INFLUENCES OF CALCIUM SOURCES AND TYPE OF SAND ON MICROBIAL INDUCED CARBONATE PRECIPITATION

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ABSTRACT

Microbial Induced Calcite Precipitation (MICP) is commonly carried out by injecting chemical solutions (e.g., urea and calcium source) and bacteria (e.g., Sporosarcina pasteurii, B. megaterium) to the soil where treatment is required.

This research aims to explore a new source of calcium chloride. The new source was the egg shells. It has been exploring the feasibility of using a new source of calcium in improving the engineering properties of two different types of fine sand (river and silica sands). Also, explore the impact of the sand type on the results of MICP.

Set of laboratory tests were conducted, including calcium carbonate content, unconfined compressive strength, soil permeability and microscopy Investigation (SEM). The results indicate that use calcium chloride produced of egg shells has the same effectiveness of that of pure calcium chloride (analytical grade) in the cementation solution. This was demonstrated clearly by measure the Precipitated Calcium Carbonate content, where the same amount of Calcium Carbonate of both calcium sources was precipitated. But it was slightly higher in the river sand.

In both cases (river and silica sands samples) the use of cementation solution contained calcium chloride made of egg shells has a significant effect on the permeability, but the effect was greater in the silica sand samples. Also, the effect of using cementation solution contained calcium chloride made of egg shells was exactly the same effect of using cementation solution contained analytical grade of calcium chloride. Finally, from SEM images, the calcium carbonate type in all cases was the calcite and the crystals sizes were relatively same. But the crystals type of calcite was changed according to the type of sand.

KEYWORDS: *Microbial-induced calcite precipitation (MICP),soil improvement, calcium chloride, permeability, UCS test, Sporosarcina pasteurii*

I. INTRODUCTION

The idea of using bio-organisms activities to enhance the properties of loose soil is encouraging and motivating to all researchers. Lately, a technique called Microbial Induced Calcite Precipitation (MICP) has appeared as an innovative and sustainable method for soil improvement.

MICP is commonly carried out by injecting or percolating chemical solutions (e.g., urea and calcium source) and ureolytic bacteria (e.g., Sporosarcina pasteurii, B. megaterium) to the soil where treatment is required. Ureolytic bacteria catalyze the hydrolysis of urea to produce ammonium and carbonate ions, which interact with calcium ions (e.g., calcium chloride and calcium acetate) to form calcite or aragonite that precipitates throughout the soil matrix [1, 2].

$$(NH_2)_2CO + 3H_2O \longrightarrow 2NH_4^+ + HCO_3^- + OH^- \quad \text{……………………………………. (1)}$$

$$CaCl_2 + HCO_3^- + OH^- \longrightarrow CaCO_3\downarrow + H_2O + 2Cl^- \quad \text{……..……………………………. (2)}$$

The researchers were investigating the feasibility of using the MICP method for the purpose of improving the engineering properties of granular soil, where it gave encouraging results with respect to cohesion, internal friction angle, stiffness, strength and permeability [3, 4].

Diverse engineering properties of soil were targeted by previous studies. The researchers suggested use MICP in biomineralized concrete [5-11], reductions in foundation settlement [12], soil stabilization prior to tunnelling construction [13, 14], wastewater treatment [15], liquefaction mitigation [16], improvement in the stiffness/strength of sandy soil [17], reduction in soil permeability [18, 19], dust control [20], slope stabilization, piping prevention for dam sand levees [14].

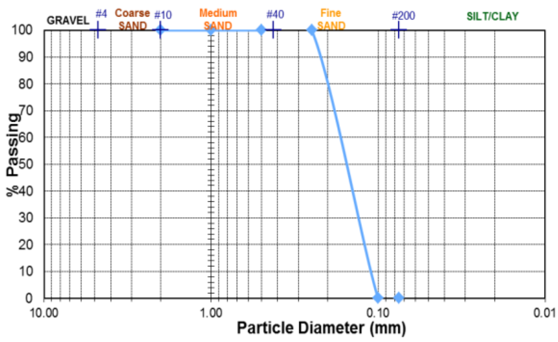
All former researchers used in their research, pure urea and calcium source (analytical grade) as a cementation solution, and this contributed directly to raising the cost of using the MICP method as a means to improve the engineering properties of sandy soils. This was somewhat, one of the reasons of delayed the adoption of this technology on a large scale to improve the soil.

This research aims to explore a new source of calcium substitute for pure calcium, which is currently used in MICP Research. The new source, which was chosen for this research was the egg shells. It has been exploring the feasibility of using a new source of calcium in improving the engineering properties of two types of soil (river and silica sand). The research investigated and compares the effects of the new and old calcium source and types of soil, on the calcium carbonate content, the crystal formation, the strength and permeability. In this paper, section 2 discuses, the materials and methods of preparation of soil specimens, bacterial culture and cementation solution In addition to laboratory tests. Section 3 discusses, results and discussion. Section 4 discusses, conclusions. Section 5 discusses, future work

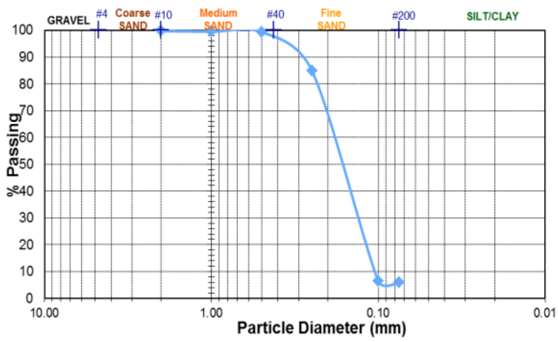
II. MATERIALS AND METHODS

2.1. Preparation of Soil Specimens

Two types of poorly graded sand were selected for the current study. The first type was fine silica sand which remaining on the sieve with an opening size of 0.1 mm (figure 1 A). While the second type was river sand taken from the Yangtze river bank (figure 1 B).The poorly graded sand column was prepared by packing the dry sand (with a unit weight of 16 kN/m3 and14 kN/m3, porosity of 37.67 % and 48.67 %, and pore void volume of about 33 ml and 43 ml, for silica sand and river sand respectively) into a polyvinyl chloride (PVC) column of 80 mm high and 37 mm inner diameter. The coefficient of permeability of the untreated sand was approximately 1.9 x 10^{-5} m/s and 5.9×10^{-5}m/s for silica sand and river sand respectively.



A



B

Fig.1 Particle size distribution of the A. Silica sand and B. River sand

2.2 Bacterial Culture and Cementation Solution

The ureolytic bacterium used in the current study was Sporosarcina pasteurii (ATCC 11859). The ATCC 11859 was cultivated under sterile aerobic batch conditions in a yeast extract medium (20 g/l yeast extract, 10 g/l ammonium sulfate, 0.13 M Tris buffer, pH = 9).The optical density OD600 of the bacterial culture varied between 1.0 and 1.3. The bio-cementation was conducted using two types of cementation solution, the first one is a highly concentrated cementation solution consisting of equal moles of pure anhydrous calcium chloride (1 M, 111 g/l) and urea (1 M, 60 g/l), following Al-

Thawadi et al. (2012) and Cheng et al. (2013) [21, 22]. While the other prepared from urea (1 M, 60 g/l) and the egg shells as a calcium source, the egg shells were crushed and mixed with hydrochloric acid (the concentration was 2M) for 24 h to produce the calcium chloride (1 M) (equation 3). Then added 0.5 g/l of NaOH to the liquid for the purpose of making the pH= 7.

$$CaCO_{3\,(egg\ shells)} + 2HCl \longrightarrow CaCl_2 + H_2O + CO_{2\,(gas)} \quad \ldots\ldots \quad (3)$$

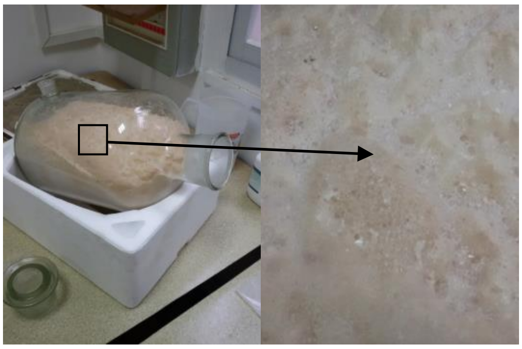


Fig.2 The reaction between egg shells and HCl acid

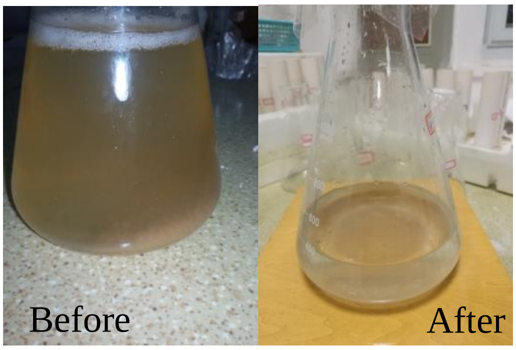


Fig.3 The egg shells solution before and after filtration by filter paper

2.3 MICP Procedure

Microbial induced carbonate precipitation for soil treatment was conducted using gravity-induced downward precipitation. Initially, the sand columns were divided into two groups. The first group was silica sand columns, and the second group was Yangtze river sand columns. The two groups were flushed with 33 ml for silica sand and 43 ml for Yangtze river sand bacterial culture, followed by 3 hours of retention time to allow the cells of bacteria installs itself in the soil granules. At this stage, each group was divided into the two subgroups. The first subgroup was flushed with a cementation solution made of $CaCl_2$ (egg shells) (equation 1) and urea, while the second subgroup was flushed with a cementation solution made of pure $CaCl_2$ and urea. The MICP reaction time was 24 h with a highly concentrated cementation solution. The flushing with cementation solution was repeated every 24 h. The whole tests were performed at a temperature of 30°C ± 2°C. After 4 days of flushing with cementation solution, the treatment was stopped and the soil specimens were put in the oven at a temperature of 60°C for 7 days.

A B

Fig.4 The soil samples after treatment A. Yangtze river sand B. Silica sand

2.4 $CaCO_3$ Content

To determine the precipitated $CaCO_3$ in the soil specimens, specimens were crushed, oven-dried and recorded the weights (M_{dry}). The dry soil was soaked in HCl solution (1 M) to dissolve precipitated $CaCO_3$, then washed with water and drained, and finally oven-dried. The weights were recorded again ($M_{residual}$). The difference between the weights before and after this process is considered to be the weight of the $CaCO_3$ precipitated in the specimen [23].

$$M_{calcite} = M_{dry} - M_{residual} \quad \ldots\ldots \quad (4)$$

2.5 Permeability, Unconfined Compressive Strength

The permeability tests were conducted using the falling head method according to ASTM D5856 – 15 before and after the MICP treatment. These tests determined the reduction in permeability and the relation with the calcium source and soil type.

Also, Unconfined Compressive Strength (UCS) tests were conducted according to the procedure reported in ASTM D2166 (ASTM, 2013) on bio-cemented soil specimens with selected diameter-to-height ratios of 1:1.5 to 1:2. Unconfined compression (UC) tests were conducted using cylindrical sample size 37.5×80 mm, at a strain rate of 1 mm/min. The samples were flushed with deionized water (about four pore volumes) and dried at 60 °C for 24 hours prior to UCS measurements. The specimen was subjected to a steadily increasing axial compression until failure. These tests were conducted to establish the relationship between the strength of the soil samples and its calcium source and soil type.

2.6 Microscopy Investigation

In order to study the calcium carbonate bonds and their distribution within the sand after treatments, the scanning electron microscope (SEM) was conducted. Additionally, the SEM provides a good comprehension of the bonding between the calcite crystals and the sand particles. An FEI Quanta 200 ESEM/VPSEM scanning electron microscope was used to conduct this test. The samples were coated with gold and carbon using a BAL-TEC / SCD 050 sputter coater. This test was conducted in the Central Laboratory at Huazhong University of Science and Technology.

III. RESULTS AND DISCUSSION

3.1 The Visual Examination

Before starting treatment of soil samples with a bacterial culture and cementation solution, the Visual examination was conducted on the cementation solution made from eggshells and compared with the cementation solution component of the analytical grade material for the purpose of ascertaining the existence of initial effectiveness reliable later when beginning the actual treatment.

The examination was conducted through the use of two cups of glass, put 25 ml of cementation solution containing urea and calcium chloride (analytical grade) in the first, while the second

contained 25 ml of cementation solution components of urea and calcium chloride which is made of egg shells. Then it was added (5 ml) of the bacterial culture to each of the two cups. Then leave the two cups for a period of 24 h to give the bacteria ample opportunity to precipitate Calcium carbonate.
Figure 5 illustrates this process. It's obvious, visually, that the bacteria were able to produce calcium carbonate in both cementation solutions, and almost the same amount (the lower part of the solution in white).
The next step in this examination was to make a comparison between the weight of calcium carbonate precipitated from the activity of bacteria in the solution containing calcium chloride is made of egg shells and urea with the weight of calcium carbonate precipitated from the activity of bacteria in the solution containing pure calcium chloride and urea. This step is performed by drying the content of the two cups in an oven warmly 100 °C. After the drought (Figure 6), the weights of the two cups with Precipitate (M_2) were recorded. The difference between the weight of the two empty cups (M_1) and the weight of the two cups with Precipitate (M_2), is considered to be the weight of precipitated calcium carbonate ($M_{calcite}$).

$$M_{calcite} = M_2 - M_1 \quad \text{……………………………………………………………………………} \quad (5)$$

This examination is clarified that the weight of precipitated calcium carbonate were 1.88.gm and 1.82 gm in each of the solution containing calcium chloride made from egg shells and the solution containing the pure calcium chloride, respectively. This shows that the cementation solution made from egg shells has the same efficiency as cementation solution made from pure materials (analytical grade).

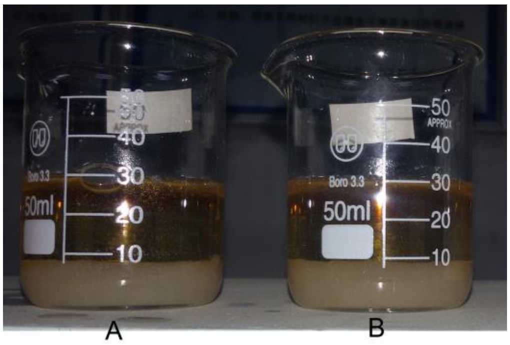


Fig.5 The calcite precipitation from two different calcium sources A. $CaCl_2$ (egg shells) and B. $CaCl_2$ (analytical grade)

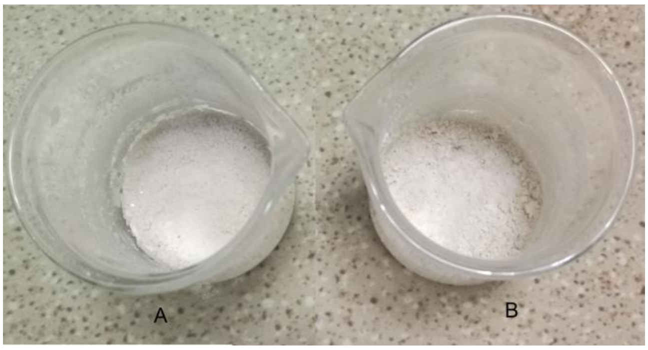


Fig.6 The calcite precipitation from two different calcium sources after drying A. $CaCl_2$ (egg shells) and B. $CaCl_2$ (analytical grade)

3.2 Calcium Carbonate Content

The calcium carbonate content (i.e., gram/gram calcite per sand) of the samples of the two types of soil which treated with two different calcium sources are shown in figure 7.

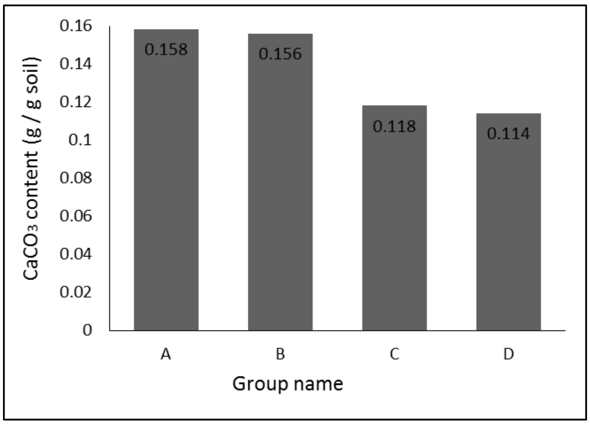


Fig.7 Effect of Calcium source and soil type on the calcium carbonate content

The two groups A and B are of river sand, the group A treated with cementation solution composed of calcium chloride which is made of egg shells and urea, while group B was treated with cementation solution composed of pure calcium chloride (analytical grade) and urea.

After four times of treatment, calcium carbonate content was (0.158) and (0.156) in group A and group B, respectively. These values showed that the bacteria cells were able to produce the same amount of calcium carbonate in both types of cementation solution. The same thing happened with silica sand groups C and D, where group C treated with the same procedure of group A treatment, while group D treated with the same procedure of group B treatment. After four times of treatment, calcium carbonate content was (0.118) and (0.114) in group C and group D, respectively. Figure 7 also showed that the calcium carbonate content of river sand (groups A and B) was generally slightly higher than the calcium carbonate content of silica sand (groups C and D. This can be interpreted as the result of different soil type.

3.3 Coefficient of Permeability

Bioclogging is a process where the soil void is filled with the product from biomicrobial-induced biochemical process. The clogging of soil as a result of formation of calcium carbonate precipitation near the soil particles contacts restricts water flow through soil, and hence reduces its hydraulic conductivity. Vandevivere and Baveye (1992) [24] and Abdel Aal et al. (2010) [25] found that the hydraulic conductivity of soil reduced significantly through the accumulation of biomass and production of exopolymeric substances. Al Qabany and Soga (2013) [26] reported that, for four injection cycles, the high concentration cementation solution produced a quicker and greater reduction in the coefficient of permeability.

The falling head permeability test was used to determine the coefficient of permeability of the cured specimens (still contained in molds).The two groups A and B are of river sand, while groups C and D are of silica sand, the groups A and C were treated with cementation solution composed of calcium chloride, which is made of egg shells and urea, while the groups B and D were treated with cementation solution composed of pure calcium chloride (analytical grade) and urea. The groups control 1 and control 2 were for river sand and silica sand without treatment, respectively. Figure 8 shows the effect of calcium source and soil type on the permeability.

From figure 8, it can be seen that in the river sand samples (groups A and B) the effect of using cementation solution contained calcium chloride made of egg shells was exactly the same effect of using cementation solution contained analytical grade of calcium chloride on the coefficient of permeability, where the average coefficient of permeability and the average decreases in the permeability of group A and group B were 3.23×10^{-5} m/s , 3.35×10^{-5} m/s and 45% , 43%, respectively. The same thing is repeated when talking about the silica sand samples (groups C and D), where the average coefficient of permeability and the average decreases in the permeability of group C and group D were 2.1×10^{-6} m/s , 2.01×10^{-6} m/s and 87% , 88%, respectively.

After all, in both cases (river sand and silica sand samples) the use of cementation solution contained calcium chloride made of egg shells has a significant effect on the permeability, but the effect was greater in the silica sand samples, where the average decreases in the permeability of the silica sand and river sand samples were approximately 87% and 45%, respectively.

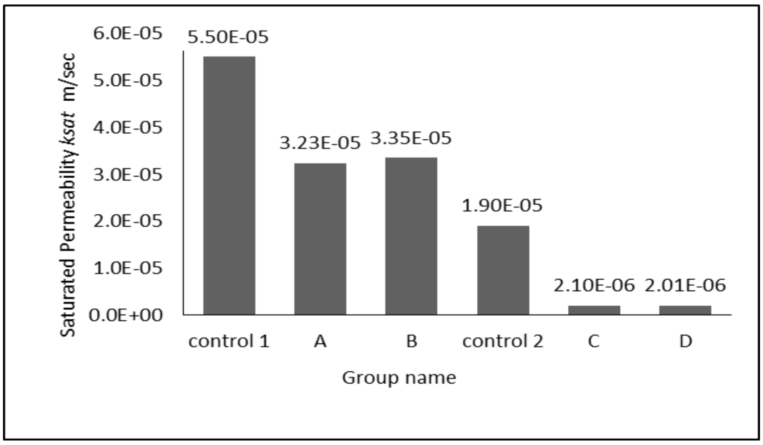


Fig.8 Effect of calcium source and soil type on the permeability

3.4 Uniaxial compressive strength

To investigate the impact of the calcium source and soil type on the strength of treated samples, unconfined compressive strength test was conducted. From figure 9, it can be seen that in the river sand samples with calcium chloride (egg shells) and calcium chloride (analytical grade) (groups A and B, respectively), the unconfined compressive strength values were very close (331KPa, 380KPa). The same thing was observed for samples of silica sand with calcium chloride (egg shells) and calcium chloride (analytical grade) (groups C and D, respectively), where the values were (638 KPa, 648 KPa).

Also, it can be seen that the sand type has the greater role in the difference in the values of the unconfined compressive strength. For the same type of calcium chloride source, the value of unconfined compressive strength of silica sand samples was approximately two times higher than that of river sand samples.

Generally, the effect of using cementation solution contained calcium chloride made of egg shells was exactly the same effect of using cementation solution contained analytical grade of calcium chloride.

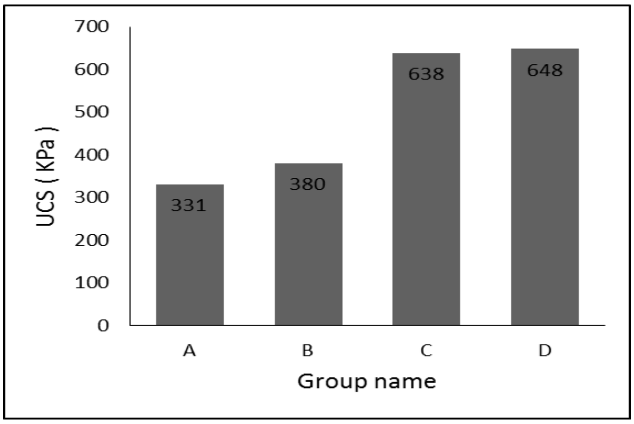


Fig.9 Effect of calcium source and soil type on the strength

3.5 Scanning Electron Microscopy (SEM)

To investigate the impact of the calcium source and soil type on the crystal formation of treated samples, SEM analysis was conducted. The SEM results shown in figure 10A–D correspond to the river sand with calcium chloride (egg shells) sample, river sand with calcium chloride (analytical grade) sample, silica sand with calcium chloride (egg shells) sample and silica sand with calcium chloride (analytical grade) samples, respectively.

Generally, the calcium carbonate crystal types include calcite, aragonite and vaterite, and calcite is the most common and steadiest among them. From figure 10A-D, it seems clear that the calcium carbonate type in all cases was the calcite and the crystals size was relatively same (about 8-14 μm in diameter).

For the river sand samples, rhombohedral crystals with smooth surfaces could be observed as a characteristic for calcite (figure 10A and B). While for the silica sand samples, the hexahedral crystals with rough surfaces as a characteristic for calcite could be observed in the case of the samples treated with cementation solution composed of calcium chloride (egg shells) and urea (figure 10C), and the hexahedral crystals with smooth surfaces of the samples treated with cementation solution composed of calcium chloride (analytical grade) and urea (figure 10D). This means that the same type of cementation solution produced two different forms of calcite crystals (rhombohedral crystals, hexahedral crystals), depending on the type of treated soil.

This leads us to the conclusion that the calcium chloride type had little effect on the crystals formed compared to the significant impact that resulted from changing the type of soil.

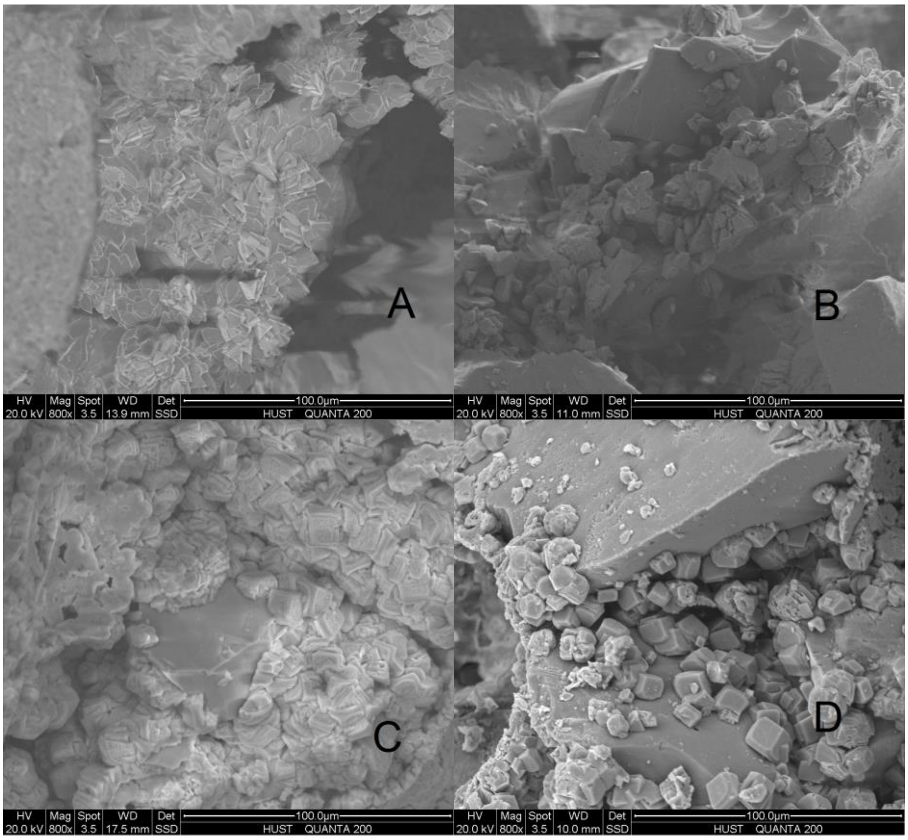


Fig.10 $CaCO_3$ crystals images (SEM) of treated samples of different calcium sources and soil types

IV. CONCLUSIONS

Microbial induced calcite precipitation (MICP) is a very complex process, particularly when it occurs among sand particles. Many factors may affect this process, like the type of soil and calcium source.

The results presented in this study revealed that:

- using the cementation solution composed of calcium chloride, which is made of egg shells and urea with MICP technique can improve the mechanical engineering properties of the fine sand (river and silica sands), exactly like the cementation solution composed of calcium chloride (analytical grade) and urea with MICP.

- The bacteria cells were able to produce the same amount of calcium carbonate in both types of cementation solution, but it was in the river sand samples slightly higher than that of the silica sand samples.
- In both types of sand, the use of cementation solution contained calcium chloride made of egg shells has a significant effect on the permeability, but the effect was greater in the silica sand samples.
- For the same type of calcium chloride source, the type of sand has played a major role with respect to unconfined compressive strength, where the unconfined compressive strength of silica sand samples was approximately two times higher than that of river sand samples.
- The calcium chloride type had little effect on the crystals formed compared to the significant impact that resulted from changing the type of soils.

V. FUTURE WORK

According to the results of paper, it’s important to further studies in this field by taking into consider changing the type of bacteria used. Also, change the soil type and the procedure of MICP treatment.

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