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The Influential Factors in the Effectiveness of Microbial Induced Carbonate Precipitation (MICP) for Soil Consolidation

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ABSTRACT

Background: Microbial induced carbonate precipitation (MICP) is a promising biological soil improvement method in geotechnical and geo-environmental engineering, which requires the recognition of the effects of various treatment methods on its applications.
Methods: To improve the efficiency of MICP by urea hydrolysis bacteria, bio-grouting experiments were conducted at low urease activity using laboratory sand columns, and injection scenarios with different procedure steps were performed. The scenarios varied in the incubation time, fixation fluid, and number of the injection steps, and their efficiency was assessed using the unconfined compressive strength test.
Results: The sand column experiments with multistep injection of bacteria and cementation solution showed that when the precipitated calcium carbonate content increased from 7.7% to 18.9%, the strength of the samples enhanced from 0.25 to 1.55 MPa, respectively. The precipitation conditions were influenced by the sand grain properties. The samples with diversified particle sizes had greater strength than those with uniform particle sizes. The multistep cementation solution injection at various concentrations had a significant clogging effect, thereby decreasing the sand column permeability.
Conclusion: According to the laboratory results, this innovative technique could be potentially practical for engineering applications, such as liquefaction prevention, clogging, and sand in oil reservoir consolidation.

1. Introduction

The use of unsuitable soil as a building material is a major cause of destruction in engineering infrastructures, which may lead to the loss of life and massive financial consequences. The mechanical properties of soil should often be improved to meet the increased demand for infrastructures [1]. The conventional soil improvement methods (e.g., chemical grouting) that are well-established in the market are frequently applied to improve the strength and stiffness of soil. However, these methods are often costly, while they require heavy machinery, disturb urban infrastructures, and may involve chemicals with significant environmental impact. In addition, these methods are not suitable for the treatment of large volumes of soil [2].

Recently, biological processes have been used to develop novel soil improvement techniques to modify various soil properties, such as strength, stiffness, and permeability. Microbial induced carbonate precipitation (MICP) is a natural biologically mediated method used for in-situ cementation and enhancing the mechanical properties of soil [3]. Owing to its simplicity and lack of excess proton production, urea hydrolysis bacteria are used in most MICP applications [4]. In the MICP by urea hydrolysis, the bacterial cells or purified urease enzyme catalyzes the urea hydrolysis into ammonium and carbonate (reaction 1), and the produced carbonate ions readily precipitate $CaCO_3$ in

the presence of a calcium source (reaction 2).

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

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

In this process, the treatment is initiated with the injection of a bacterial suspension to the soil, followed by the injection of several batches of a urea-calcium chloride solution [5].

To date, MICP has been used for wastewater treatment [6], improvement of resistance to liquefaction [7], foundation bearing capacity, and slope stability [8], healing of cracks in concrete and masonry [9], creating a water-impermeable crust on sand surface [10], immobilizing heavy metals [11], shallow carbon sequestration [12], enhancing the compressibility and shear strength of organic soil [13], and controlling the wind erosion of sandy soil [14].

The main technical challenges in the scale-up of MICP for soil improvement include biochemical factors (e.g., rate and concentration of the chemical reactants) [15,16] and geotechnical factors (e.g., soil gradation, texture, and porosity), which control the timing and rate of calcite precipitation to strengthen soil homogeneously [17]. Despite the studies regarding the effects of the mentioned factors, the effects of biochemical parameter has not received enough attention. In particular, the proper injection procedure at low levels of urease activity to control the efficiency of MICP must be taken into account. In such case, the proper injection procedure could be significant due to the impact of the homogeneity of the formed precipitates.

The present study aimed to investigate the effects of the design of a bacterial mix injection and level of precipitated calcium carbonate on the efficiency of biological treatment. A set of bio-grouting experiments with laboratory sand columns were conducted, and the effect of soil grain size on the unconfined strength was also evaluated based on the obtained appropriate injection procedure. Based on the results, the main influential factors in efficient MICP treatment and the necessitation of its practical applications were further discussed.

2. Materials and Methods

2.1. Bacterial Suspension and Cementation Solution

Although the urease activity of pathogenic Helicobacter pylori is extremely high [18,19], Sporosarcina pasteurii (PTCC 1645) was used as the non-pathogenic urease-positive bacterium in this study. The cultivation of the microorganism was performed in a medium containing a yeast extract (20 g/l^{-1}), NH4Cl (10 g/l^{-1}), and $NiCl_2$ (10 µM) at the pH of 8.5 [16]. S. pasteurii was grown in 200 millilitres of a growth medium in aerobic batch conditions. In addition, broth cultures were incubated in an incubator shaker (model: 3020 DR, Fanavaran Sahand Azar Co., Iran), operated at 200 rpm. The cementation solution consisted of 1 M $CaCl_2$ and 1 M urea. All the experiments were performed at the ambient temperature of 28 ± 2°C.

2.2. MICP Experiments in Sand Environments

The efficiency of MICP in the strengthening of porous media was evaluated in packed sand columns, in which sand was flushed with a bacterial suspension. The columns consisted of polycarbonate tubes (ID = 5 cm, H = 30 cm) and packed with the top and bottom of the column covered with a layer of approximately one centimetre of gravel filter and a layer of scouring pad (Figure 1). The midsection of the sand columns (20 cm) was packed via compaction to the dry density of approximately 1.7 g/cm^{-3}. To ensure the efficiency of the proposed injection procedure, poorly graded dry coarse sand was assessed (grain size diameter: 200-350 mm; porosity: 40%). The columns were closed and positioned vertically, and a peristaltic pump (TakBioTech, Tabriz, Iran) was attached to an injection point at the bottom of the column to regulate the flow rate.

In order to induce MICP in the soil subsurface, a bio-cementation solution (bacteria and urea-$CaCl_2$) had to be injected into the location where strength enhancement was required. In an attempt to extend the injection distance, the injection of the bio-cementation solution was carried out with the approximate pore volume of 1.1. It is also notable that the minimum urease activity requirement for calcium carbonate precipitation is 10 mM urea/min^{-1} [18].

2.3. Analytical Methods

During the experiments, the biomass concentration was determined through the measurement of optical density (OD) at 600 nanometres using a spectrophotometer (model: Spectroquant Pharo 300, KGaA Merck, Germany), and pH was measured using electrodes SP10B and a consort multi-parameter analyzer C20-30. In the absence of calcium ions, the conductivity of the bacterial suspension was used to determine the urease activity using electrodes SK10T and a consort multi-parameter analyzer C20-30 as previously described [4]. In the measured range of the activities, 1 mS min^{-1} was correlated with the hydrolysis activity of 11 mM urea/min^{-1}. The calcium carbonate content of the cemented samples was measured using a gravimetric acid washing technique [1]. In order to determine the entire strength of the biologically cemented columns, the unconfined compression strength (UCS) method was employed in accordance with the ASTM D2166. The axial load was applied at the constant rate of 1.0 mm/min, and the permeability of the porous media was calculated using Darcy's equation (Equation 3) [20], as follows:

$$\mathbf{Q} = \frac{\mathbf{KA\Delta P}}{\mu \mathbf{L}} \quad (3)$$

With a constant pressure difference, Equation 3 could be used to obtain the ratio of the final and initial permeability from the measured values of the flow rate. The effect of MICP on the permeability of the porous media was evaluated using various concentrations of Urea-$CaCl_2$ (0.25 and 0.5 equimolar). Notably, the columns were preserved at room temperature.

3. Result and Discussion

3.1. Injection Procedure

Considering the low level of the urease activity in the present study (1.5 mM urea/min^{-1}) and in order to increase the efficiency of the biological cementation during the precipitation process, various injection procedures were performed, which were developed to achieve an acceptable strength level in the samples (Table 1).

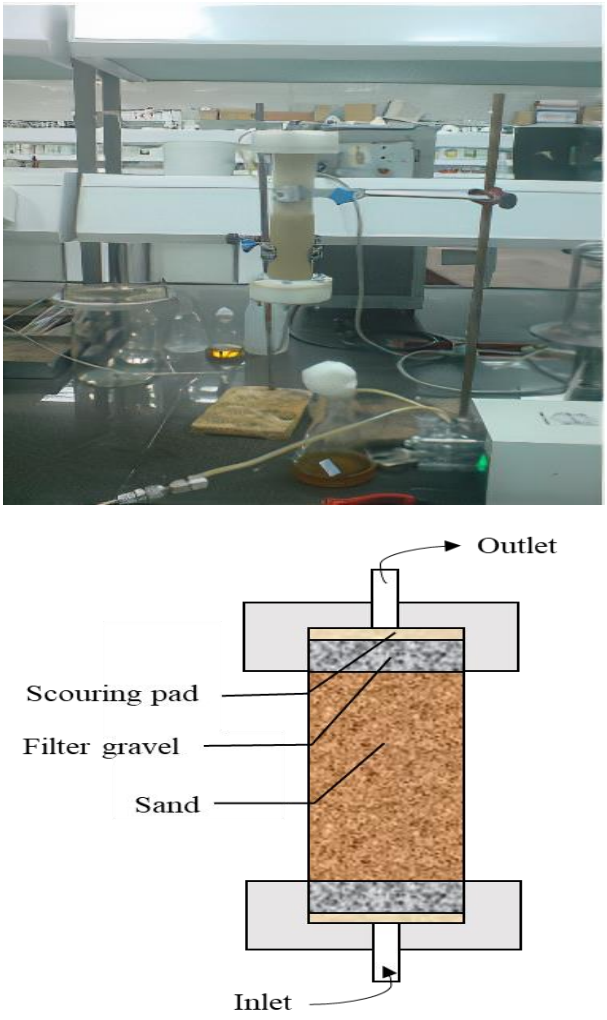

Figure 1: Schematic and photograph diagram of packed column setup

The UCS of the cemented column was also used to assess the effectiveness of the proposed injection procedure (Figure 2).

In all the injection procedures after the flushing of tap water, the columns were flushed with the pore volume of 1.1 (200 ml) of the bacterial suspension with the activity level of 90 mM urea/hr-1 at the flow rate of 200 ml/h^{-1}. In row one, the same amount of the cementation solution containing 1 M CaCl2 and 1 M urea was flushed through immediately after bacteria injection, and the flow was discontinued. After the reaction time (two hours), the second batch of the cementation fluid was injected through the column (procedure two). Unfortunately, the samples did not increase in strength after mold extraction even after the second batch of the cementation solution injection (Figure 2). In fact, due to the low bacterial attachment, the biological improvement of the sand was extremely poor after the flushing of the bacterial suspension and cementation fluid once or twice, and the samples were crushed during the mold extraction (procedures 1 and 2).

The obtained results from procedure three suggested that the mixing of the bacteria with calcium chloride directly led to the formation of small flocculation near the injection point, so that no bacterial cell could be observed in the effluent (OD_{600} = 0). After the injection of the cementation solution, the entrance of the sand column was blocked, and cementation was only carried out in the gravel filter. These findings are in line with the previous studies in this regard [21]. In procedure four, 200 millilitres of the fixation fluid (0.05 M $CaCl_2$) was injected after the bacteria injection. The presence of the Ca^{2+} ions could facilitate the bacterial flocculation and improve the bacterial retention and attachment to the sand grains [16].

According to procedure four, the incubation time increased from two to 16 hours. In other words, the fluid in the column was allowed to react for 16 hours. In this case, the mean strength was estimated at 200 kPa, and the reaction time (incubation time) had to be prolonged to allow all the reagents to be converted even at the sites with minimal urease activity so as to form uniform $CaCO_3$ precipitation.

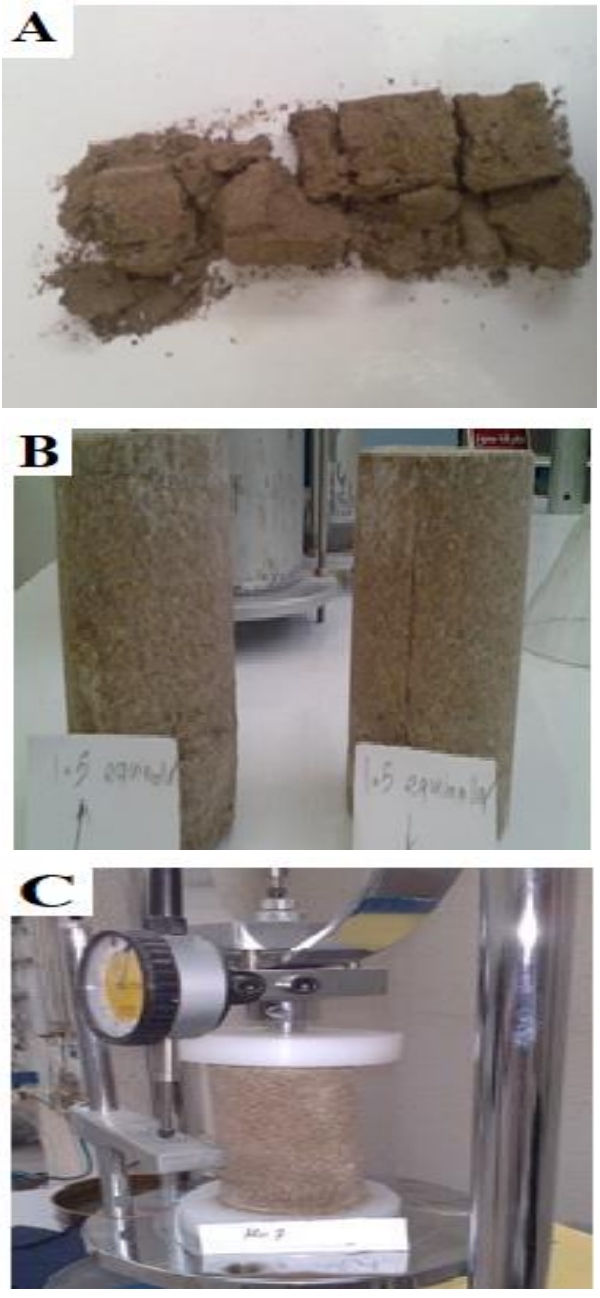

Figure 2: The obtained samples by injection procedure 3 (A), injection procedure 6 (B) and unconfined compressive strength (UCS) apparatus

Table 1: Details of injection procedure

Solution Constituent	CS*		FS**	Number of CS Injections	Injection Volume of CS (L)	Retention Time (h)	Strength of top part (kPa)	Strength of bottom part (kPa)
	$CaCl_2$ (M)	Urea (M)	$CaCl_2$ (M)					
Procedure 1	1	1	0	2	400	0	unconsolidated	unconsolidated
Procedure 2	1	1	0	2	400	2	unconsolidated	unconsolidated
Procedure 3	1	1	0	2	500	2	Clogging ***	unconsolidated
Procedure 4	1	1	0.05	3	600	16	170	244
Procedure 5	1	1	0.05	4	800	16	323	366
Procedure 6	1	1	0.05	6	1200	16	723	766

*Cementation solution and **Fixation solution *** Clogging at the injection entrance due to the deposition of $CaCO_3$

With the initial urease activity of 60-100 mM urea/h^{-1} in the current research, 1 M urea was hydrolysed within a maximum of 16 hours. As a result, the incubation time should not be considered less than 16 hours.

In procedure five, the same procedure was carried out, with the exception of the introduced cementation solution volumes (800 ml for the sand column). When the number of the cementation solution injections increased to six (procedure six), an enhancement in the UCS was expected (Figure 2). Moreover, the addition of the cells or cementation solution could increase the point-to-point contact and strength by up to 1.6 MPa.

3.2. Correlation between UCS and $CaCO_3$ Content

After completion of biological cementation, the column was flushed with excess water and the treated sand sample was cut with a saw into two parts for evaluation of the mechanical properties. Calcium carbonate content value was determined from at least 3 samples for each section.

To investigate the effect of calcium carbonate precipitation on the mechanical properties of the biologically treated material, the strength results were correlated with each sample calcium carbonate content (Figure 3).

Accordingly, the UCS was exponentially associated with the amount of calcium carbonate precipitation (R^2 = 0.9542), so that the increased calcium carbonate precipitation resulted in higher unconfined strength (Figure 4). On the other hand, the distribution of calcium carbonate in the soil voids directly impacted the resulting strength.

It is also notable that the rate of strengthening would continue as long as all the voids between the grains were filled by the precipitated calcium carbonate crystals. Apparently, the rate decreased after precipitation exceeded a certain level, with the strength finally reaching a constant value [2]. Evidently, if the carbonate calcium was not homogeneously distributed, the strength would be governed by parts of the sample with the minimum calcium carbonate. In such case, although the mean precipitated calcium carbonate in the sand samples might be high, the samples would show low strength, which highlights the importance of achieving a homogeneous sample.

As is depicted in Figure 4, the strength of the samples enhanced as the precipitated $CaCO_3$ content increased. No tangible difference was observed between the strength of the samples to 0.0015 of the axial strain. However, the samples with the higher $CaCO_3$ content showed higher axial strength, while the increased $CaCO_3$ content caused stronger inter-granular bonding between the soil grains. As a result, the samples with higher precipitated calcium carbonate also had higher strength. This is consistent with the previous studies in this regard [2]. The samples had larger elasticity modulus, which again confirms the stronger bonding. On the other hand, the stronger inter-granular bonding caused the samples to show brittle behaviour, which has also been observed in the soil samples improved by other materials, such as cement [22]. In other words, the samples were crushed after reaching their peak strength since the samples with 7.69% of $CaCO_3$ has ductile behaviour; such example is the samples that were not crushed immediately after reaching the peak strength. Therefore, it could be inferred that inter-granular bonding would not form between all soil grains.

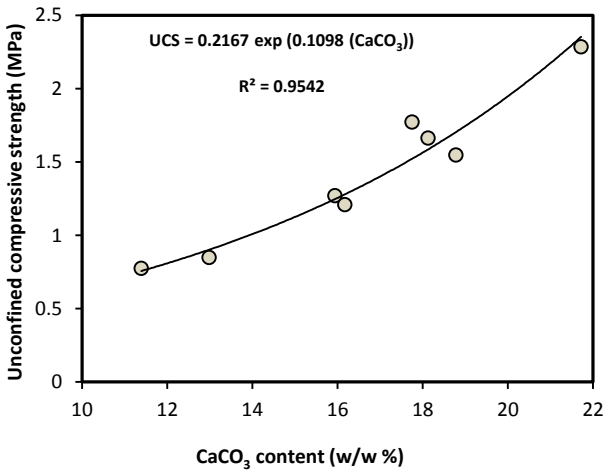

Figure 3: Correlation between unconfined compressive strength and $CaCO_3$ content

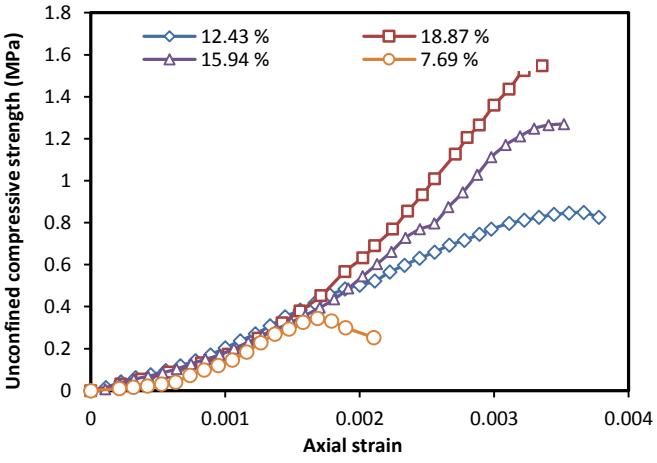

Figure 4: Stress-strain curve of biologically treated soil samples at different $CaCO_3$ contents

3.3. Effect of Soil Grain Size on the UCS

To evaluate the effect of soil grain size on the UCS, the soil samples were prepared (Table 1) and enhanced through an injection procedure similar to procedure six. The UCS was measured at the top and bottom of the sand columns.

In general, larger particle sizes were associated with fewer bacteria trapped through the pore throat between the soil particles. Due to the lower surface area and more distance between the soil grains in this case [23], the cementation bonds were hardly formed, implying that for the soils with coarser grains, a greater percentage of precipitation crystals are formed around the soil particles at the same precipitation level rather than via particle-particle contacts [3]. Due to high porosity, the smaller crystals could be easily withdrawn from the sand columns, and a small number of precipitated crystals would be formed between the sand grains.

Table 2 shows the unconfined strength of the bottom and top sections of the samples. Accordingly, the unconfined strength at the bottom section, which was closer to the injection point, was higher compared to the top section in all the samples. In addition, precipitation decreased with the increased distance from the injection point, highlighting the importance of multistep injections to achieve homogeneous samples.

According to the results of the present study, the samples with diversified particle sizes (well-graded soils) showed higher strength compared to those with uniform particle sizes (poorly graded soils). The most suboptimal result in this regard was obtained where the sand was coarse and poorly graded due to the key role of sand grains in maintaining the bacteria and cementation solution, as well as the crystal formation between the soil particles. In the poorly graded soil, the pore size between the soil grains was so large that the bacteria could not be retained and were settled over the sand bed length of 18 centimetres [3, 23]. Another possible explanation for the low strength in the samples with poorly graded coarse sands involves the kinetics of $CaCO_3$ precipitation and transport of the crystals. When the solution is not sufficiently oversaturated, the crystals remain small or are not even formed, and there would be no nucleation; this causes the easy transport of the crystals through the soil porous medium [2, 5].

During the mold extraction in the current research, clogging was observed near the inlet in the samples with medium grain size (coarse sand). In these samples, the crystals were formed around the grains with no bonding between the sand grains. By moving away from the injection point, precipitation gradually decreased, and no precipitated crystal was observed near the outlet. This confirms the withdrawal of the primary formed calcium carbonate crystals, as well as the relatively lower bacterial concentration in the areas close to the outlet. Conversely, higher strength was observed due to the smaller size of the pores in the well-graded finer sand.

3.4. Clogging Effect

Another aspect of MICP in soil is the reduction of soil permeability. Previous studies have been focused on the reduction of soil permeability, while overlooking the strength of the samples [10, 24]. In most of these experiments, the one-step injection technique has been employed, in which a layer of crystals form a crust on the surface of the sample. In the current research, a multistep injection treatment was performed to enhance the strength and incline permeability simultaneously.

Figure 5 shows the effect of the multistep injection treatment on the permeability of the packed column at various concentrations of CS. According to the findings, the mean permeability of the untreated fine sand (Dave =210 μm) was 942 md. After the first injection of CS, a significant decrease was observed in the permeability of the sand column. Following the reaction period, the permeability decreased from 942 to 685 md (permeability ratio: 0.73). Furthermore, the second injection of the cementation solution led to the further reduction of permeability from 658 to 365 md. The decrease in permeability after the first and second injections was 27% and 47%, respectively. In other words, the overall reduction in permeability after two injections was 61%. As can be seen, the increased CS concentration had a significant effect on the extent of clogging, which was observed as a decrease in the permeability of the packed column due to the formation of $CaCO_3$ crystals [25]. At the CS concentration of 0.5 M, the permeability of the porous media decreased from 907 to 98 md (permeability ratio: 0.12; 88% reduction in permeability) after the two CS injections.

According to the results of the present study, the multistep injection of the CS solution could enhance the soil permeability efficiently, which could be due to the fact that during multistep injection, the bacteria were propagated more evenly in the soil samples, and the formed crystals resided in the same manner. Every step of the injection caused more crystals to form; as a result, the entire sample, rather than only near the inlet, was filled by the crystals, which inevitably enhanced the strength of the samples with higher homogeneity. Figure 5-B shows that with the increased CS solution concentration, the strength of the mentioned process improved.

Table 2: Strengths of sand samples at different grain size distribution

Grain size distribution based on USCS	Clay content (%)	Silt content (%)	D50	Strength of top part (kPa)	Strength of bottom part (kPa)
SW	-	-	0.6	607	640
SW	-	-	1.2	586	612
SW	-	-	2	435	472
SP (Coarse Sand)	-	-	3.35	unconsolidated	unconsolidated
SP (Medium Sand)	-	-	1.18	280	307
SP (Fine Sand)	-	-	0.27	195	255
SP-SM	-	9	1.075	322	334
SP-SC	9	-	1.075	400	445

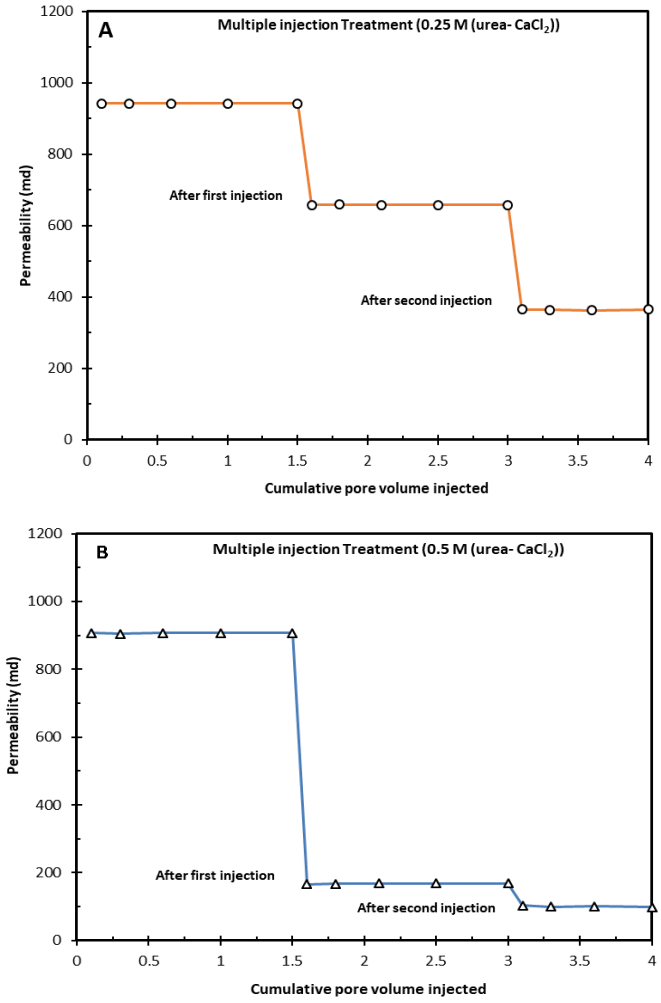

Figure 5: Effect of multistep injection of CS on permeability reduction: CS = 0.25M (A) and CS = 0.5M (B)

4. Conclusion

This study was conducted with low injection pressure and low urease activity, in which soil strength could be successfully enhanced. Although a significant improvement was observed in the mean compressive strength of the samples, the strength of the bottom section of the samples increased more significantly compared to the top section, which may be due to the up-flow of the bacterial mix through the soil columns.

According to the results, engineering parameters were correlated with the $CaCO_3$ content. In addition, the critical aspects of the process (e.g., grain size distribution of sand and injection procedure) were identified. The multistep injection of CS at various concentrations had a significant effect on the clogging rate, which was observed as the decreased permeability of the packed column. The obtained outcomes provided following suggestions for further investigations or industrial-scale applications: firstly, various treatment procedures should be tested to find the optimal combination between the number and time of the batch treatment, flow rate, and flushed volume. Secondly, the column walls in the experiment bounded the flow conditions to 1-D. In real applications, the flow will be 3-D, and fluid properties and injection times play a more pivotal role in this regard.

Authors' Contributions

M.M., M.Y., and M.R.Y., design of the experiments; M.M., and A.N., performing the experiments; M.M., M.Y., M.R.Y., and A.N., data analysis; M.M., and A.N., contributing with the reagents, materials, and analysis tools; M.M., M.Y., M.R.Y., and A.N., drafting of the manuscript.

Conflict of Interest

The Authors declare that there is no conflict of interest.

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