# Enhancement of Shear Strength Characteristics by Microbial Cementation in Sand

S. Muthu Lakshmi *[1], J. Mathura [2], U. Gayathri [2], V. J. Vedhanayaghi [1]

[1] *Faculty of Civil Engineering, Rajalakshmi Engineering College, Chennai, Tamil Nadu, India*

[2] *Student of Civil Engineering, Rajalakshmi Engineering College, Chennai, Tamil Nadu, India*

*Corresponding Author e-mail: muthulakshmi.s@rajalakshmi.edu.in

***Abstract*— In the present work, enhancement of soil strength characteristics of 2 types of soil available in and around Chennai area by Microbially Induced Calcite Precipitation (MICP) has been studied. MICP is a relatively green and sustainable technique which utilizes a biochemical process in the soil to improve its engineering properties such as strength and impermeability. Bacteria are capable of performing metabolic activities which leads to precipitation of calcium carbonate ($CaCO_3$) in the form of calcite. In this study, a specific microorganism Bacillus Pasteurii which is abundantly available soil microorganism plays an important role in the cementation process and the precipitation of $CaCO_3$ occurs as a by-product of common metabolic processes such as urea hydrolysis. The bacteria were cultured under controlled conditions to maintain most likely environment for its better multiplication. Improvement in shear strength of treated soil by microbial cementation was quantified based on Unconfined Compression (UCC) test performed at different relative compactions of 60%, 75% and 90% and compared with virgin soil. Also the extent of microbial cementation that has occurred in 2 soil samples was studied using a microscope. From the results, it was observed that MICP treated soil showed increase in UCC strength compared to that of virgin soil and also the UCC strength was found to increase with increasing density of soil.**

***Keywords*— MICP, biocementation, Bacillus Pasteurii, urea hydrolysis, shear strength**

## I. INTRODUCTION

In recent times, number of innovative and evolving ground improvement techniques such as biogrouting, biocementation, bioclogging, use of bio-enzymes etc. are available to improve the shear strength, bearing capacity, permeability characteristics, and also to reduce settlement, erosion etc. Biocementation in soil using microbes is the latest and budding technique in geotechnical engineering used to enhance the engineering properties of soil. Bioclogging process is similar to biocementation except that in bioclogging process, production of pore filling material results in reduced porosity and permeability of soil whereas in biocementation process, production of binding materials in the void spaces between the soil grains binds them to form a hard rock like mass. One of the most promising biogeochemical treatments for soil is microbial-induced calcite precipitation (MICP), which effectively precipitates Calcium Carbonate ($CaCO_3$) in the soil, thereby increasing its strength, stiffness and reducing water permeability ([10]-[13]). As per Reference [2], physical properties of soil can be modified by MICP which is an innovative technique applied to various fields of civil engineering such as geotechnical engineering, rehabilitation of structures, environmental engineering etc.

Large number of microorganisms are available in the soil out of which bacteria are dominant microorganisms in soil. As the size of microorganisms is in microns, they are capable of freely moving through the void spaces in soil. If these microorganisms are provided with suitable nutrients, they multiply and flourish in soil resulting in precipitation of inorganic minerals which changes the mechanical properties of soil. One such process is hydrolysis of urea. Although a number of bacteria can be found in the soil, Bacillus Pasteurii has been commonly used in producing microbial cementation of soil particles. This is because Bacillus Pasteurii uses urea as an energy source and produces ammonia, which increases pH in the neighbouring environment, causing $Ca_2$ and $CO_3$ to precipitate as $CaCO_3$ ([1], [16]-[17]). The bacteria used in the present study is Bacillus Pasteurii which is available abundantly in the uppermost part of the earth's crust. Calcite precipitation results from the interaction of Bacillus Pasteurii and urease through a series of complex biochemical reactions. Calcite is the most abundant soil carbonate source and is mostly formed in the root zones. The most efficient biogeochemical pathway for MICP involves the microbial hydrolysis of urea, which is catalysed by the microbial enzyme urease ([14]-][15]). According to Reference [6], Bacillus Pasteurii precipitates $CaCO_3$ as a by-product of common metabolic processes such as urea hydrolysis and thus plays an important role in cementation by producing urease, which hydrolyses urea to ammonia and carbon dioxide. MICP binds soil particles together at particle-particle contacts and thus results in increased strength and stiffness of the soil. Subsurface microbes can promote MICP by increasing the alkalinity accomplished by reaction networks like urea hydrolysis.

Precipitation can be attained by simply mixing together moderate concentrations of soluble $Ca_{2+}$ and $CO_{32-}$ ions, as soon as the concentrations of both $Ca_{2+}$ and $CO_{32-}$ ions exceed 3.3 nmol.$L^{-1}$ at 25°C, calcite will precipitate. If the rate is too fast, little strength is achieved, but with a slower rate of formation, the binding strength of calcite can be significantly improved.

Many researchers have worked on MICP technique to improve the geotechnical properties of soil. Reference [2], studied the interactions between ureolytic species (Sporosarcina Pasteurii) and non-ureolytic species (Bacillus Subtilis) and its effect on MICP. Reference [3] introduced Bacillus and $CaCl_2$ into loose sand and soft silt and studied the formation of $CaCO_3$ (calcite precipitation) in the voids of soil after 7 days curing leading to cementation of soil particles. It was observed that higher calcium carbonate depositions were observed in poorly graded soil as compared to well graded soil and the quantity of $CaCO_3$ produced in sand specimens were about two times more than those for silt specimens. Reference [4] studied the calcite precipitation using Bacillus Pasteurii, and observed that shear strength of microbial soil increased while permeability reduced. MICP is a very economical method and can replace other methods of soil stabilization like the more energy demanding mechanical compaction methods or the expensive and environmentally unfriendly chemical grouting methods [4]. Reference [5] studied the BioCementation technology by using pure microorganisms (B. Pasteurii) under complete sterilization conditions during the cellular growth and found that cost of BioCementation technology was reduced by using enrichment culture (mixture of bacteria) to cement the sand particles. Further reduction in cost was achieved by performing the BioCementation process under non-sterile conditions. Reference [7] studied the effectiveness of MICP in improving the shear strength and reducing the hydraulic conductivity for residual soil and sand. A species of Bacillus group, B. megaterium was used to trigger the calcite precipitation and the improvement varied with soil densities, soil types and treatment conditions. Improvement in shear strength of residual soil specimens were significantly higher than those of the sand specimens whereas the reduction in hydraulic conductivity was found to be more in sand specimens than in residual soil specimens [7]. According to Reference [8], bacterial species, reactant concentration, reaction time, and depth were among the factors affecting $CaCO_3$ formation. In S. Pasteurii treated columns, mean calcium carbonate precipitation increased significantly toward the upper layers with increasing reactant concentration through time. Microbial calcium carbonate showed bridging, coating, and / or infillings of sand particles in thin sections, thus leading to reduction in soil porosity. According to Reference [9], calcium carbonate precipitation in soil reduces porosity, increases the strength and stiffness of the soil. The decreasing porosity influences the permeability and therefore the flow. As per Reference [3], permeability of soil was found to be reduced by 30% thus leading to reduced flow of water in soil. According to Reference [9], $CaCO_3$ can precipitate in several ways. It can attach to sand grains but can also form crystals. When these crystals are large enough, they will stick in the pore throats and are not transported. However, when these crystals are small, probably they can be transported. $CaCO_3$ connecting sand grains, will give a contribution to strength, while loose crystals will hardly contribute to strength.

### *A. Bacteria*

A urea-hydrolysing bacteria named Sporosarcina Pasteurii, formerly known as Bacillus Pasteurii (NCIM-2477) was obtained from 'National Collection of Industrial Microorganisms, National Chemistry Laboratory, Pune, India'. It was provided in glass ampule as slant culture which contains millions of bacteria grown at 30°C in nutrient agar medium. The details of the components of nutrient agar and the mixing proportions are given in Table I. This solution is referred to as the test media or growth media. The bacteria were inoculated in the test media from the stock culture and incubated at 30°C inside the incubator for 24-36 hours.

TABLE I
COMPONENTS OF NUTRIENT AGAR AND THE MIXING PROPORTIONS

| **Composition** | **Quantity** |
|---|---|
| Beef extract | 10g |
| NaCl | 5g |
| Peptone | 10g |
| Distilled water | 1L |
| Agar | 20g |
| Adjust pH to 7.0-7.5 | |

### *B. Making Bacterial Solution*

The preparation of the nutrient broth is explained in the following steps:
Step 1: With definite percentages of various of components of Nutrient media, nutrient broth was prepared in a 1L conical flask with cotton plug at its mouth.

Step 2: Ethanol 70% was used for sterilising a 100ml conical flask
Step 3: Nutrient broth was then transferred into the 100ml conical flask and was kept in Laminar Air Flow Chamber for 24-36 hours.
Step 4: It was then autoclaved resulting in a nutrient broth medium.
Step 5: One loopful was taken from the stock bacteria obtained from NCIM (National Collection of Industrial Microorganisms) called Slant Culture and was added to the conical flask and was incubated for 48-72 hours at 30°C.
Step 6: Once the incubation was done, conical flask was centrifuged at 8000 rpm for 10 minutes in order to separate the supernatant. The supernatant was removed by pouring it into a separate flask, and the remaining bacterial pellet was used for the bacterial treatment process applied to the soil sample.

### *C. Making Nutrient Solution*

The nutrient solution was prepared in the following way:
Step 1: The following ingredients were mixed to create the urea medium solution: nutrient Bacto (3 g), urea (20 g), NH4Cl (10 g), NaHCO3 (2.12 g), and 500 ml distilled water.
Step 2: Each of the solid ingredients were mixed thoroughly in 500 ml of distilled water until they dissolved.
Step 3: More distilled water was then added to reach the final required volume (1 L). After autoclaving, the pH of the urea medium was measured and found to be 8.0.
Step 4: After autoclaving, the resulting 1 litre solution was divided into 100 ml batches. The 100 ml of aerated solution was used for the experiment.
Step 5: Laboratory reagent Calcium Chloride ($CaCl_2$) was used for preparation of Calcium Chloride solution.

## II. Experimental Work

In this paper, 2 types of soil samples obtained from in and around Chennai area in Tamil Nadu was used in the experimental work. Tests such as wet sieve analysis, specific gravity test etc. were conducted in the laboratory to determine the index properties of the soil and the soil was classified as per Indian Standard Soil Classification System (ISCS).

Standard Proctor Compaction Test (SPCT) was conducted on the 2 soil specimens to determine the maximum dry density (MDD) and optimum moisture content (OMC). Remoulded soil specimen for UCC test was prepared at 3 different relative densities of 60%, 75% and 90% based on the OMC & MDD obtained from SPCT. UCC test on MICP treated soil were conducted after a curing period of 3 days, 7 days and 14 days to determine the optimum period of curing for the biocementation process. From the experimental results, the effectiveness of precipitation of $CaCO_3$ in the form of calcite for soil compacted at different relative densities and for varying curing periods was studied. Percentage increase in UCC strength for MICP treated soil compared with that of virgin soil for different curing periods was also found.

## III. Results and Discussion

Result of the tests such as specific gravity test, wet sieve analysis etc. are listed below. OMC and MDD obtained from SPCT and the results of UCC strength test conducted on treated and untreated soil prepared at different densities and cured for varying periods are also tabulated below.

### *A. Soil Classification*

Results of the tests conducted in the laboratory on the 2 soil samples to determine the index properties of soil are presented in Table II. Soil sample was classified as per ISCS based on the index properties of the soil.

TABLE III
SOIL CLASSIFICATION AS PER ISCS

| Name of the test | Sample 1 | Sample 2 |
|---|---|---|
| Specific Gravity test (G) | 2.62 | 2.61 |
| Sieve Analysis | | |
| Percentage of Gravel | 0 % | 6.12 % |
| Percentage of Sand | 98.05 % | 93.76 % |
| Percentage of Fines | 1.95 % | 0.12 % |
| D10 | 0.2 | 0.33 |
| D30 | 0.38 | 0.6 |
| D60 | 0.5 | 1.5 |
| $C_U$ | 2.5 | 4.5 |
| $C_C$ | 1.44 | 0.72 |
| Standard Proctor Test | | |
| OMC | 9.4 % | 11.28 % |
| MDD | 1.928 gm/cc | 2.04 gm/cc |
| Soil Classification as per ISCS | SP (Poorly graded sand) | SP (Poorly graded sand) |

Both the soil samples used for the experimental work were collected from two different locations around Chennai area and both were found to be SP (poorly graded sand), classified based on the index properties as per ISCS. In the present work, soil from location 1 was referred to as Sample 1 (S1) and soil from location 2 was referred to as Sample 2 (S2). Fig. 1 shows the particle size distribution curve for the two soil samples tested by wet sieve analysis. Fig. 2 shows the moisture dry density relation for the two soil samples obtained from SPCT.

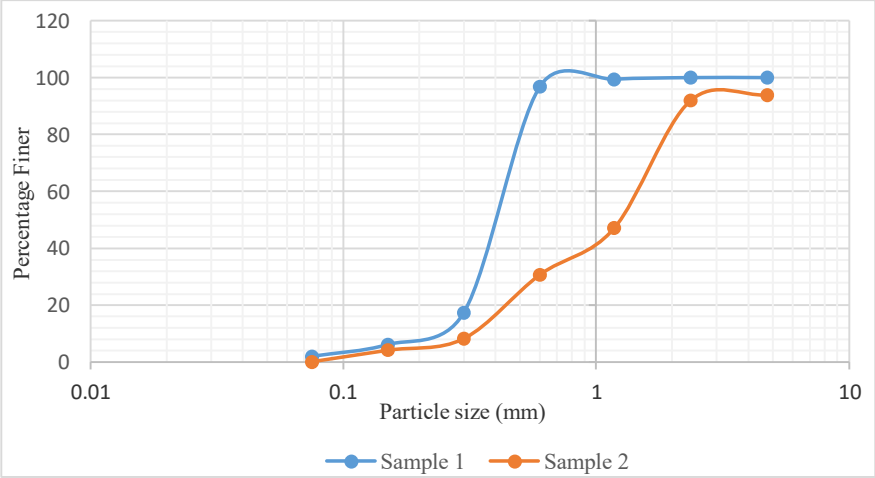


Fig. 1 Particle Size Distribution Curve by Wet Sieve Analysis

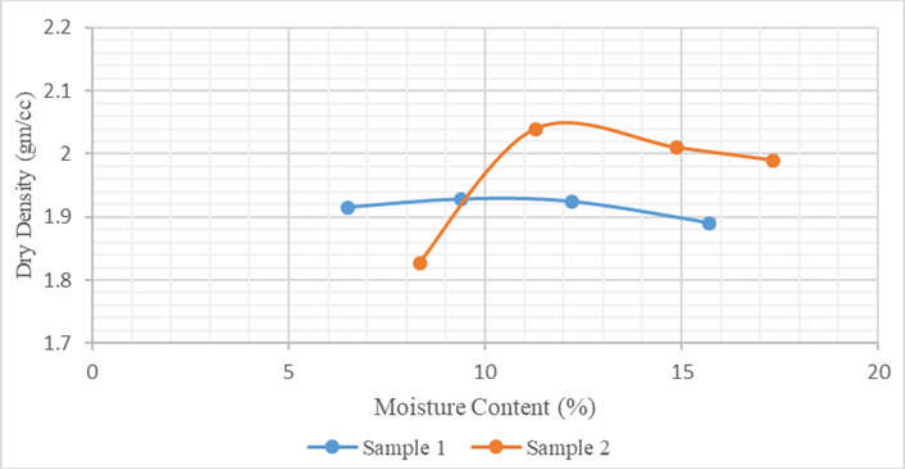


Fig. 2 Moisture – dry density relation from SPCT

*B. Microscopic Study*

Both the soil samples classified as poorly graded sand were treated with bacterial solution and different combinations of nutrient solutions such as urea and $CaCl_2$. Depending on urease activity and the ability of bacteria to produce calcite, they were viewed under microscope and the calcite crystals formed were studied further using Light Microscope Analysis. The microscopic analysis revealed that $CaCO_3$ crystals were embedded with bacteria. This explained that the bacteria served as the nucleation sites for the process. For further affirmation of the carbonate as calcite crystals, different combinations facilitating bacteria enhanced growth was adopted and microscopic analysis was performed.

High calcium amounts in the bacterial sample indicated that calcite was present in the form of $CaCO_3$. Fig. 3 shows untreated sand and bacterial treated sand with the formation of calcite within the voids.

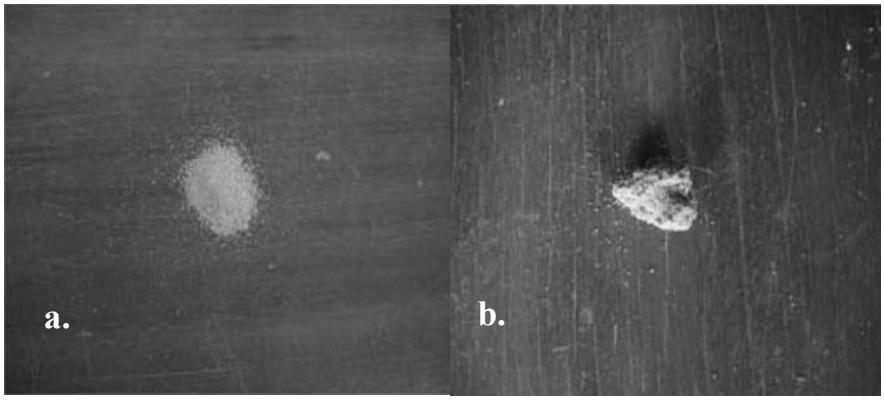


Fig. 3 a. Un-treated sand; b. Treated sand

Fig. 4 and Fig. 5 shows the microscopic analysis of calcite crystal formation along the periphery and within the voids of both the soil samples - S1 & S2 respectively, treated with bacterial solution and different combinations of nutrient solutions such as urea and $CaCl_2$.

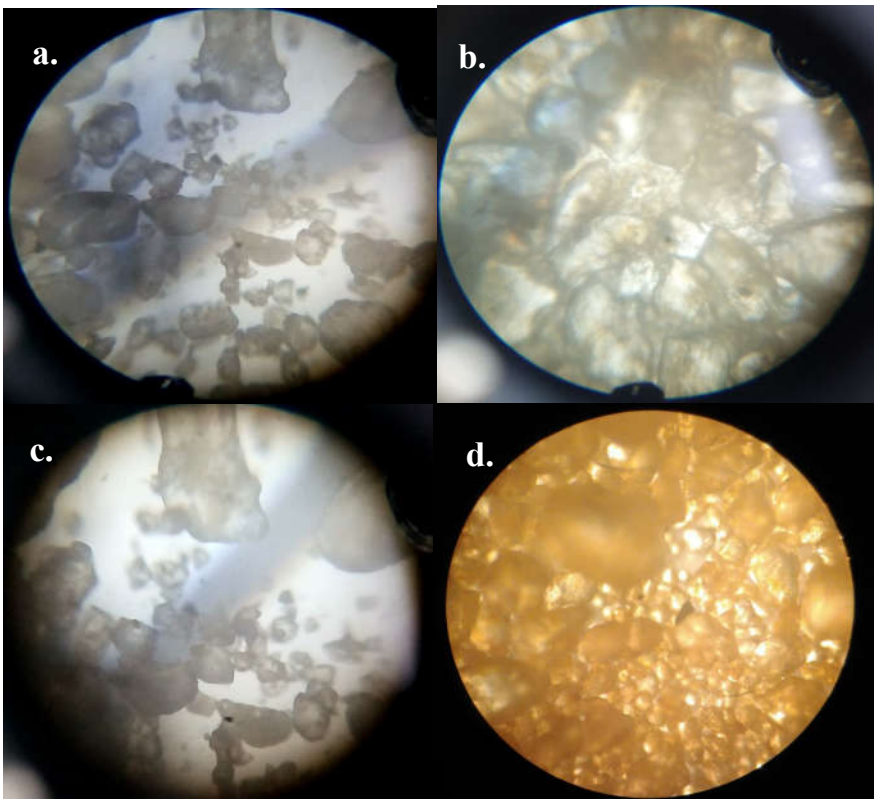


Fig. 4 a. Culture + S1; b. Culture + S1 + Urea; c. Culture + S1 + $CaCl_2$; d. Culture + S1 + Urea + $CaCl_2$

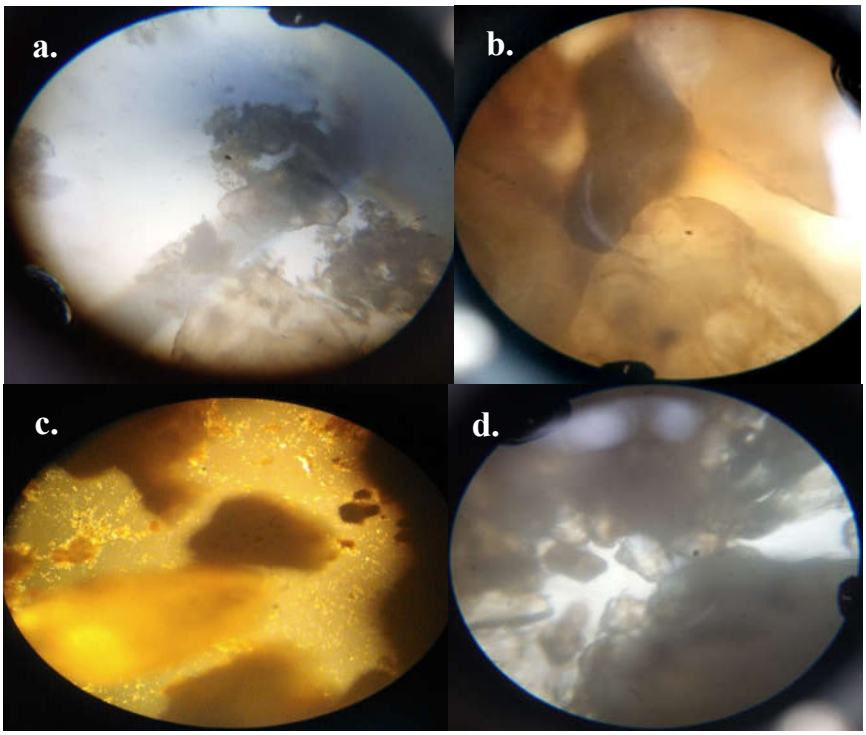


Fig. 5 a. Culture + S2; b. Culture + S2 + Urea; c. Culture + S2 + $CaCl_2$ d. Culture + S2 + Urea + $CaCl_2$

Microscopic observations are qualitative and not quantitative. However, the thin sections studied under microscope clearly showed the difference in relative amounts of calcite crystals formed among different treatments. From the microscopic analysis, it was observed that for both the soil samples S1 & S2 treated with urea showed better calcite crystal formation compared to the soil sample treated with $CaCl_2$. Also it was observed that maximum calcite crystal formation was observed for soil treated with both urea and $CaCl_2$.

*C. Unconfined Compression Test (UCC)*

UCC test was performed on virgin soil/untreated soil of samples S1 and S2 prepared at 90% relative compaction and the results were compared with that of MICP treated soil. MICP treated soil of S1 and S2 were prepared by mixing sand with calcium chloride solution (nutrient solution) and microbial solution in the ratio of 1:1 as shown in Table III. S1 and S2 mixed with bacterial solution and nutrient solution were prepared at relative compactions of 60%, 75% and 90% and were tested for UCC strength after curing for 3 days, 7 days and 14 days to determine the increase in UCC strength with respect to virgin soil.

TABLE IIIII
QUANTITY OF BACTERIAL + NUTRIENT SOLUTION ADDED TO SOIL FOR DIFFERENT RELATIVE COMPACTION

| Soil Sample | Relative Compaction | Weight of Soil (gm) | Weight of Bacterial + Nutrient Solution (ml) |
|---|---|---|---|
| S1 | 60% | 129.12 | 12.14 |
| | 75% | 161.47 | 15.17 |
| | 90% | 193.72 | 18.21 |
| S2 | 60% | 139.63 | 16.42 |
| | 75% | 174.43 | 20.51 |
| | 90% | 209.24 | 24.61 |

MICP treated soil specimen for UCC test were prepared as follows

Step 1. Based on the OMC obtained from SPCT, the quantity of bacterial solution and nutrient solution was added to soil to obtain a homogeneous soil mixture. Water was completely replaced by Bacterial solution and Nutrient solution mixed in the ratio of 1:1.

Step 2. This soil mixture was compacted into PVC moulds of 38mm diameter and 90mm length in three layers, each layer tapped with a tamping rod and compacted with an energy corresponding to SPCT. The surface of each layer was lightly scarified before another layer was compacted.

Step 3. Treated soil specimens were then put inside a plastic bag and cured for 3, 7 and 14 days inside a room with controlled humidity.

Table IV shows the results of the UCC test conducted on treated and untreated soil specimens. Fig. 6 shows the UCC strength of untreated and treated soil compacted at 90% relative compaction.

TABLE IVV
UCC TEST ON TREATED AND UNTREATED SOIL

| Soil Sample | Relative Compaction | UCC Strength (kPa) | | |
|---|---|---|---|---|
| | | 3rd Day | 7th Day | 14th Day |
| S1 (Untreated soil) | 90% | 14.89 | | |
| S1 (MICP treated soil) | 60% | 17.37 | 17.28 | 17.39 |
| | 75% | 17.39 | 17.35 | 17.4 |
| | 90% | 17.4 | 17.39 | 17.42 |
| S2 (Untreated soil) | 90% | 13.47 | | |
| S2 (MICP treated soil) | 60% | 17.22 | 17.19 | 17.33 |
| | 75% | 17.24 | 17.21 | 17.37 |
| | 90% | 17.33 | 17.24 | 17.43 |

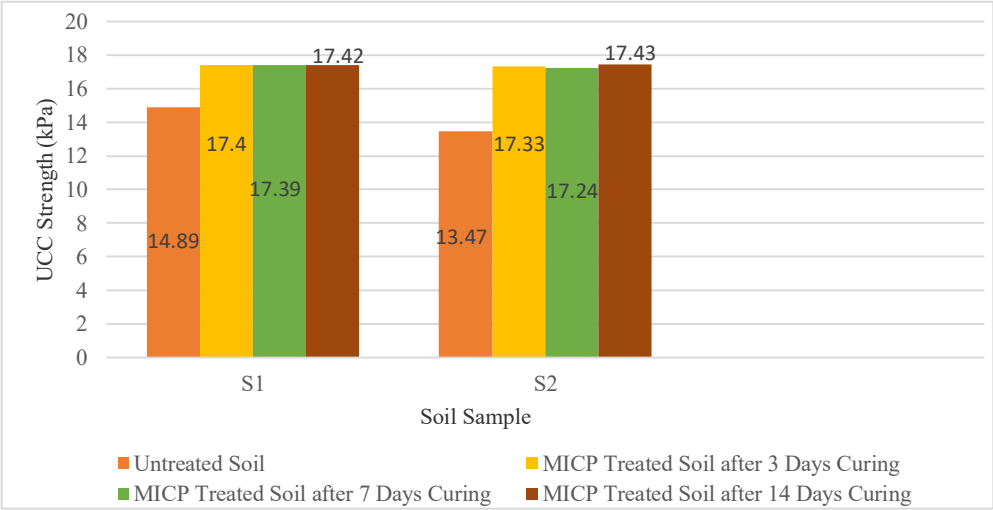


Fig. 6 UCC strength of soil specimens compacted at 90% relative compaction

From the results it was observed that for both the soil samples S1 and S2, treated soil showed increase in UCC strength compared to that of virgin soil. Maximum UCC strength gain was observed for treated soil cured for 14 days and percentage increase in UCC strength with respect to virgin soil was found to be 17% for S1 and 29.4% for S2. Increase in strength may be attributed to the formation of calcite crystals within the voids of both the soil samples forming a bond between the sand grains and thus increasing the strength and stiffness of soil compared to that of the virgin soil.

Fig. 7 and Fig. 8 shows the UCC strength of MICP treated soil samples S1 and S2 respectively compacted at relative compaction of 60%, 75% and 90% after curing for a period of 3, 7 and 14 days.

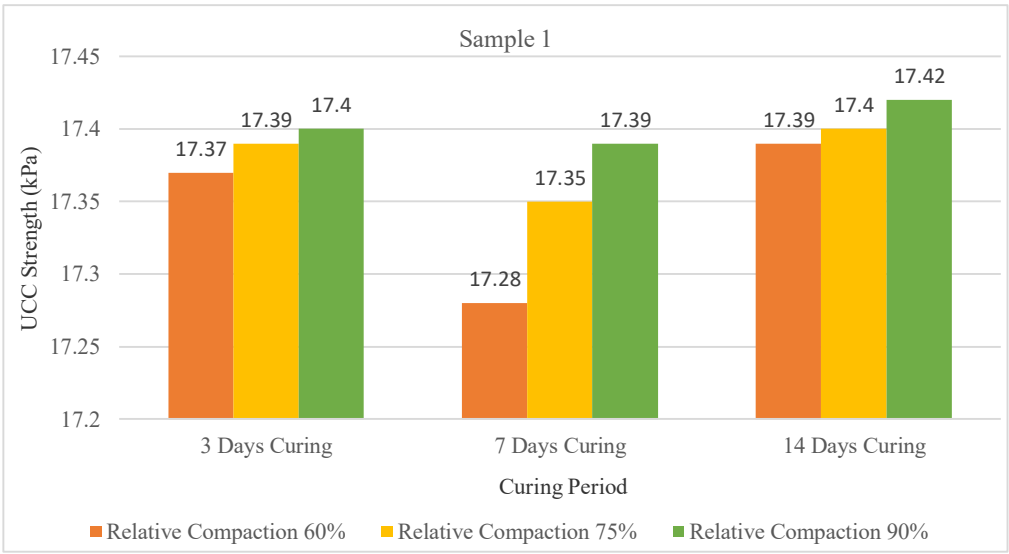


Fig. 7 UCC strength of MICP treated soil sample S1

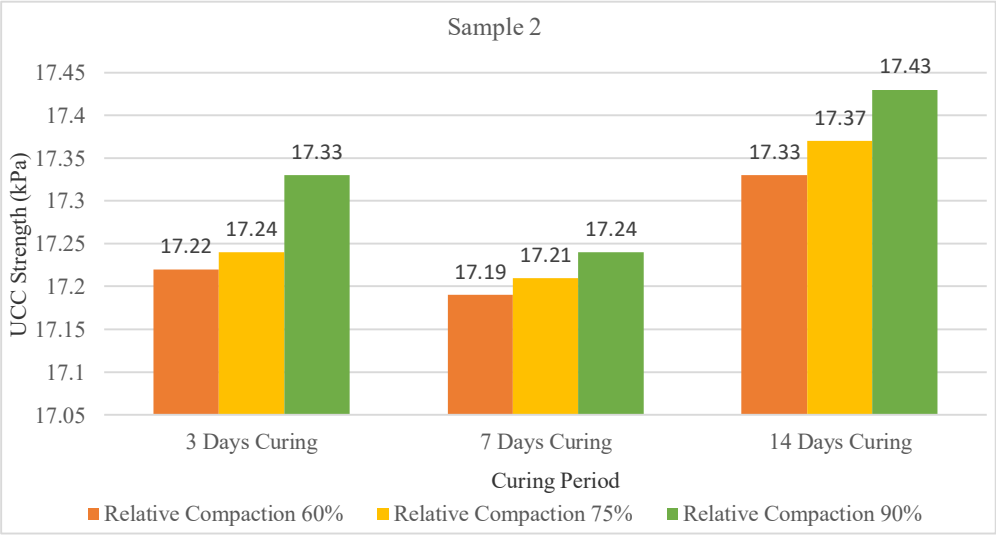


Fig. 8 UCC strength of MICP treated soil sample S2

Results showed that for both the MICP treated soil samples S1 and S2, UCC strength was found to increase with increasing density of soil observed at 3 days, 7 days and 14 days curing. Thus it can be inferred that increased microbial activity resulting in calcite precipitation within the voids was observed more in soil having dense state with less void ratio compared to soil in loose state having more number of voids. Also much variation cannot be observed in the UCC strength of MICP treated soil compacted at varying relative densities cured for 3, 7 and 14 days thus indicating that minimum curing period of 3 days is sufficient from shear strength point of view for the formation of $CaCO_3$ within the voids of the soil. It was also observed that, UCC strength slightly decreased at 7 days curing compared to that of 3 days and 14 days curing for all densities of soil and maximum UCC strength gain was observed for soil having 90% relative compaction after 14 days of curing.

## IV. Conclusions

Based on the microscopic analysis, both the soil samples S1 & S2 treated with urea showed better calcite crystal formation compared to the soil sample treated with $CaCl_2$. Maximum calcite crystal formation was observed for soil treated with both urea and $CaCl_2$.

MICP treated soil showed increase in UCC strength compared to that of virgin soil due to the formation of $CaCO_3$ within the voids of soil thus binding the individual sand grains and increasing the shear strength of soil. UCC strength was found to increase with increasing density of soil. Maximum UCC strength was observed for soil at 90% relative compaction compared to that of 60% and 75% relative compaction thus indicating that increased microbial activity occurs in soil having dense state than in soil having loose state. Much variation cannot be observed in the UCC strength of the MICP treated soil cured for 3, 7 and 14 days thus indicating that minimum curing period of 3 days is sufficient from shear strength point of view for the formation of $CaCO_3$ within the voids of the soil. Maximum UCC strength gain was observed for soil having 90% relative compaction after 14 days of curing and the percentage increase in UCC strength with respect to virgin soil was found to be 17% for S1 and 29.4% for S2.

## References

[1] J. T. Dejong, B. M. Mortensen, B. C. Martinez, and D. C. Nelson, "Bio-mediated Soil Improvement," *Journal of Ecological Engineering*, vol. 36, pp. 197-210, 2010.

[2] D. Gat, M. Tsesarsky, D. Shamir, and Z. Ronen, "Accelerated microbial-induced CaCO3 precipitation in a defined coculture of ureolytic and non-ureolytic bacteria," *Biogeosciences*, vol. 11, pp. 2561–2569, 2014.

[3] Daehyeon Kim and Kyungho Park, "An Environmentally Friendly Soil Improvement Technology with Microorganism," *IJR International Journal of Railway*, vol. 6, No. 3, pp. 90-94, Sept. 2013.

[4] P. Pawar Shahaji, "Assessments of Soil Properties by Using Bacterial Culture," *International Journal of Innovations in Engineering Research and Technology [IJIERT]*, vol. 2, Issue 6, pp. 1 - 7, June 2015.

[5] Salwa M. Al-Thawadi, "Consolidation of Sand Particles by Aggregates of Calcite Nanoparticles Synthesized by Ureolytic Bacteria under Non-Sterile Conditions," *Journal of Chemical Science and Technology*, vol. 2, Issue. 3, pp. 141-146, July 2013.
[6] Waleed Siddik, Henifi Canakci, and Ibrahim Kilic, "Effect of bacterial calcium carbonate precipitation on the geotechnical properties of soils," in *Proc. 2nd International Balkans Conference on Challenges of Civil Engineering (BCCCE)*, Epoka University, Tirana, Albania, pp. 802-809, May 2013.
[7] Ng Wei Soon, Lee Min Lee, Tan Chew Khun, and Hii Siew Ling, "Improvement in Engineering Properties of Soils through Microbial Induced Calcite Precipitation," *KSCE Journal of Civil engineering*, vol. 17(4), pp. 718-728, 2013.
[8] M. Sarmast, M. H. Farpoor, M. Sarcheshmehpoor, and M. K. Eghbal, "Micromorphological and Biocalcification Effects of Sporosarcina pasteurii and Sporosarcina ureae in Sandy Soil Columns," *J. Agr. Sci. Tech*., vol. 16, pp. 681-693, 2014.
[9] W. K. Van Wijngaarden, F. J. Vermolen, G. A. M. Van Meurs, and C. Vuik, "Modelling Biogrout: A New Ground Improvement Method Based on Microbial-Induced Carbonate Precipitation," *Transp Porous Med*, Springer, 2010.
[10] J. T. DeJong, B. M. Mortensen, B. C. Martinez, and D. C. Nelson, "Bio-mediated soil improvement," *Ecol. Eng*., vol. 36, pp. 197–210, 2010.
[11] M. P. Harkes, van L. A. Paassen, J. L. Booster, V. S. Whiffin, and van M. C. M. Loosdrecht, "Fixation and distribution of bacterial activity in sand to induce carbonate precipitation for ground reinforcement," *Ecol. Eng*., vol. 36, pp. 112–117, 2010.
[12] M. Nemati, and G. Voordouw, "Modification of porous media permeability, using calcium carbonate produced enzymatically in situ," *Enzyme Microb. Technol*., vol. 33, pp. 635–642, 2003.
[13] V. S. Whiffin, van L. A. Paassen, and M. P. Harkes, "Microbial carbonate precipitation as a soil improvement technique," *Geomicrobiol. J*., vol. 24, pp. 417–423, 2007.
[14] W. De Muynck, N. De Belie, and W. Verstraete, "Microbial carbonate precipitation in construction materials: A review," *Ecol. Eng*., vol. 36, pp. 118–136, 2010.
[15] A. B. Lloyd, and M. J. Sheaffe, "Urease activity in soils," *Plant Soil*, vol. 39, pp. 71–80, 1973.
[16] S. Stocks-Fisher, J. K. Galinat, S. S. Bang, "Microbiological Precipitation of CaCO3," *Soil Biology and Biochemistry*, vol. 31, pp. 1563-1571, 1999.
[17] K. L. Bachmeier, A. E. Williams, J. R. Warmington, and S. S. Bang, "Urease Activity in Microbiologically-induced Calcite Precipitation," *Journal of Biotechnology*, vol. 93, pp. 171-181, 2002.