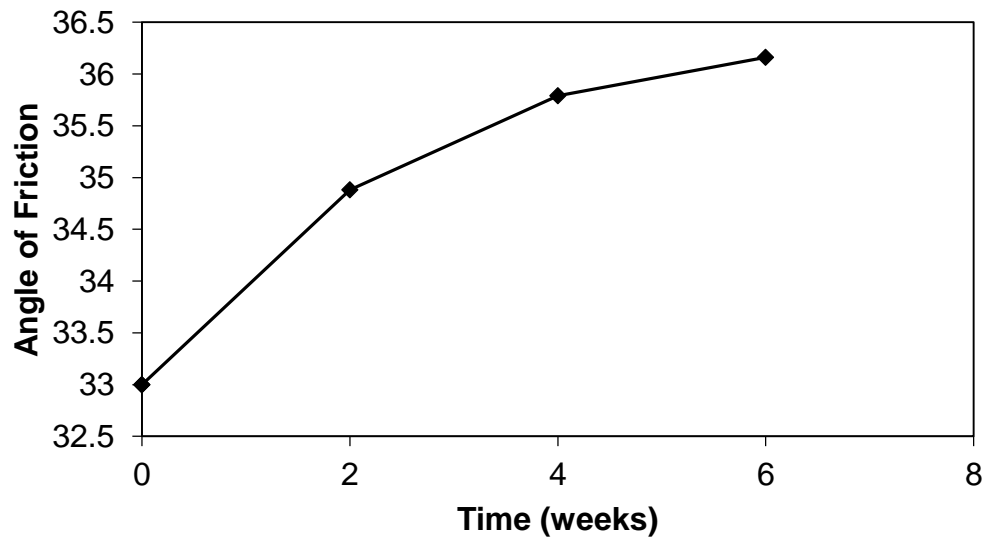


Fig 58: Angle of Friction Vs Time

This graph shows the results obtained after direct shear. At the vertical stresses of 50 KPa the increment is about 18 KPa, at the vertical stresses of 100 KPa the increment is about 20 KPa and at the vertical stresses of 200 KPa the increment is about 28 KPa.

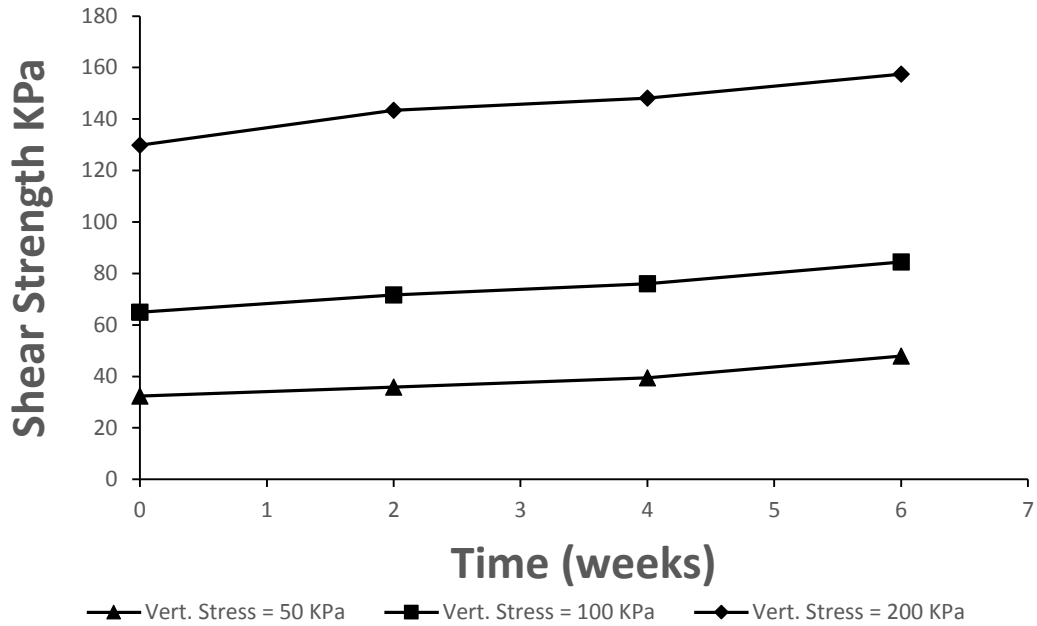


Fig 59: Shear Strength Vs Time

5.4 Change in Permeability with time:

The permeability of soil has been changed from 7.14×10^{-5} m/s to the value of 4.14×10^{-5} m/s. this change in permeability is not much but this slight change is in our favor. If the permeability decreased up to the level to clays and silts, it will lead to settlement issues.

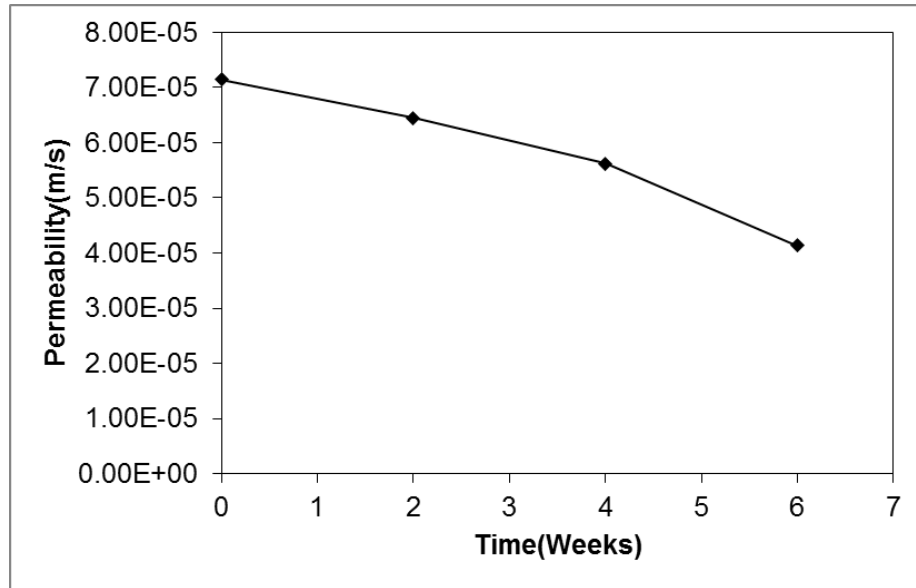


Fig 60: Permeability Vs Time

5.5 Change in %age of Volume of Voids with time:

This graph shows decrease in volume of voids in soil sample. This decrement is about 5% and the produced amount of CaCO_3 is also 5%. So it can be concluded that the amount produced is not wasted in any case.

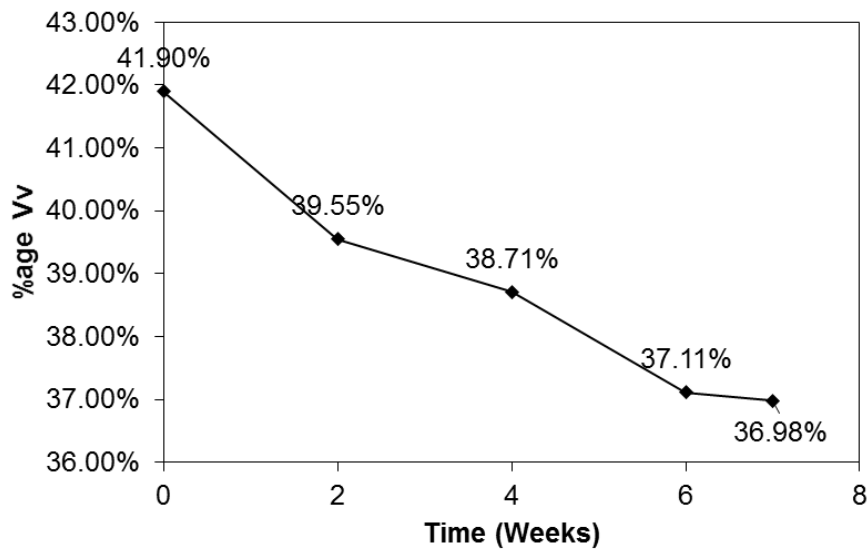


Fig 61: Percentage of Volume of Voids Vs Time

5.6 Verification Example:

After the results, a verification example has been done to check whether this technique can be implemented in field or not. A hypothetical model of an embankment is made with a same homogenous soil that we have treated. It is constructed at an angle 3 degree steeper than angle of repose. Than this model has been tested with the properties before injection and failed at FOS= 0.88. After that bio-cementation is done to the depth of 5 feet and safety factor is again calculated. Now it comes to be 1.3. Hence we can increase the FOS with an increment in Depth of treated area.

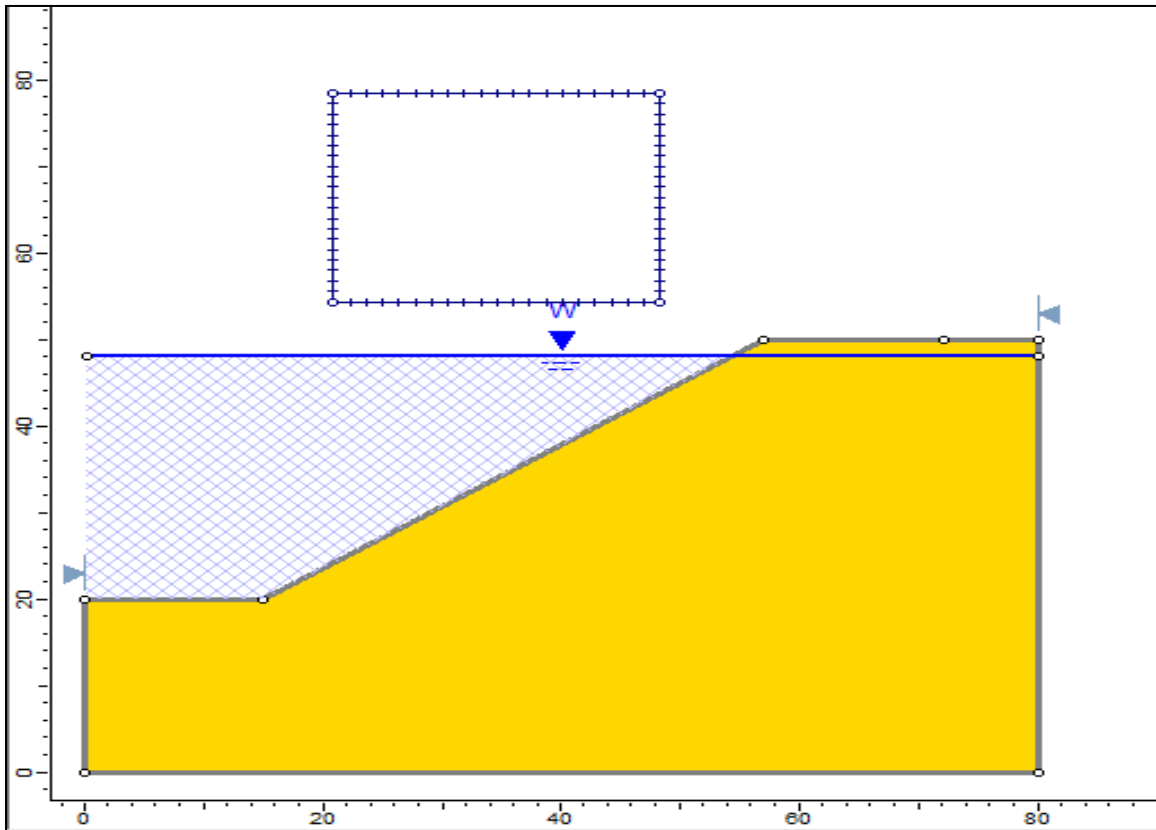


Fig 62: Hypothetical Model

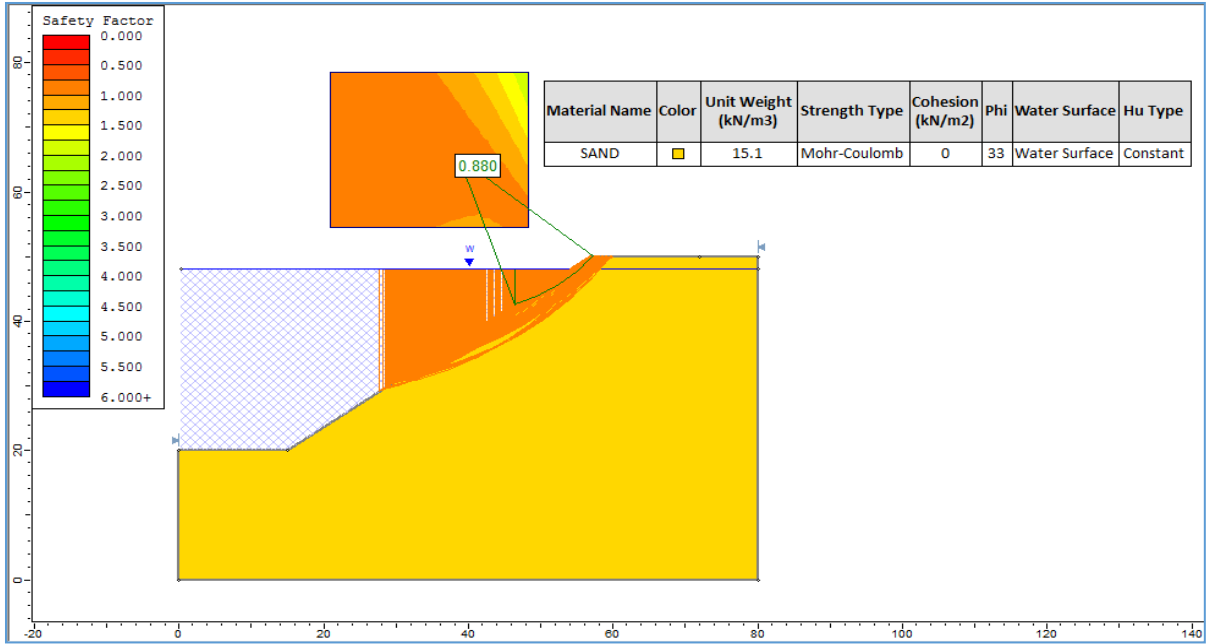


Fig 63: FOS before Bio-Grouting

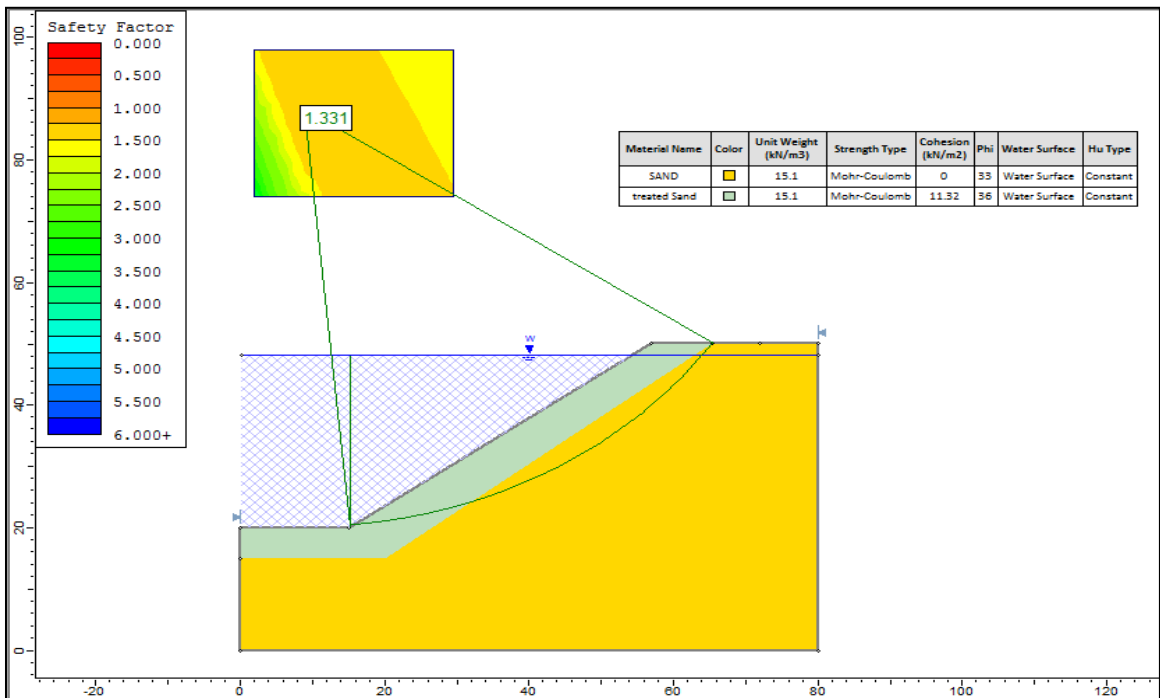


Fig 64: FOS after Bio-Grouting

5.7 Limitations:

Though it is very economical and environmental friendly process but it has certain limitations

- Bacillus pasteurii was used for bio cementation in all the literature we read. But Bacillus Pasteurii was not available to us. So, we made a little change we used another member of Bacillus family for our research.
- All the specified ingredients of bio-mineralized solution were not available i.e.(Corn steep liquor replaced by vitamins)
- We could not inject bacteria in bulk to our sample rather we injected to each of our test samples, because process was affected by soil disturbance.
- Syringes and pooling methods were used because of unavailability of peristaltic pump for injection of enrichment and bio-mineralized solution.
- It is slow process.
- Limited number of moulds for preparation of test samples.
- Limited availability of testing equipment of SEM and XRD.
- Microbial process is complex process as it depends on many factors such as bacteria type, bacteria and grain size compatibility, PH, Temperature, nutrients, metabolism activity of bacteria and many more.
- Bio-clogging and bio cementation requires data of
 - Growth
 - Biosynthesis
 - Biodegradation
 - Bio reduction
 - Bio oxidation
 - Specific enzymatic activities
 - Precipitation
 - Crystallization
 - Adhesion.
- All the testing is done in laboratory. It is not yet used for large ground improvement in the field.

- Limited literature was available due to lack of research.
- Uncertainty of results.
- Unavailability of mediums to detect the nomenclature of selected bacteria.

5.8 Findings:

- Instead of *Bacillus pasteurii*, we can also use other bacteria of bacillus family.
- Bacteria used for urease activity are non-pathogenic, Thus aquifer or ponds of water would not be polluted.
- Indigenous bacteria wouldn't be affected by environment because they were part of that environment.
- Bio-cemented can be carryout by stimulating the growth of bacteria in soil instead of extracting them and making their culture in laboratory.
- Undrained injection of bio-mineralized solution will precipitate more calcium carbonate than drained injection.
- Shear strength, Un-confined compressive strength and hydraulic conductivity of soil can be improved.
- Natural resources can be used as a source of urea and calcium chloride.
- Organic matter already present in soil can be used to stimulate the process.
- Disturbance of soil will affect the results.
- Pooling will affect the uniform precipitation of calcium carbonate (CaCO_3).
- Improvement of mechanical properties of soil wasn't linear.
- Increase in shear strength and unconfined compressive strength was mainly observed from 2nd week to 6th week.
- Decrease in permeability was not large enough to make the settlement issues of sand comparable with clay.
- Change in amount of CaCO_3 with time.
- By scanning electron microscope images showed the precipitation of CaCO_3 on sand particles.

5.9 Suggestions:

- With different concentration of CaCl₂, Strength changes must be checked.
- For uniform injection of solution other methods must be considered.
- Un-drained testing is required for comparison.
- This technique must be preferred on the basis of its economic and environmental benefits.
- More field research is required.
- Must be done on different gradations of soils
- No injection of bacterial culture.

5.10 Conclusions:

By keeping in mind all the biological, ecological, geo-chemical and geo-technical aspects in mind, we came to following conclusion.

- The soil under observation can bear three times more load. Before treatment it was able to bear load of 250 psf while after treatment it can bear load up to 750 psf. Load transformation has been shown in SLIDE model available in appendices.
- The results of mechanical properties under consideration are
 - (a) In Direct Shear, Drained cohesion changed from 0 to 11.32 kPa and friction angle has changed from 33 to 36. On vertical stresses of 50 kPa the increase in Shear strength is 16 kPa, at vertical stresses of 100 kPa the increase in Shear strength is 20 kPa and at vertical stresses of 200 kPa, the increase in Shear strength is 28 kPa.
 - (b) In Permeability test values has changed from 7.14×10^{-5} m/s to 4.14×10^{-5} m/s. the difference is that great enough and it is in favor of our technique. If the decrement was great in such a way that it enters into the limits of clays and silts, it may lead to settlement issues.
 - (c) In UCS, undrained compressive strength changed from 0 kPa to 149.36 kPa. Undrained shear strength changed from 0 kPa to 74.68 kPa. This mechanical property has shown the most effective results of this technique.

- From economic point of view, to mitigate the sand volume of 1 m³ with the strength of 150 kPa, Bio-grout costs about Rs. 439.45, Cement Grout costs about Rs. 1998.00 and the cheapest chemical grout costs about Rs. 525.00.
 - (a) So it is concluded that bio-grouting is 4.5 times cheaper than cement Grouting and 1.19 times cheaper than cheapest chemical grout.
- From Environmental point of view Bio grout has many advantages over cement and chemical grouting.
 - (a) The end product precipitated is non-toxic and insoluble in water.
 - (b) No acidification of soil because of usage of no acidic product.
 - (c) Bio-diversity leads to the variety in eco-system.
 - (d) Natural resources are used in bio-grouting while in cement and chemical grouting artificial and man-made resources are used.
 - (e) Moreover, this technique can be done in the presence of organic matter. This organic matter can be used as food for bacteria as well as it leads to the reduction in cost of nutrients that we have to provide to bacteria for rapid reproduction.

APPENDICES

APPENDIX A

TABLES AND FIGURES

Table 20(a): AASHTO Classification of Soil

General classification	Granular materials (35% or less of total sample passing No. 200)						
	A-1		A-3	A-2			
Group classification	A-1-a	A-1-b		A-2-4	A-2-5	A-2-6	A-2-7
Sieve analysis (percentage passing)							
No. 10	50 max.		51 min.	35 max.	35 max.	35 max.	35 max.
No. 40	30 max.	50 max.	51 min.	35 max.	35 max.	35 max.	35 max.
No. 200	15 max.	25 max.	10 max.	35 max.	35 max.	35 max.	35 max.
Characteristics of fraction passing No. 40							
Liquid limit	6 max.		NP	40 max.	41 min.	40 max.	41 min.
Plasticity index	6 max.		NP	10 max.	10 max.	11 min.	11 min.
Usual types of significant constituent materials	Stone fragments, gravel, and sand		Fine sand	Silty or clayey gravel and sand			
General subgrade rating	Excellent to good						
General classification	Silt-clay materials (more than 35% of total sample passing No. 200)						
Group classification	A-4		A-5	A-6		A-7	
				A-7-5 ^a		A-7-6 ^b	
Sieve analysis (percentage passing)							
No. 10			36 min.	36 min.	36 min.	36 min.	
No. 40			36 min.	36 min.	36 min.	36 min.	
No. 200			36 min.	36 min.	36 min.	36 min.	
Characteristics of fraction passing No. 40							
Liquid limit			40 max.	41 min.	40 max.	41 min.	
Plasticity index			10 max.	10 max.	11 min.	11 min.	
Usual types of significant constituent materials			Silty soils			Clayey soils	
General subgrade rating	Fair to poor						

^aFor A-7-5, $PI \leq LL - 30$

^bFor A-7-6, $PI > LL - 30$

Table 20(b): AASHTO Classification of Soil

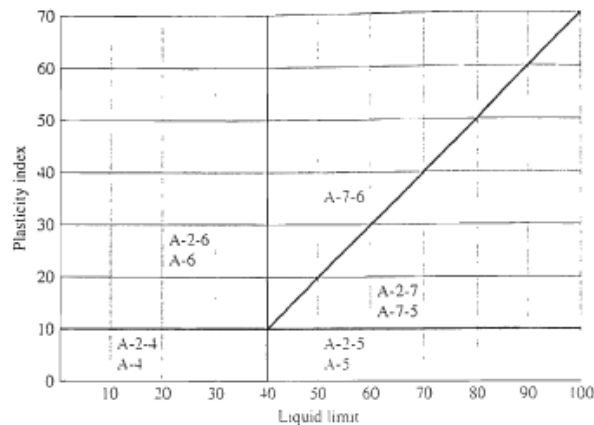


Table 21(a): Soil Classification according to ASTM Standards

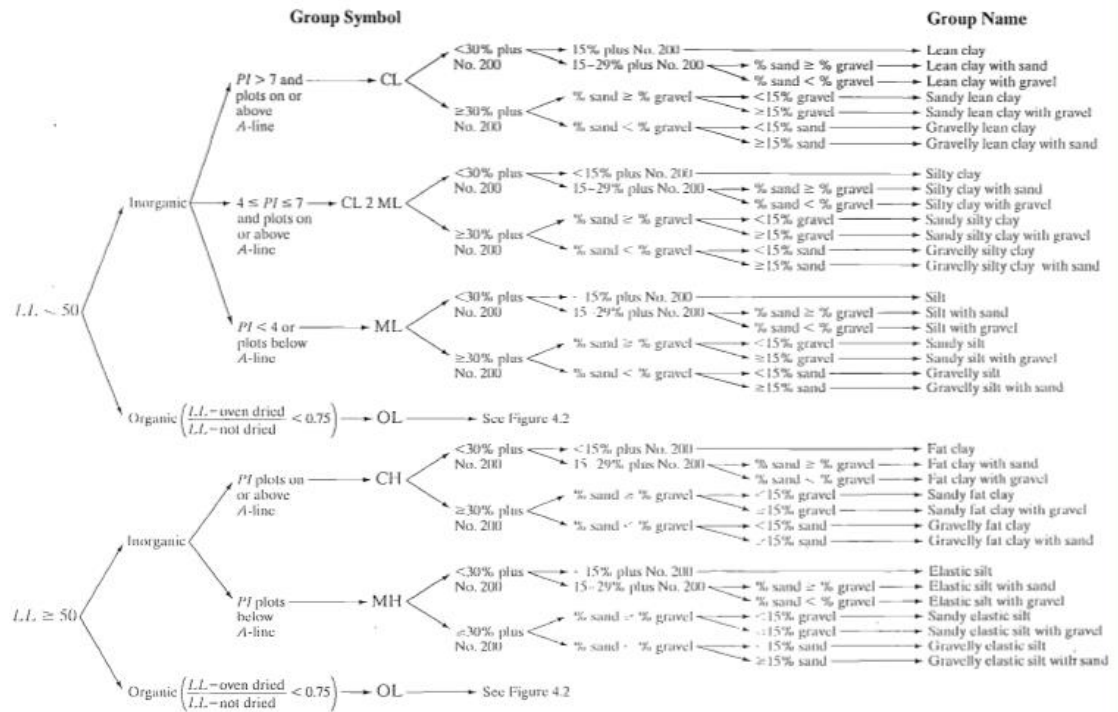


Table 21(a): Soil Classification according to ASTM Standards

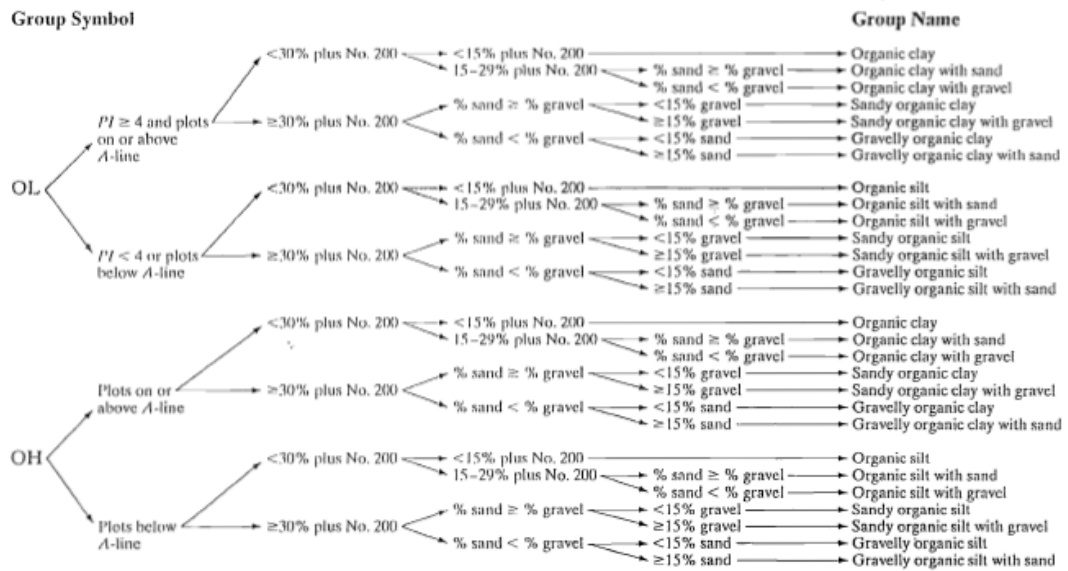


Fig 65: Graph showing Soil Gradation

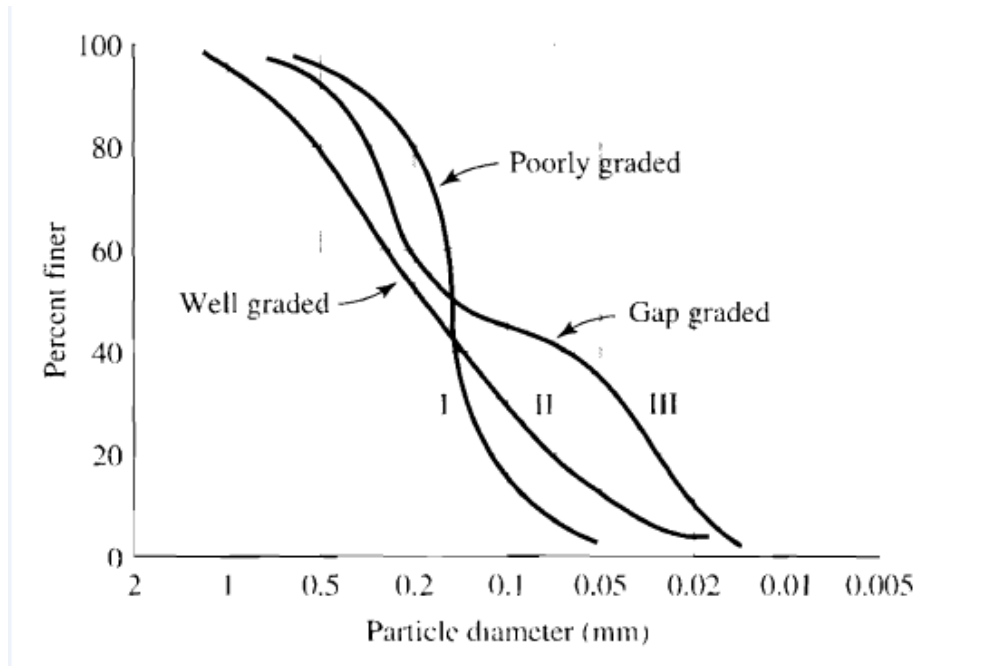


Table 22: Hydraulic Conductivity of Different Soil Types

Soil type	k	
	cm/sec	ft/min
Clean gravel	100-1.0	200-2.0
Coarse sand	1.0-0.01	2.0-0.02
Fine sand	0.01-0.001	0.02-0.002
Silty clay	0.001-0.00001	0.002-0.00002
Clay	<0.000001	<0.000002

Table 23: Angle of Friction of Different Soil Types

Soil type	ϕ' (deg)
<i>Sand: Rounded grains</i>	
Loose	27-30
Medium	30-35
Dense	35-38
<i>Sand: Angular grains</i>	
Loose	30-35
Medium	35-40
Dense	40-45
<i>Gravel with some sand</i>	34-48
<i>Silts</i>	26-35

Table 24: Void Ratio, Moisture Content and Dry Unit Weight for some typical Soils in Natural State

Type of soil	Void ratio, e	Natural moisture content in a saturated state (%)	Dry unit weight, γ_d	
			lb/ft ³	kN/m ³
Loose uniform sand	0.8	30	92	14.5
Dense uniform sand	0.45	16	115	18
Loose angular-grained silty sand	0.65	25	102	16
Dense angular-grained silty sand	0.4	15	121	19
Stiff clay	0.6	21	108	17
Soft clay	0.9–1.4	30–50	73–93	11.5–14.5
Loess	0.9	25	86	13.5
Soft organic clay	2.5–3.2	90–120	38–51	6–8
Glacial till	0.3	10	134	21

Table 25: Qualitative Description of Granular Soil Deposits

Relative density (%)	Description of soil deposit
0–15	Very loose
15–50	Loose
50–70	Medium
70–85	Dense
85–100	Very dense

Fig 66: Load Bearing Capacity of Soil before Bio-Grouting

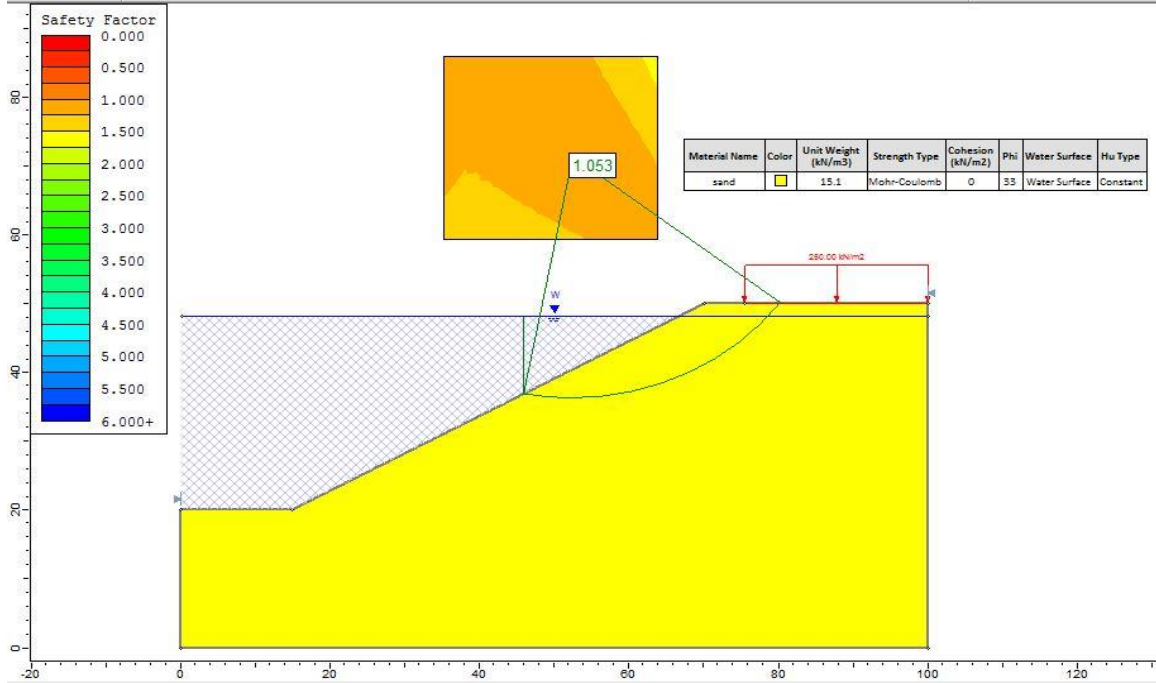
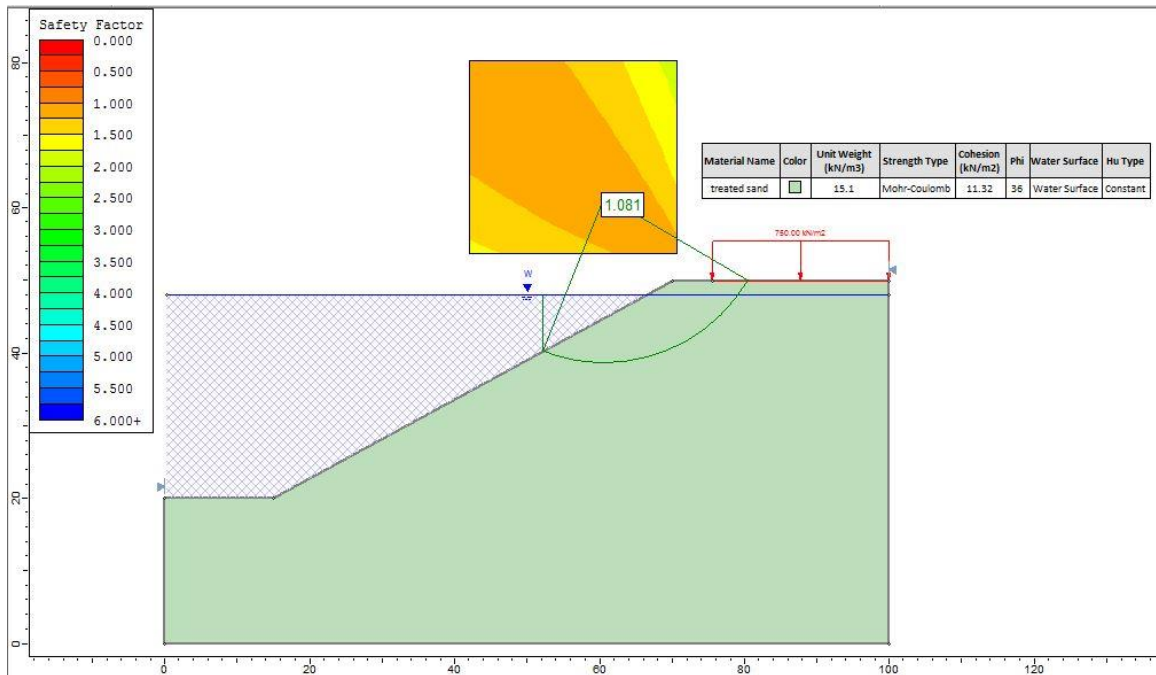


Fig 67: Load Bearing Capacity of Soil after Bio-Grouting



APPENDIX B

NOTATIONS

C' Drained Cohesion of Sand

Φ Angle of Friction of Soil

S_u Undrained Cohesion of Sand

q_u Unconfined Compressive Strength of Soil

k Hydraulic Conductivity of Soil

e Void Ratio

n Porosity

γ Unit Weight of Soil

G_s Specific Gravity

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