SOIL STABILIZATION BY BACTERIAL CEMENTATION

KRISHNA KUMARI[1], A.AISHWARYA[2], RUBINA SWEETLIN[2]
PROFESSOR[1], STUDENT[2], DEPARTMENT OF CIVIL ENGINEERING,
PANIMALAR ENGINEERING COLLEGE, CHENNAI.

ABSTRACT: The challenges to enhance the weak soil constantly lay a need for research and exploration to flourish a new and advance method for soil stabilization. The feasible technique is Bacterial cementation which has evolved recently and raised a sustainable technique for soil improvement. This paper aims to analyze the potency of MICP *(*Microbiologically induced calcium carbonate precipitation) in developing shear strength and lowering the hydraulic conductivity of soil. Laboratory has been done to certify the effect of Biogrout *(*Bacterial Cementation) process on the strength of 2 types of sand *(High Plastic clay and Low plastic clay*). The species of *Bacillus* group*, B.magaterium* was utilized to activate the calcite precipitation. The strain of Bacillus group was utilized to trigger and catalyze the calcite precipitation due to chemical reaction of calcium chloride and urea. MICP utilizes bacteria to hydrolyze urea and provide carbonate ions and that reacts with the calcium chloride solution which provides calcium carbonate *(calcite*) that binds and holds the soil particles together leads to increase the strength of soils and raise stiffness. It was also found that the strength developed with advancement in treatment duration. The outcome of this experiment was evidently shows from the pictures of Scanning Electron Microscope *(SEM*).

Keywords: *B.megaterium*, Calcite precipitation, Bacterial cementation, Shear strength, Hydraulic Activity.'

1. INTRODUCTION

Presently, construction on controversial soil is unavoidable on account of the increasing paucity of land worldwide. Consequently growth on the controversial soils is extremely susceptible to hazards on soil comprises imprudent settlement of embankment or foundation debris flow and catastrophic landslide. The majorcharacteristics of soil development comprise: developing the shear strength of soil, decreasing the potentiality for total and differential settlement, decreasing the time duration which the settlement occur, lowering the potential for liquefaction in saturated fine sand or hydraulic fills,

decreasing the hydraulic conductivity of soil, removing water from the soil etc (Kazemian and Huat, 2009; Krebs andWalker, 1971; Leonards, 1962).Traditionally, the rule is to remove low strength soil deposits with engineering fill. Chemical stabilizers are typically used in conjunction with shallow and deepcombining or injecting chemical grouts that can penetrate through soils to artificially cement soil particles for soil stabilization. Physical characteristics of soil can be changed by the utilization of mechanical compaction or compaction grouting while chemical characteristics of soil can be changed by the utilization of chemicalstabilizers includes Portland cement, lime and fly ash. Mechanical compaction isproposed for sandy soils and is cost- effective to a depth less than 10 m (Ivanonv & Chu, 2008). Chemical stabilization is usually suggested for expansive soils (Petry & Little, 2002). Environmentally secured practices include pre-wetting and moisture barriers are only practicable for small restricted spaces, and are not fit for larger construction works includes highways and railways which spread for miles. Traditional ground improvement processes have many restrictions. Peak pressures are essential to inject the grouts because of their rapid hardening time or high viscosity. Freezing isof course not a definite solution in the course of construction. Most of these techniques are high-priced, muddling urban infrastructure, require heavy machinery, and demand chemicals with notableenvironmental impact. Accordingly, these standard techniques are not fit for treating huge volumes of soil. Artificial cementation methods are never possible and eco-friendly.However, decline in the utilization of artificial cementation methods can be done by inclusion of eco-friendly techniques.

One of the techniques of soil stabilization is,Microbial Induced Calcite Precipitation (MICP). MICP technique is a finer and moreeco-friendly substitution to the conventional technologies. This method uses microbes asa primary element for stabilization. Calcium carbonate precipitation has been persuaded into the soil structure by microorganism through their metabolic activity to develop the engineering properties of soil. Accordingly, this method is called as microbial induced carbonate precipitation or MICP. Successful application of MICP will have its application in an ample mix of civil engineering fields includes firmness of retaining walls, embankments and dams, regulating soil erosion, sustaining cohesion less soils to enable the firmness of underground constructions, raising bearingcapacity of shallow and piled foundation anddecreasing the liquefaction potential of soil.

2. THEORETICAL BACKGROUND

Nutrients, temperature, pH, H_2O, presence of the organic contaminants and heavy metals, space of solids, the concentration of dissolved organic carbon, and concentration of Ca ions are the factors governing the growth of bacteria and the precipitation of $CaCO_3$. Stocks-Fisher et al. (1999) have that$CaCO_3$ precipitation came to high level at a pH of 8. Nemati and Voordouw (2003) expressed that, at reduced concentration of enzyme (0.03 g=1), a raise of temperature from 20 to 50^{0}C enhanced the precipitation of calcium carbonate. It can be abridged thatthe calcite=$CaCO_3$ precipitation method relays upon 4 things: Ca ion concentration; dissolved inorganic carbon concentration; pH; and accessibility of nucleation sites. Moreover, environmental circumstances include salinity, temperature, and nutrients also control $CaCO_3$ precipitation. The bacterial precipitation of $CaCO_3$ can be abstracted as follows: Numerous bacteria make enzyme urease from their bacterial activity. The enzyme urease generated bybacteria decomposes urea (CO $(NH_2)_2$) inthe soil through a chemical reaction calledas hydrolysis of urea:

$$(CO(NH_2)_2 + 2H_2O \rightarrow 2NH_4^+ + CO_3^- \quad (1)$$

The ammonium (NH_4^+) freed from urea hydrolysis cause raise in pH and activates the precipitation of calcium carbonate. The high pH raises the propensity for bacteria to work as a nucleation area for $CaCO_3$ crystals. Calcite is precipitated through the amalgam of carbonate ions (CO_3^{2-}) from the hydrolysis of (CO $(NH_2)_2$) and the $Ca2^+$ ion from calcium chloride nutrients. Since the bacteria's cell wall is negatively charged,the bacteria draw out the cations from the environment, together with Ca^{2+}, to deposit them on the surface of the cell, as shown in Eq. (2), directing to the precipitation of calcium carbonate.

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

3. MATERIALS REQUIRED

A. SOIL SPECIMEN

The two varieties of soil involved in this research were tropical residual soil and sand.The soil specimen was acquired from a area in University Tunku Abdul Rahman, whereas the sand specimen was of typical concrete sand. Table 1 represents the values of physical indices acquired from the standard soil properties experiments. The particle size distributions of soil are represented in Fig. 1. Depending on the BS soil classification system, the tropical residual soil specimen was categorized asSandy Silt, whereas the sand specimen was categorized as Well Graded Sand.

Table 1. Physical Properties of the Soil Specimens

	Residual Soil (Sandy Silt)	Sand
Composition		
Gravel (%)	0	2
Sand (%)	29	96
Silt (%)	55	2
Clay (%)	16	0
LL (%)	58.0	-
PL (%)	44.3	-
PI	13.7	-
Soil Classification BSCS	MHS	SW
Maximum Dry Density (MDD)	1563 kg/m^3	-
Maximum Index Density (ρ_{max})	-	1842 kg/m^3
Minimum Index Density (ρ_{min})	-	1439 kg/m^3

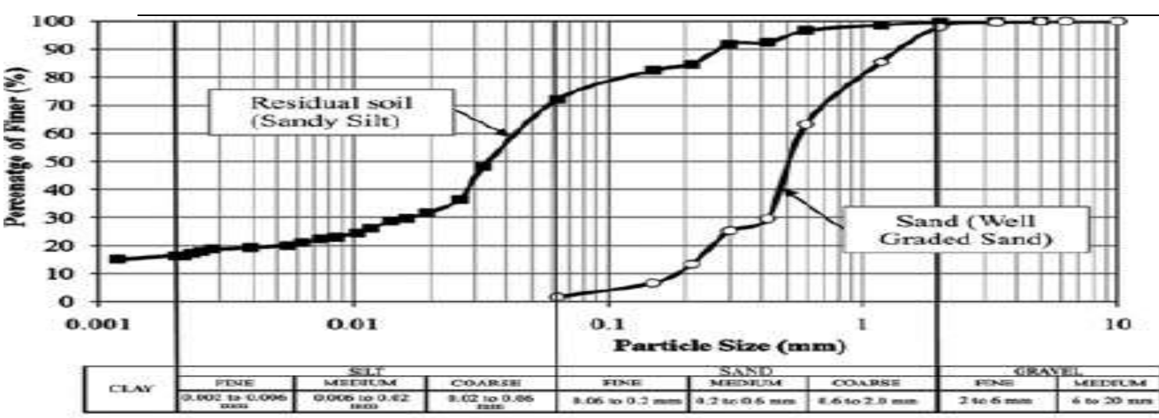


Fig. 1. Particle Size Distributions of the Soil Specimens

B.MICROORGANISM

The urease-fabricating microorganismutilizes in this experiment was *B.megaterium ATCC 14581*. *B.megaterium*, Gram positive, rod shaped soil bacterium of diameter 2 µm to 5 µm. It can be seen in various habitats from rice paddies to dried food, seawater sediments, fish, and even in honey bee. The duty of *B. megaterium* is to manufacture urease enzyme through its metabolic activity under right cultivation method. The urease enzyme activates the microbiologically induced calciumcarbonate precipitation by hydrolyzing urea by the following reaction:

$$CO\,(NH_2)_2 + 3H_2O \rightarrow 2NH_4^+ + HCO_3^- + OH^-$$

The ammonium (NH^{4+}) raises the pH and makes the bicarbonate (HCO^{3-}) to precipitate with ($Ca2+$) from the calciumchloride furnished in order to obtain the $CaCO3$:

$$Ca^{2+} + HCO_3^- + OH^- \rightarrow CaCO_3 + H_2O$$

The calcite produced is accountable for cementing and clogging of the soil sample. Bacterial cementation is obtained when the CaCO3 crystals precipitate on the surface or construct bridges between the prevailing soilgrains. These CaCO3 crystals, bind the soil particles and prohibit motion of the grains, and consequently increase the strength and stiffness characteristics of the soil (Harkes *etal*., 2010). Bioclogging, on the flip side, is the evolution of pore-filling materials (calcite) through bacterial activity and later results in a declination of soil porosity and hydraulic conductivity.

C. CEMENTATION REAGENTS

The MICP process uses cementation reagents as the raw materials for calcite formation. The cementation reagents used inthis analysis were a 0.25M solution and calcium chloride (CaCl2). Besides, the cementation reagents also have 3 g nutrient broth, 10 g ammonium chloride, and 2.12 g sodium bicarbonate per litre of deionized water. Every chemicals products utilized were of Analytical Reagent grade.

4. PREPARATION AND TREATMENTPROCESS

The soil samples were air-dried in the laboratory at room temperature for 1 week prior to MICP treatment. The desired densities were obtained for the residual soil specimens by compacting the soils into prefabricated moulds with the moisture content corresponding to the desired density calculated from the compaction curve. The desired densities for the sand specimens were achieved by layering a predetermined amount of sand specimens. All of the soil specimens were saturated after compactionto ensure a constant flow of cementation reagents through them during the MICP treatment.

MICP can be accomplished by combining urease-producing microorganisms with external sources of urea and calcium chloride solution in the soil. The technology has been successfully applied in a variety of engineering fields, including sand column consolidation, concrete strengthenhancement, and concrete crack repair, among others. To prevent rapid calciteprecipitation and subsequent clogging near the specimen mould's inlet, the rate of calcite precipitation should not be too high. Since the specimen would take a long timeto cement, the precipitation rate should not be too low. At least 60 kg calcite per meter cube of soil must be precipitated to achieve a substantial increase in strength for loose granular sand. This equates to around 2 mol CaCO3 precipitate per litre of pore space, and researchers discovered that this amount of precipitated calcite can be obtained by injecting one pore volume of 0.25 M CaCl2/ urea solution into the soil specimen at 6- hour intervals for a treatment period of 38- 48 hours. In this analysis, B. megaterium was grown in nutrient broth (pH 7.0 prior to autoclaving) at 130 rpm and 37oC incubation temperature. The spread plate technique was used to assess theconcentration of B. megaterium. On the incubated culture, serial dilution was performed. After 16 hours of incubation, the diluted culture was placed on the surface of an agar plate, and the colonies were counted.Following that, the soil mixture was compacted to the desired densities with the addition of sufficient moisture material. To saturate the soil specimens and fix the B. megaterium homogeneously in the soil specimens, a fixation fluid (50mM CaCl2) was flushed through them. Over the course of 48 hours, one pore volume of 0.25M CaCl2/urea solution was injected into the soil specimens at 6-hour intervals. The flow velocity was kept steady at about 1.7 105 m/s. This was achieved by adjusting the air compressor's pressure. The reagent solution in the pressure tank was compressed by the pressurized air, resulting in a hydraulic gradient between the inlet and outlet of the soil specimen mould. For all soil specimens, this treatment configuration was used.

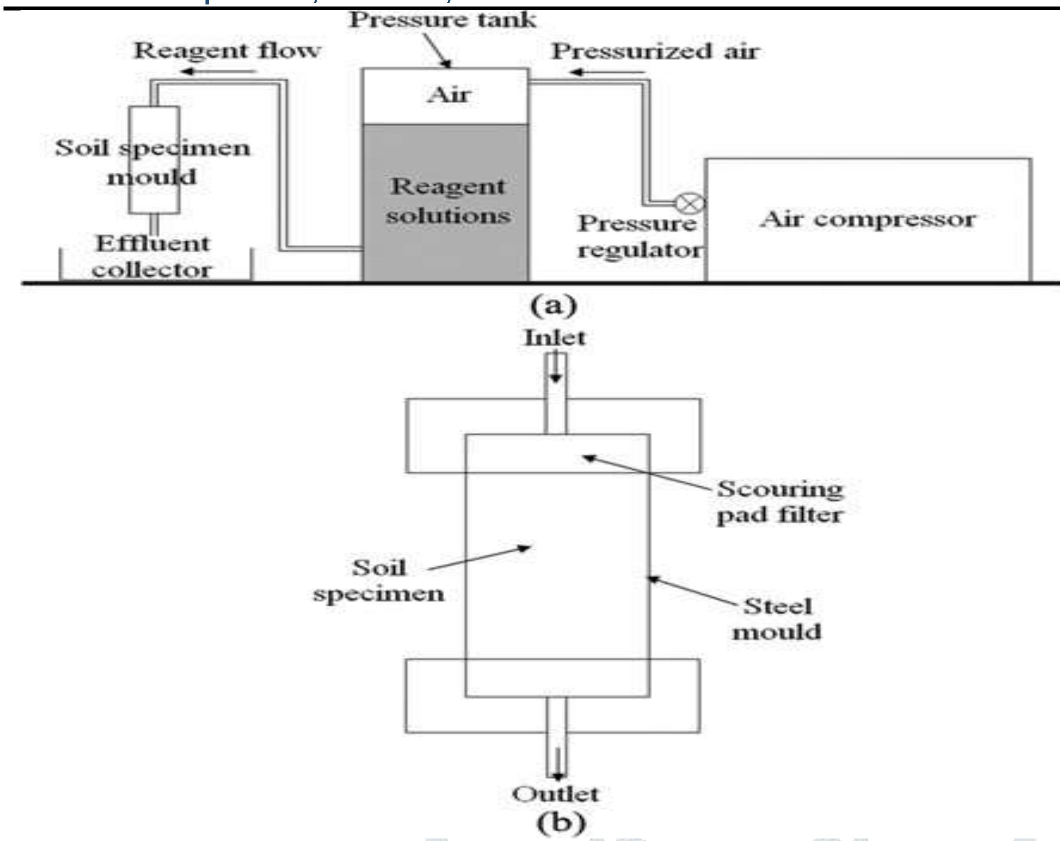


Fig. 2. (a) Schematic Diagram of Laboratory Setup
(b) Soil Specimen Mould

5. EXPERIMENTAL RESULTS

A. SHEAR STRENGTH OF RESIDUAL SOIL

The peak stress of the soil that yields the axial strain of 20% is defined as theunconfined compressive strength, (qu) of thesoil. In study the residual soil's shear strength is characterized by undrained shear strength parameter which is taken as half of the UCC strength. 0.85RT, 0.90RT 0.95RT are the improvised densities of the residual soils treated by the shear strength of MICP. The improvement ratio of shear strength is

increased with density. The improved parameter in undrained shear strength is 1.11, 1.25 and 1.33 for the specimen of 0.85RR, 0.90RR and 0.95RR respectively. The naturally inhibited microorganisms of soil deposit trigger the result of MICP. The specimen treated by B. megaterium and cementation reagents shows lower improvements. Hence, this shows that thebiomass is ineffective in improving the residual soil's shear strength

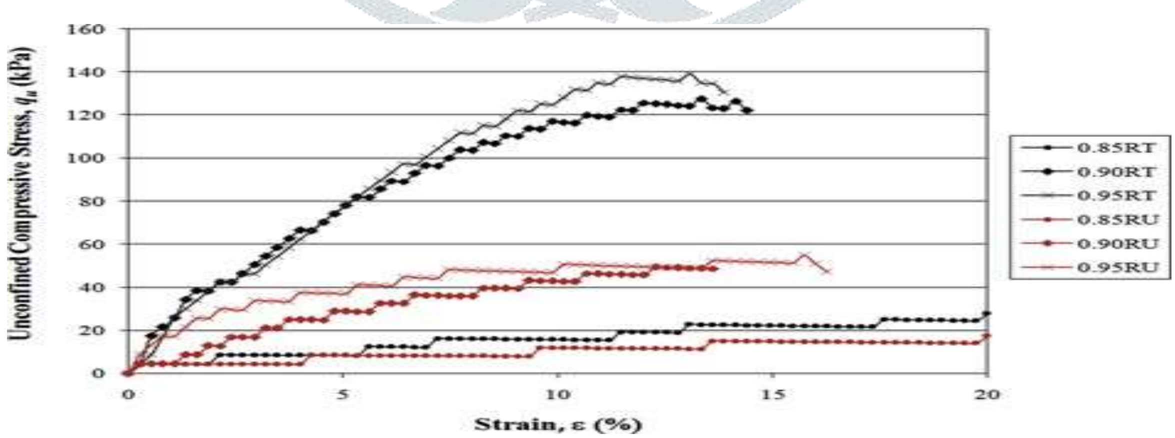


Fig. 3. Stress-strain Curve for Residual Soil Specimens

B. SHEAR STRENGTH OF SAND

The effective internal friction angle (φ ') is used in the study of dry sand's shear strength. Between 39.90 and 48.80 are the effective internal friction angles for untreated sand specimen. The MICP-treated dry sand specimen internal friction angles are higher than untreated specimen. The ratio of improvement decreases with increase in density. The observation of sand specimen is opposite to the observation of residual soil.

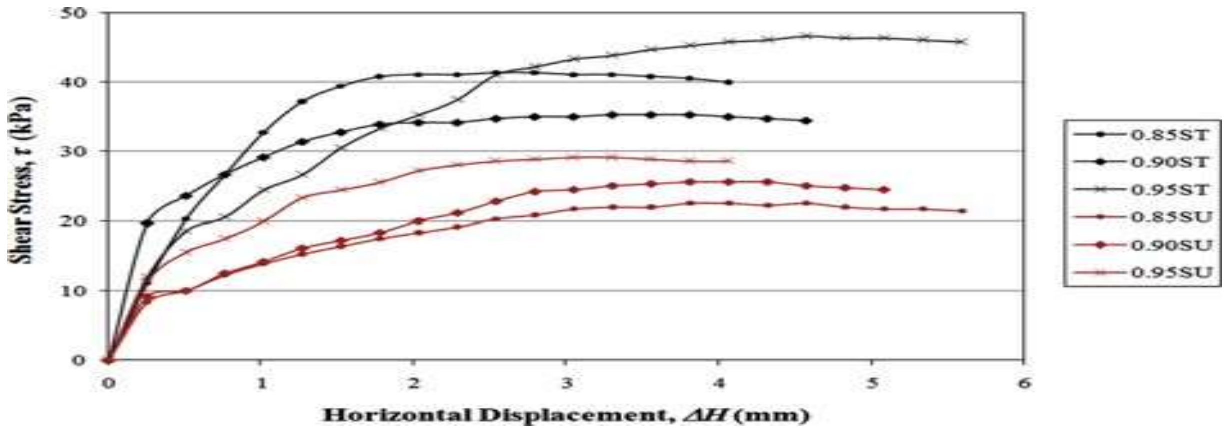


Fig. 4. Stress-strain Curve for Sand Specimens

C. HYDRAULIC CONDUCTIVITY OF RESIDUAL SOIL

The MICP-treated soil of saturated hydraulicconductivity has reduction for all density.The disparity in hydraulic conductivity between untreated saturated hydraulic conductivity specimens (0.85RU, 0.90RU,and 0.95RU) and specimens treated with B. megaterium and cementation reagents (0.85RT, 0.90RT, and 0.95RT) shows that calcite infliction decreases hydraulic conductivity. The ratio of reduction for 0.90RT and 0.855RT specimens is 60% and 55% respectively. The result confirms that the calcite precipitating microorganism is found naturally in the residual soil. The specimen without cementation reagent supply but treated with microorganism only has no significant change in hydraulic conductivity. This is due to the clogging of pores in the formation of calcite of residual soil.

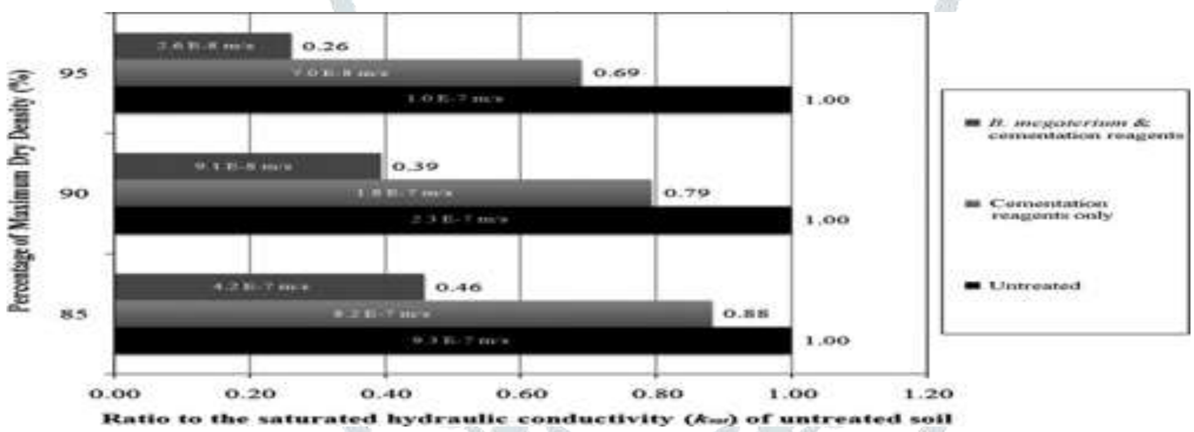


Fig. 5. Saturated Hydraulic ConductivityReductions of Residual Soil Specimens

D. HYDRAULIC CONDUCTIVITY OF SAND

By observing the sand specimen's shear strength there is less reduction in hydraulic conductivity with increase in density. The hydraulic conductivities are reduced to an average of 6% to 7%. Therefore, when treated only with microorganism the reduction in hydraulic conductivity of sand becomes negligible.

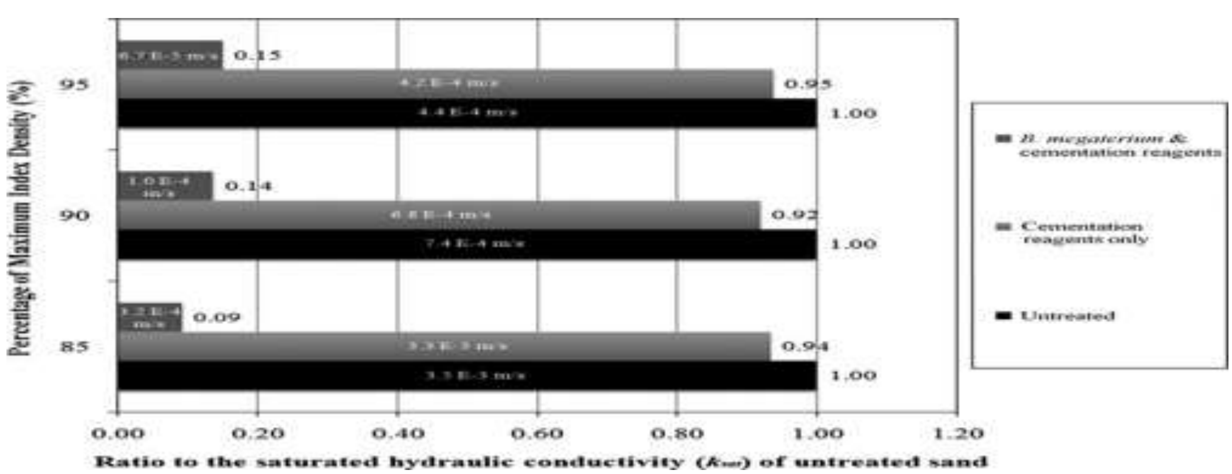


Fig. 6. Saturated Hydraulic ConductivityReductions of Sand Specimens

E. SCANNING ELECTRON MICROSCOPY ANALYSIS

The calcite precipitation in the soil specimens was visualized using Scanning Electron Microscope (SEM) imagery on the selected soil specimens. The Hitachi S- 3400N Scanning Electron Microscope was used to capture the SEM images. The tests on the formation of calcite crystals after MICP treatment were of particular interest. The SEM images of residual soil specimens treated under three different conditions, namely untreated, cementation reagents only and B. megaterium and cementation reagents. The untreated specimen had relatively smooth particle surfaces. Some calcite crystals were observed on the soilparticles after treatment with cementation reagents only. Calcite crystals were abundant in specimens treated with B. megaterium and cementation reagents. Close inspection revealed rod-shaped B. megaterium in close proximity to the calcite crystals. SEM images of sand specimens revealed similar patterns. In comparison, the amount of precipitated calcite crystal in the sand specimen treated only withcementation reagents was less than that observed in the residual soil specimen. Closeinspection revealed rod-shaped B. megaterium in close proximity to the calcite crystals. SEM images of sand specimens revealed similar patterns. In comparison, theamount of precipitated calcite crystal in the sand specimen treated only withcementation reagents was less than that observed in the residual soil specimen. This observation confirmed the results of the shear strength and hydraulic conductivity tests, which showed that naturalmicroorganisms only existed in traceamounts in the sand specimens and thus induced minor changes in their properties.

Fig. 7. Scanning Electron Micrograph (SEM) showing the formation of Crystals of $CaCO_3$

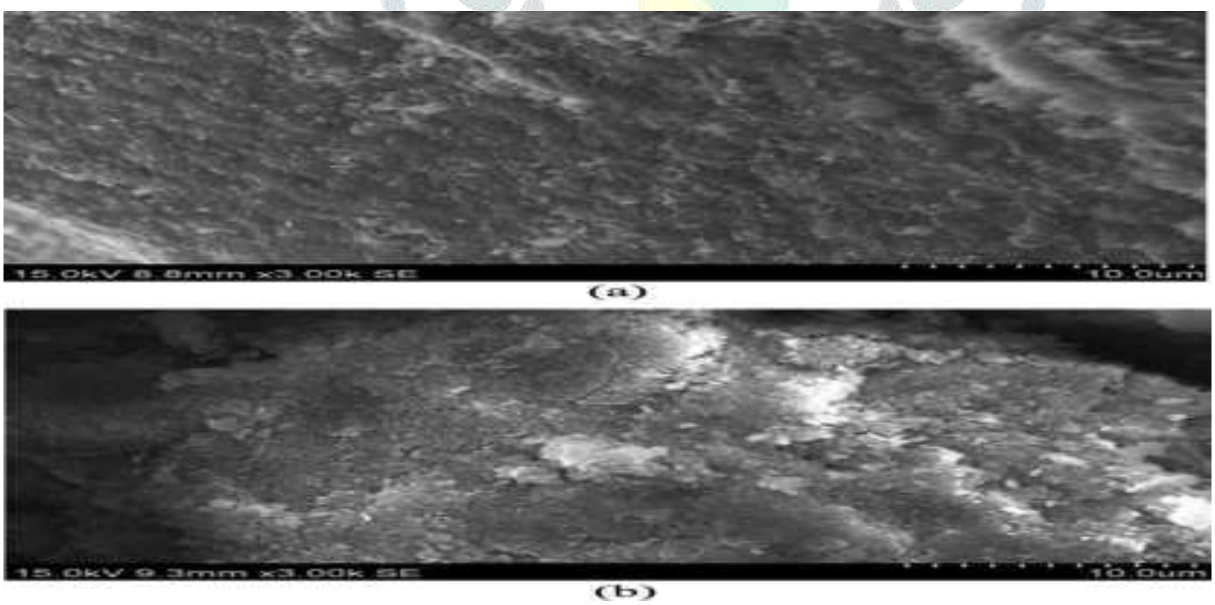
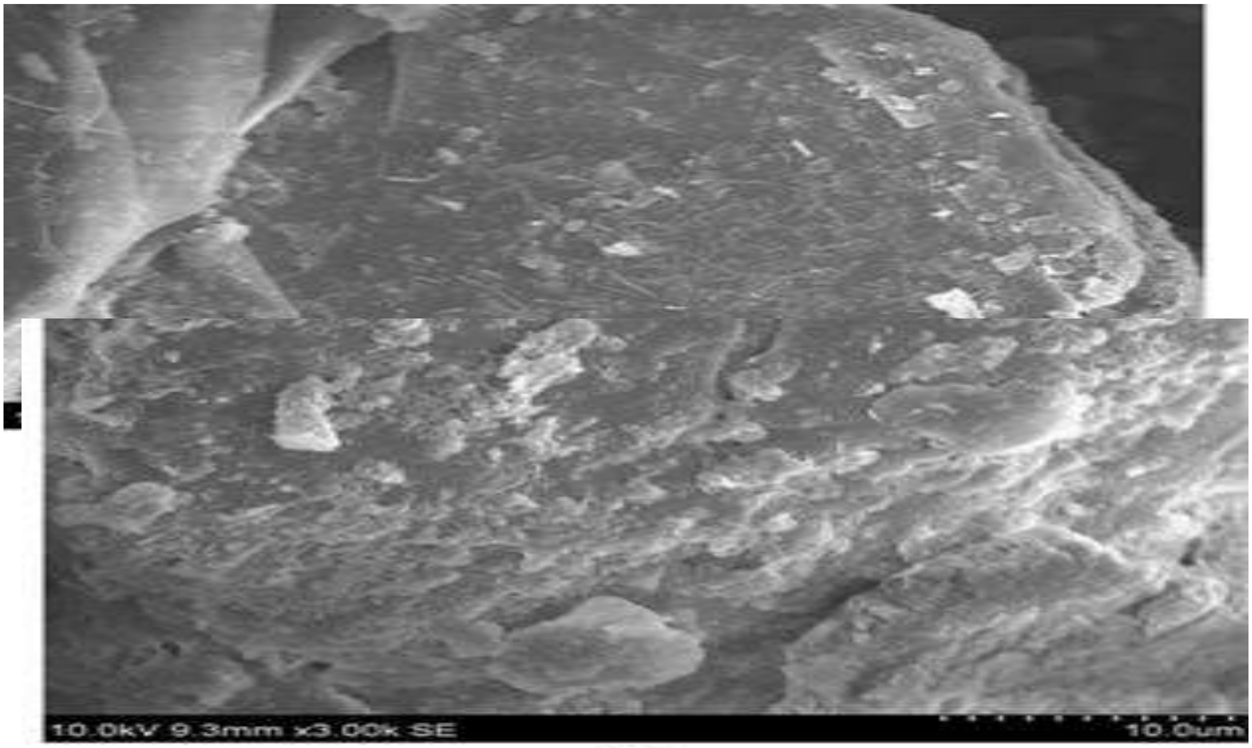
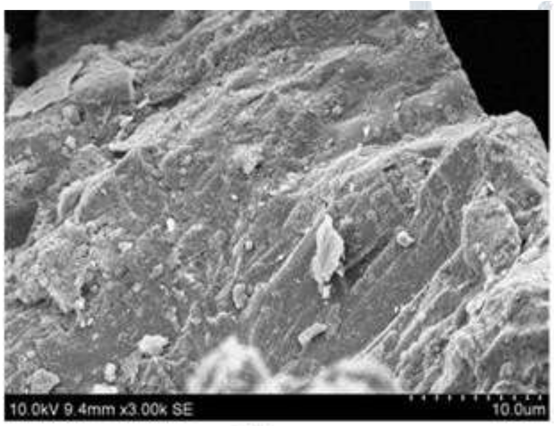


Fig. 8. SEM Images for the Residual SoilSpecimens: (a) Untreated, (b) Treated with Cementation Reagents Only.



(c)



(b)

Fig. 9. SEM Images for the Sand Specimens:
(a) Untreated,
(b) Treated with Cementation Reagents Only,
(c) Treated with *B. megaterium* and CementationReagents

6. RESULTS AND CONCLUSIONS

The use of microbially induced calciteprecipitation to improve soil shear strength and minimize hydraulic conductivity has been demonstrated. Increased soil strength can help to increase ground bearing capacity, while decreased hydraulic conductivity can help to reduce settlement, shrink well propensity, seepage, and rainfall penetration into soils. MICP was found to bemore effective in improving shear strength in residual soil than in sand in the experiments. The sand specimen was found to be more effective than the residual soil in terms of reducing hydraulic conductivity. Increased density resulted in increased shearstrength and reduced hydraulic conductivity of residual soil. The sand specimens, on the other hand, showed the opposite pattern. The hydraulic conductivity reduction ratio of the sand specimen is higher than that of the residual soil. Sand has a higher porosity, which explains this. Greater porosity means more pore space is sufficient for B. megaterium to deposit calcite, resulting in a lower hydraulic conductivity. The inadequate concentrations of particle- particle contacts per unit volume can explain the relatively low improvements in shear strength of sand compared to residual soil. This is due to the presence of coarser granular particles in the sand specimen. In comparison to the residual soil specimen,there are less interactions between coarseparticles. The particle-particle interactions may also explain why increased density of residual soil improves shear strength and reduces hydraulic conductivity. In comparison, the residual soil specimensimprove marginally more than the sand specimens. This is because the residual soil specimen was collected in-situ from a site potentially rich in natural microorganisms, whereas the sand specimen was typical concrete sand exposed to the extreme tropical climate. As a result, the number of naturally inhibited microorganisms in sand specimens is lower than in residual soil specimens.

7. OTHER APPLICATIONS

A. MATERIAL SCIENCE

MICP has been described as a long-lasting remediation method that has been manifested peak potential for crack, cementation of diverse structural formations such as granite and concrete.

B. TREATMENT OF CONCRETE

MICP has been manifest to prolong concretelife-span because of $CaCO_3$ precipitation. The $CaCO_3$ cures the concrete by hardening of the cracked concrete surface, imitatingthe activity by which bone fractures in human body are cured by osteoblast cells that mineralize to rectify the bone. Two procedures are presently being analyzed: injection of $CaCO_3$ precipitating bacteria and by employing bacteria and nutrients as asurface treatment. Rise in strength andduration of time of MICP treated concrete has been described.

C. BRICKS

2010 Metropolis Next Generation Design Competition was won Architect Ginger Krieg Dosier for her work using microbial- induced calcite precipitation to make bricks whereby reducing CO_2 emissions. Ginger Krieg Dosier has since forasmuch as bioMASON, Inc., a company that uses microorganisms and chemical reactions toproduce building materials.

D. FILLERS FOR RUBBER, PLASTICS AND INK

Filler in rubber and plastics, fluorescentparticles in stationery ink, and a fluorescent marker are made by the MICP technique for biochemistry applications, such as westernblot.

8. CONCLUSIONS

The bacterial induced cementation method was attained by urea hydrolysis with B. *megaterium* which were able to catalyze the hydrolysis of urea. The victory of this process lies in the nutrient treatment flushes,level of the pH, and the bacterial activation. The SEM images shows clearly about the bacterial induced cementation. The time sequence of nutrient relates the amount of cementation.

The following are some of the other conclusions that can be taken from this study:

1. Calcite processing is greatly aided by the sand's mineralogical composition.
2. Bearing strength is proportional to the rateof calcite formation over time.
3. The power of the bacteria-treated specimens is around five times that of the untreated specimens.
4. Adding more bacteria to the mix has no effect on calcite production or precipitation.

9. REFERENCE

1. Al Qabanny, A., Soga, K., Santamarina,C., 2012. Factors affecting efficiency of microbially induced calcite precipitation. J.Geotechn. Geo-environ. Eng. 138, 992-1001.

2. Animesh Sharma, Ramkrishnan R., 2018. Effect of Microbial Induced Calcite Precipitates on strength of fine grained soils, Perspectives in Science at Elsevier 2018.

3. 549.Author, F., Author, S., Author, T.: Booktitle. 2nd edn. Publisher, Location (1999).

4. DeJong, J.T., Fritzges, M.B., Nüsslein,K., 2006. Microbial induced cementation to control sand response to undrained shear. J.Geotechn. Geo-environ. Eng. 132 (11), 1381-1392.

Greene, E.A., Hubert, C., Nemati, M.,Jenneman, G.E., Voor-douw, G., 2003. Nitrite reductase activity of sulphate- reducing bacteria prevents their inhibitionby nitrate reducing, sulphide-oxidizingbacteria. Environ. Microbial. 5 (7), 607-617.

5. Harkes, M.P., Van Paassen, L.A.,Booster, J.L., Whiffin, V.S., 2010.Fixationand distribution of bacterial activity in sandto induce carbonate precipitation for ground reinforcement. Ecol. Eng. 36(2), 112-117.

6. Karol, H. R. (2003), Chemical grouting and soil stabilization. 3rd edition. Marcel Dekker, Inc., New York

7. Lee Min Lee, Ng Wei Soon, Tan Chew Khun, Hii Siew Ling, 2012.Bio-mediated soil improvement under various concentrations of cementation reagents. Appl. Mech. Mater. 204-208 (2012), 326- 329.

8. Ng Wei Soon, Lee Min Lee, Tan Chew Khun, Hii Siew Ling, 2013. Improvements in engineering properties of soils through microbial-induced calcite precipitation. KSCE J. Civ. Eng. 17 (4), 718-728.

9. Siddhartha Mukherjee, R. B. Sahu,JoydeepMukherjee, Suchandra Sadhu.,2016.Application of Microbial Induced Carbonate Precipitation for Soil Improvement via Ureolysis, Indian Geotechnical ConferenceIGC 2016.

10. Wei-Soon Ng, Min-Lee Lee, Siew-Ling Hii, 2012. An overview of the factors affecting microbial-induced calcite precipitation and its potential application in soil improvement. World Acad. Sci. Eng.Technol. 62, 723-729.