



An Investigation on Biological Treatment of Sandy Soil

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ABSTRACT: Due to environmental problems of desert expansion as well as dust storms, looking for more efficient and comprehensive methods to stabilize dune sands seems to be an essential necessity. Microbial-induced CaCO_3 precipitation (MICP) is an innovative technique that harnesses bacterial activities to modify the physical and mechanical properties of soils. This method produces calcium carbonate precipitation in the soil pores by fracturing urea in the presence of calcium ions. An important factor in achieving uniform calcite deposition (and hence consistent enhancement of geotechnical properties) throughout the treated soil mass is the protocol adopted to inject the reagents of ureolytic bacteria, urea, and calcium. In this study, an urease microorganism was prepared in the laboratory and injected into cylindrical dune sand samples. After required and appropriate curing time, the samples were subjected to unconfined compression and falling-head permeability tests. The test results showed a significant strength improvement and the reduction of permeability of the treated samples in comparison with those of untreated soil. The research results verified the capability of the biological treatment of dune sand which may be regarded as a potential technique to control desert expansion and dust storms.

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1- Introduction

Conventional soil improvement techniques mostly include injecting cement and chemical grouts into the soil, however, it is believed that all the chemical grouts are to some extent toxic and dangerous except Sodium Silicate (U.S. Army Corps of Engineers, Engineering Manual NO. EM 1110-1-3500). Therefore, such products must be used with caution from the environmental point of view. Thus, some restrictions have been made on the usage of the chemical grouts or their consumption has been inhibited [1, 2].

Microbial geotechnology is a new branch of geotechnical engineering which deals with the applications of microbiological methods to geomaterials used in engineering. The aim of such applications is to improve the mechanical properties of soil for construction or environmental purposes. In this context, two notable applications, bio-clogging, and bio-cementation have been developed [3].

As soil contains a lot of nutrients and moisture within its pores, it includes more genera and species of microorganisms than other microbial habitats. Some species of microorganisms exist in large numbers while some others are not so, possibly due to the lack of factors necessary for their survival and growth. Microorganisms are greatly adaptive to varying conditions both genetically and physiologically, as they have been in existence for over 3.5 billion years [4, 5].

There are about 10^9 - 10^{12} micro-organisms per kilogram of a soil mass within zones near to the ground surface. Bacteria, archaea, and eukarya are among the microorganisms present in the soil. Some of the important features of bacteria and archaea include simple cell structure without a membrane-encased nucleus, more than one chromosome and pertinent

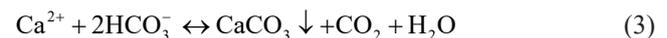
chemical composition which are more distinguished than structure. Identification, characterization, and classification of microorganisms are usually carried out based upon the type of cell wall, shape, nutrients, type of biochemical transformation, and DNA and RNA sequences [6,7].

In the early 1980s during the North-West Shelf gas platform commercialization, a problem came up in the structure foundation. It was discovered that the foundation piles had not acquired enough bond resistance with the surrounding deposits. In this context, Price conducted a study on the structure and rheology of carbonate deposits around the piles. Finally, he proposed stimulating a synthetic calcite precipitation process using urease bacteria present in the natural deposits [8].

In calcite precipitation, the overall equilibrium reaction is [9]:



Microbiologically induced calcite precipitation occurs according to the following reactions [9]:



The high pH environment is provided by the decomposition of urea according to the reaction [9]:



Stocks-Fischer et al., (1999) examined physical and biochemical properties of calcite¹ precipitation induced by *Bacillus pasteurii*, an alkalophilic soil microorganism with X-ray diffraction analysis and scanning electron microscopy.



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They showed the process of calcite precipitation within the soil pores and bonding the soil particles to each other [10]. Whiffin et al., (2007) injected bacteria and process reagents into a five-meter-long cylinder. After treatment, they showed that strength and stiffness have been improved without making the soil impermeable to fluids [11].

Anima (2007) and Seena (2008) examined the impact of *Bacillus pasteurii* on two types of soils and found that biocementation exhibited an increase in the unconfined compressive strength of soil [12, 13]. Ivanov and Chu (2008) examined the application of biocementation techniques to improve the soil in situ [14]. Harkes et al., (2010) introduced a methodology to distribute and fix bacteria (with their enzyme activity) relatively homogeneously in a sand bed, before supplying cementation reagents [15].

De Jong et al., (2010) studied the effects of calcium carbonate biological deposition on shear and compression waves velocity of a sandy soil [16]. Soon et al., (2012) studied the effectiveness of microbial induced calcite precipitation (MICP) on the enhancement of the shear strength and reduction of the hydraulic conductivity of residual soil and sand with *B. megaterium*. They showed that shear strength and permeability varied with soil densities, soil types, and treatment conditions [17].

Lee et al., (2012) examined the effect of microbial induced calcite precipitation on shear strength enhancement and reduction of permeability of sandy soils and residual soils (Sandy Silt). The research results showed that microbial induced calcite precipitation could efficiently enhance the shear strength (1.41-2.64 times) and decrease permeability (1.14-1.25 times) of both soil types [18].

Modaresnia et al., (2013) compared the performance of microbial induced calcite precipitation (MICP) method on the strength of sandy soil with the other two methods of mixing treatment for soil improvement, including resin stabilization and fiber/resin reinforcement [19]. Montoya et al., (2015) examined the stress-strain behavior of sands cemented by MICP with *Bacillus pasteurii*. They made triaxial tests on the soil samples in both undrained and drained conditions and measured the shear wave velocity through the samples. The results showed a transition from strain hardening to strain softening behavior and thus corresponding transition of global to localized failure as calcite cementation increased [20].

Neupane et al., (2015) evaluated the potential of improving an in situ calcite grouting technique. They examined the distribution of the grout materials (urea, calcium chloride¹, and urease enzyme) and precipitated calcite within sand columns with a diameter of 5cm and a height of 100 cm. It was found that a uniform distribution of the grout materials up to a distance of 1m from the inlet is achievable. It was also concluded that it is possible to obtain a relatively uniform calcite precipitation as long as the rate of calcite precipitation is well controlled [21].

Carmona et al., (2016) used urease enzyme instead of urease bacterial to avoid complex and sensitive processes of cultivation and storage of the bacteria, which may make field applications difficult. Results of unconfined compressive strength tests and XRD² showed the precipitation of

calcite and the effectiveness of this methodology for the improvement of a sandy soil. It was also found that the urea-CaCl₂ concentration increase may inhibit the activity of urease [22]. Sharma and Ramkrishnan (2016) applied the species of *Bacillus* group, *B. pasteurii* and urea-calcium chloride to improve two different types of fine grain soils. They examined the effects of *B. pasteurii* concentration, cementation reagent and duration of treatment on the soils improvement using unconfined compressive strength tests. The results showed that MICP application can enhance the unconfined compressive strength (1.5-2.9 times) for both types of soils. It was also found the soil strength increased with an increase in treatment duration [23].

Generally, experimental results of soil improvement through microbial calcite precipitation system show that the production of calcite due to bio-calcification promotes the bonds between the soil particles and stick the particles to each other resulting in the enhancement of both soil strength and stiffness. Also, the calcite precipitation process decreases the void ratio of soils leading to permeability reduction.

The biological stabilization seems to be a promising technique to control the expansion of dune sand deserts and in turn encountering the problem of dust storms. This paper reports the likely potentials of application of biological treatment on dune sand samples taken from a desert region near Rafsanjan city. It was found that the stabilization procedure could efficiently stabilize such soils in terms of noticeable strength enhancement as well as permeability reduction. In this context, a bacteria of *Bacillus* group i.e. *B. pasteurii* was used to produce calcite precipitation within the soil samples through an injection setup.

2- Materials and Testing Procedures

2- 1- Soil Type

Sandy soil used in this study was taken from a dune field near Rafsanjan. Figure 1 and Table 1 show respectively the gradation curve and physical properties as well as the classification of the selected soil.

Table 1. Physical properties of soil

Properties	Value
Unified System	SP
AASHTO System	A-3
C _u	1.50
C _c	0.92
G _s	2.70
e _{max}	0.78
e _{min}	0.60

2- 2- Bacterial cell solution

A seed culture was prepared by transforming the *B. pasteurii* lyophilised culture into 20 ml of LB sterilized medium and allowing the culture growth temperature at 28 °C over a 24 h period. A little amount of this mixture was then cooled and saved at 4 °C prior to its use. The main culture medium was prepared by transferring the seed culture into LB³ fresh

1 CaCl₂
2 X-Ray Diffraction

3 Liquid Broth

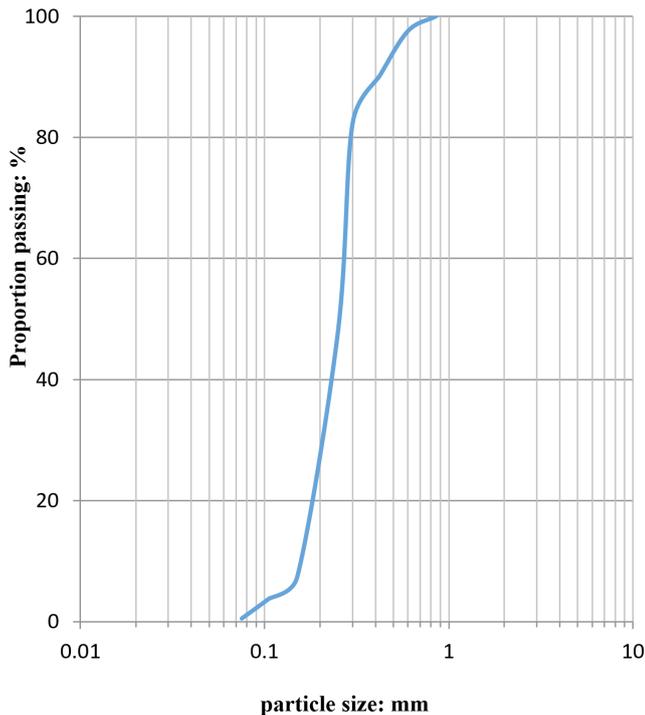


Fig. 1. Physical properties of the testing soil (dune sand)

medium (10% v/v) and incubated under agitation at 28 °C over a 16 h period. After the curing process, the required bacterial cells in the culture medium were harvested by centrifuging at 4 °C and 4500 g for 15 minutes [24].

To remove metabolic wastes generated during the bacterial growth phase, sodium phosphate buffer 0.1 M (pH=7) was used two times to rinse the harvested cells in the culture medium. The metabolism process refers to all chemical reactions that occur in living organisms, including the digestion and transport of substances into and between different cells, in which the set of reactions within the cells is called intermediate metabolism [24].

The harvested cells were re-suspended in nutrient broth-urea solution. This solution contains 3 g of nutrient broth (NB), 20 g of urea, 10 g of ammonium chloride, and 2.12g of sodium bicarbonate per liter of distilled water [25]. The pH of the bacterial cell solution was regulated to 5.8 by using 1 M hydrochloric acid prior to autoclaving. Concentration of bacterial cell adjusted in laboratory at OD_{600} (optical density at 600 nm wave length) value of 1.2 equal to 10^8 cel/ml. Figure 2 shows the spectrophotometer used for this purpose.

2- 3- Cementation solution

Reactive substances in biological calcium carbonate precipitation process are urea and calcium chloride. In this study, the concentrations of urea and calcium chloride were considered 2 M and 1 M, respectively. Based on the required magnitude of molecular weight and concentration, proper amounts of urea and calcium chloride were weighted and after dissolving in distilled water reached to the required volume. This solution is known as cementation solution.

2- 4- Specimen preparation

To prepare the specimens, regarding the volume of mold, 282 g dry soil with 60 ml bacterial suspension and 10ml



Fig. 2. Spectrophotometer used to indicate concentration of bacterial cell

cementation solution was well mixed and poured into the molds of 47 mm diameter and 100 mm height (height to diameter ratio 2.1). After sealing molds with o-ring gasket, 60 ml cementation solution at approximate rate of 90 ml/h was injected into each specimen. The injection procedure was exactly repeated after 12 hr. Figures 3 and 4 show the typical mold and injection set up used for this purpose, respectively. The schematic procedure of injection has also been shown in Figure 5.

After the injection, the specimens were kept for 24 hours in saturated conditions and then removed from set up and kept for 7 days (a total of 8 days) in the laboratory environment at a temperature of 25 ± 3 °C. At the end of a seven-day-period, the specimens were placed in an oven at 70°C for 24 h, in order to stop the calcite precipitation process within specimens and obtain sufficient stability for an unconfined compression test. Calcite precipitation increases with time which continues to achieve its utmost rate as the pH of medium reaches to an appropriate value. It has been found that such optimum pH level (8.3 to 9.3) is achieved within four to seven days [26, 27]. Thus, the curing time of eight days was selected to ensure the achievement of the high rate of calcite precipitation.

The injection setup and procedure were precisely designed to obtain uniform distribution through a gravitational permeation mechanism within the soil sample. The mechanism has the pronounced advantage in comparison to previous pressurized injection procedures as it has no need to use a peristaltic pressure pump. In this investigation, the dry density and density index of soil samples were adopted as 1.63 gr/cm^3 and 67%, respectively.

2- 5- Testing Procedure

To examine the mechanical and physical properties, the unconfined, triaxial compression and permeability (falling head method) tests were carried out on the cured specimens. These tests were conducted in accordance with the ASTM standard. The compression tests speed was 1 mm/min.

Finally, scanning electron microscopy (SEM) was used to examine the calcium carbonate (calcite) sediment and procedure of connecting sand grains to each other.



Fig. 3. Typical mold used for sample preparation



Fig. 4. Set up for gravitational injection at controlled rate

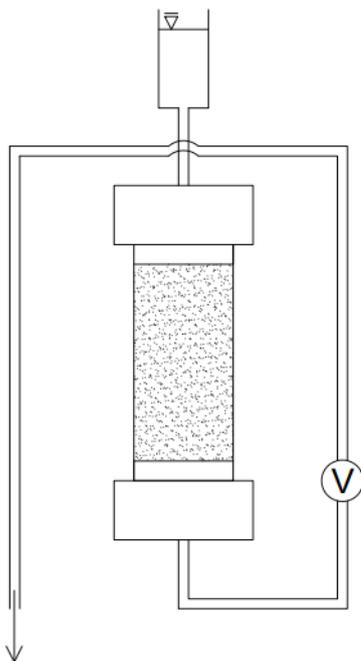


Fig. 5. Schematic view of gravitational injection into the sample

3- Results and Discussion

In this study, *B. pasteurii* was employed to stable dune sand. The geotechnical properties of the microbially treated soil specimens are described and discussed in the following sections.

3- 1- Unconfined compression strength and stiffness

Figure 6 shows the axial stress-strain of treated soil samples derived from unconfined compression tests along with the stress-strain curve of untreated soil derived from a triaxial compression test. As observed, the unconfined strength of the treated soil with *B. pasteurii* is obtained as 1391 kPa which is significantly greater than the strength of untreated soil.

It should be mentioned that since conducting unconfined compression tests on the sand samples was not possible, a set of triaxial compression tests was carried out on the sand sample to obtain the strength of untreated soil for comparing with the unconfined strength of the treated soil. The untreated soil (dune sand) strength was determined at a relatively low confining stress of 50 kPa. The failure deviator stress of 256 kPa was obtained through the triaxial test on the sand sample at $w=16\%$ and $\gamma_d=1.74 \text{ gr/cm}^3$ (proctor compaction optimum conditions).

The high unconfined strength of *B. pasteurii* medium in comparison with that of the untreated soil is clearly due to the calcite precipitation made of biochemical reactions. The cementation process caused the soil particles to bound with each other and creating a firm mass.

Figure 7 shows the amount of both elasticity modulus and deviation stress of untreated and treated sandy soil.

The stress-strain modulus was determined as a secant method corresponding to half of failure stress. Table 2 shows the result summary of unconfined and triaxial compression tests. As observed, a significant enhancement has been achieved in both the strength and stiffness of the dune sand due to the biological treatment.

Table 2. Results of unconfined and triaxial compression tests

Soil Condition	Strength (kPa)	Failure Strain (%)	Es (MPa)
untreated	255.7	3.6	25.5
Treated with <i>B. past.</i>	1391	0.9	136.37

3- 2- Coefficient of permeability

Figure 8 shows the permeability coefficient of both untreated sand and biologically treated samples through falling head permeability test setup. The permeability of treated samples was measured using the setup used for injecting treatment solution. According to Figure 8, permeability coefficient of *B. pasteurii* medium, i.e. treated sample is significantly less than that of untreated sand. This may be interpreted as an indication of calcite precipitation within soil pores caused by biochemical reactions. An alternative verification of calcite precipitation within the soil voids due to the biological treatment was made using Scanning electron microscopy (SEM) as explained in the next section.

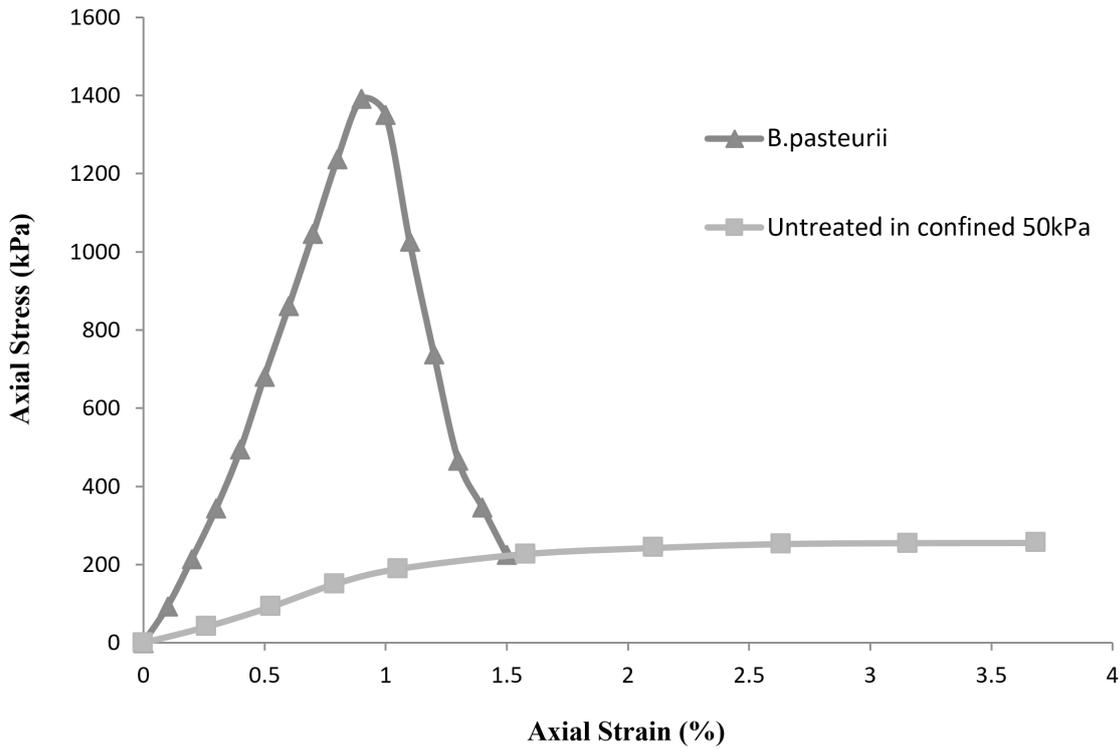


Fig. 6. Stress-strain diagram of untreated and treated sand

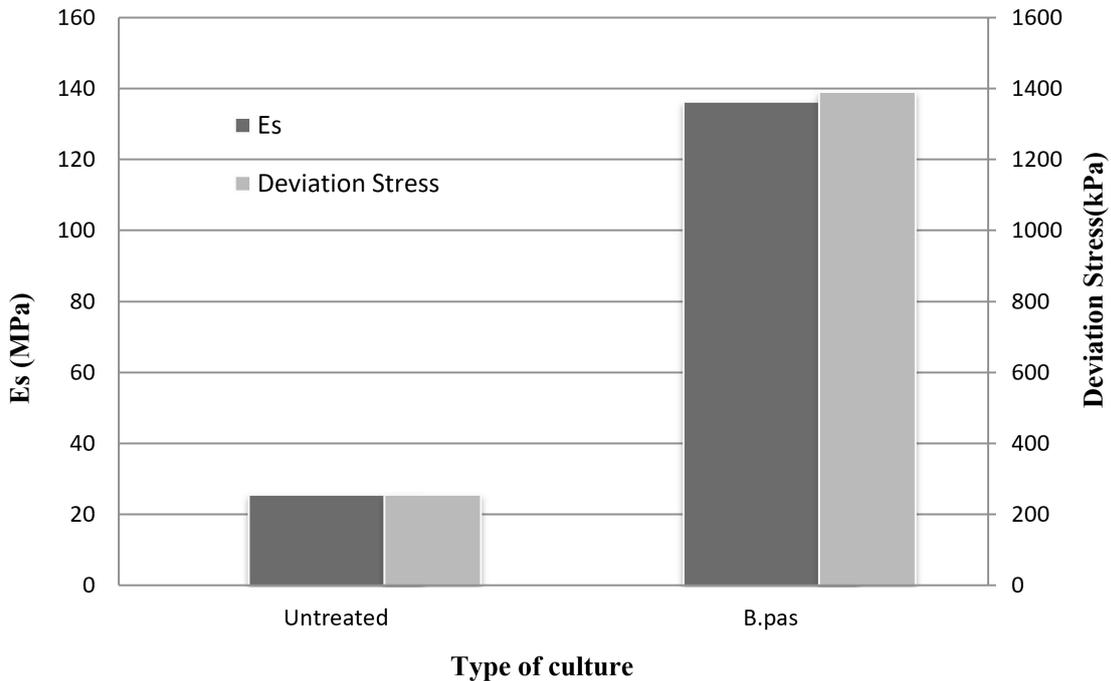


Fig. 7. Comparison of both stiffness modulus and deviation stress of treated sand with *B. pasteurii* and untreated soil

3- 3- Comparison of the results with those of other studies

In order to compare the results obtained and their validation, Table 3 shows some MICP studies reported in the literature. Unconfined strength in the current investigation is 1391kPa that is compatible with results in the literature. Also, reduction permeability of treatment with *B. pasteurii* in the current study seems to be reasonable in comparison with that of

previous results as shown in Table 3. The observed differences in the compression strength and permeability values may be attributed to the type of soil, nutrients, bacteria, geometric compatibility of bacteria, bacteria cell concentration, fixation and distribution of bacteria in the soil, temperature, reagents concentration, pH, and injection method [28].

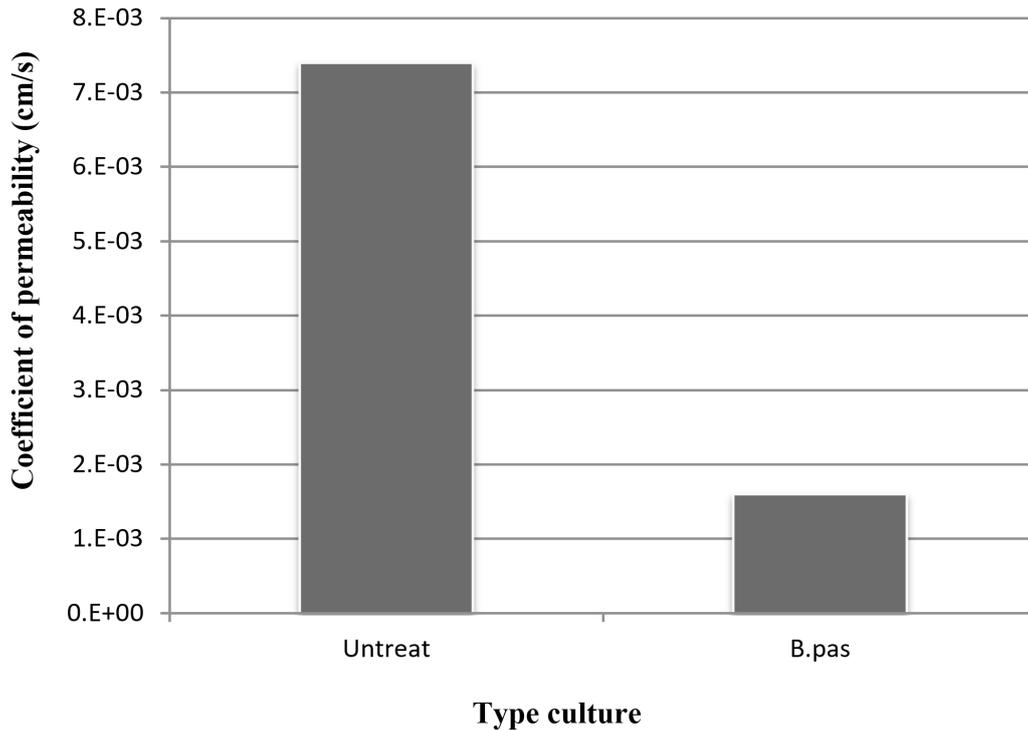


Fig. 8. Permeability coefficient of untreated soil and treated sand with *B. pasteurii*

3- 4- Verification calcite precipitation using SEM

Typical images of untreated and treated samples provided by SEM technique are presented in Figures 9 and 10, respectively. Figure 10 clearly shows the shape of CaCO₃ crystals on the surface of sand grains and filling voids between the soil particles. Calcite precipitation bounds particles with each other which, in turn, leads to the strength enhancement as well as permeability reduction.

4- Conclusion

This article reports a laboratory investigation on biologically stabilizing dune sand samples utilizing calcite precipitation mediated by enzyme-driven mineralization. In this context, representative samples of dune sand were prepared from a desert area near Rafasanjan city. The stabilization process was carried out using some species of Bacillus group, *B. pasteurii* (OD=1.2 equal to 10⁸ cel/ml) and urea-calcium chloride (2 to 1 M, respectively) leading to calcite precipitation on the sand grain surfaces and filling the soil pores. The stabilization efficiency was evaluated in terms of the soil compressive

strength, secant stiffness, and permeability with unconfined compression and falling head tests, respectively. As observed from the experiment results, the calcite precipitation through MICP improves the mechanical and physical properties of the sandy soil. Comparing the current study with the previous investigation (Table 3), the research outcome seems to be quite promising and may be regarded as an indication of the applicability of such biological methods to encounter dust storms and desert expansion. The following conclusions may be drawn from the above study.

- The unconfined strength of the biologically treated dune sand was evaluated to be about 5.5 times greater than the strength of untreated sand under 50 kPa confinement stress.
- The soil stiffness was also increased significantly due to biological treatment so that the stiffness of treated samples found to be 5 times of that of untreated soil.
- The treated sand was found to be more brittle than untreated sand as the failure strain of the stabilized decreased 75% relative to that of untreated samples.

Table 3. Some previous MICP results in comparison with current study

Reference	Soil type	Cementation concentration: M	Number of injection cycles	UCS: kPa	Permeability reduction: %
Whiffin et al. (2007) [11]	Itterbeck sand, D ₅₀ = 0.165mm	1.1	1	0-500	22-75
Yasuhara et al. (2011) [29]	Sand	0.5, 1.0	4-8 for UCS 1-4 for permeability	373-1500	60-70
Palmen (2012) [8]	Quartz sand, D ₅₀ = 0.85 mm	NR	1-5	200-2600	NR
Current investigation	Dune sand, D ₅₀ = 0.25 mm	0.5 (urea/CaCl ₂)	2	1391	78.3

* NR, not reported; UCS, unconfined compressive strength.

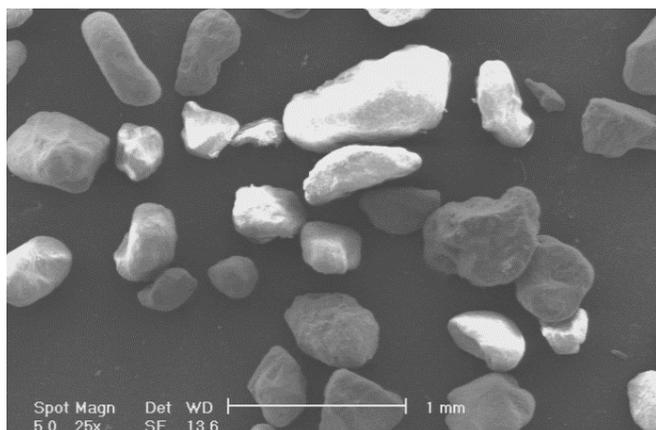


Fig. 9. SEM image of Untreated soil (sandy soil)

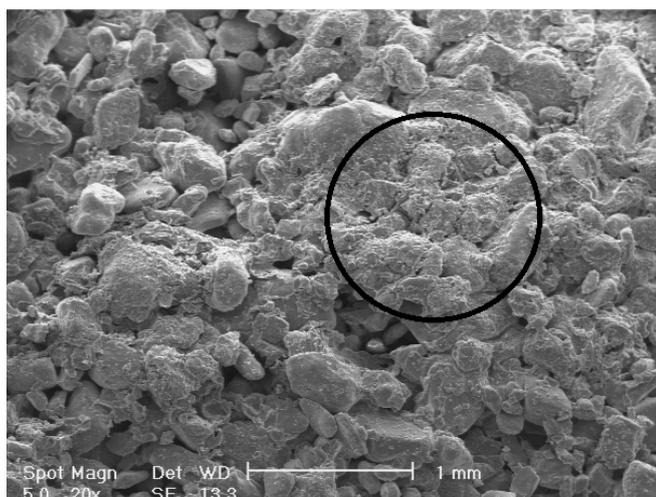


Fig. 10. Treated soil with *B. pasteurii*

- The permeability of the treated sand through biological precipitation showed 78.3% reduction in comparison with untreated sand.
- The efficiency of the applied biological treatment was also verified through Scanning electron microscopy (SEM).

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