Journal of Civil Engineering and Construction 2019;8(4):149-156
https://doi.org/10.32732/jcec.2019.8.4.149

A Model-Scale Investigation for Microbially Induced Calcite Precipitation in Sand

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Received: 8 July 2019; Accepted: 22 August 2019; Available online: 30 September 2019

Abstract: New, exciting opportunities for utilizing biological processes to modify the engineering properties of the soil (e.g. strength, stiffness, permeability) have recently emerged. Enabled by interdisciplinary research at the confluence of microbiology, geochemistry, and civil engineering, this new field has the potential to meet society's ever-expanding needs for innovative treatment processes that improve soil supporting new and existing infrastructure. Ureolytic bacteria are one of the most efficient organisms in producing amounts of carbonate that easily react with the free calcium ions available in the environment. *Sporosarcina pasteurii*, a robust microbial alkaline environment was used in this work for its high potential in the biocementation process that involves the biomediated calcite precipitation. This study presents the results of a model-scale laboratory investigation conducted on bio-cemented siliceous sand. Chemicals used in this study are commercially available in order investigate the viability of implementing this technique in the field at larger scales. To make it more practical, the microbial cells are directly used with neither sterilization nor utilization of a centrifuge process for the growth medium. Blocks of the bio-treated soil were excavated from the model and were tested to examine the strength and durability parameters of the improved soil. The results show that the unconfined compressive strength (UCS) and slake durability index significant increased upon biological treatment. However, due to the downwards permeation of the fluid due to gravity, samples obtained from the bottom and the center of the treated column gave larger UCS and slake durability indices than those obtained from the top and the sides of the column.
Keywords: Bio-cementation; *Sporosarcina pasteurii*; Soil improvement; Model-scale; Unconfined compressive strength; Slake durability.

1. Introduction

Siliceous sands are widely available across the arid land of Egypt. Aeolian sand deposits cover significant area of the Egyptian deserts. Such deposits are characterized by very low densities and are found in a very loose state. Furthermore, many applications, such as the construction of water canals and embankments, are introduced on weak siliceous sand formations. The geotechnical behavior of siliceous sands is usually connected with various interdependent problems, such as high permeability, low bearing capacity, unstable slopes and erosion by wind and water if it exists as a natural slope or if formed by the excavation of water canals. Construction of canals and embankments in such formations necessitates the need for soil improvement as one of the most economic engineering solutions to overcome stability, erosion and seepage problems [1-3]. Soil improvement or stabilization can take place using mechanical, biological or chemical methods.

One of the common technique or usual approach for soil stabilization was by removing the loose soil and replacing it with denser and stiffer material such as gravel or crushed rock. The cost of replacing loose deposits is significantly high, thus it led various researches to investigate other methods in order to solve this problem [4]. Over the past decades, bio-cementation has emerged an advanced and sustainable technique for soil improvement [5,6].

$$CO(NH_2)_2 + 3H_2O \xrightarrow{\text{Urease}} 2NH_4 + CO_3^{2-} \quad (1)$$

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

Cementation is a process whereby calcium carbonate either forms or deposits on a surface. When it is the product of a biological process it can be called biocementation. Ureolytic bacteria, especially *Sporosarcina pasteurii* (formerly *Bacillus pasteurii*), has generated a lot of interest where it produces the urease enzyme which has the ability to convert urea into ammonia and carbonate. The produced carbonate precipitates with calcium (Equations (1) and (2)) [7-12].

Yang and Cheng [13] reported that the microbial cementation reinforcement is used when traditional grouting materials, such as lime, cement and epoxy, cannot be employed. This new material is superior to conventional methods in enhancing strength and durability of soil. Bio-cementation takes place through the application of a bacterial culture to a soil volume. The treated sand volume of loose sand turns into a stiff medium in few days [14-16].

Urease is produced via hydrolyses of urea by soil microorganisms, producing carbon dioxide which forms calcium carbonate in presence of calcium ions. Calcium carbonate precipitates on the surface of soil particles forms bonds between the grains which enhances the mechanical properties of cemented materials [17]. This study investigates the bio improvement of sand based on microbial carbonate precipitation in a laboratory model

2. Materials and methods

An experimental program was conducted at the laboratories of the Construction Research Institute of the National Water Research Center, in Egypt. The experimental program included soil characterization before and after treatment, and strength and durability measurements.

2.1 Soil

Figure 1 shows the grain size distribution of the natural soil used in this study. The used soil is classified as poorly graded uniform sand according to USCS classification system with a mean diameter (D_{60}) of 0.675 mm, effective diameter (D_{10}) of 0.211 mm, a uniformity coefficient (C_u) of 3.196, and a coefficient of curvature (C_c) of 1.385. Fine sand constitutes 26.2% of the soil, medium sand 67.5%, coarse sand 3.9% and gravel 2.4%. The index and engineering properties of the tested sand were determined according to ASTM [18].

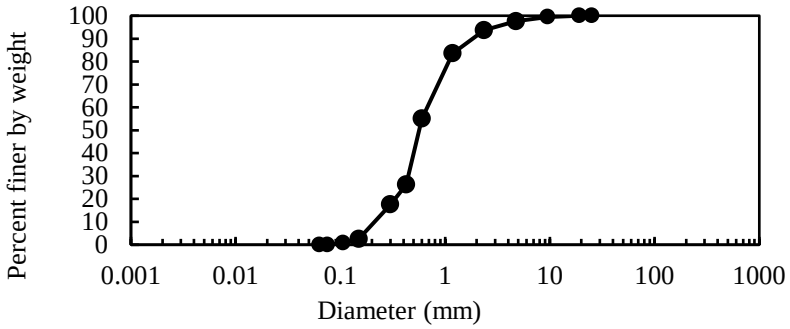

Fig. 1. Grain size distribution curve of the natural tested siliceous sand.

2.2 Bacteria and growth conditions

The soil bacterium utilized in this study was *Sporosarcina paseurii* (DSM 33). It was obtained from Deutsche Sammlung van Mikroorganismen und Zellkalturen, Germany by the National Research Center, Egypt. Cultures were grown in medium consisting of 20 g/L yeast extract and 10 g/L ammonium chloride. The cells were precultured in a 100 mL medium at 30°C and stirred at160 rpm for 24 hours. Ten milliliters (10 mL) of the preculture were inoculated into 3000 mL of fresh medium then incubated for approximately 15 hours at room temperature under aerobic conditions. Aerobic conditions were enabled by an air pump (Diaphragm vacuum pump; type/s [m3/h] :ME2/1.9; Germany). It was observed that increasing the air flowing into the container of the nutritious media leads to increasing the growth of bacteria and exit out of the container (Fig.2). Therefore, holes were created in lines connecting the pump to the container to reduce the air incoming to the container. In this study, microbial cell were incubated in unsterilized growth media. The growth media were used directly without centrifugation.

2.3 Cementation solution

The cementation solution consisted of urea (1M) and calcium chloride (1M). All chemicals used in this investigation are commercial except for yeast extract which was purchased from LAB-M, a Neogen company, United Kingdom.

Fig. 2. Overflow of bacteria out of the container due to the excess presence of air

2.4 Model description

A laboratory model was constructed from a Perspex cylinder. The cylinder had a height of 2.0 m and a width 0.5 m and was open from top and bottom. The model was filled with siliceous sand without compaction. The sand was air-dried naturally in an open area. The solutions were added to the center of the model. Dispersion and distribution of solutions within the model depends on the hydraulic properties of sand.

2.5 Treatment

The bacterial solution was added to the sand by percolation (i.e. unrestrained flushing of fluid from top to bottom) for a more homogeneous distribution of *S. pasteurii* cells throughout the column before cementation started. The columns were then left static (no flow) for 20 minutes to promote bacterial attachment to sand grains. The cementation solution was then added to the sand by percolation. The model was treated for 8 days with one batch per day. Each batch consisted of 3Lof the nutrient media and 3L of the cementation solution. Figure 3 shows the time required for the solutions to percolate the entire length of the sand column during the treatment period. A sample was taken from the effluent through the sand column after about 145 days of application.

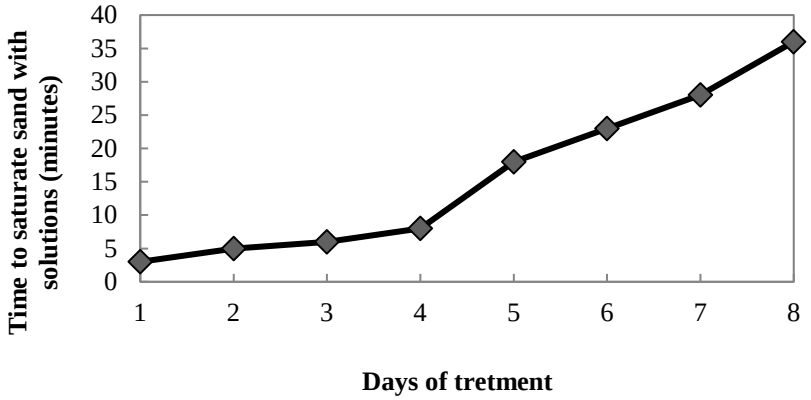

Fig. 3. Time required for solutions to percolate sand during the treatment period

3. Experimental tests

Blocks of the bio-treated sand were extracted from the model after 145 days. Samples were cored out of the blocks and a number of tests were performed to investigate the strength and durability of the collected samples.

The model was virtually split into two zones, the surficial zone and the bottom zone. A number of blocks were collected from each zone. Each block was approximately 30x40x30 cm. Figure 4 and Figure 5 show pictures for some the extracted blocks from the model.

During sample collection, each block was inspected for macroscopic defects to ensure it would provide test specimens free from fractures and joints. Samples were then extracted from different locations of the block. Table 1 presents a description of the location of each sample and Figure 6 and Figure 7 show the locations on the extracted blocks.

Sand grain size analyses were performed in accordance with the ASTM [18] test method for particle size analysis of soils on samples extracted from the model. Characteristic is classified according to the Unified Soil Classification System.

Fig. 4. Surface block after cementation.

Fig. 5. Bottom block after cementation.

Table 1 Summary of the location from the block.

Sample ID	Description of sample location
SR	Right corner of the surface block
SL	Left corner of the surface block
SC	Center of the surface block
BR	Right corner of the bottom block
BL	Left corner of the bottom block
BC	Center of the bottom block

Fig. 6. Sampling location for surface block.

Fig. 7. Sampling location for bottom block.

One of main concern in the biocementation of soil is the effect of water on the strength of the bio-cemented soil. Therefore, stability in water tested was investigated by submerging the samples in tap water at room temperature for 24 hours [6,7]. Samples from similar locations to those described in Table 1 were submerged then visually checked for loss of integrity.

Unconfined compression tests were also conducted to examine the effects of the bio-treatment on the stiffness and strength of treated sand samples. The unconfined compression tests were performed according to ECP [19].

The slake durability test was an important check and its results are closely related to its mineralogical composition and hence its resistance to degradation (weakening and disintegration). The slake durability index

was measured using standard cycles of drying and wetting. The test was carried out in accordance with ECP [20]. Samples from similar locations to those described in Table 1 were tested. Each sample comprised of nine lumps, roughly spherical in shape, each weighing 50 ± 10 g. The lumps were placed in a drum and dried in an oven at 105°C until constant weight was obtained. For the slake durability test the drum was mounted on a trough coupled to the motor. The trough was then filled with water to a level of 20 mm below the drum axis and the temperature was maintained at 25°C. After the drum had been rotated at 20 rpm for a period of 10 min, it was removed from the trough and dried at a temperature of 105°C for 4 h. During the test, the finer products of slaking pass through the mesh and into the water bath. The slake durability index (Id) is the ratio of the final to the initial dry weight of remaining in the drum.

$$\text{Slake durability index (Id)} = (W_t / B) \times 100 \quad (3)$$

where, W_t is a sample dry weight after second cycle and B is the initial sample weight before testing

Calcium carbonate content of the consolidated the treated samples was determined by adding 2 mL of 2 M HCl solution to a 1–2 g of dried sample and then measuring the volume of CO_2 gas with a U-tube manometer under standard conditions (25 ^oC, and 1 atm) [21]. The calibration was made with analytical grade $CaCO_3$ powder.

4. Results and discussion

4.1 Sand grain size distribution

Grain size distribution curves of biocemented sand samples are illustrated in Figure 8. Biocemented samples were disintegrated using a mortar and pestle just to break the particles apart and ensure minimal impact of calcite-coated sand grains. It is noticed that the fine grains of all biocemented sand samples are higher than the raw sand. The gradual increases in the fine grains of biocemented sand are due to filling the voids of the raw sand by precipitation of fine calcite. The fine grained fraction is indication of the amount of the precipitated fine calcite. Figure 8 shows that more calcite has been precipitated in samples collected from the bottom of the model compared to that precipitated closer to the surface. Fines contents in samples collected from the top and bottom blocks were approximately 8% and 13%, respectively; compared to 2.2% fines in the untreated raw sand.

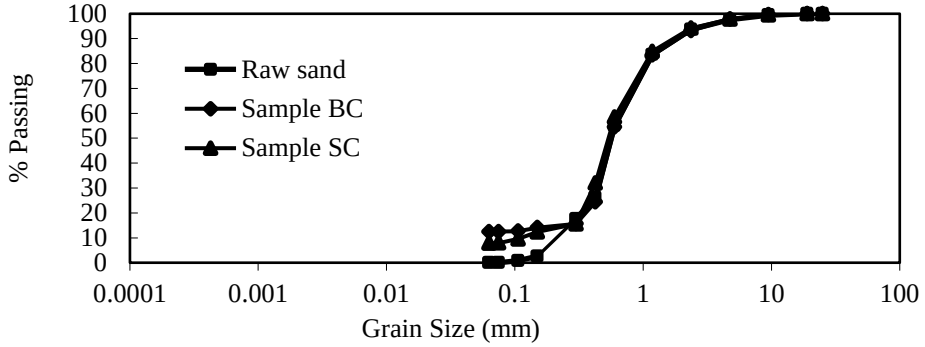

Fig. 8. Grain size of untreated sample, biocemented samples BC and SC.

4.2 Stability of bio-cemented sample in water

The results of the stability of bio-cemented sample in water test clearly illustrated the improvement of the structural stability of the soils when totally submerged in water. Bottom lumps were found to be more stable when submerged in water than surface lumps. No breakdown was observed for biocemented soil samples in center (BC and SC). However, the edge samples showed more signs of disintegration when submerged in water. Sample SR followed by Sample SL recorded lowest stability in water when compared with all samples (Figure 9).

4.3 Unconfined compressive strength

Stress strain curves of bio-cemented sand are shown in Figure 10. The results of UCS tests shows clearly that the strength of the bottom blocks is higher than that of the surface blocks. The highest value of UCS was exhibited by in sample BC (7.41 kg/cm^2) while the sample SL recorded the lowest values (2.83 kg/cm^2). Some locations on the edge of both blocks have a relatively lower strength and stiffness. The strength increases along the sand column downwards. As precipitation of calcite occurs in a random fashion, some calcite minerals may be less effective in bonding soil matrix [22].

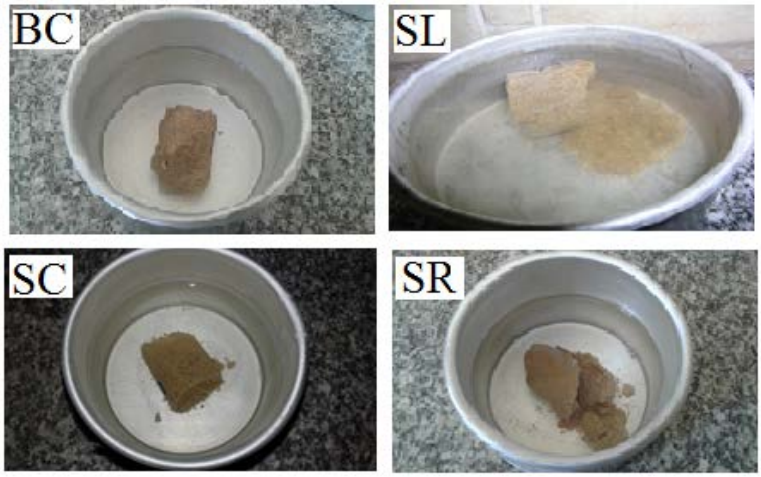

Fig. 9. Bio-cemented samples after submergence with water for 24h

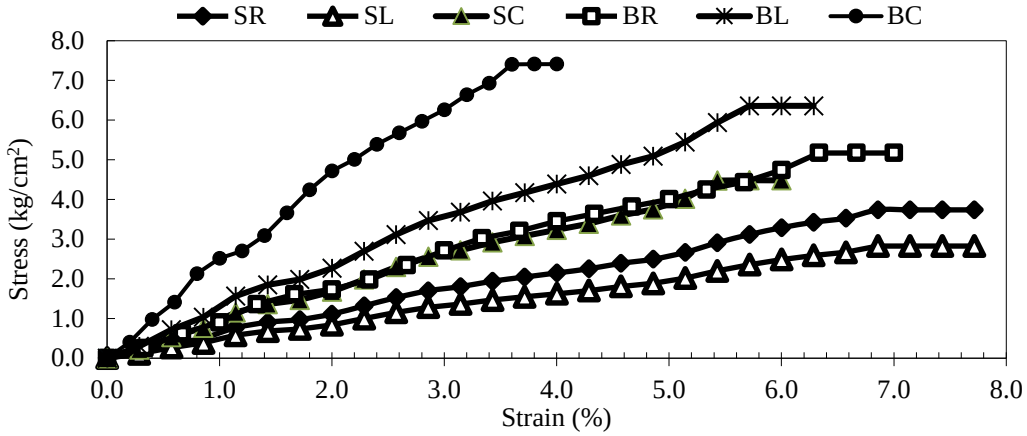

Fig. 10. Unconfined compressive strength for samples from surface and bottom blocks.

4.4 Slake durability index (SDI)

Standard slake durability tests were performed on bio improved sand. Two cycles were performed to determine the robust slake durability index. Table 2 presents a summary of SDI results. Slake durability values ranged from 25% in sample SL to 40% in sample BC. The bottom blocks offered higher resistant to slaking compared to those from the surface blocks. Higher durability indices for the bottom blocks are in agreement with UCS values obtained

Table 2. Unconfined compressive strength, Slake durability index and calcite percentage values of bio improved samples.

sample	Unconfined compressive strength(Kg\ cm^3)	Slake durability index (%)	Calcite percentage(%)
SR	3.74	28	5.7
SL	2.83	25	4.3
SC	4.69	33	7.6
BR	5.54	35	8.8
BL	6.36	38	10.7
BC	7.41	40	12.6

4.5 Calcite content

Results showing percent of calcite for bio improved sand are presented in Table 2. The carbonate content that existed naturally in the untreated soil was 2.3%. The initial carbonate content of the untreated specimen could be contributed by the presence of in situ calcite, dolomite ($CaMg(CO_3)_2$) or siderite ($FeCO_3$) [20]. The carbonate contents could be arranged in descending order as follows: in sample BC (12.6%), sample BL (10.7%), sample BR (8.8%), sample SC (7.6%), while sample SR and SL recorded slightly lower carbonate contents (5.7 % and 4.3%, respectively).

Trends of calcite concentration along the length of the model indicating relatively smaller concentrations of calcite at the edges of both blocks (5.7%; 4.3%; 8.8 and 10.7 % for SR, SL, BR and BL, respectively) compared to those at the centers of the blocks. This is consistent with the results of UCS and Slake durability testing.

As the ratio of calcite increase, the values of compressive strength and slake durability index increased. Figures 11a and 11b show the correlation between calcite content and (a) unconfined compressive strength and (b) slake durability index. The results suggest that calcite content is linearly proportional with compressive strength and slake durability index for all samples with linear regression coefficients R^2 of 0.90 and 0. 89, respectively.

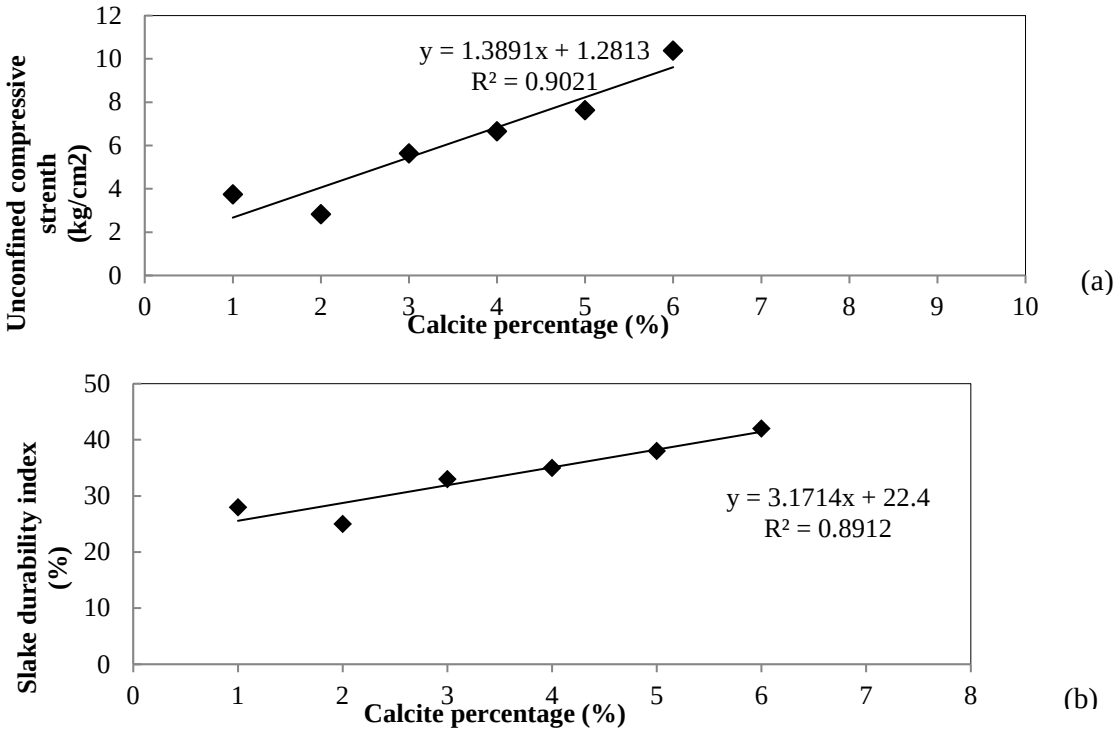

Fig. 11. Correlations between calcite content and (a) unconfined compressive strength; and (b) slake durability index.

5. Conclusions

An experimental program based on a model-scale laboratory investigation was conducted on bio-cemented siliceous sand through microbially induced carbonate precipitation. Chemicals used in this study are commercially available in order investigate the viability of implementing this technique in the field at larger scales. To make it more practical, the microbial cells are directly used with neither sterilization nor utilization of a centrifuge process for the growth medium. The improvement in the mechanical and durability properties of the tested sand was investigated through a series of unconfined compressive strength (UCS); slake durability and stability in water tests. Based on the results presented in this research, it was found that using a media with neither sterilization nor centrifugation processes and incubating bacteria for 12 h gives significant improvement to the treated sand. This procedure leads to a more simplified biological treatment procedure compared to conventional biological treatment. Such simplification can lead to implementing bio-cementation for large area or soil volumes on site with less effort and relatively shorter time. Bacteria *S. pasteurii* plays a significant role to improving the physico-mechanical properties of silica sand due to the precipitation of calcium carbonate by the bacterial activity. The unconfined compressive strength and slake durability index showed significant increase upon biological treatment. However, due to the downwards permeation of the fluid due to gravity, samples obtained from the bottom of the treated column gave larger UCS and slake durability indices than those obtained from the top of the column. Likewise, samples obtained from the center of the column shown more improvement than those obtained from the sides.

6. References

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