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Biological improvement of sandy soil by microbial induced carbonate precipitation

Mashaalah Khamehchiyan, Karim Rowshanbakht, Mohammad Reza Nikudel
Tarbiat Modares University, Department of Engineering Geology
Jalal Ale Ahmad Highway, P.O.Box: 14115-111, Tehran, Iran
khamechm@modares.ac.ir; rowshanbakht@gmail.com; nikudelm@modares.ac.ir

Reza H. Sajedi
Tarbiat Modares University, Department of Biochemistry
Jalal Ale Ahmad Highway, P.O.Box: 14115-111, Tehran, Iran
sajedi_r@modares.ac.ir

Abstract

Microbial-induced calcite precipitation (MICP) is a relatively green and sustainable soil improvement technique. It refers to a chemical reaction network that is managed and controlled within soil through biological activity and whose byproducts alter the engineering properties of soil. To treat soil, first, the microbial population in-situ is augmented by the injection of additional urease positive bacteria and then reagents are added. A series of laboratory test was carried out to investigate the potential application of the technique in improving the strength and impermeability of a sand specimen and utilized techniques, materials, methods and empirical process during the test are explained. The results showed that as a result of the calcite precipitation, shear wave velocity increased up to 1000m/s and UCS strength increased to about 300Kpa and permeability of soil decreased significantly upon MICP treatment.

***Keywords*:** Microbial-induced calcite precipitation, Bio grout, Biological improvement, Geotechnical properties.

1. Introduction

Rehabilitation and expansion of civil infrastructure is required to meet ever-growing societal needs and is directly limited by the availability of competent soils upon which they can be constructed [1].

A wide range of products is available on the market for the improvement of soil properties (permeability, internal friction angle, bearing capacity etc). Some of the products used for treatment of soil are not considered as environmentally friendly due to the pollution effect and poison effect during manufacturing and application [2].

Microbial-induced calcite precipitation (MICP) is a relatively green and sustainable soil improvement technique [3]. It refers to a chemical reaction network that is managed and controlled within soil through biological activity and whose byproducts alter the engineering properties of soil [1].

MICP has been used to mitigate several engineering problems such as crack repair in concrete [4,5], permeability reduction [6], repairing calcareous monuments [7,8], concrete compressive strength improvement [4,9], concrete durability improvement [10,11], selective plugging for enhanced oil recovery [12], wastewater treatment [13], soil improvement [12,14,1], bricks durability [15], bioconcret [16].

Ivanov and Chu [14] performed an approximate cost comparison between the raw materials for microbial grouting and the conventional chemical grouting. They suggested that the cost for microbial grouting ($0.5 – $9 / m3 of soil) is significantly cheaper than that of chemical grouting ($2 – $72/ m3 of soil.

In this research, increase in strength and decrease in permeability proved by uniaxial compressive strength and constant head permeability test. Scanning electron microscopy (SEM) indicated the precipitated materials and their composition investigated by XRF (X-ray Fluorescence Spectroscopy).

2. Biocementation

Biocementation improves the shear strength of soil through the production of soil particle-binding materials, as the result of introducing bacteria and cementation reagents into the soil. The soil cementation materials are mostly carbonates, silicates, phosphates, sulphides and hydroxides [14]. Calcium carbonate (calcite) is an attractive element to be studied in biocementation because calcite formation is commonly found in nature. Bioclogging is a process where the soil void is filled by the product from microbial-induced biochemical process [3].

The microbial urease enzyme hydrolyzes urea to produce dissolved ammonium, dissolved inorganic carbon and CO2, and the ammonia released in the surroundings subsequently increases pH, leading to accumulation of insoluble CaCO3 in a calcium rich environment. Quantitatively, 1 mol of urea is hydrolyzed intracellularly to 2 mol of ammonium (Eqs. 1 and 2)

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

$$Ca\text{^}(2+) + CO\_3\text{^}(2-) \rightarrow\rightarrow CaCO\_3 \quad (2)$$

These reactions occur under the influence of natural environmental factors that control the activity of the urease enzyme. Factors such as the type of bacteria, bacteria cell concentration, temperature, urea concentration, calcium concentration, ionic strength, and the pH of the media may have a significant impact on MCP. The bacteria should possess high ureolytic efficiency, alkalophilic (optimum growth rate occurs at pH around 9, and no growth at all around pH 6.5), non-pathogenic, and possess the ability to deposit calcite homogeneously on the substratum [17].

Several factors affecting the performance of MICP which some of them are mentioned as; Nutrients, Types of Bacteria, Geometric Compatibility of Bacteria,

Bacteria Cell Concentration, Fixation and Distribution of Bacteria in Soil, Temperature, Reactant Concentration, pH and Injection Method [3].

3. Materials and methods

3.1. Calcite forming bacteria

Ureolytic bacteria especially *Sporosarcina pasteurii* (formerly *Bacillus pasteurii*) have generated a lot of interest in this area, and have been studied extensively [17,5,12]. The bacteria provided from Persian Type Culture Collection PTCC1645.

3.2. Determining the soil characteristics

The soil type used in experiments was sandy soil which its specifications were determined. Grain size distribution was determined according to [18,19], minimum density according to [20] and maximum density according to [21].

3.3. Culture medium composition and reagents concentration

Different growth medium were tested. Bacterial growth monitored by optical density (OD 600nm) measurements [23,17,12], in order to choose a suitable one. Final selected medium components presented in table 1. Medium pH set at 9 and growth lasted for 18 hours in 27 Celsius degree. In order to ensure the survival of bacteria for improvement, they harvested at the end of growth phase. Concentration of reactants including urea and calcium chloride presented in Table 1.

Table. 1. Culture medium composition and reagents concentration

Solution		Concentration
Culture medium	Yeast Extract	20gr/Lit
	NH_4Cl	10gr/Lit
	$NiCl_2$	10μmol/Lit
Fixation fluid	Calcium chloride	0.05 Molar
Cementation fluid	urea	1.1 Molar
	Calcium chloride	1.1 Molar

3.4. Sample column preparation

Samples prepared in columns with 3cm diameter and 60cc volume.To achieve a certain relative density, the weight of dry sand required to achieve the desired density calculated and weighed separately in three equal parts. Then, each part placed in column and reached to the calculated volume by tamping.

Each end of the column was fitted with filter material consisting of 3 layers of scouring pad (Scotch Brite) at the outside and approximately 1 cm of filter gravel on the inside, next to the sand (Figure 1). The column was positioned vertically with upward flow direction to avoid any settling of the packing material and generation of preferential flow paths that may occur if the column was positioned horizontally.

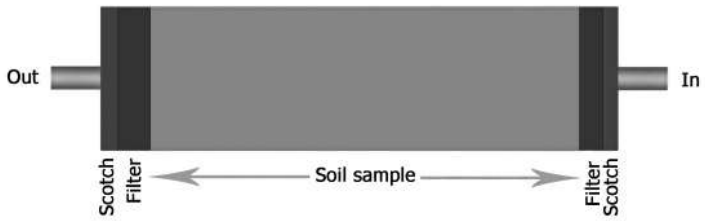

Figure 1. Column setup schematic

Columns were installed in such a manner that allows injection from down the column and the effluent to be collected from top of the column and columns remain saturated during the test.

3.5. Treatment procedure

To treat soil by the MICP process, the procedure which derived from [12] was applied to the columns.

Columns saturated with autoclaved deionized water and rinsed about twice the pore volume from the bottom. Then each column injected by bacterial suspension at an optical density (OD 600nm) of about 1.

Approximately 1 pore volume of bacterial suspension was injected into the column and then immediately followed by approximately 1 pore volume of fixation fluid. The fixation fluid contained 50mM CaCl2 (same as [12,1,23]). After 4 hours from the fixation fluid injection, a third fluid -the cementation fluid- containing 1M equimolar CaCl2 and urea was flushed through. After the first batch of cementation fluid was fully loaded into the column, the flow was stopped and the fluid in the column was then allowed to react for 24 h. The next day, the column was flushed with a 2nd batch of cementation fluid. During all flushes, the flow rate was kept constant at approximately 200 ml/h (same as [12,23]).

Samples were taken periodically from the effluent to analyze optical density and ammonium concentration.

according to the stoichiometric calculations of the mass of products, assuming complete conversion of reactant(s), to achieve 160 gram calcite per cubic meter, about three times injection of pore volume with 1molar cementation solution are required.

At each step of injection, samples were taken sequentially from effluent to monitor changes.

Finally, the columns were rinsed with distilled water for about 1.5 pore volume. Columns were saturated for two days. The water was then allowed to drain by weight and samples were stored in moist condition until the experiments.

3.6. Measurement of the ammonium concentration

Nessler reagent was used to measure the ammonium concentration [24]. In order to determining the calibration curve, solutions with different concentrations

from 0.05 to 5 ml per litter of ammonium prepared. 0.1 cc of Nessler reagent added to 2 ml of solution. Optical density at 425 nm (OD425) measured after 5 min. optical density was plotted against the ammonium concentration and the relationship between these two parameters obtained for next measurements.

Samples were diluted to a concentration in the range of valid measurement by Nessler method and ammonium concentration calculated according to the OD and dilution factor.

3.7. Tests on improved samples

The tests were done after the completion of the improvements include permeability testing, wave velocity, uniaxial compressive strength, calcimetry and electron microscopy.

The constant head permeability test method used to determine the permeability of samples before and after the improvement.

In this study P wave velocity determined in improved samples and then shear wave velocity calculated based on relations between shear and compressional wave velocity [25].

In order to evaluate the strength of improved samples, uniaxial compressive strength tests were performed on samples. Samples prepared with a length to diameter ratio of 2. Tests were performed according to standard methods [22].

Calcite precipitated in columns determined by Bernard Calcimetry method. In this method, calcite percentage is calculated based on the amount of gas released during the reaction of calcite with dilute hydrochloric acid.

Scanning electron microscopy (SEM) performed on sample prepared from improved columns.

4. Discussion

4.1. Soil properties

Poor graded sandy silica soil with negligible calcite used in this study. Grain size distribution curve produced from the results of laboratory tests on the soil is provided in Figure 2. Physical properties of soil are presented in Table 2. Samples prepared with relative density of 85%.

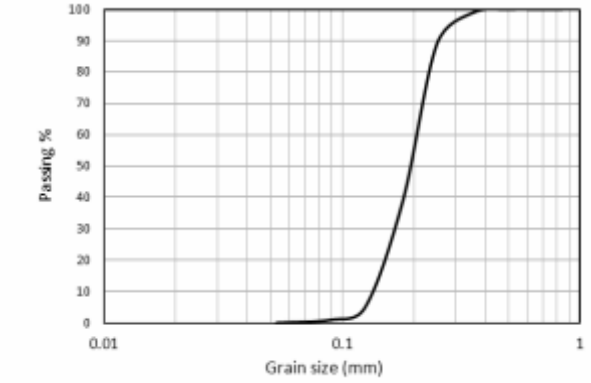

Figure 2. Grain size distribution of material

Table. 2. Physical properties of material

Soil class	Cc	Cu	D_{50} (mm)	e_{min}	e_{max}	G_s
SP	0.83	1.46	0.2	0.89	0.59	2.65

4.2. Bacterial growth

Bacterial growth is calculated by measuring optical density at 600nm [23,12,17] and simultaneously the production rate of ammonium, which represent the bacterial activity is measured and are presented in Figure 3 in order to determine the most appropriate time for the injection of bacteria.

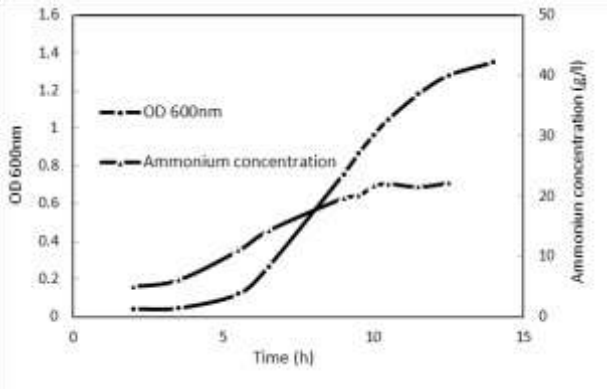

Figure 3. Bacterial growth curve and ammonium production

4.3. Ammonium concentration

Nessler reagent was used to measure the ammonium concentration according to the described method [24]. Starting the injection phase, samples were taken from effluent in samples with volume of 4cc, periodically. About 6 to 7 samples were taken from each injection phase.

Changes in Ammonium concentration was studied in successive samples from each phase. As an example, a graph is presented in Figure4a. In the first sample due to the greater distance from injection point, the ammonium concentration is less and in the latter case, due to lack of sufficient accuracy in estimating the pore volume and their change with calcite precipitation and the effect of the filter material, the measured concentration is less too. In other cases, the measured values are approximately equal which indicates that the activity is nearly uniform throughout most of the sample length.

In addition, the average value of the ammonium concentration at each injection stage is compared and presented in Figure4b.

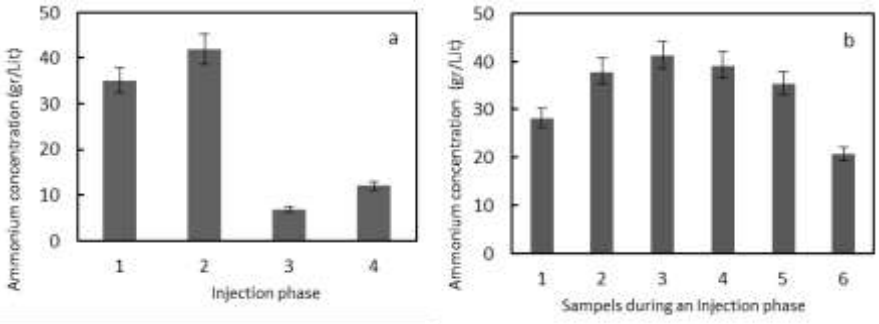

Figure 4. Ammonium concentration; in different injection phases (a), during an injection phase (b)

It is obvious that after two injection stage, ammonium concentration which represents the bacterial activity and calcite precipitation has reduced significantly. According to the ammonium concentration and stoichiometry calculations, major part of the injected urea in the first and second stages of the injection have participated in reaction (over 95%) and it is expected to consume the same amount of calcium ion as well. But in 3rd injection, this has reduced to about 20%.

4.4. Permeability test

Constant head permeability test was performed on the samples before and after improvement. Results showed a decrease in permeability from $1.15*10^{-2}$ to $2.5*10^{-3}$ cm/s.

4.5. Wave velocity measurement

The shear wave velocity is a property of the soil that can help identify density and more directly stiffness. It is used directly in liquefaction analysis and to identify the general characteristics of a soil in both the lab and in situ testing [26]. Standard loose sand may have a shear velocity between 100-200 m/s. A liquefiable soil is any soil falling under a shear velocity of 500 m/s. The goal of the MICP is to raise that shear wave velocity above 500 m/s and to stay in the range of 500-1000 m/s with the properties more associated with that of sandstone [26].

In this study P-wave velocity measured in improved columns and based on the relationships between compressional and shear wave velocity, shear wave velocity is calculated. Equations proposed by various researchers to establish the relationship between shear and compressional wave velocity are studied. Some of them are introduced in Figure 5. Results of the wave velocity measurements are presented on this figure. According to the calculations, average shear wave velocity increased up to about to 1000 m/sec.

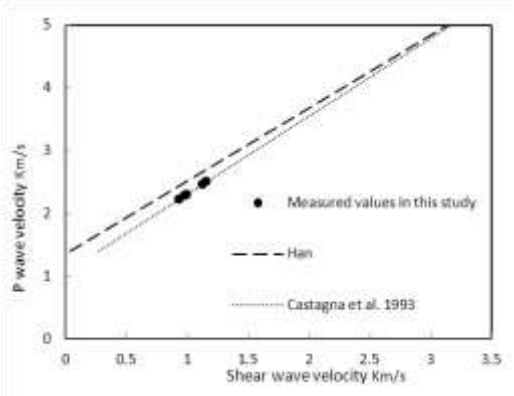

Figure 5. Relations between shear and compressional wave, (Vs is calculated as shown by [25])

4.6. Uniaxial compressive strength testing

Experiments were performed on 4 specimens. The results showed an increase in strength of samples up to 300 kPa. One of the stress-deformation curves which obtained during uniaxial strength testing are presented in Figure 6.

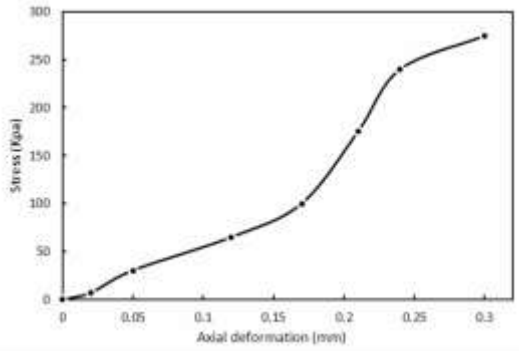

Figure 6. An example of stress-deformation curves of UCS test

4.7. Calcimetry

Precipitated Calcite percentage in the columns was measured using Bernard calcimetry test. Small pieces of the specimen from 3 parts (near the entrance, middle, near the outlet) of columns prepared and their calcite content determined. Results presented in Figure 7. According to the results, measured calcite percentage varies from at least 4%, near the outlet up to 7%, near the inlet. Average of these values is 4.6% near the outlet and 6.7% near the inlet. A decrease in precipitated calcite is clear in all samples with the distance from the column inlet to the injection point.

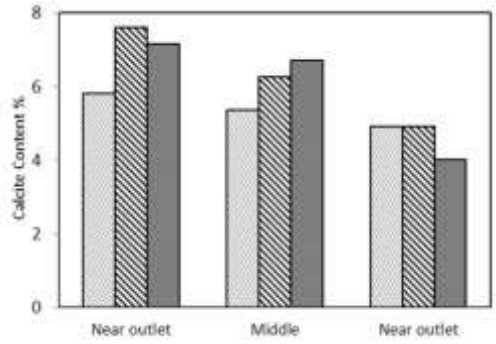

Figure 7. Measured percentage of precipitated calcite in the columns

4.8. Scanning electron microscopy

Results from scanning electron microscopy (SEM), shows the calcite precipitation. Calcite deposition concentration in samples taken from closer to the injection point is more and decreases with the distance from entrance Images show there is a greater concentration of calcite at the particle–particle contacts (Figure 8a). X-ray Fluorescence Spectroscopy (XRF) used to detect the composition of precipitated material. Results which presented in Fig 8b prove silica grain and calcite cement.

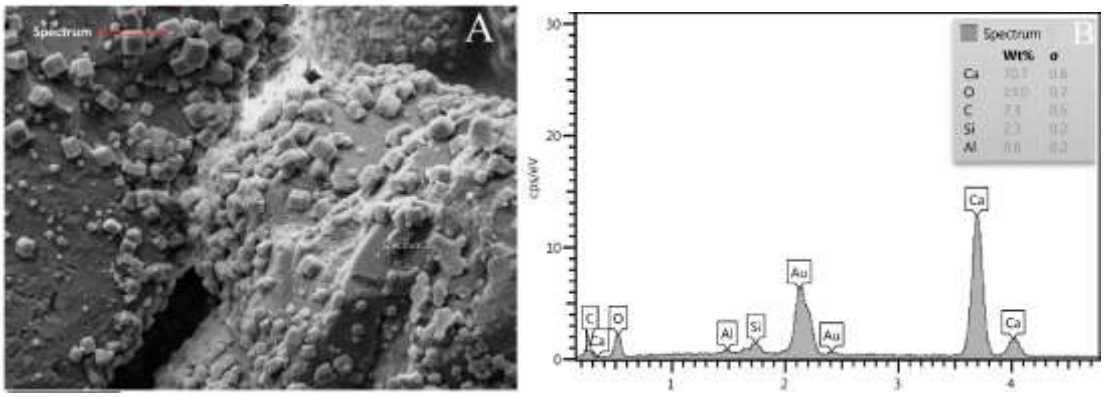

Figure 8. SEM image of calcite concentration at grain contacts (a), XRF analysis (b)

5. Conclusion

Microbial-induced calcite precipitation (MICP) is a relatively green and sustainable soil improvement technique. Phase-injection of bacterial suspension, fixation and cementation fluid expanded the cementation over the column length by calcite precipitation. Precipitated calcite percentage varies from 4 to 7 percent. Permeability is decreased and compressional strength is increased in Laboratory specimens as a result of biological improvement. Shear wave velocity increased after improvement too. Although electron microscopy studies shows calcite precipitation throughout the column, however, the density of calcite precipitation is more, closer to the injection point. It also has a greater tendency to precipitate in particles contacts.

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