THE ROLE OF BACILLUS PASTEURII ON THE CHANGE OF PARAMETERS OF SANDS ACCORDING TO TEMPERATURE COMPRESION AND WIND EROSION RESISTANCE

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

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

ARTICLE DETAILS

ABSTRACT

Microorganisms have played a critical role in geological processes and in the formation of soils throughout geological time. It is hypothesized that biological activity can also affect soil properties in short engineering time-scales. Nowadays, Bio-grout is a new soil improvement method based on microbiologically induced precipitation of calcium carbonate. For this purpose, bacteria, which are able to convert urea into ammonium and carbonate, are injected into the soil, followed by a solution containing urea and calcium chloride and therefore it is produced carbonate precipitates with calcium. The calcium carbonate crystals form bridges between the sand grains, which increases the strength of the sand mass. In this study, focus is placed on bio-mediated calcite precipitation of sands and *Sporosarcina pasteurii* was chosen for the experimental part of the study. This study has examined the effect of temperatures and the soil compaction amount on optimising calcite precipitation induced by sporosarcina pasteurii. The result demonstrated that most bacterial activities were seen on $60°C$ and according to the tests result, medium compaction (Dr 56%) is the best case for compatibility between the soil grain characteristics and bacteria size for transportation. It seems bio-mediated treatment techniques can result in the improvement of soil properties and increased the soil strength in wind erosion resistance test.

1. INTRODUCTION

A kind of dust suppressive includes chemicals which bind fine particles together or onto larger particles. These chemicals fall into several groups, such as petroleum-based, organic methods, electrochemical stabilizers, synthetic polymers and microbial induced calcite precipitation. The traditional dust control methods including spraying water, chemicals, and petroleum products onto the surface of dust particles can be rather expensive and environmentally unfriendly [1]. Recently with increasing attention on an environmentally method for improving soil properties novel grouting technic have emerged to treat soils by simulating natural processes by depositing calcite ($CaCO_3$) on the soil grains thereby increasing the material's stiffness, strength and reducing its erodibility. One of these methods, termed bio grouting, has shown some promise in soil cementation via microbial induced carbonate precipitation (MICP). The microbiological process relies on uratolytic bacteria such as *Bacillus pasteuri*i to hydrolyze urea in the presence of calcium ions, resulting in the precipitation of calcite crystals.

S. pasteurii produces the urease enzyme, which hydrolyzes urea to produce both ammonium and carbonate ions [2,3]. This reaction raises the pH of the surrounding environment, ultimately precipitating calcite from carbonate and calcium ions [4,5].

The microbial method of soil improvement generally involves three steps:
• Urea is hydrolyzed by microbial urease to form ammonium and carbonate ions (Eq. (1)).

$$CO(NH_2)_2 + 2H_2O \rightarrow 2NH_4^+ + CO_3^{2-} \quad (1)$$
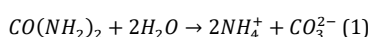
• The produced carbonate ions react with calcium ions and precipitate as calcium carbonate crystals (Eq. (2)).

$$Ca^{2+} + CO_3^{2-} \rightarrow CaCO_3(s) \quad (2)$$
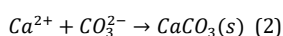
• Sand grains are bound together by the calcium carbonate crystals.

This approach has experienced an increased level of interest in recent years for applications such as restoration of calcareous stone materials bioremediation, wastewater treatment, strengthening of concrete and remediation of cracks, selective plugging for enhanced oil recovery, improvement in the stiffness/strength of sandy soil, reductions in foundation settlement, soil permeability, liquefaction mitigation and dust control [4-26]. Furthermore, calcium carbonate ($CaCO_3$) precipitation induced by *Sporosarcina pasteurii* is a potentially long-lasting, environmentally innocuous process that can be used to suppress dust from landfills, open pit mines, unpaved roads, and construction sites. In particular, *S. pasteurii* has recently been explored as a potential dust suppressive when applied to the surfaces of different soils including poorly-graded commercial sandblasting sand, silt, and clay soils [27].

In this study, we have introduced a biological dust control technique utilizing a naturally occurring soil microorganism, *Sporosarcina pasteurii*, which is capable of inducing calcium carbonate precipitation in the environment. To evaluate the dust suppressive potential of this microbial calcite, *S. pasteurii* was suspended in medium and applied to sand. The treated soil samples were tested via a wind erosion at intervals and mass losses were measured. In order to identify the optimum conditions of microbial dust suppression, we examined the effects of temperature and soil compaction amount. the type of the soil samples treated with *S. pasteurii* formed a crust-like layer on the surface and showed a significant reduction in mass loss. Our study demonstrated the potential of this microbial mediated process as an effective, environmentally friendly means of dust control.

2. MATERIALS AND METHODS

2.1 Soil Preparation and Analysis

Compatibility between the soil grain characteristics and bacteria size is an important factor for MICP treatment. The soil pores should be of sufficient size to allow the transportation of bacteria 0.5 –3.0 μm in length therefore the particle size of 50–400 μm can be considered as the most favorable range for bacterial activity in the pores [28]. In the present study, a

medium sand was investigated and comprised angular to sub-angular quartz grains.

In this study, the soil was obtained from Dasht-e Loot desert in Iran. Percentages of soil components of various sizes were determined through sieve analysis. Particulate matter is nearly poorly graded, with a uniformity of coefficient of 2.2 and a coefficient of curvature of 0.77 (Table 1). This size distribution is the soil designation of 'SP' (poorly-graded sand) based on the Unified Soil Classification System (ASTM D 2487).
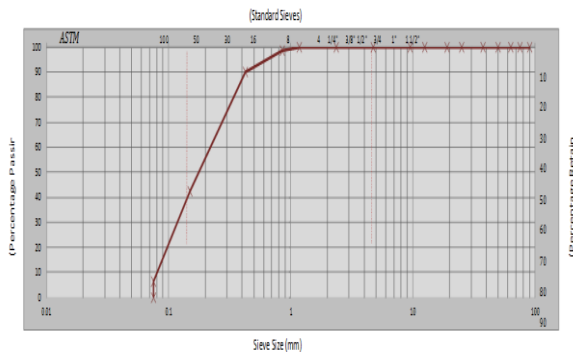

Figure 1: Grading curve

Table 1: Soil Properties

Soil type	D_{50} (mm)	C_u	C_c	G_s	Shape	USCS
Sand	0.18	2.2	0.77	2.63	Round	**SP**

2.2 Preparation and Treatment of Bacteria

For the MICP process, *Sporosarcina pasteurii* (PTCC 1645) purchased from the persian type culture collection was used as the urease-positive bacterium. *Bacillus pasteurii* (*Sporosarcina pasteurii)* is a bar and Gram-positive bacteria with a spherical spore which is formed at the end of the cell. This bacterium can be separated from different soils and wastewater. According to the available compounds in medium, the colonies with different forms are created which are usually spherical and opaque. The cultures are very active in converting urea to calcite and the optimal PH for bacteria growth is alkaline about 9. *Sporosarcina pasteurii,* a urea hydrolyzing bacterium, were cultivated under aerobic batch conditions in a medium containing yeast extract 7 gL-1; urea 20 gL-1; tryptone 5 gL-1 and meat peptone 5 gL-1. Before the cultivation medium was sterilized at 121 oC and pH of the medium was adjusted to 9. The growth medium was inoculated with the *Sporosarcina pasteurii* stock culture at 30°C in a shaker (160 rpm) for approximately 48 hours before harvesting and then the bacteria were stored suspended in their growth medium in the fridge at 4oC prior to use.

2.3 Preparation of cementation solutions

In this study, the non-equimolar combinations of urea and calcium chloride concentrations were investigated as cementation solutions for sand. Considering that the molecular weights of anhydrous urea and calcium chloride are 60 and 111 g/mol, respectively. For these cementation solutions the urea molarity was 1.85 times that of the $CaCl_2$. Hence, to produce cementation combination (1.85 M urea–1 M calcium chloride), it has been used 111 g of urea and 111 g of calcium chloride that was dissolved per liter of distilled water (i.e. molecular weight ratio of 1.85).

3. EXPERIMENTAL METHODS

3.1 Specimen preparation

Test specimens of sand were prepared for MICP treatment followed by strength testing using the method adopted from Ismail [29]. All columns used in this study were made of Poly Vinyl Chloride (PVC) tubes with 60 mm internal diameter by 200 mm long, with the two halves of these moulds held together during the preparation and curing stages using fastener. A rubber membrane was fitted in contact with the inner wall surface of each assembled mold and a layer of filter paper was placed at the bottom of each mold. Then, the dry sand was air pluviated into each specimen mould in three layers of equal thickness. And finally, 10 mm-deep gravel filters were placed above each sand specimen prepared. For uniform distribution of bacteria solution, a container with 60 mm external diameter was used. At the bottom of this container, some holes were

created to transfer the solution to the soil for having a uniform distribution for the solution.

3.2 Injection strategies

Successful MICP treatment in sand requires the injection of the bacterial cell and cementation solutions followed by their permeation through the entire sand specimen/bed. The most important factor in achieving an even deposition of precipitated calcite throughout the sand mass is the uniform distribution of the bacterial cells [30]. Different MICP injection strategies consisted of the following steps:

(a) Mixing the bacterial cell and cementation solutions together before injection. However, the reagents flocculate immediately, and while this approach may be considered for coarser soils, rapid clogging of the pore voids generally occurs for fine/ medium sand, rendering the treatment ineffective [31,32].

(b) Two-phase injection, in which the bacterial cell solution is injected first, followed by the cementation solution [33].

(c) Staged injection, with or without retention periods between the injection phases [34]. Using this approach, excessive crystal accumulation close to the injection point can be prevented from occurring and a more uniform distribution of calcite crystal formation can be achieved over a greater distance in the sand specimen. More effective MICP treatment is achieved using retention periods between injection phases that allow more bacteria to be fixed into the pore void space. Retention periods also facilitate greater numbers of reactions to occur between the bacterial cell and cementation solutions [35].

(d) Single-phase injection or reagents injected simultaneously into the sand [30]. In the present study, for bio-cementation under unsaturated conditions the percolation method adapted from staged injection with retention periods was used. Sand columns were positioned vertically. Bacterial suspension and cementation solution was introduced from the top of the columns. The transport of liquid was the result of gravity and capillary forces. For the main testing program, the MICP treatment was applied to lose specimens of sands A and B using staged injection with downward flow, including retention periods.

The stage injection steps in this study included as:
● A bacterial cell solution of volume 2Vv was injected into each specimen, where Vv is the volume of the specimen pore voids, which was 98.4 ml for the 120 mm-long specimens of sand that were prepared in the manner described earlier.

● After this volume of bacterial cell solution has been introduced into each specimen, the flow was stopped for a 6 hours period, allowing the bacterial cells to be adsorbed by the sand grains. At the end of this retention period, the bacterial cell solution was allowed to drain under gravity from the specimen base.

● Reservoirs containing the urea–$CaCl_2$ solutions were placed above the specimens and cementation solution of volume Vv was injected into each specimen.

● After the urea–$CaCl_2$ cementation solution of volume Vv had entered each specimen, the flow was stopped for a 24 hours period to allow the bacteria to react with the cementation solution. At the end of this 24 hours retention period, each sand specimen was allowed to drain under gravity from its base.

3.3 Unconfined Compressive Strength (UCS) Tests

Effect of MICP treatment using of urea–$CaCl_2$ cementation solutions and bacteria cell were assessed in terms of measured strength and stiffness values. Measures of the absolute improvements in strength and stiffness under confined compression achieved by the MICP treatment could not be determined since the untreated sand specimens mobilized no shear resistance [30]. Hence, the effects of bacterial cell and urea–$CaCl_2$ solution were evaluated by comparing the treated specimens' UCS and stiffness values. The UCS values were determined as the peak deviatoric stresses mobilized and the stiffness values were determined as the gradients of the steepest, approximately linear portions of the deviatoric stress– strain plots.

For investigation on compressive strength of treated soil to evaluate the effects of other environmental conditions such as the amount of soil compaction in remediation, five different modes of soil compaction were

used (Table 2)

To quantify the strength imparted into the MICP treated silica sand under different samples, the unconfined compressive strength (UCS) tests were conducted on cemented specimens. The uniaxial compressive tests were conducted in accordance with ASTM D2166 using standard UTM (Universal Testing Machine) device (Unconfined Compression Machine, Motorized, 110V/60Hz, 500 LB, MODEL#: S8631). In this experiment, the axial load was applied at a constant rate of 1.0 mm/min.

Table 2: Five different density of sand

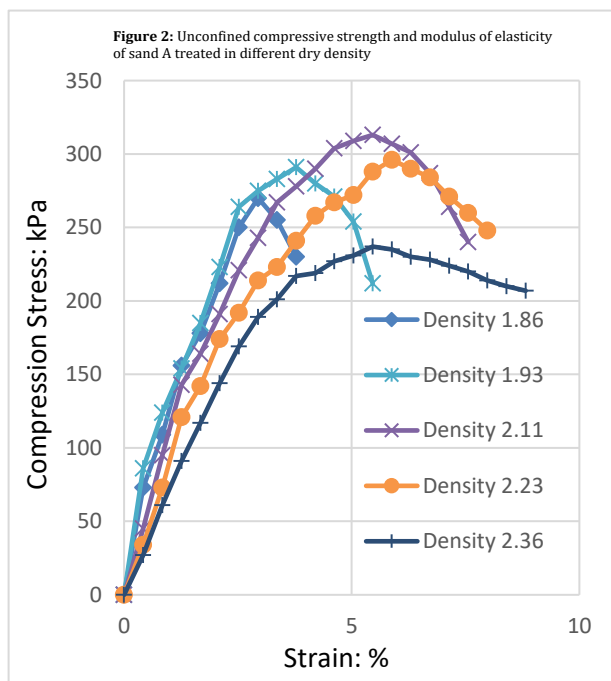| Different modes	Soil Unit Weight (gr/cm³)	Dr %
Mode 1	1.86	0
Mode 2	1.93	17
Mode 3	2.11	56
Mode 4	2.23	78
Mode 5	2.36	100

3.4 Temperature

In this study we used Sporosarcina pasteurii at 10, 15, 21, 35, 50, 60 and 80℃ to evaluate that urease-catalyzed ureolysis is temperature-dependant like any other enzymatic reaction and investigated that microbial activity and temperature are one of the key factors promoting calcite precipitation. The effect of temperature on urease activity was measured in experiments where a constant number of bacteria was supplied with 1.85 M urea and 1 M $CaCl_2$ and the temperature was varied. In order to evaluate temperature as an effect of microbial induced carbonate precipitation on urease activity and soil strengthening process uniaxial test was used for measuring unconfined compressive strength. Notice that the rate of speed for the unconfined compression test set at 1 mm per minute.

3.5 Dust Suppression

Soil wind erosion tests were performed by using a desert wind simulator, with a height of 0.42 m, a width of 0.45 m, and a length of 5.4 m. Wind speed was measured using a handheld turbo meter wind speed detector to determine the correct blower input voltagecorrelating with 80 km/hr according to the maximum local wind speed. Incoming air was filtered by adding fiberglass filters to the panel in front of the blower fan. In this simulation, soil erosion was studied in three representative samples wwhich include as:

1- MICP treated with 1500 cc cementation solution in which urea molarity was 1.85 times that of the $CaCl_2$ and 1500 cc suspension of bacteria;
2- Treated with 1500 cc water solution;
3- Untreated (control) sample

Figure 2: Unconfined compressive strength and modulus of elasticity of sand A treated in different dry density

and each of them was wind erosion tested at intervals of 1, 2, 3, 4, and 5 days. Three replicate samples were placed into the plates with the sizes: 40 cm in width, 60 cm in length, and 5 cm in height and placed side-by-side at 290 cm downstream of the blower fan in the center of the wind erosion simulator, with 20 mm spacing between each. To account for uneven erosion across the soil surface, 7 cm tall spires were constructed at 0.46 m in front of the soil cups to disperse the wind flow according to the method adopted from Meyer [26]. Samples were subjected to 80 km/hr wind for 30 min each day. Following wind erosion testing, final masses were recorded. Mass loss of the soil sample was calculated by taking the difference between the masses measured before and after wind tunnel testing.

4. EXPERIMENTAL RESULTS

4.1 Uniaxial compression test

Each UCS and stiffness value reported in Table 3 and Figure 2 is the mean of the respective values measured for the three specimens tested for each experimental condition, with good reproducibility achieved. Hence, the effects of bacterial cell and urea–$CaCl_2$ solution were evaluated by comparing the treated specimens' UCS and stiffness values. Thereby, the uniaxial compressive strength of five different density of soil was studied at density of 1.86, 1.93, 2.11, 2.23 and 2.36 g/cm³ and the results are presented in Figure 2.

Figure 2 indicates changes for uniaxial compressive strength, so that the maximum amount of uniaxial strength and stiffness in the density of 2.11 gr/cm³ was equal to 313 kPa and 11.3 Mpa, respectively. Then, the amount of uniaxial strength and hardness reach to 240 kPa and 6.7 MPa with a significant drop. This reduction in strength and modulus of elasticity in high densities is due to the decrease of calcite precipitation which resulted from the lack of bacteria transferability in soil high densities. Furthermore, based on the studies of DeJong et al, calcite precipitation is normally formed around the intersections of particles [21]. In higher densities, soil particles are replaced by large bonds of calcite deposition and lead to the formation of smaller and more calcite precipitation in one area. The softer behavior of soil and increased flexibility are the consequences of this effect.

Table 3: UCS and stiffness value

Different modes	Soil Unit Weight (gr/cm³)	Stiffness: (MPa)	UCS (q_u) kPa
Mode 1	1.86	8.7	270
Mode 2	1.93	10.5	291
Mode 3	2.11	11.3	313
Mode 4	2.23	9.6	296
Mode 5	2.36	6.7	237

4.2 Temperature result

Temperature is one of the most important factors of MICP process and that affecting the interaction between sand grains and calcite precipitation. In this study, the compressive strength of the treated soil was investigated at

temperatures of 10, 15, 21, 35, 50, 60 and 80°C and soil with dry density of 1.86 g/cm^3 was used to make sand columns. Figure 3 shows that by an increase in temperature until 60 degrees, the unconfined compressive strength increased along the increase of bacterial activity; in fact, this increase in temperature increased the solubility of the solution and enhanced rates of calcite precipitation than lower temperatures. Due to the solubility of gases in liquids reduced with the increase of temperature, the amount of dissolved air in water reduced from 60 to 80 degrees and as result compressive strength reduced slightly.
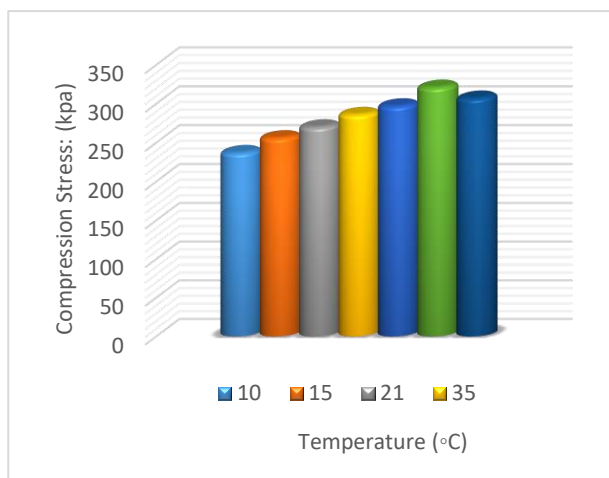

Figure 3: The effect of temperature on unconfined compressive strength

4.3   Wind erosion experiment

Figure 4. shows that the microbial-induced calcite precipitation method considerably improved the strength of soil against wind

5.   CONCLUSIONS

This study was conducted to investigate the influence of MICP method on the improvement of sand which has made a lot of diseases for the people living in the east of Iran. The effects of microbial treatment method to resolve these problems were studied through a series of experiments. The soil density and temperature conditions were studied to achieve the optimal conditions of bacteria reaction. Then, a model was made for the wind erosion experiment by using the optimal conditions obtained at the previous step to study the sample performance against the wind.

The results indicated that MICP method is very appropriate for the regions where need dust suppression. The uniaxial compression tests results showed that there were various factors affecting on forming resistant bonds among sand grains. The highest stiffness and compressive strength were obtained for the density of 2.1 gr/cm^3, while the higher values of density of soil prevented the bacteria from transferring easily among the sand particles. Another factor is a temperature that can change the compressive strength of samples treated by MICP. The optimum temperature for bacteria activities was 60 °C, and curing under higher temperature (80 °C) reduced the compressive strength because of a drop in the amount of dissolved air in water for anaerobic bacteria.

Moreover, the results obtained from wind erosion experiments showed the appropriateness of this method to be used as the stabilization of desert sands and replacement for traditional methods.

Overall, it can be concluded that MICP method can be alternative for traditional methods and materials in improving and controlling the harmful sand existing in deserts of Iran.

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