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Optimum Bacteria Suspension Volume for Stabilizing Silty Sand Soils by *Sporosarcina pasteurii* Bacteria

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ABSTRACT

Recently, the bio-mediated soil improvement techniques have gained an increasing attention. In this method, the bacteria was cultivated aerobically in the laboratory and added to the soil with reactant solutions such as urea and calcium chloride. Most of the existing studies are on sandy soils and a few researches have done on silty sandy soils. However, most soils in nature are compounds of fine-grained and coarse-grained soils. In fine-grained soils, silt does not have very good resistance due to the lack of adhesion between its particles. Hence, in this study *Sporosarcina pasteurii* bacterium was aerobically cultivated for stabilizing the sand with different percentages of silt to determine the optimum bacteria suspension volume. After some bacterial tests such as measuring bacterial growth, standard plate count, gram staining, pH determination, growth without urea, and urease test, geo-technical tests like soil sieve, compaction, and Atterberg limits were also done. Standard plate count was estimated $2.5*10^8$ through serial dilution plating and culture media pH was determined 8.64 from different samples. Moreover, to achieve the best results, different sampling methods were compared. As the calcium carbonate creates a network of calcified bridges of calcite between sand grains, an electron microscope was used for scanning the surface with a focused beam of electrons. Results of triaxial tests showed that by adding optimum bacteria suspension volume, the maximum strength for samples with 0, 10, 20, 30 and 40% of silt was improved from 700, 900, 750, 600 and550 to 1100, 1400, 1550, 1600, and 1500 kPa, respectively.

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1. INTRODUCTION

Microbial geo-technology is considered as a branch of geo-technical engineering. Surface soil may contain 109- 1012 cells per gram several in situ conditions such as light, heat, pH, oxygen, toxic substances, organic matter, and pressure**.** The bio-geochemical byproducts change the engineering properties of soil; hence the bio-mediated soil improvement techniques have gained an especial attention [1]. Mitchell and Santamarina [2] introduced microbiological concepts and illustrated their potential roles in soils and rock. DeJong et al. [3] reported the outcomes of the workshop entitled "Geological and Geotechnical Engineering in the New Millennium: Opportunities for Research and Technological Innovation". Moreover, there are some reviews of the existing or potential applications of microorganisms in geo-technology to discuss their advantages and disadvantages [4-6].

There are two approaches in microbial geo-technology: bio-clogging and bio-cementation. Bio-clogging is the process of filling the soil voids with microbial biomass thus reducing porosity and hydraulic conductivity [7]. Bio-cementation is to enhance the shear strength and stiffness of soil through the production of particle-binding materials via microbial means. It is also

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diminishing the permeability of tropical residual soil and sand [8].

One of the bio-mediated soil improvement techniques is Microbially Induced Carbonate Precipitation (MICP) which is used for various geotechnical engineering applications such as improving foundation bearing capacity, remediation of liquefaction potential during earthquakes, diminishing foundation settlements, and reducing excavation and tunnel support requirements. MICP can be achieved by various processes [9] and among these processes urea hydrolysis is more favorable which has come to prominence in the late nineteenth [10, 11]. The urease is cultivated aerobically in the laboratory and added to the soil with a solution of urea and calcium chloride. The microbial urease catalyzes the hydrolysis of urea to produce carbonate and ammonium (Eq. 1). That action may increases the pH which accelerates calcium carbonate production in the presence of calcium ion [12, 13].

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

The produced carbonate ions precipitate in the presence of calcium ions as calcium carbonate crystals, which form cementing bonds between the existing sand grains (Eq. 2)

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

The precipitated calcium carbonate will be durable under natural groundwater conditions and in the absence of acidifying processes in the pores. A sufficient quantity of calcium carbonate significantly increases soil stabilization [14, 15].

Certain bacterial species cause urease enzyme which catalyzes the hydrolysis of urea to ammonia and carbonic acid. Mwandira et al. [16] used the microbially induced calcium carbonate precipitation technique in conjunction with the bacterium *Pararhodobacter* sp. to bioremediate lead. DeJong et al. [17] used *Sporosarcina pasteurii* to engineer a cemented soil matrix within initially loose and collapsible sand. They demonstrated that the MICP-treated specimens exhibit a noncollapse strain-softening shear behavior, with a higher initial shear stiffness and ultimate shear capacity than untreated loose specimens. Chou et al. [18] carried out direct shear and California Bearing Ratio (CBR) tests on sand specimens subjected to treatment by growing, resting, and dead *S. pasteurii* cells. The peak strength and the friction angles were significantly increased for the loose sand with the growing-cell treatment. Martinez et al. [19] compared two injection methods and expressed that the injection of urea- and calcium-rich solution produces a more uniform calcite distribution as compared to a continuous injection method. AL Qabani et al. [20] assessed the optimum use of *S. pasteurii* to induce precipitation and determined the time required for the process. Tirkolaei and Bilsel [21] examined environmental factors on the microbial urea hydrolysis process for bio-cement production. Kim and Youn [22] employed five microbes (which urease activities of the four of them were higher than *S. pasteurii*) to precipitate calcite in cohesionless soils of two different relative densities (60% and 80%). They showed that the relative density of cohesionless soils significantly affects the amount of calcite precipitation and that there is a weak correlation between urease activity and calcite precipitation. Li et al. [23] studied the effects of microbial-induced carbonate precipitation on fine sand samples by adding some additives. They showed that the precipitated calcium carbonate in the core increased 1.6 times through this method. Jalili et al. [24] expressed that bio-cementation method compared with the conventional injection method has many features such as lower cost, less pressure, more radius of influence, in accordance with the environmental conditions that would justify the use of this method. They showed that bio-cementation method in addition to increasing uniaxial compressive strength causes to increase the Stiffness of the soil granular samples. Kahani et al. [25] used inexpensive nutrients and water resources in composition of the culture media and examined sanitized media instead of sterilized ones in order to reducing the cost of production of urease bacteria and achieving an ecofriendly process.

Given the above and the environmental benefits of soil biological improvement, this issue has received much attention in recent years. It is worth mentioning that the effect of this bacterium on sandy soil has been studied so far, but there are few studies about silty sand soils with different combinations and comparison between sand and silty sand soils. However, most soils in nature are compounds of fine-grained and coarse-grained soils. In fine-grained soils, silt does not have very good resistance due to the lack of adhesion between its particles. In addition, for biologically soil improvement, the volume and amount of bacterial suspension added to the soil is important both in terms of increasing resistance and in terms of economics. Therefore, for any type of soil composition, it is necessary first to determine the optimal volume of bacterial suspension for soil remediation. In this study, the samples were sand with different percentages of silt (0 to 60%) and for further accuracy the experiments were also done for pure silt samples. Moreover, the optimum bacteria suspension volume for different percentages of silt was determined by triaxial tests. For this purpose, bacterium *S. pasteurii* was used for soil improvement after basic bacterial tests such as measuring bacterial growth by conducting an optical density test, Standard Plate Count (by serial dilution experiments), gram staining, pH determination, growth without urea, and urease test. Geotechnical tests like soil sieve, compaction, and Atterberg limits were also done on samples. Moreover, several methods were tested for

sampling construction to accomplish the homogenous distribution of bacterial culture and cementation solution within the soil grains. As the calcium carbonate creates a network of calcified bridges of calcite between sand grains, an electron microscope was used to produce images of a sample by scanning the surface with a focused beam of electrons. At last, the optimum bacteria suspension volume for different percentages of silt was determined by triaxial tests.

2. MATERIALS AND METHODS

The most important things in microbial geo-technology are type of the bacteria and preparing suitable culture medium for it. After cultivation, some bacterial tests are needed to make sure the bacteria are healthy. At first, the optical density (OD) method was used for cell density measurement. Distilled water was used as a blank and the bacterium was tested some days after culturing. Then, the gram staining method and the pH was tested as well. Moreover, two samples from each solid and liquid medium were prepared for the urease test.

Then, the geotechnical tests were done for investigating the effect of different values of bacteria suspension values and determining the optimum amount for mixing with soil. For this purpose, the soil was seeded in two different manually and mathematical methods. Compaction tests were performed on all samples with different percentages of silt and optimum moisture percentage and maximum specific dry weight were obtained. In order to achieve the best sampling method due to the presence of bacteria, nutrients and silt in the soil, various methods were tested. After preparing the samples, they were kept at normal temperature for stabilization and one day before the test they were placed in an oven (60°C) in order to dry. Then, since the surfaces of the samples were not perfectly flat, a thin layer of clay was drawn on them before testing. All experiments were performed at 200 kPa confining stress 7 days after sample preparation.

The experimental setups are described in the following sections.

2.1. Culture Medium

The most common growth media for microorganisms are nutrient broths (liquid medium) and agar plates (solid medium). The compositions of the culture medium are pepton, meat extract, agar, distilled water and urea (Table 1). Agar is not added or used while preparing the liquid medium. If agar is added to a nutrient broth, it becomes solid medium for petri-dish studies.

TABLE 1. Composition of media for *S. pasteurii* strain

Item	Unit	Amount
Pepton	g/l	5.0
Meat Extract	g/l	3.0
Agar	g/l	15.0
Distilled water	l	1.0
Urea	%	2

All materials except urea were mixed and then sterilized in an autoclave at 121°C for 20 minutes. For liquid medium, the lid was opened under the laminar hood and the urea was added by using sterilized filter. The bacteria are then spilled, and the resulting solution incubated at 30°C in a shaker at 200 revolutions/min for some days in accordance with the growth time of the strain.

For solid medium, after sterilizing and before reaching 50°C, the urea was added under the laminar hood. It stayed inside the hood until it became solid at normal temperature after about 24 hours. It was then ready for cultivation.

In addition, suitable pH is an important requirement for optimum microbial growth in culture media. The bacteria grow at pH higher than 6.5 and the optimum pH is in the range of 8.5 to 9 [26, 27]. The initial pH of nutrient broth is 7 and adding urea the pH increased. To ensure that pH reaches the desired level, it was measured from different samples at different stages of culture and bacterial growth. For testing, the apparatus was first calibrated by solutions with pH 4 and 7, and then a bacterial culture medium was tested. The pH obtained from this experiment was 8.64 which is good for the growth of specific strain of bacteria.

2.2. Standard Plate Count by Serial Dilution Experiments (SPC)

It is essential to determine the number of microorganisms in a given sample. Estimation of Standard Plate Counts (SPC) through serial dilution plating is the most common technique for monitoring cultivable bacteria. In our experiment, the sample was serially diluted and then plated out on an agar surface in such a manner that single isolated bacteria form visible isolated colonies. After two days, the SPC is determined by multiplying the number of colonies on a dilution plate by the corresponding dilution factor. It should be noted that for statistical reasons, only the data from plates that had between 30 and 300 colonies was used in this calculation. The SPC obtained from this experiment was $2.5*10^8$ which is suitable.

2.3. Growth without Urea

Since urea increases pH, a lack of bacterial growth in urea-free media indicates a proper cultivation process. Bacteria were cultured in urea-free medium and disappeared after 4 days.

2.4. Sample Construction

The samples were sand with different percentages of silt (0, 10, 20, 30 and 40%). As previously mentioned, the experiments were also done for samples with 50 and 60% of silt and pure silt in order to provide further accuracy. The following methods were tested for sampling construction:

1. Injection of bacteria and reactant solution: The soil sample was made inside the mold at a specified moisture content, which varied from the lowest moisture to optimum moisture, then the bacteria and after that, reactant solution were injected into the soil sample.
2. Injection of reactant solution: As the previous method, but the bacterial suspension was mixed with the soil instead of adding water as moisture, and then reactant solution was injected into the sample.
3. Mixing all materials while making samples: All materials, including bacterial suspension and reactant solution, were mixed with the soil and then the sample was made.
4. Put the sample in the aquarium containing nutrients and reactant solution: The bacterial suspension was mixed with the soil. The sample was placed into the aquarium containing nutrients and reactant solution [28]. This method was done in several ways, including the time of sample placement in the aquarium and the aeration in the aquarium solution while the sample was in it.
5. Injection of calcium chloride: In this method, the bacterial suspension was mixed with the soil with a portion of reactant solution and the sample was made. The calcium chloride was then injected into the sample by controlling pressure and discharge.

In the injection method, the silt particles in the sample were displaced by flow of bacteria suspension or reactant solution and caused heterogeneity. Naveed et al. [29] stated that when the bacteria are injected within the soil, they are likely to be filtered within the soil particles with a reduction of bacterial population along the path of injection. Hence, it is difficult to accomplish the homogenous distribution of bacterial culture and cementation solution within the soil grains. Mixing all material for making samples produces lower strength because of rapid reaction of the bacteria with the reactant solution. It causes loss of cementitious bonds between the soil particles. Finally, in the aquarium method, the exact amount of nutrients and reactant solution imported into the sample cannot be controlled. The samples made by this method achieve the same strength with the injection method; however, it is not practicable in field. Hence, injection calcium chloride was considered as the final sample preparation method. To prevent silt leaching in the samples, calcium chloride was injected 2 times (each time 6 hours) with 0.2 bar hydrostatic pressure [30].

3. RESULTS OF BACTERIAL TESTS

Results and discussions for the microcosm experiments are described in the following sections.

3.1. OD Test

The measurement of bacterial cell numbers is a critical process during bacterial cell culture. The OD method is widely used among the different methods which have been developed for cell density measurement. It measures the absorbance/scattering of the media containing bacterial cell particles. When a fresh medium is inoculated with a given number of cells, and the population growth is monitored over a while, plotting the data will yield a typical bacterial growth curve. The exponential phase of growth is a pattern of balanced growth wherein all the cells are growing. Exponential growth cannot be continued forever, and population growth is limited. After the population reaches the stationary phase, a death phase follows, in which the viable cell population declines. The best time for using bacteria is the end of the growth stage.

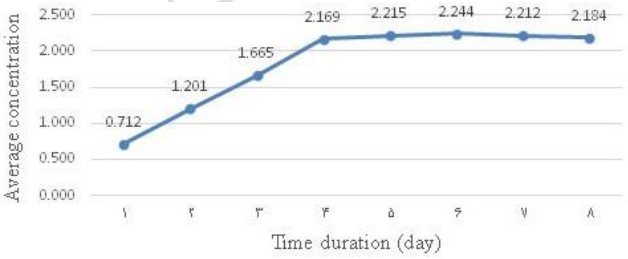

Figure 1. Measured average population variation with time duration from an optical density test Not Acceptable, y-axces

Since the experimental aim was to compare the population of bacteria with respect to time, distilled water was used as blank. The bacterium was tested the day after culturing. As shown in Figurere 1, the population of the tested bacteria reaches to the maximum after four days so the best time to use the bacteria is four days after cultivation.

3.2. Gram Staining

Gram staining is one of the most important and widely used bacteriological laboratory methods used to distinguish and classify bacterial species into two categories based on the physical properties of their cell walls. Gram-positive bacteria have a thick mesh-like cell wall and as a result, are stained purple by crystal violet. Alternatively, gram-negative bacteria stain red and have a thinner layer [31]. The bacterium *S. pasteurii* is gram-positive, so it should naturally be purple after the staining. The result of the experiment is shown in Figure 2 with two different magnifications and purple rod bacteria are visible under the microscope.

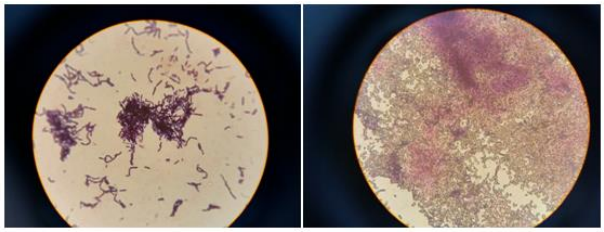

Figure 2. Gram staining result

3.3. Urease Test

The urease test is used to find out the ability of an organism to split urea, through the production of the urease. When urea undergoes hydrolysis, it produces ammonia and carbon dioxide. Urea is acidic but the formation of ammonia turns the medium into alkaline. The change in pH is indicated by the changes in color from yellow to purple. Therefore, an organism that tests positive for urease makes the medium magenta-colored. The bacterium *S. pasteurii* is a positive urease bacterium. The urease test compounds are urea, Na_2HPO_4, KH_2PO_4, yeast extract and phenol red (Table 2). Explain that these compounds belong to the liquid medium, 15 g/l agar is added to provide a solid medium.

TABLE 2. Urease test compounds

Item	Amount (g/l)
Urea	20
Na_2HPO_4	9.5
KH_2PO_4	9.1
Yeast extract	0.1
Phenol Red	0.01

Two samples from each solid and liquid medium were prepared, one for control and the other one for bacterial inoculation. Figure 3 shows the colors after four days. Figure 3-a is a liquid culture medium, as it can be seen the left inoculated sample was changed to purple however the right one retains its natural color. Figure 3-b is a solid culture medium; the color was changed where the bacteria were inoculated.

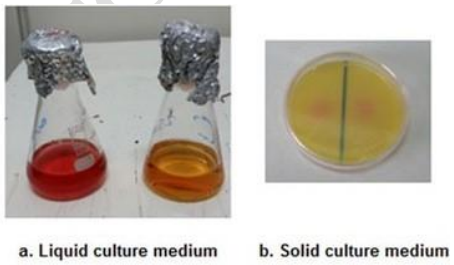

Figure 3. Urease test; the color was changed where the bacteria were inoculated

3.4. Scanning Electron Microscope (SEM) Image

The calcium carbonate creates a network of calcified bridges of calcite between sand grains, while crystals can also get fixed on sand surfaces. A scanning electron microscope is a type of electron microscope that produces images of a sample by scanning the surface with a focused beam of electrons [32]. There are many ASTM standards that specify procedures in microscopy, and several of them are specific to electron microscopes. E 766 is a standard practice for calibrating the magnification of an SEM. It describes calibration in both the x- and y-directions as well as calibration of the scale marker [33]. E 986 is a standard practice for SEM beam size characterization. It describes an experiment for measuring the electron beam diameter with suitable precautions to achieve reproducible results [34].

Figure 4 demonstrates the SEM images showing calcite formation. As it can be seen in Figure 4, some of the pure sand particles form cement bonds with increasing the bacteria and the calcium carbonate deposition between the particles is evident. In the samples with 10, 20 and 30% silt, with increasing the silt, the pores between sands are filled with silt due to the particle size, which results in better and more cementitious bonds between the soil particles. By increasing the silt to 40%, the silt particles are found to lose the cohesion in part of calcium carbonate to the sand particles and to be seen as a non-bonded part in the soil, which reduces the strength in the soil containing 40% silt to that in the soil with 30% silt.

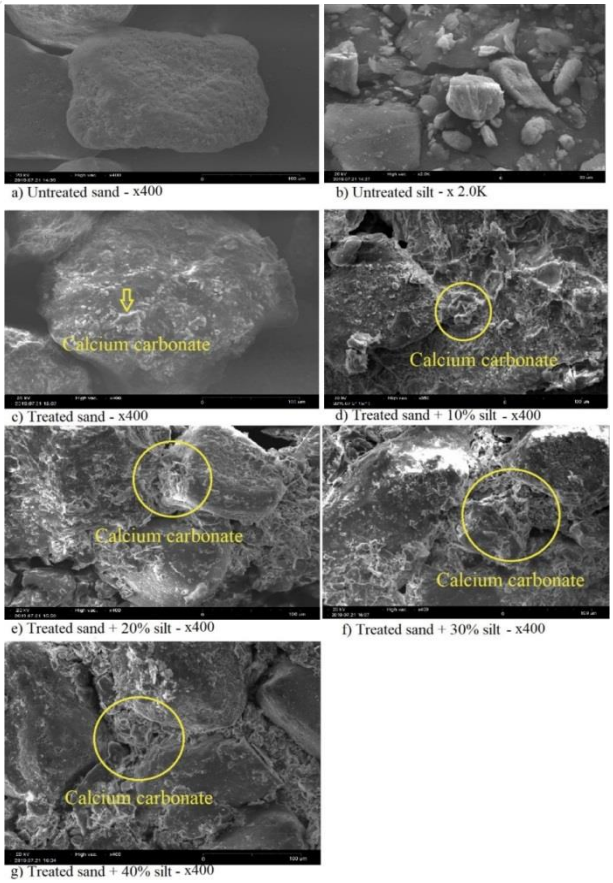

Figure 4. Photos of SEM analyse of untreated and treated soil

4. RESULTS OF GEOTECHNICAL TESTS

Results and discussions for the geotechnical tests are described in the following sections.

4.1. Grading Curve

The soil was seeded in two different ways. The tested soil is manually made by combining different percentages of silt with sand. Hence, sand and silt were graded separately and then the grading curve was plotted using mathematical methods. Also, each sample with a specified percentage of silt was made at first and then the new sample was seeded (Figure 5).

After grading tests, the specific gravity of the solid matter of the soil was determined (Gs=2.7).

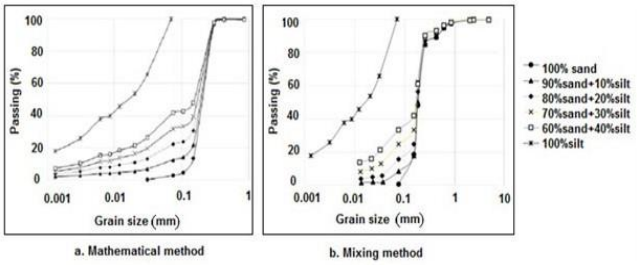

Figure 5. Grain size distribution curve

4.2. Compaction Test

The compaction tests were performed on all samples with different percentages of silt (0, 10, 20, 30 and 40%). For further accuracy, the experiment was also done for samples with 50 and 60% of silt and pure silt. The specific dry weight of samples for different moisture percentages is shown in Figure 6. Optimum moisture percentage and maximum specific dry weight for different silt percentages are illustrated in Figure 7. Increasing fine grained size in sandy soil decreases the void ratio and arranges soil skeleton in more compacted form. This soil needs less moisture and achieves higher density in compared to poorly graded sand. Hence by adding silt particles in sandy soils, maximum dry weight increases and optimum moisture of content decreases. This trend continues up to the special percent of fine grained size particles. After that, by increasing the special surface of soils, the trend is reversed [35].

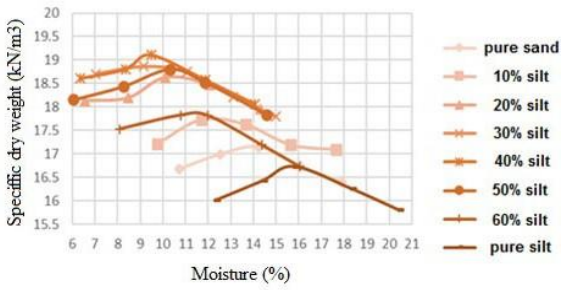

Figure 6. Dry unit weight variation with moisture content

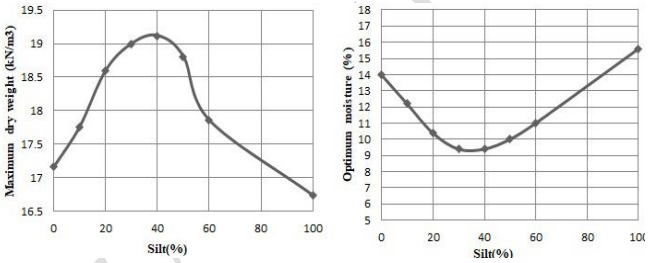

Figure 7. Maximum dry unit weight and optimum moisture content variation with silt percentages

4.3. Effect of Optimum Bacteria Suspension Volume on Stress-Strain Variation

According to the results of the compaction test, to determine the optimum bacterial for soil stabilization, the required fluid for optimum water content is determined first. The volume of the mold was calculated to be 188.44 cubic centimeters. Then, for determining the optimum bacteria suspension volume for different percentages of silt, 5 samples are made with higher and fewer bacteria in comparison to water content. In other words, one sample with 5 g higher and three samples with 5 to 15 g lower than the required fluid are also made. It should be noted that for samples with bacterial levels below the required fluid, distilled water is added to the bacterial suspension.

The samples were kept at normal temperature for stabilization and one day before the test they were placed in an oven (60ºC) to dry. Then, since the surfaces of the samples were not perfectly flat, a thin layer of clay was drawn on them before testing. All experiments were performed at 200 kPa confining stress 7 days after sample preparation. As expected, untreated samples failed in barrel shape (Figure 8-a) but treated samples were first deformed into barrel shape and then broke at an angle of about 65 degrees (Figures 8-b and 8-c).

To determine the effect of bacteria on increasing resistance, triaxial experiments were performed on untreated soil samples initially. After that, triaxial tests were done with 5 different values of bacteria suspension to determine optimum volume for each soil (pure sand or sand with different silt percentages). Results are illustrated in Figures 9 to 13. The numbers written next to the graph are bacterial values (g). As can be seen from

all samples, the optimum bacterial is equal to the optimum moisture at 95% of relative density. From figures, in each series with different content of silt, the strength of untreated samples is much less than the treated samples; and this is higher in samples with 0% and 40% of silt in comparison of others. For pure sand samples (0% silt), the maximum strength is improved from 700 kPa to 1100 kPa and it is improved from 900 to 1400 kPa, 750 to 1550, 600 to 1600, and 550 to 1500 kPa for samples with 10, 20, 30 and 40% of silt, respectively. As can be seen in the figures, the soil behavior more brittle when it is treated.

Figure 8. Broken sample in triaxial test: a. Ductility behavior (barell shape failure), b. Brittle bihavior (barell shape and linier failure), c. Failure angle

As it can be seen the maximum triaxial strength in stabilized soil increases from 1097 (0% silt) to 1671 kPa (30% silt) then, decreases again to 1488 kPa (40% silt). The maximum strength is obtained 1671 kPa in stabilized soil while it is near 900 kPa in unstabilized soil. Hence, the strength is improved by almost 80% by stabilizing.

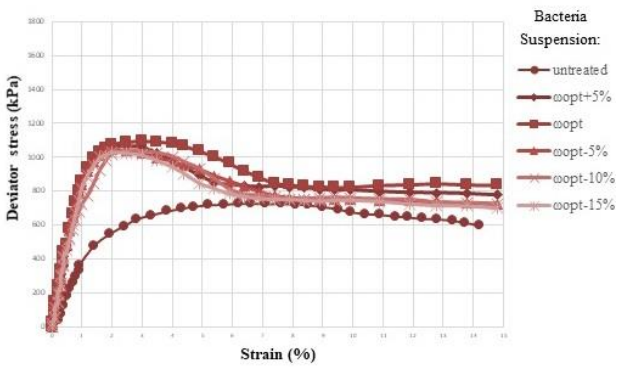

Figure 9. Deviator stress variation with strain for sand and different percent of bacteria suspension

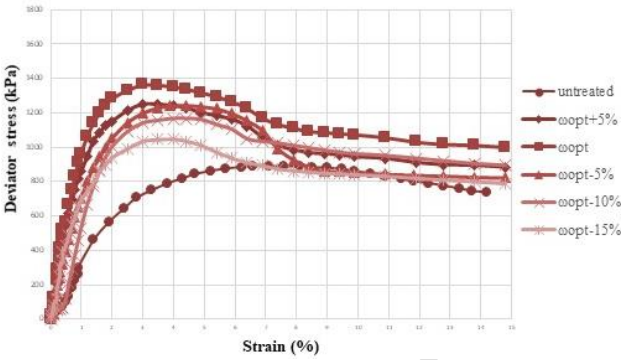

Figure 10. Deviator stress variation with strain for sand with 10% silt and different percent of bacteria suspension

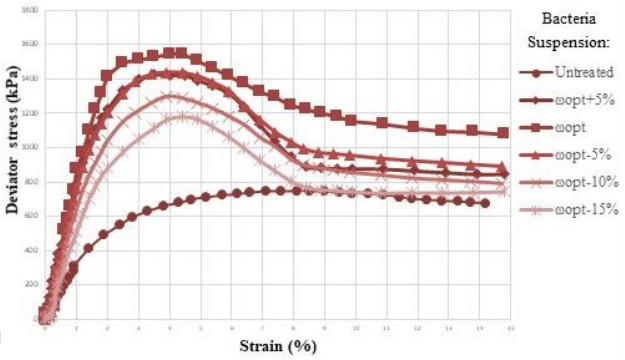

Figure 11. Deviator stress variation with strain for sand with 20% silt and different percent of bacteria suspension

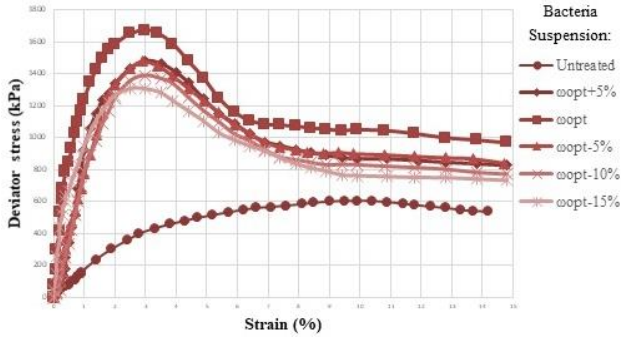

Figure 12. Deviator stress variation with strain for sand with 30% silt and different percent of bacteria suspension

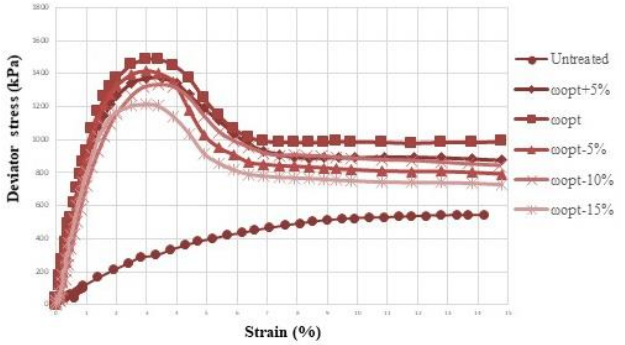

Figure 13. Deviator stress variation with strain for sand with 40% silt and different percent of bacteria suspension

The variation of maximum Deviator stress for all stabilized samples with different bacteria suspension is shown in Figure 14. The figure illustrates that maximum major stress for all samples is obtained at bacteria suspension equal to optimum moisture and by increasing moisture (Wopt+5%), it decreases. As well as, the strength reduces when the bacteria suspension in samples is less than optimum amount. It is worth noting that variation of maximum major stress in sandy samples with different bacteria suspension is neglectable. Moreover, the maximum major stresses are very close in samples with 20 and 40% silt.

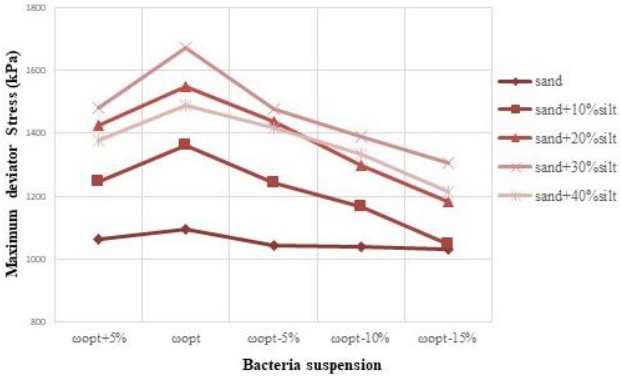

Figure 14. Maximum deviator stress variation with different bacteria suspension for all stabilized samples

5. CONCLUSION

In this study bacterium *Sporosarcina pasteurii* is used for soil improvement. The samples were sand with different percentages of silt (0, 10, 20, 30 and 40%). For further accuracy, the experiments were also done for samples with 50 and 60% of silt and pure silt. At first, the OD method was used for cell density measurement. Distilled water was used as a calibration liquid and the bacterium was tested some days after culturing. The cell density of the tested bacteria reached maximum after four days. Also, the SPC was obtained $2.5*10^8$ from serial dilution experiment. Then, the gram staining method was done, and purple rod bacteria were observed under the microscope. Moreover, two samples from each solid and liquid medium were prepared for the urease test. It was shown that the colors after four days were changed to purple. Results of triaxial tests can be summarized as follows:

- The samples were first deformed into barrel shape and then broke at an angle of about 65 degrees.
- The optimum bacteria suspension volume for different percentages of silt is determined by triaxial tests and results showed that this is equal to the optimum moisture.
- By adding silt into the soil, the maximum triaxial strength in stabilized soil increased from 1097 (0% silt) to 1671 kPa (30% silt). However, by adding more silt (40% silt), the resistance decreases again to 1488 kPa.
- The maximum strength was obtained 1671 kPa in stabilized soil while it was near 900 kPa in untreated soil.
- The strength was improved by almost 80% by stabilizing.

The main limitation in this study was the use of bacteria as living organisms and sensitive to environmental conditions. In biological methods the most important point is providing the necessary and required conditions for bacteria. Other limitation of this study was the method of combining bacterial suspension with soil and preparing suitable samples. The best method was selected by performing several experimental samples. It is worth mentioning that in future studies, the native bacteria in the soils of each region can be studied to investigate their effects on increasing soil resistance. This can reduce the environmental impacts and costs**.**

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Persian Abstract

چکیده

یکی از مسائل مهم در مهندسی ژئوتکنیک که اخیرا مطالعات زیادی در رابطه با آن انجام شده است، بهسازی بیولوژیکی خاک می‌باشد. در این روش باکتریهای اوره آز مثبت به همراه مواد مغذی نظیر اوره و کلسیم کلراید به خاک اضافه میشوند و باعث رسوب کربنات کلسیم و ایجاد چسبندگی بین ذرات خاک و در نتیجه بهبود خواص فیزیکی و مکانیکی خاک میشود. با توجه به اینکه اکثر مطالعات انجام شده بر روی خاک های ماسه ای می باشد و تحقیقات اندکی بر روی خاک های ماسه ای لای دار انجام شده است، در این مطالعه از نوعی باسیل به نام اسپورسارسینا پاستیوری که به صورت هوازی کشت داده می شود برای تثبیت خاک ماسه ای لای دار با درصدهای مختلف لای (0 تا 60) استفاده شده است تا حجم بهینه سوسپانسیون لازم برای بهسازی خاک برای درصدهای مختلف لای تعیین شود. برای این منظور، ابتدا آزمایشهای بیولوژیکی برای بررسی، صحت سنجی و اطمینان از سلامت باکتری انجام شد که این آزمایشها شامل آزمون تعیین جمعیت باکتری، آزمایش سری رقت، رنگ آمیزی باکتری، آزمایش pH، کشت باکتری بدون افزودن اوره و آزمون اوره آز می باشد. پس از انجام آزمایش های بیولوژیکی و کشت باکتری، آزمایش های فیزیکی و اندکس نظیر دانه بندی، تعیین حدود

اتربرگ و تراکم به روش پروکتور بر روی خاک مورد استفاده انجام شد. همچنین، برای دستیابی به مناسب ترین روش نمونه سازی برای تثبیت خاک با درصد های مختلف لای با حضور سوسپانسیون باکتری و مواد مغذی، روش های مختلف نمونه سازی مورد آزمون قرار گرفت و روش مناسب انتخاب گردید. علاوه بر آن، جهت بررسی عملکرد قابل مشاهده باکتری در داخل خاک و به تصویر کشیدن شبکه منسجم حاصل از کربنات کلسیم، تصویر برداری از نمونه های تثبیت شده و تثبیت نشده خاک با استفاده از میکروسکوپ الکترونی انجام شد. نتایج حاصل از آزمایش های سه محوری نشان می دهد که با افزودن حجم بهینه سوسپانسیون باکتری و انجام آزمایش های سه محوری، حداکثر مقاومت برای نمونه های حاوی 0، 10، 20، 30 و 40 درصد لای به ترتیب از 700 به 1100، 900 به 1400، 750 به 1550، 600 به 1600 و 550 تا 1500 کیلو پاسکال افزایش یافته است. این موضوع بیانگر افزایش 80 درصدی مقاومت خاک تثبیت شده با استفاده از باکتری نسبت به نمونه های تثبیت نشده می باشد.