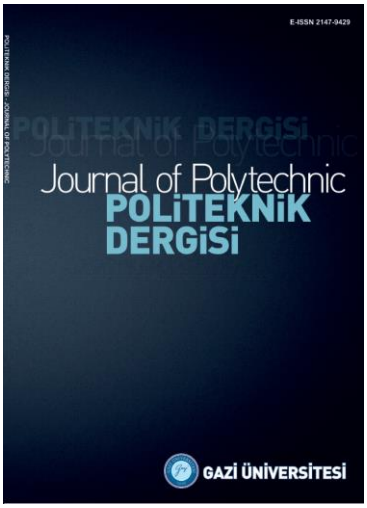

POLİTEKNİK DERGİSİ

JOURNAL of POLYTECHNIC

ISSN: 1302-0900 (PRINT), ISSN: 2147-9429 (ONLINE)
URL: http://dergipark.org.tr/politeknik

Mikrobiyolojik yöntemle çimentolanmış kum örneginin üç eksenli kesme davranışı ve mikroyapısının incelenmesi

Triaxial shear behavior and microstructure of microbially treated sand specimens

Ayşe ÖZDOĞAN DÖLÇEK[1]

ORCID[1]*: 0000-0001-9740-2273*

To cite to this article): Ozdogan Dolcek A., "Triaxial shear behavior and microstructure of microbially treated sand specimens", ***Journal of Polytechnic***, 26(4): 1675-1682, (2023).

Bu makaleye şu şekilde atıfta bulunabilirsiniz: Ozdogan Dolcek A., "Triaxial shear behavior and microstructure of microbially treated sand specimens", ***Politeknik Dergisi***, 26(4): 1675-1682, (2023).

Erişim linki (To link to this article): http://dergipark.org.tr/politeknik/archive

DOI: **10.2339/politeknik.986565**

Triaxial Shear Behavior and Microstructure of Microbially Treated Sand Specimens

Highlights

- *Use of bacteria induced calcite cementation for ground improvement as the state-of-the art*
- *This technique uses non-pathogenic organisms (Sporosarcina Pasteurii)*
- *Calcite cementation are formed at the particle-to-particle contact of sand specimen*
- *Isotropically consolidated undrained (CU) test on bio-treated sand specimens*
- *Perform Scanning electron microscope (SEM) imaging to examine the soil microstructure*

Graphical Abstract

The current study focused on cementation that is caused by calcite formation aided by the presence of calcium carbonate producing bacteria. The effects of this microbial calcite cementation on cohesionless soils was examined in detail using triaxial tests conducted under isotropically-consolidated undrained shear conditions.

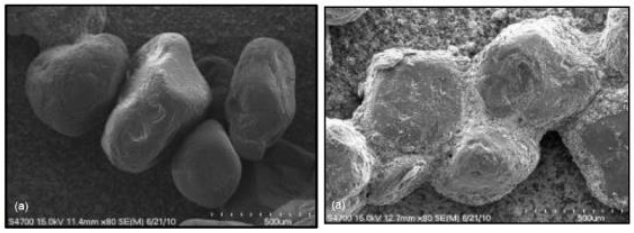

Figure 1. Images of untreated sand samples (left side) and bio-treated samples (right side) with a scale of 500μm

Aim

This study aims to describe the microbial calcite cementation that occurs in soil medium using common soil microorganism Sporasarcina pasteurii. While a number of significant factors can affect the success of the microbial treatment, this study focused on the effects of: relative density, strength of the soil, and length of curing time.

Design & Methodology

Isotropically consolidated undrained (ICU) tests were performed to define shear behaviour of bacteria cemented soils. Scanning electron microscope (SEM) imaging was performed to examine the soil microstructure over a range of specimen curing periods to assess the nature of any cementitious bonds that may have formed.

Originality

This project is a valuable first step for developing techniques that can be used to control a natural biological process, in order to reduce the liquefaction potential of a collapsible sand soil.

Findings

The bio-treated (bacteria-cemented) specimens did not exhibit a brittle type of failure mechanism; instead, much more ductile behavior was observed as contrary to expected brittle behavior. SEM imaging results that a clear image of the voids between sand particles was observed in the untreated sand matrix. Additionally, a significant amount of microbial calcite cementation was observed at the contact points between sand particles of the bacteria-cemented specimen and the shape of individual sand particles is readily apparent.

Conclusion

The average relative densities of tested specimens was achieved as of 44%, resulting in loose specimen. During testing, it was observed that the shear strength of the bacteria-cemented soil could be sensitive to the amount of back pressure applied during saturation. The SEM observations described herein indicate that the specimen curing time had an effect on the soil cementation process. A clear image of the voids between the sand particles was observed in the untreated sand matrix. Additionally, a significant amount of microbial calcite cementation was observed at the contact points between sand particles of the bacteria-cemented specimen.

Declaration of Ethical Standards

The author of this article declare that the materials and methods used in this study do not require ethical committee permission and/or legal-special permission.

Triaxial Shear Behavior and Microstructure of Microbially Treated Sand Specimens

Araştırma Makalesi/Research Article

Ayşe ÖZDOĞAN DÖLÇEK

Balıkesir University, Dept.of Geological Engineering, 10145 Balıkesir, Turkey

(Received : 25.08.2021 ; Accepted : 07.02.2022 ; Early View : 15.03.2023)

ABSTRACT

The use of bacteria induced calcite cementation for ground improvement presents a relatively new option for geotechnical engineers, one that has the potential to revolutionize the way that we improve soils to prevent liquefaction-induced damage. This technique uses non-pathogenic organisms which are found naturally in the soil environment to cement sand particles together at their particle-to-particle contacts. There is significant potential for a reduction in environmental concerns on various types of projects; in the long-term, this technique may also prove to be an extremely sustainable form of ground improvement. Consequently, the goal of the research described herein is to enhance the state-of-the-art with respect to our understanding of controlling biological cementation processes in soil. Bio-treatment of sand specimens was performed using a commonly encountered urea-producing soil microorganism called *Sporosarcina Pasteurii* (ATCC-6453). Microorganisms that were suspended in solution were introduced to the soil, and over time the microorganisms were supplied with necessary nutrients via cycling with a peristaltic pump. After bio-treatment, the specimens were back pressure saturated, isotropically consolidated, and sheared under undrained conditions. Scanning electron microscope (SEM) imaging was performed to examine the soil microstructure over a range of specimen curing periods to assess the nature of any cementitious bonds that may have formed.

Keywords: Biocementation, shear strength of sand, ground improvement.

1. INTRODUCTION

The application of bacteria for microbial carbonate cementation has been used for a decade in a number of industries for procedures such as selective mineral plugging for enhanced oil recovery [1], immobilizing calcium and contaminants in surface and ground water [2,3], restoration of calcareous stone materials [4], and bioremediation [5]. It has also been used in civil and geotechnical engineering applications, for: remediating cracks in granite and concrete [6,7,8], increasing the bearing capacity of soil [9], bioclogging (pore filling) to reduce the soil porosity and hydraulic conductivity, and biocementation (particle binding) [10,11,12].

Biocementation is of particular interest to geotechnical engineers, as it has been shown to increase the shear strength of granular soils [13,14,15,16]. Filling of cracks in concrete has also been investigated [17,18,19]. Sarda et al. [20] reported successful biocalcification in brick, and showed the favorable effect that microbes can have on improving the durability of bricks by reducing water absorption. Gomez et al. [21] conducted an experimental test for large-scale sand specimens (1.7 m x 0.3 m) and measured shear wave velocities throughout the treatment process. Their results showed a significant increase in cone penetration tip resistance after treatment. Jiang et al. [22] demonstrated the applicability of biocementation for controlling erosion in a sand-clay mixture, with characteristics that are generally similar to what is used to construct an earth embankment dam.

The current study focused on cementation that is caused by calcite formation aided by the presence of calcium carbonate producing bacteria. The effects of this microbial calcite cementation on cohesionless soils was examined in detail using triaxial tests conducted under isotropically-consolidated undrained shear conditions. This project is a valuable first step for developing techniques that can be used to control a natural biological process, in order to reduce the liquefaction potential of a collapsible sand soil.

*Sorumlu Yazar (Corresponding Author)
*e-posta: aodolcek@balikesir.edu.tr

2. MATERIAL and METHOD

2.1. Microbial Process

An isolated bacterial culture of Sporosarcina pasteurii ATCC-6453 was used in this study. This bacteria was cultured under aerobic batch conditions in an ammonium yeast (NH4-YE) medium. A tris buffer solution was prepared to adjust the pH of the bacteria culture medium for optimum bacterial growth conditions. Specific details regarding microorganism preparation and culturing are available in Ozdogan [23]. The components of the bacterial treatment solution that was utilized are shown in Table 1.

2.2. Soil and Specimen Preparation

Ottawa sand was used for all of the tests in this experimental study. This sand conforms to the requirements for standard density testing sand outlined in ASTM D 1556-07 [24], the standard test method for density and unit weight of soil in place by the sand-cone method. The grain size distribution of this sand was determined in general accordance with ASTM D 422 [25], the standard test method for particle size analysis of soils. This sand classifies as a poorly graded sand (SP), according to the unifisoil classification system [26].

Pertinent classification data and other data of interest for this sand which was collected from the technical literature are summarized in Table 2.

method was measured as 35 mm (1.4 in.) in diameter and 76 mm (3.0 in.) in height, dimensions which are in accordance with ASTM 4767-04 [32]. The average of

Table 1. Components of the bacterial treatment solution

Solution	**Constituents**	**Amounts**
Urea Medium*	Bacto nutrient broth powder Urea ($NH_2(CO)NH_2$ NH_4Cl $NaHCO_3$ Distilled Water	3 g 20 g 10 g 2.12 g 1 L**
Bacterial	*S. pasteurii* Urea medium $CaCl_2$ solution	1 pellet*** 100 mL 2 mL (25.2 mM)
Nutrient	Urea medium $CaCl_2$ solution	100 mL 2 mL (25.2 mM)

*Adjust pH of urea medium to 6.0 with 5N HCl prior to autoclaving
**Distilled water added to solids to achieve a total volume of 1 L; consequently, the total volume of water that is added is just under 1 L
***Bacteria pellet created using the preparation process described in detail in Ozdogan [23].

Table 2. General characteristics of Ottawa sand

Specific gravity, G_s[a]	2.65
Max. void ratio, e_{max}[b]	0.82
Min. void ratio, e_{min}[b]	0.51
D_{50} (mm)	0.4
C_u	1.61
C_c	0.86
Soil Description	Poorly graded sand (SP)

Notes: [a] Assume Gs for Ottawa sand = 2.65 from Chen [27].

[b] Assume emax and emin from Yamamura [28]; these minimum and maximum void ratio values are in the same range as those observed for Ottawa sand by Gallagher and Mitchell [29] and Fritzges [30], even for Ottawa sand samples of varying grain sizes. Variations in grain size do not appear to significantly affect these values. In any case, the effect of this assumption does not significantly affect the conclusions that are drawn from this research study, so this type of assumption is believed to be a reasonable approach.

Air-pluviation was chosen as one of the specimen preparation methods for this study, because this approach simulates natural deposition methods and a collapsible sample with desired relative density more directly than other commonly used sample preparation methods such as compaction, tamping, or vibration [31]. Autoclaved soil weighing between 120 and 125 grams was poured into the triaxial specimen split-mold using a mini-pluviator, while lifting the pluviator towards to top of the split-mold to maintain a consistent (and relatively low) level of drop energy. The triaxial specimen split-mold was then stretched with a latex membrane; this split mold was used as the forming jacket for the specimens, which was necessary given the cohesionless nature of the sand that was used in this study. Further details regarding to specimen preparation method can be found in study by Ozdogan [23]. The approximate size of all of the specimens that were prepared using the air pluviation

the measurements, combined with the weight of the specimen, was used to calculate the volume and density. Relative density for each specimen was calculated using the following equation;

$$D_r = \frac{e_{max}-e}{e_{max}-e_{min}} x\ 100\ (\%) \quad (1)$$

where e_{max} = maximum void ratio, e_{min}= minimum void ratio, e= specimens void ratio (measured).

As stated earlier e_{max} and e_{min} was assumed from the literature [28,29,30]. It should be noted that determination of void ratio for this specific specimen preparation method (air pluviated soil) is not easy. Although results are highly variable and operated-dependent, average relative density of 44% was achieved.

2.3 Testing Procedure

Isotropically consolidated undrained (ICU) tests were performed in general accordance with the guidance provided in ASTM D 4767-04 [32]. The ICU triaxial tests that were conducted had three major stages: back pressure saturation, consolidation, and shear. After completion of microbial treatment, back pressure saturation of each triaxial specimen was performed to ensure that the pore pressure measurements at the end of the specimen could be considered to be reasonably representative of the pore pressures on the specimen failure plane.

Periodically, to assess the progression of saturation of the specimen as the back pressure was being increased, a "B-check" process was used. Following this procedure, incremental values of Skempton's pore pressure parameter B were calculated using the following equation:

$$B = \frac{\Delta u}{\Delta \sigma_3} \qquad (2)$$

Where B = Skempton's pore pressure parameter, Δu = changes in pore pressure resulting from changes in all-around stress, and $\Delta \sigma_3$ = an incremental change in all-around stress (total confining pressure). After completion of back pressure saturation, each specimen was isotropically consolidated to an effective confining pressure of 69 kPa (10 psi). During consolidation, the cell pressure was increased slowly, using small pressure increments (e.g., 3.5 kPa) that were applied gradually over the course of one hour, to minimize the breakage of any cementitious bonds that may have been formed in the specimen during the microbial treatment process. The consolidation process for each specimen was consequently completed within approximately 60 minutes.

After completion of consolidation, each specimen was subjected to axial compression using displacement-controlled loading that was applied at a strain rate of 5% per hour. This shear was conducted under undrained conditions (both drainage valves were closed), with pore pressure measurements taken throughout the shearing process. Each specimen was sheared until an axial strain of 15% had been reached. Bio-treated specimens and untreated specimens (control tests) were carried out simultaneously to check the effectiveness of microbial calcite cementation for improving the shear strength of loose specimens. An overview of the complete triaxial testing setup with microbial treatment is shown in Fig. 1.

3. RESULTS AND DISCUSSION

3.1 Shear Response of Bio-Treated Specimens

Figure 2 presents the deviator stress versus strain response from tests conducted on eight bio-treated specimens. Surprisingly, the bio-treated specimens did not exhibit a brittle type of failure mechanism; instead, much more ductile behavior was observed. In general, as has been observed by other researchers (e.g., Ismail 2002; Dejong et. al., 2010), specimens cemented by chemical or microbial methods typically have a greater strength than untreated specimens, and typically exhibit a brittle stress-strain response. This type of cemented-specimen behavior was not observed for the bio-treated specimens herein shown in Figure 2.

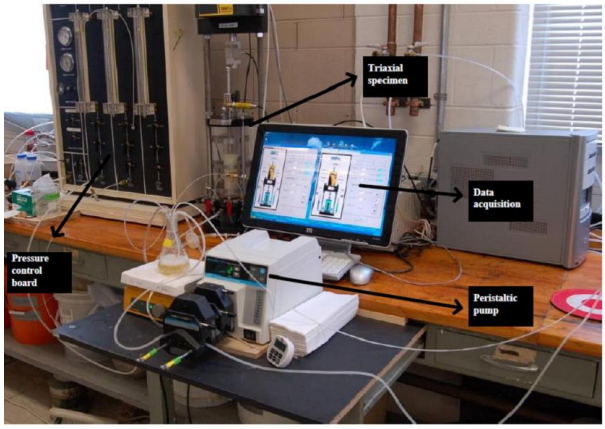

Figure 1. Schematic view of setup for ICU triaxial testing with microbial treatment

As shown in Figure 2, the bio-treated specimens also exhibited significantly different stress-strain behavior during shear, particularly at larger strains, even though they were each prepared and tested in a similar manner. One possible explanation for this behavior is the variation of relative density of each specimen that is inherent to the air pluviation sample preparation method that was used. The results show that the bio-treated specimens (the "Bio Tests"), began to fail at an approximate deviator stress of 45 kPa, around an axial strain of 0.4%, and that they each then exhibited strain hardening behavior for the rest of the test (Figure 2).

On the other hand, the untreated specimen (Bio Control-4) showed a slightly higher failure point (in terms of deviator stress), which was about 50 kPa at an axial strain of 0.4%, and also exhibited the most significant amount of strain hardening of all of the tests that were conducted. Since the stress-strain response was ductile, and changes in specimen pore pressure were believed to play a significant role in specimen behavior, "failure" of the specimens was defined as the point corresponding to the maximum effective principal stress ratio (σ'_1/σ'_3); fo evaluation purposes, principal stress ratio vs. strain plots for each of the tests that were conducted are shown in Figure 3. The maximum effective principal stress ratio and the corresponding deviator stress, axial strain, and pore water pressure for all of the bio-treated and untreated triaxial tests are summarized in Table 3.

Figure 4 shows the pore water pressure responses of specimens, Δu, during shear. As can be observed, the general trend in the pore water pressure responses – a small increase in pore pressure at small strains, followed by a significant decrease in pore pressure at larger strains – was the same for all of the specimens. The magnitude

of this response, however, was often substantially different from specimen to specimen.

The untreated specimen (Bio Control-4) exhibited the largest changes in pore pressure, increasing to a value of +5 kPa at an axial strain of 0.4%, and then decreasing gradually for the rest of the test to a value of -210 kPa. The changes in pore pressure for all of the bio-treated specimens exhibited a similar trend, whereby pore pressures gradually increased to a maximum value in the range of 5 to 30 kPa at small axial strains (e.g., 0.8 %), and then gradually decreased to values ranging from -55 to -180 kPa at an axial strain of 15%.

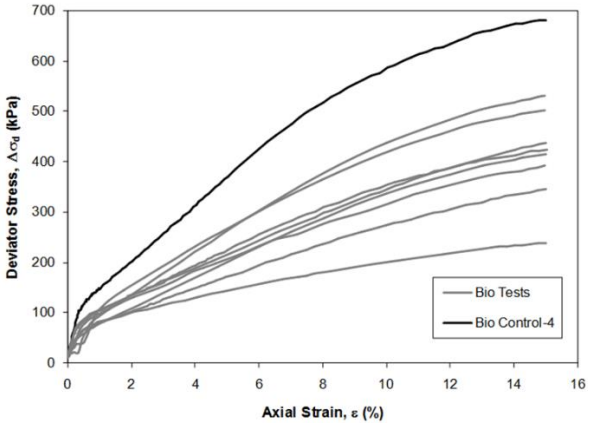

Figure 2. Deviator stress versus axial strain, ICU triaxial, σ'_3=69 kPa (10 psi)

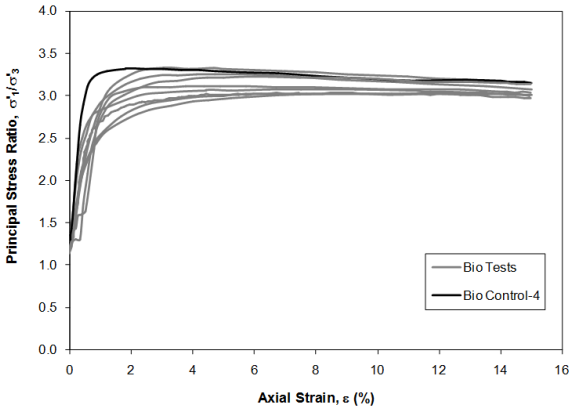

Figure 3. Principal stress ratios versus axial strain, ICU triaxial, σ'_3=69 kPa (10 psi)

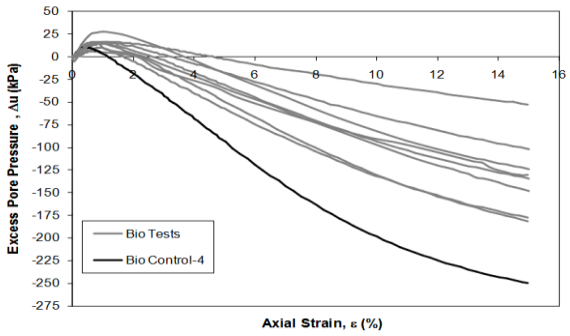

Figure 4. Pore water pressure change versus axial strain, ICU triaxial, σ'_3=69 kPa (10 psi)

Table 3. Specimen properties and stress-strain relationships at failure for all specimens tested (failure selected as the point of maximum effective principal stress ratio)

Specimen	Relative Density D_r (%)	Axial Strain at Max. PSR ε (%)	Max. Principal Stress Ratio (PSR) σ'_1/σ'_3	Deviator Stress at Max. PSR $\Delta\sigma_d$ (kPa)	Pore Water Pressure at Max. PSR Δu (kPa)	B- Value
Bio Control-4	47	0.9	3.3	144	5	0.97
Bio-1	42	4.5	3.0	160	-12	0.93
Bio-2	43	2.9	3.0	152	-15	0.92
Bio-3	47	2.9	3.2	174	-11	0.91
Bio-4	46	1.7	3.1	146	-1.4	0.91
Bio-5	49	3.4	3.3	176	-10	0.96
Bio-6	38	3.4	3.1	170	-16	0.93
Bio-7	42	2.1	3.3	112	20	0.96
Bio-8	42	3.1	3.0	114	9	0.94

3.2 Microscopic Examination of Microbial Calcite Cementation in Soil Specimens

In order to investigate the relationship between the observed soil shear behavior and the apparent microstructure of the soil specimens, a variety of SEM imaging tests were conducted on specimens having various curing times after the triaxial test. Representative samples taken from different triaxial specimens were imaged after 14, 19 and 25 day curing periods. A number of bio-treated specimens that were not subjected to a curing period were imaged immediately after the triaxial test. Figure 5 shows the imaging results that are typical when examining an untreated (control) test specimen. It can be observed that the image is quite clear, and the shape of individual sand particles is readily apparent, with the gaps and voids that are present in the untreated sand matrix being clearly visible, along with no evidence of any cementation at the particle contacts (Bio Control-4). Another sample from a bio-treated specimen (Bio-8) that was imaged immediately after the triaxial test, without being subjected to any curing period, exhibited light evidence of microbial calcite cementation at the particle contacts, as shown in Figure 6.

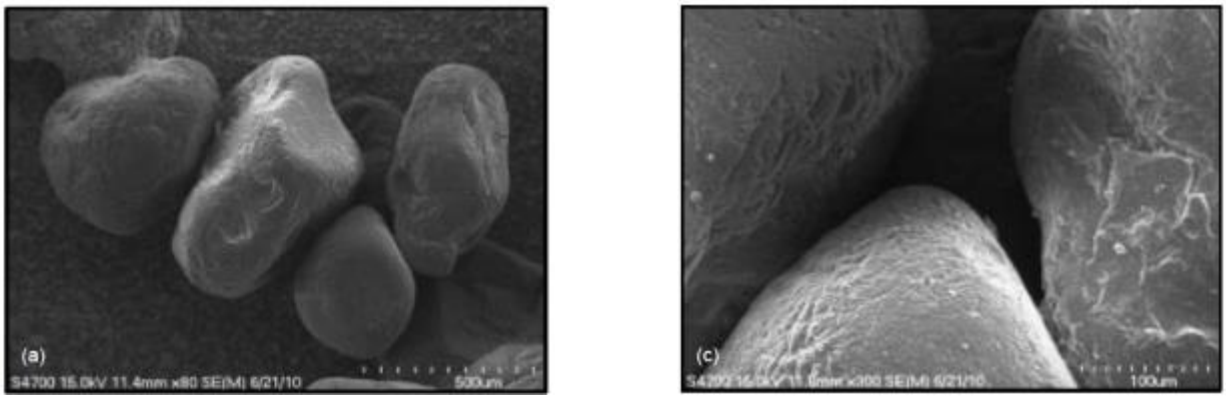

Figure 5. Images of untreated sand samples, Bio Control-4, with different scales; 500µm and 100µm

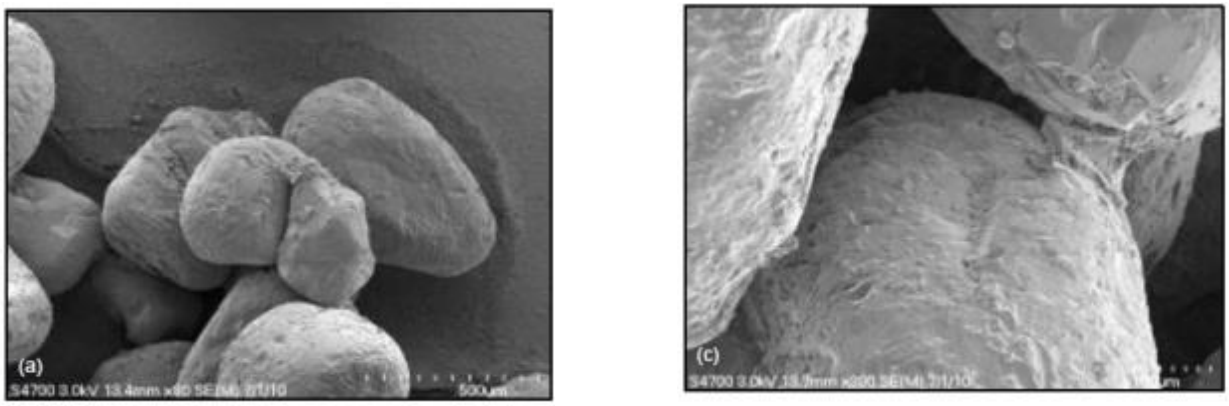

Figure 6. Images of biotreated sand samples, Bio-8, with different scales; 500µm, 100µm (no curing period)

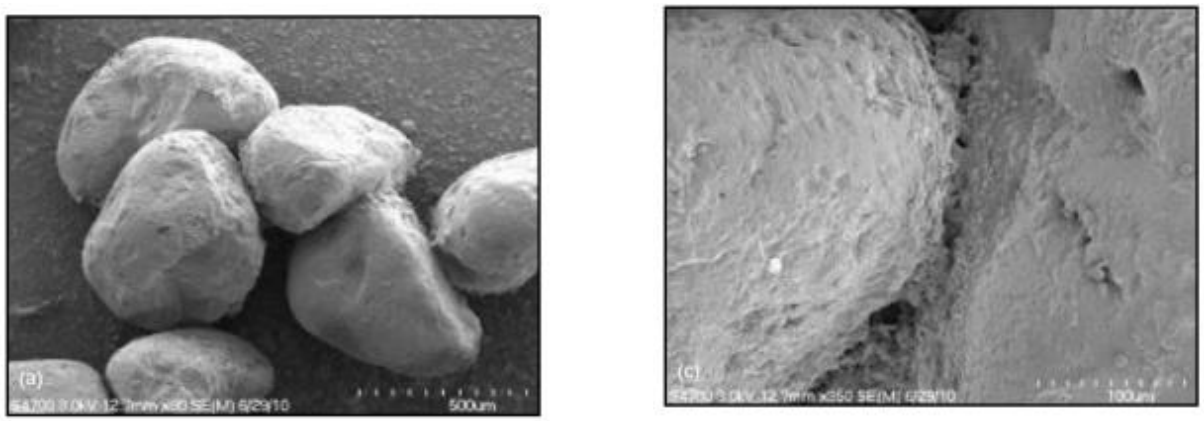

Figure 7. Images of biotreated sand samples, Bio-7, with different scales; 500µm,100µm (14 day curing period)

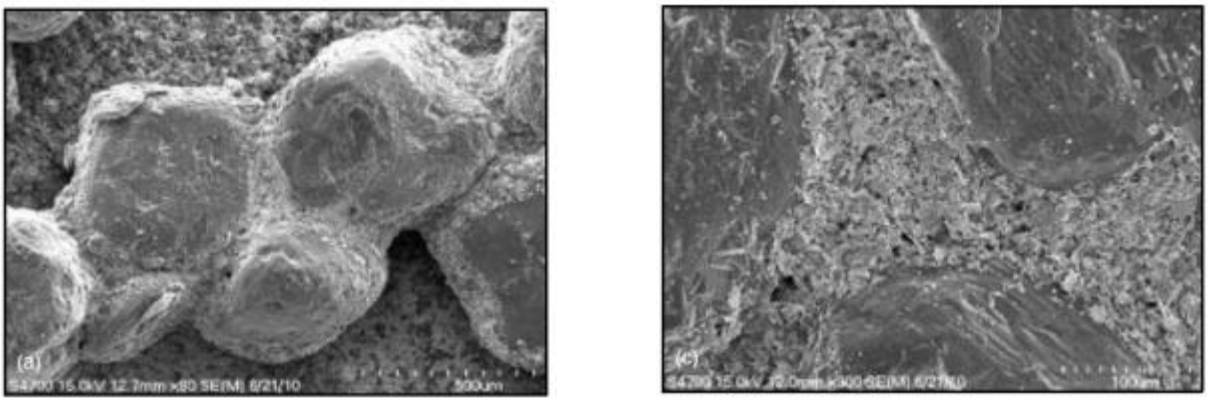

Figure 8. Images of biotreated sand samples, Bio-6, with different scales; 500µm, 100µm, (19 days curing period)

A biotreated sample cured for 14 days (Bio-7) also showed light evidence of microbial calcite cementation at the particle contacts, and a significant difference from the control specimens, as shown in Figure 7. Another specimen cured for 19 days (Bio-6), exhibited a significant increase in calcite cementation at the particle contacts, with much of the void space between particles also being filled up with the cementing agent (Figure 8). The SEM observations described herein indicate that the specimen curing time had an effect on the soil cementation process.

4. CONCLUSION

Microbial calcite cementation was performed using the common soil microorganism *Sporasarcina pasteurii* and a liquid growth medium containing urea and calcium chloride. While a number of significant factors can affect the success of the microbial treatment, this study focused on the effects of: relative density, microbial calcite cementation on strength of the soil, and length of curing time. In the testing procedure that was conducted,

specimens were prepared using air pluviation methods. The average relative densities of tested specimens was achieved as of 44%, which resulting in loose specimen.

The bio-treated specimens generally did not exhibit stress-strain behavior that was significantly different than the control specimens. During testing, it was observed that the shear strength and behavior of the cemented soil could be sensitive to the amount of back pressure applied during saturation. It is believed that any cementitious bonds that may have formed during bio-treatment were broken during the back pressure saturation and consolidation phases of the test; scanning electron microscope (SEM) imaging of specimens post-test support this observation.

The bio-treated specimens were imaged using a SEM after triaxial testing was completed, and after being subjected to a range of curing periods. As expected, the SEM images showed that the rod-shaped *S. pasteurii* could be clearly observed in the sand matrix. Additionally, a significant amount of microbial calcite cementation was observed at the contact points between sand particles. A clear image of the voids between the sand particles was observed in the untreated sand matrix. As evidenced by the SEM microscopy, a longer curing time after triaxial testing resulted in increased cementation, with a sometimes significant amount of void filling between particles being observed. This increase is attributed to interaction between the bacteria and the surrounding air as the specimen was cured, yet no experimental evidence has been recorded to support this hypothesis. The concentration of calcium ions during microbial treatment may also play a significant role in the cementation process that occurs. Future testing is warranted to explore these issues further; the authors plan to continue their work in this area.

ACKNOWLEDGEMENT

The author thanks Prof. Christopher L. Meehan for his valuable advisory and guidance during the study. Soil microstructure analyses using a scanning electron microscope were performed at the Delaware Biotechnology Institute (DBI) of the University of Delaware. This study was funded by the national scholarship program by the Ministry of National Education of Turkish Republic.

DECLARATION OF ETHICAL STANDARDS

The author of this article declare that the materials and methods used in this study do not require ethical committee permission and/or legal-special permission.

AUTHORS' CONTRIBUTIONS

Ayşe Özdoğan Dölçek: Performed the experiments, analysed the results and wrote the manuscript

REFERENCES

[1] Ferris, F. G., & Stehmeier, L. G. Bacteriogenetic mineral plugging. Patent number: 5143155.US. Patent Office. Washington DC. , 19, (1992).

[2] Hammes, F. S., Van Hege, K. , Van de Wiele, T., Vanderdeelen, J., Siciliano, S., & Verstraete, W.

"Calcium removal from industrial wastewater by biocataltic CaCO3 precipitation", ***J Chemical Technology Biotechnology*** , 78, 670-677, (2003).

[3] Mitchell, A. C., & Ferris, F. G. "The Influence of Bacillus pasteurii on the Nucleation and Growth of Calcium Carbonate", ***Geomicrobiology Journal***, 23, 213-226, (2005).

[4] Levrel, G. L., Castanier, S., Orial, G., Loubiere, F., & Perthuisot, J. P. "Application of bacterial carbonatogenesis to the protection and regeneration of limestones in buildings and historic patrimony", ***Sedimentary Geology,*** Vol. 126, 25-34, (1999).

[5] Ferris, F. G., Phoenix, V., Fujita, Y., & Smith, R. W. "Kinetics of calcite precipitation induced by ureolytic bacteria at 10◦C to 20◦C in artificial groundwater", ***Geochem Cosmochim Acta*** , 67, 1701-1722, (2003).

[6] Gollapudi, U. K., Knutson, C. L., Bang, S. S., & Islam, M. R. "A New Method For Controlling Leaching Through Permeable Channels", ***Chemosphere,*** Vol. 30, 695-705, (1995).

[7] Ramachandran, S. K., Ramakrishnan, V., & Bang, S. S. "Remediation of concrete using micro-organisms", ***ACI Materials Journal***, Vol. 98 , 3−9, (2001).

[8] Achal, V, Mukerjee, A., Reddy, M.S. "Biogenic treatment improves the durability and remediates the cracks of concrete structures", ***Constraction and Building Materials***, 46, 1−5, (2013).

[9] Lo Bianco, A., & Madonia, G. "B.U.L.M. technique for increase of the bearing capacity in the pavement layers subjected to biological treatment", University of Palermo, ***4th International Siiv Congress***, Palermo (Italy),16, (2007).

[10] Ivanov, V., & Chu, J. "Applications of microorganisms to geotechnical engineering for bioclogging and biocementation of soil in situ", ***Reviews in Environmental Science and Biotechnology***, 7, 139-153, (2008).

[11] Chou, C. W., Seagren E. A., Aydilek A. H., and Lai M. "Biocalcification of sand through ureolysis", ***Journal of Geotechnical and Geoenvironmental Engineering***, 137(12), 1179-1189, (2011).

[12] Chu, J., Stabnikov, V., and Ivanov, V. "Microbially induced calcium carbonate precipitation on surface or in the bulk of soil", ***Geomicrobiology Journal***, 29(6), 544-549, (2012).

[13] Canakci, H., & Cabalar, A. F. "Improvement of a Sand Matrice Using Biopolymer-Forming Bacteria", ***Proc. of International conference on new developments in soil mechanics and geotechnical engineering***. Lefkose, N. Cyprus, (2003).

[14] Dejong, J. T., Fritzges, M. B., & Nusslein, K. "Microbially Induced Cementation to Control Sand Response to Undrained Shear", ***Journel of Geotechnical and Geoenvironmental Engineering*** ASCE 1090-0241, 1381-1392, (2006).

[15] Al Qabany, A., and Soga, K. "Effect of chemical treatment used in MICP on engineering properties of cemented soils", ***Géotechnique***, 63(4), 331–339, (2013).

[16] Venda Oliveira, P.J., Costa, M.S., Costa, J.N.P., Nobre, M.F. "Comparison of the ability of two bacteria to improve the behavior of a sandy soil", ***Journal of Materials in Civil Engineering***, 27, 1943−5533, (2015).

[17] Bang, S. S., Galinat, J. K., & Ramakrishnan, V. "Calcite precipitation induced by polyurethane-immobilized Bacillus pasteurii", ***Enzyme and Microbial Technology***,28 , 404-409, (2001).

[18] Ramakrishnan, V. "Performance characteristics of bacterial concrete-a smart biomaterial", ***Proceedings of the First International Conference on Recent Advances in Concrete Technology***, Washington, DC , 67−78, (2007).

[19] Jonkers, H. M., Thijssen, A., Muyzer, G., Copuroglu, O., & Schlangen, E. "Application of bacteria as self-healing agent for the development of sustainable concrete", ***Ecological Engineering***, 3-6, (2009).

[20] Sarda, D., Choonia, S., Sarode, D. D., & Lele, S. S. "Biocalcification by Bacillus pasteurii urease: a novel application", ***Jeo Industrial Microbiol Biotechnol*** , 36, 1111-1115, (2009).

[21] Gomez, M.G., Anderson, M., Graddyy, C.M.R., Dejong, J. T., Nelson, D.C., Ginn, T. R. Large-scale comparison of bioaugmentation and biostimulation approaches for biocementation of sands. ***Journal of Geotechnical and Geological Engineering***, 143 (3), 1943-5606, (2016).

[22] Jiang, N.J., Soga, K., Kuo, M. "Microbiologically induced carbonate precipitation for seepage-induced internal erosion control in sand-clay mixture", ***Journal of Geotechnical and Geoenvironmental Engineering***, 143 (3), 1943-5606, (2017).

[23] Ozdogan, A. 2010. Ozdogan, A. "A Study on the Triaxial Shear Behavior and Microstructure of Biologically Treated Sand Specimens", ***Master Thesis***, University of Delaware, US, (2010).

[24] ASTM. Designation: D 1556-00, "Standard Test Method for Density and Unit Weight of Soil in Place by the Sand-Cone Method", West Conshohocken, PA, (2000).

[25] ASTM. Designation: D 422, "Standard Test Method for Particle-Size Analysis of Soils", West Conshohocken, PA, (1998).

[26] ASTM. Designation: D 2487-06, "Standart Practice for Classification of Soils for Engineering Purposes (Unified Soil Classification System)", West Conshohocken, PA, (2006).

[27] Chen, Y. "An experimental investigation of the behavior of compacted clay/sand mixtures", ***Master's Thesis***, University of Delaware, Newark, DE, (2010).

[28] Yamamuro, J. A., & Lade, P. V. "Static liquefaction of very loose sands", ***Canadian Geotechnical Journal***, 34, 905−917, (1997).

[29] Gallagher, P. M., & Mitchell, J. K. "Influence of Colloidal Silica Grout on Liquefaction Potential and Cyclic Undrained Behavior of Loose Sand", ***Dynamics and Earthquake Engineering*** , 22, 1017-1026, (2002).

[30] Fritzges, M. B. "Biologically Induced Improvement of the Response of Sands", ***Master's Thesis***, University of Massachusetts, Amherst, MA, 111, (2005).

[31] Baxter, C. D., Bradshaw, A. S., & Veyera, G. E. "Liquefaction Potential of Inorganic and Organic Silts", URITC Project No.00060, University of Rhode Island, 42, (2005).

[32] ASTM. Designation: D 4767-04, "Standart Test Method for Consolidated Undrained Triaxial Compression Test for Cohesive Soils", West Conshohocken, PA, (2004)