Contents List available at RAZI Publishing
Journal Clean WAS
Journal Homepage: http://www.razipublishing.com/journals/journal-cleanwas/
https://doi.org/10.26480/jcleanwas.02.2017.01.05

ISSN: 2521-0912 (Print)
ISSN: 2521-0513 (Online)

IMPROVING SAND PROPERTIES USING MICROBIAL-INDUCED CALCITE PRECIPITATION METHOD

Maysam Bahmani[1*], Ali Noorzad[1], Javad Hamedi[2], Fatemeh Salimi[2]

[1]Faculty of Civil, Water, and Environmental Engineering, Shahid Beheshti University, Tehran, Iran.
[2]Department of Microbial Biotechnology, School of Biology, College of Science, University of Tehran, Tehran, Iran.
**Corresponding Author Email: bahmanimaysam@gmail.com*

ARTICLE DETAILS

Article History:

Received 3 July 2017
Accepted 3 October 2017
Available online 1 November 2017

Keywords:

Bacillus pastteurii, calcite precipitation, durability, soil compaction, *sporosarcina pasteurii*, temperature, wind erosion resistance

ABSTRACT

Nowadays, despite the accepted standard soil improvement methods or utilization of artificial materials such as cement and lime, these procedures are criticized due to detrimental environmental influences caused during their production process and applications; thus, such problems led to increased demand for new environmentally-friendly methods to improve soil properties. The experimental section of the study used *sporosarcina pasteurii (bacillus pastteurii)* to evaluate microbial-induced calcite precipitation (MICP) on two distinct grains of sand. The effect of temperature and saturation level on the optimization of calcite precipitation produced by *Sporosarcina pasteurii* was also examined. The findings revealed that most bacterial activities occurred at a temperature of 35 °C and that saturation reduces the efficacy of remediated sand. The results also show that despite their particle size, remediated sands have a better performance in the durability test. These findings indicate that *sporosarcina pasteurii* in MICP as a bio-mediated treatment approach improved sandy soil's mechanical characterization and improved soil characteristics, and enhanced wind erosion resistance.

1. INTRODUCTION

Soil improvement has caught the interest of numerous academics due to population growth and a scarcity of land with adequate ground conditions. On the other hand, some soil enhancement techniques are not environmentally friendly or need significant energy to create or install components [1, 2]. It's also worth mentioning that most standard techniques aren't viable in locations with buildings or subterranean structures. Microbial-induced calcite precipitation is a multidisciplinary technology that combines geotechnical engineering, ecology, and microbiology to enhance soil qualities. It was first proposed in 1999 that the bacteria *sporosarcina pasteurii* can induce calcite precipitation in soil [3]. MICP uses ureolytic bacteria to hydrolyze urea and form calcium carbonate crystals, which bond soil particles in the presence of enough calcium ions, improving and reinforcing the soil [4]. The following simplified chemical reactions which lead to calcium carbonate precipitation are as follows [4]:

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

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

Urea is converted to 2 mol of ammonium carbonate ions by the urease enzyme [5, 6]. Calcite precipitates when calcium and carbonate are combined in the presence of Ca^{2+}.

This research attempted to use *sporosarcina pasteurii* bacteria to apply a biological dust control strategy to sand, resulting in calcium carbonate precipitation in the soil. The impacts of saturation degree, optimal concentration of cementation solution, and temperature were explored to discover the best conditions for microorganism growth and performance. Finally, uniaxial compressive strength, wind erosion test, and freeze-thaw durability were performed to evaluate the bacterial potential and performance in dust suppression and soil characteristic improvment.

2. MATERIALS AND METHODS

2.1 Soil Classification

Particle size distribution is one of the most significant factors in influencing the geotechnical behavior of soils. This study used two different varieties of silica sand (Sand A and Sand B). The sands were from Iran's Dasht-e Loot Desert, and the particle size distribution was determined using sieve analysis. Figure 1 depicts the particle-size distribution curves of sand A and sand B. Based on sieve examination, sand B was coarser than sand A. Both sands are classified as poorly graded sands (SP) by the Unified Soil Classification System (USCS) because more than 95 percent of the sand grains were larger than 0.075 mm and less than 2 mm [7]. Due to undesirable engineering qualities such as uniform soil gradation, lack of cohesion among soil particles, rapid settlement, and low mechanical strength, poorly graded sands were chosen for this study. Table 1 shows sand A and sand B parameters in detail; the specific gravity and median particle size for sand A were 2.63 and 0.18 mm, respectively, and 2.67 and 0.87 mm for sand B.

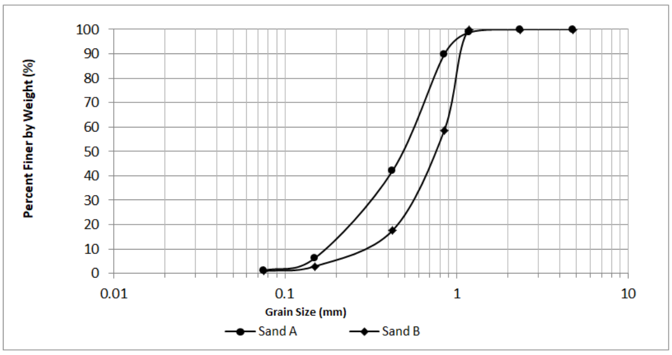


Figure 1: Grading curve of sand A and Sand B

Table 1: Properties of sand A and sand B

Soil type	USCS	Dry density (gr/cm^3)	pH	Shape	G_s	C_c	C_u	D_{60} (mm)	D_{50} (mm)	D_{30} (mm)	D_{10} (mm)
Sand A	SP	1.92	8.6	Round	2.61	0.76	2.3	0.21	0.19	0.12	0.09
Sand B	SP	1.63	8	Round	2.68	1.15	2.8	0.88	0.74	0.56	0.31

2.2 Biological Material

Sporosarcina pasteurii with the Persian type culture collection (PTCC) code 1645 was utilized in the MICP technique and was purchased from Iran's Industrial Scientific Research Center's bacteria collection. For bacteria cultivation, the aerobic batch conditions of yeast extract 7 g/L, urea 20 g/L, tryptone 5 g/L, and meat peptone 5 g/L were sterilized at 121 ^{o}C and pH of 9 [8]. Before harvesting, the bacteria stock culture was introduced into the growth media for 48 hours at 28°C in a shaker (100 rpm), and the bacteria were kept suspended in their growing medium at 4°C until they were used [8, 9].

2.3 Optimal Concentration of Cementation solution:

Six different concentrations of calcium chloride and urea were chosen to obtain the best concentration for isolated bacteria, as shown in Table 2. The selected isolate was inoculated from pre-cultivating liquid with a ratio of 10% to liquid culture after 3.5 days. Sampling was done in a volume of 25 mL, and three sampling flasks and one control flask were considered for each bacteria. From the 3rd day to the 11th day, the sampling was carried out every two days. In order to measure the deposition of calcite, after measuring the pH of samples, they were dried and titrated based on the back titration method. The urea–calcium chloride medium was autoclaved for 20 min at 121°C before adding to the specimens.

Table 2: Calcium chloride and urea concentration

No.	Urea (M)	Calcium Chloride (M)
1	0.2	0.2
2	0.4	0.4
3	0.6	0.6
4	1.5	1
5	1.85	1
6	2	1

3. EXPERIMENTAL METHODS

3.1 Preparing sand column subjected to urea– calcium chloride and culture medium

The bacterial injection approach is critical for achieving homogeneous calcite precipitation distribution in soil particles. A three-step mixing process was adopted for the bacteria and cementation solution [10]. For the unconfined compressive strength (UCS) test, soil column samples (Poly Vinyl Chloride (PVC) tubes) with a height and internal diameter of 12 cm and 6 cm, respectively, were used [8].

The bacteria and cementation solution were blended into the soil column through the top of the sand column. According to a study, the injection steps are as follows [11]:

- With an input rate of about 1 l/h, bacterial suspension and cementation solution with double the size of Vv (the volume of sand column pores) were introduced to the sand column. After that, a vacuum pump was used to remove any extra solution from the column to maintain the saturation degree constant.
- Following adding the bacteria solution to the sand column, the flushing was paused for 12 hours at 25 degrees Celsius, allowing the bacteria to attach to the soil particles.
- Finally, for a 12-hour curing period, the cementation solution was flushed at the same flow rate. The most important aspect of the above procedure is to maintain a steady saturation degree throughout the testing [11]. The saturation degrees were measured at the top and bottom of the 12 cm long sand columns, as well as every 2.5 cm between the top and bottom, according to Cheng et al.2013. Between 2.5 and 9.5 cm, the local degrees of saturation were generally homogeneous, with a range of less than 5%. As a result, specimens were prepared for mechanical property testing.

3.2 Unconfined Compressive Strength (UCS) Tests

The UCS tests were done to measure the strength of sand samples treated by MICP to the compressive strength of treated samples, according to ASTM D2166 [12]. To study the effect of saturation degree, sand samples were produced with a mixture of 1.85 M urea and 1 M calcium chloride and treated at 21°C under 20, 40, 60, and 80% degree of saturation. At a constant speed of 1.0 mm/min, the experiments were repeated three times for each sample.

3.3 Freeze-thaw durability

At various saturation levels, UCS experiments were utilized to determine the durability of treated sand A and sand B. For this, a mold with a diameter of 6 cm and a height of 12 cm was used. The treated samples were subjected to ten freeze-thaw cycles, each involving a 12-hour freeze at -10°C and a 12-hour thaw at 30°C [8].

3.4 Temperature Role in Bacteria Growth:

Sporosarcina pasteurii was tested for ureolysis-driven microbially generated carbonate precipitation or urease-catalyzed ureolysis at 10, 15, 21, and 35 degrees Celsius. *Sporosarcina pasteurii*, like any other enzymatic process, is temperature-dependent, and microbial activity and temperature are both crucial variables in calcite precipitation. A constant number of bacteria were controlled 1.85 M urea and 1 M calcium at various temperatures to study the influence of temperature on urease activity. The uniaxial test was used to quantify UCS to assess the effect of temperature on urease activity and the soil strengthening process due to microbial carbonate precipitation.

3.5 Wind Erosion Test

A wind tunnel with dimensions of 0.42 m height, 0.45 m wide, and 5.4 m long was used to test the effect of bioremediation on wind erosion. Trays (40*60*5 cm) filled with sand that went through sieve No. 30 were put in a wind tunnel at a speed of 80±2 km/h, according to the maximum local wind speed, for soil erosion test specimens. Soil erosion was examined in three typical samples in this simulation:

1- Sand that was treated with a 1500 cc cementation solution containing 1.85 times the molarity of calcium chloride and a 1500 cc bacterial suspension.

2- Sand that was treated with a 1500 cc of water

3- Sand that was not treated as a control sample

At 1, 3, and 5-day intervals, each sample was exposed to the flow for 30 minutes each day and evaluated for wind erosion. The wind erosion test calculated the final masses by subtracting the beginning and final masses of soil in the trays.

4. EXPERIMENTAL RESULTS

4.1 Temperature influence on bacteria activity

Temperature is one of the most critical MICP process variables influencing calcite precipitation. The treated soil's compressive strength was measured at 10, 15, 21, and 35°C, and sand columns were constructed from soil with a dry density of 1.86 g/cm^3. Figure 3 demonstrates that increasing the temperature to 35 degrees increased the UCS along with the rise in bacterial activity; in fact, compared to lower temperatures, this increase in temperature enhanced the solubility of the solution and the rate of calcite precipitation.

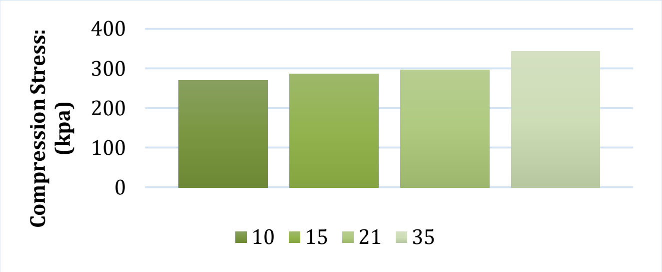


Figure 2: The effect of temperature on the UCS

4.2 The optimal concentration of calcium chloride precursor and urea for precipitation

Obtaining the optimal conditions to produce calcite precipitation is one of the main factors which needs to be considered. To this end, six different concentrations of cementation solutions (Table 2) were chosen to evaluate the optimum calcium chloride and urea concentration for sand bio-cementation with *sporosarcina pasteurii*. Based on the back titration technique duration, the amount of $CaCO_3$ precipitation was determined, and the results are shown in Figure 3. Each of the three experimental conditions was carried out three times. As depicted in Figure 3, the highest calcium chloride precipitation was obtained when 1.85 M urea and 1 M calcium chloride were used for the sand treatment of 8.9 g/l on the third day. Although urea and calcium chloride are necessary for calcite precipitation, the amount of precipitation decreased when cementation media concentration increased from the optimum. The reduction in precipitation might be due to the calcium ion concentration being higher than optimal, which causes them to better link with cell surfaces and reduce urea penetration speed. Moreover, when urea concentration increases from its optimum, the lack of enough water for surrounding urea at the active site of the urease bacteria enzyme will occur and result in the impaired enzyme activity. As shown in figure 3., 2 M urea and 1 M calcium chloride decrease the amount of calcium carbonate precipitation.

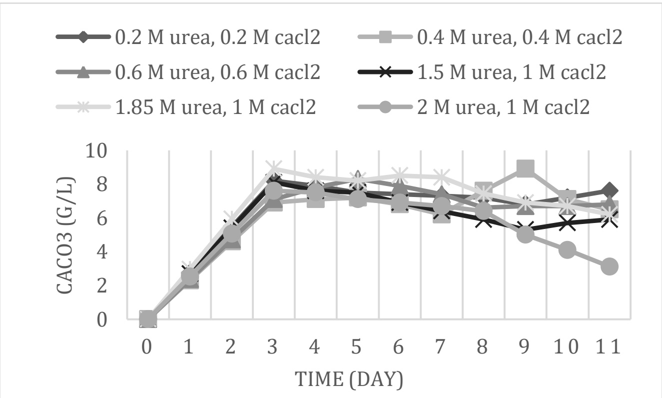
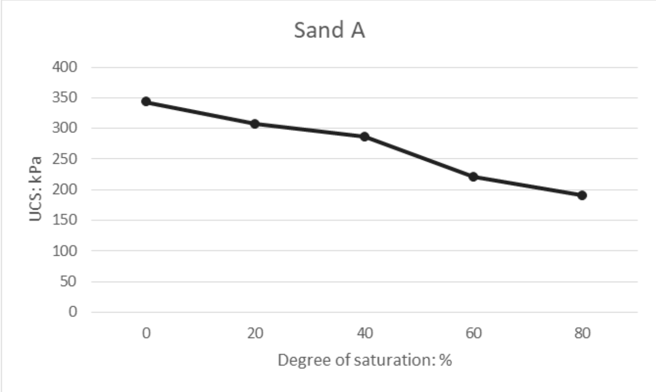


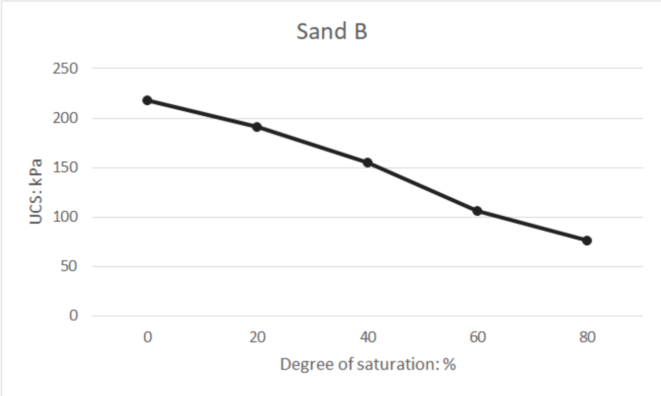
Figure 3: The amount of calcite precipitation during the period for the different concentrations of cementation solution.

4.3 Effect of degree of saturation on UCS

It was shown that the degree of saturation influences the dispersion of calcium carbonate crystals [13]. Thus, the UCS tests were conducted on the samples treated by *sporosarcina pasteurii* under various degrees of saturation: 0, 20%, 40%, 60%, and 80%. In this study, the influence of saturation degree was studied in equal calcium carbonate precipitation and all specimens made at the best ratio of urea to calcium (1.85) and OD600, 0.61 for *sporosarcina pasteurii*. Based on the result for sand A (Figure 4a), the highest amount of UCS is 343 kPa for dried treated sand and the UCS reducing by increasing saturation degree. For sand B (Figure 4b) treated samples also followed this process, and treated samples show a better performance in lower amounts of saturation degree.



(a)



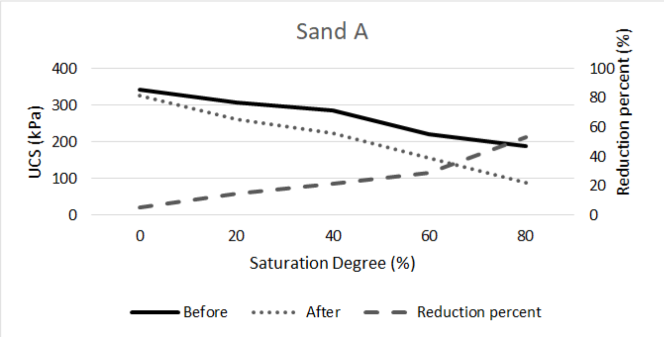
(b)

Figure 4: Variation of UCS with different degrees of saturation for a) sand A, b) sand B.

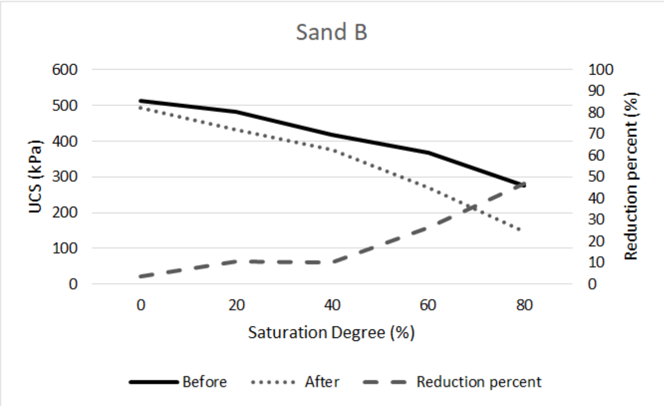
4.4 The effect of MICP treatment on the freeze-thaw durability of sand at various saturation degrees

Many porous materials have been destroyed by freezing and thawing (FT). Researchers were quite concerned more than 200 years ago, especially in frigid locations [11]. The impact of 10 FT cycles on bio-mediated sand A samples are shown in Figure 5a. UCS tests were carried out before and after 10 FT cycles at varying saturation degrees of 0, 20%, 40%, 60%, and 80%. A decrease in UCS for sand A treated by microorganisms was found after 10 cycles. After FT cycles, UCS reduced by an increase in the degree of saturation. The samples treated at a lesser degree of saturation of 0 and 20% had the lowest level of decrease while treating at an 80 percent degree of saturation resulted in the maximum reduction of 59 percent for treated sand A. A change in the phase of water absorbed in the soil pores spaces is the principal cause of degradation of exposed porous materials.

For the strengthening performance of MICP-treated sand B samples after FT cycles, the results were shown in Figure 5b. As expected, the reduction process of compressive strength after FT cycles were followed by bio-cemented sand A, and also higher degree of saturation provided the higher amount of reduction. By comparing the UCS of treated sands after cycling, it can be seen that sand B had a better performance which is because of larger soil grains and higher permeability; in fact, the high permeability and porosity permit more air and water to go through the sand matrix, which results in increasing the FT resistance.



(a)



(b)

Figure 5: UCS of treated sands after FT cycles. a: sand A; b: sand B.

4.5 Wind erosion test

To determine the MICP method's performance in reducing soil erosion, a wind erosion test was conducted. To test dune sand performance against wind erosion, various samples were employed, including MICP treated with 1500 cc cementation solution, treated with 1500 cc water solution, and untreated sample. As figure 6. shows, MICP treated samples considerably reduce soil erosion even after five days with about ten percent loss. Meanwhile, samples treated with water just showed good resistance on the first day, but the erosion amount after five days was about 83 percent. Figure 6 also indicates that more than 95 percent of sand without any king treatment does not perform well against erosion. The sample treated by calcite precipitation bunded sand particles and made a layer formed on the soil's surface that prevents the dust particles from spreading into the air by resistance at the outer surface of the sand [9].

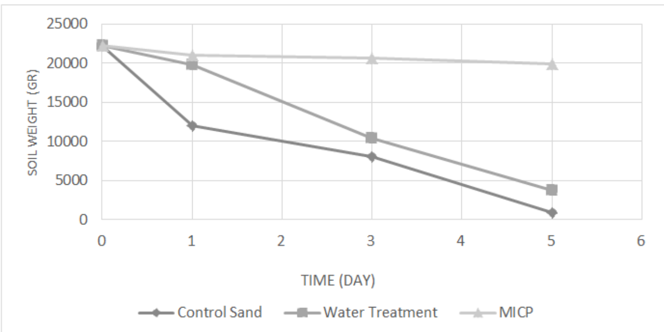


Figure 6: Weight of soil remaining in the tray over time in wind erosion test[9]

5. CONCLUSIONS

This study shows the MICP influence on improving sand properties. Therefore, microbial-induced calcite precipitation can be considered an alternative method for traditional approaches. Many studies have been carried out to investigate the impact of the microbial treatment approach in resolving soil stability issues. The degree of saturation and temperature conditions were investigated in order to obtain the best bacteria response circumstances. The sample performance against the wind was then studied using the ideal circumstances established in the previous phase to create a model for the wind erosion experiment. The results showed that the MICP technique is ideal for dust suppression in areas where it is required. The results of the uniaxial compression experiments revealed that various variables influence the formation of resistant connections between sand grains. The dry sample without moisture had the maximum stiffness and compressive strength in the UCS test, whereas greater saturation degrees inhibited the bacteria from successfully inducing calcium carbonate among the sand particles.

The compressive strength of MICP-treated samples can also be influenced by temperature. Curing at lower temperatures resulted in a loss in compressive strength, but the ideal temperature for bacteria activity was 35°C. The use of precipitated calcite as a cementing agent will reinforce the problematic granular soils, and optimal conditions must be considered in the MICP process. To this end, different quantities of cementation solution were tested to determine the optimal quantity of urea and calcium chloride, which was found to be 1.85 M urea and 1 M calcium chloride. For higher concentrations, calcite precipitation decreased because of the harmful effects of extra urea and calcium chloride. Furthermore, the results of wind erosion studies demonstrated that this approach is suitable for stabilizing desert sands and replacing existing methods. Overall, the MICP technique can be used to improve the problematic sand found in deserts as an alternative to standard methods and materials.

REFERENCES

[1] DeJong, J.T., 2010. Bio-mediated soil improvement. Ecological Engineering, 36 (2), Pp. 197-210.

[2] Fatehi, H., Bahmani, M., and Noorzad, A., 2019. Strengthening of dune sand with sodium alginate biopolymer. in Geo-Congress 2019: Soil Improvement. American Society of Civil Engineers Reston, VA.

[3] Stocks-Fischer, S., Galinat, J.K., and Bang, S.S., 1999. Microbiological precipitation of CaCO3. Soil Biology and Biochemistry, 31 (11), Pp. 1563-1571.

[4] Mujah, D., Shahin, M.A., and Cheng, L., 2017. State-of-the-art review of biocementation by microbially induced calcite precipitation (MICP) for soil stabilization. Geomicrobiology Journal, 34 (6), Pp. 524-537.

[5] Stocks-Fischer, S., Galinat, J.K., and Bang, S.S., 1999. Microbiological precipitation of CaCO 3. Soil Biology and Biochemistry, 31 (11), Pp. 1563-1571.

[6] Warren, L.A., 2001. Microbially mediated calcium carbonate precipitation: implications for interpreting calcite precipitation and for solid-phase capture of inorganic contaminants. Geomicrobiology Journal, 18 (1), Pp. 93-115.

[7] ASTM, D., 2006. Standard Practice for Classification of Soils for Engineering Purposes (Unified Soil Classification System). ASTM International, West Conshohocken, PA, USA.

[8] Bahmani, M., 2019. Biological soil improvement using new environmental bacteria isolated from northern Iran. Environmental Geotechnics, 40(XXXX), Pp. 1-13.

[9] Bahmani, M., 2019. The Role Of Bacillus Pasteurii On The Change Of Parameters Of Sands According To Temperature, Compression And Wind Erosion Resistance Maysam Bahmani, 1 Ali Noorzad, 2. Environmental Planning and Management, Pp. 239.

[10] Ferris, F., 2004. Kinetics of calcite precipitation induced by ureolytic bacteria at 10 to 20 C in artificial groundwater. Geochimica et Cosmochimica Acta, 68 (8), Pp. 1701-1710.

[11] Cheng, L., Cord-Ruwisch, R., and Shahin, M.A., 2013. Cementation of sand soil by microbially induced calcite precipitation at various degrees of saturation. Canadian Geotechnical Journal, 50 (1), Pp. 81-90.

[12] ASTM, D., 2003. Standard test method for unconfined compressive strength of cohesive soil. Annual book of ASTM Standards, American Society for Testing and Materials, Philadelphia, 4 (08).

[13] Cheng, L. and Cord-Ruwisch, R., 2012. In situ soil cementation with ureolytic bacteria by surface percolation. Ecological Engineering, 42, Pp. 64-72.