
MICP-Reinforced Soil Under Three-Dimensional Grout Injection Condition

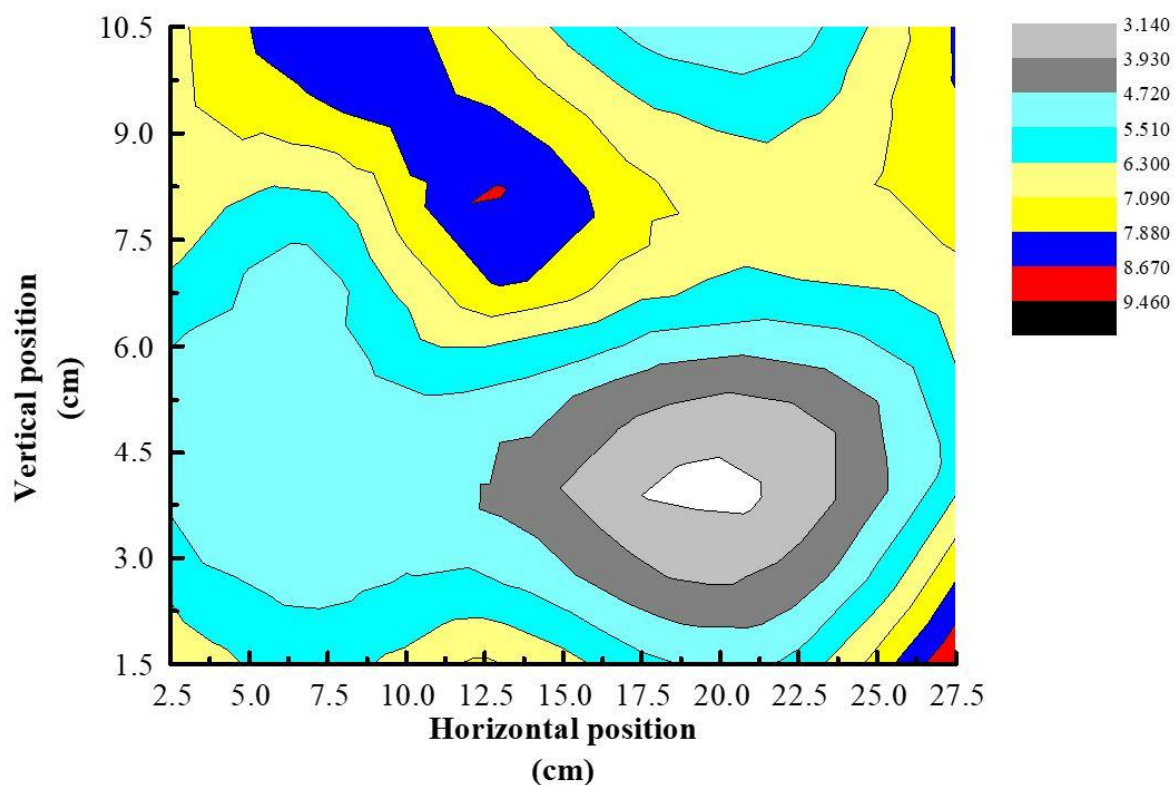
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GRAPHICAL ABSTRACT



Abstract

The use of microbial-induced carbonate precipitation (MICP) in soil reinforcement has attracted attention in the academic field in recent years. However, most of the existing studies have been conducted based on one-dimensional (1D) grout injection condition. The present study conducted in vitro and sand column experiments of MICP using a ureolytic bacterium (ATCC 11859) (three-dimensional (3D) and 1D models were considered in the sand column experiments) as well as the feasibility and reinforcing effect of the 3D MICP grout injection method. A comparison of the 3D and 1D grout injection methods showed that the specimens reinforced using the 3D grout injection method had higher strength, better homogeneity, a greater C_aCO_3 content, and a larger permeability coefficient compared with the specimens reinforced using the 1D grout injection method. The limitations of the 1D model should be considered in future practical applications.

1. Introduction

Soil reinforcement is an important topic in the geotechnical engineering field. Conventional soft foundation reinforcement methods, such as preloading consolidation and chemical grout injection, are generally disadvantageous due to long construction periods, high energy consumption, and high costs. In addition, the majority of grouts used in the chemical grout injection method are harmful to the environment (DeJong et al. 2010). With requirements for sustainable and environmentally friendly development being introduced by various countries, ecological environmental protection and energy conservation should be given more consideration when selecting soil reinforcement methods. Under this background, the application of microbial-induced carbonate precipitation (MICP) in soil reinforcement has been receiving attention and is being investigated increasingly more.

MICP, referring to the process of calcium carbonate (C_aCO_3) synthesis, in which the metabolites of a specific bacterium react with matter in the surrounding environment (DeJong et al. 2013), (Mohammad Khari, 2019). For example, ureolytic microorganisms generate urease over the metabolic procedure. To increase the pH of the surrounding solution, the urease catalyzes the hydrolysis of the urea and induce the formation of ammonium ions and carbonate ions (CO_3^{2-} ions). There are negative charges on the bacterial surface, offering opportunities for the absorption of calcium ions. Combined with cells, structure of crystal nuclei exists once oversaturated CO_3^{2-} and C_a^{2+} ions form C_aCO_3 crystals in the surroundings (Ivanov and Chu 2008), (Rawat, 2020) (Fig. 1). Within the MICP process, C_aCO_3 crystals fill the pores in the soil and cement soil particles together, along with reinforcing the soil by increasing the strength of the soil, reducing the porosity of the soil, and decreasing the permeability coefficient of the soil and hence it can be considered as an excellent cementing material (Choi et al 2016), (Cheng and Cord-Ruwisch 2014).

Since Boquet et al. (1973) used the bacteria *Bacillus* genus and *Pseudomonas aeruginosa* to induce the formation of C_aCO_3 precipitates in the laboratory for the first time, the following few types of microorganisms have all been discovered to have the capacity to form C_aCO_3 precipitates: ureolytic microorganisms (e.g., *Sporosarcina pasteurii* (*S. pasteurii*)), denitrifying microorganisms, sulfate-reducing microorganisms, and iron-reducing microorganisms (Whiffin 2004), (Hammes and Verstraete 2002). Researchers have studied the application of various types of microorganisms to repair historic buildings and brick material surfaces, to repair cracks on granite and concrete objects, and for seepage control (Ramachandran et al. 2001), (Jiang et al. 2017), (Jiang and Soga 2017). Whiffin (2004), (Achille and Enow, 2020) used MICP in soil reinforcement for the first time. Whiffin used *S. pasteurii* to induce the precipitation of $CaCO_3$ in loose sand. The $CaCO_3$ precipitates significantly increased the shear strength of the sand. DeJong et al. (2010) used X-ray diffraction (XRD) to determine that the cementing material between the sand particles was C_aCO_3 crystals in calcite form. Later, researchers further investigated the application of MICP in soil reinforcement (Lu et al. 2010), (Abdikar et al., 2018). Because the existing research results demonstrate that ureolytic microorganisms are advantageous due to their excellent adaptability to the environment, their capacity to produce large amounts of C_aCO_3 , and their ability to precipitate C_aCO_3 at high rates (Mortensen et al. 2011), (De Muynck et al. 2010), ureolytic microorganisms have become the microorganisms most used in studies that investigate the use of MICP in soil reinforcement.

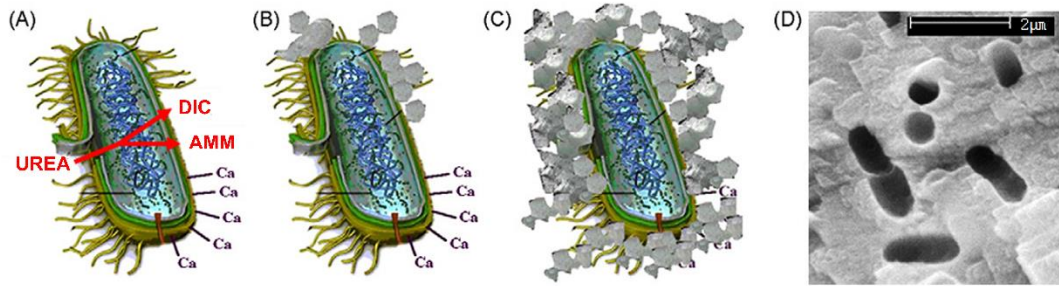


Figure 1. Figure of MICP

It can be observed from Figure 1, DIC and AMM are released in the micro-environment of the bacteria due to the additional urea (A). Calcium carbonate precipitate heterogeneously on the cell wall of bacteria based on the appearance of calcium ions induced local super saturation (B). Then the whole cell was encapsulated (C), resulting in the limitation of the nutrition supply and the death of the cell. Fig. 1D depicts the imprints of bacterial cells involved in carbonate precipitation (De Muynck et al. 2010).

It remains challenging to develop effective method to transport microorganisms and the relevant chemical reagents (e.g., urea and $CaCl_2$) to soil where in need of reinforcement, shedding lights of MICP usage in soil reinforcement (Sari 2015), (Soon et al. 2012), (Gomez et al. 2017). Initially, researchers directly mixed a microorganism suspension with chemical reagents and injected the mixed solution into the soil. However, it was found that the mixed solution rapidly flocculated, and crystal precipitates were formed, resulting in the blockage of the pores in the soil near the injection point, which, in turn, obstructed further injection of the mixed solution. Whiffin et al. (2007) proposed a two-phase grout injection method in which solutions are injected separately and successively: to begin with, the soil is injected with bacteria solution, following by fixation solution injection (e.g., $50mM$, $CaCl_2$ solution); the chemical reagents needed to form precipitates are added in the last step. A portion of the bacteria injected at first is retained in the soil due to adsorption and filtration. After the fixation solution is injected, because they carry positive charges, Ca^{2+} ions are more easily adsorbed onto the soil particle surface that carries negative charges. In addition, Ca^{2+} ions can also adsorb bacteria that carry negative charges. Therefore, Ca^{2+} ions have a certain fixation effect. Using the two-phase grout injection method, Whiffin extended the effectively treated sand column length to $5m$. Later, many researchers improved the two-phase grout injection method. Cheng et al. (2012; 2013), (Gelleh et al., 2018) directly sprayed the bacterial solution and chemical reagents onto the specimen surface successively to reinforce the unsaturated soil. While studying the use of MICP in tropical residual soil reinforcement, Soon et al. (2013) transported the nutrient solution using a pressure pump. Thus far, the related studies were all based on one-dimensional ($1D$) experiments, which may be relatively significantly different from actual engineering applications. van Paassen (2010) conducted a large-volume ($8m \times 5.6m \times 2.5m$) MICP experiment. The authors repeatedly injected the bacterial solution and reagents successively at one side and used a pumping well to transport the bacterial solution and reagents at the other side. A reinforced body with a volume of approximately $100m^3$ was formed. However, this study only performed $1D$ experiments. Clearly, the grout injection method in actual engineering is not a $1D$ problem. Therefore, studying the three dimensions of the MICP solution and relevant chemical reagents, the three-dimensional ($3D$) grout injection method and its reinforcing effect, and the difference between the $3D$ and $1D$ grout injection methods has important theoretical and application value for guiding the future practical application of MICP.

To study the $3D$ grout injection method and its effectiveness, the present study conducted $3D$ and $1D$ model chamber experiments to investigate the use of MICP in soil reinforcement and comparatively investigate the reinforcing effect and grout consumption of the $3D$ and $1D$ grout

injection methods. The results showed that it was feasible to use MICP in 3D sand reinforcement. Compared with the 1D-reinforced specimens, the 3D-reinforced specimens had higher strength, a higher C_aCO_3 content, and better homogeneity. The 3D grout injection method had a better overall reinforcing effect than the 1D grout injection method. Recent studies provide insightful information for the improvement of MICP application in practical engineering in the future.

2. Material and Methods

2.1. Bacteria

S. pasteurii (ATCC 11859) was grown at $30^{\circ}C$ in a culture medium ATCC 1376, which contained the following per liter of deionized water: $0.13mol^{-1}$ tris-3 buffer ($PH=9.0$), $10gL^{-1}$ $(NH_4)_2SO_4$ and $20gL^{-1}$ yeast extract. The ingredients were autoclaved separately and mixed together post sterilization. The culture medium was inoculated with the *S. pasteurii* stock culture and incubated aerobically at $30^{\circ}C$ in a shaking water bath with 200 rev min⁻¹ for approximately 40h before harvesting at a final optical density (OD_{600} , 600nm) of 0.8–1.2 (10^7 – 10^8 cell/mL) (Al Qabany et al. 2012), (Aunsary and Chen, 2019). The bacteria and growth media were stored in centrifuge vials at $4^{\circ}C$ until used (Mortensen et al. 2011).

2.2. Cementation media

In recent studies, a mixed urea- C_aCL_2 solution was used as the cementation media. Urea, the nitrogen source, was mainly responsible for microorganism growth, while C_aCL_2 was the calcium source during the MICP process (Mortensen et al. 2011), (Martinez et al. 2013), (Arslan et al., 2018). Table 1 summarizes the components, concentrations and sterilization methods. All the components, concentrations and sterilization methods are listed in Table 1.

Table 1. Recipe of cementation media

Chemical name	Chemical concentration (mM / L)	Sterilization	Molar mass	Amount (g / L)
Urea	1500	Filter	60.06	90.09
NH ₄ Cl	187	Autoclave	53.49	10.00
Tris	82.5	Autoclave	121.14	10.00
CaCl ₂	500	Autoclave	110.98	55.49
Nutrient Broth	3 g/L	Autoclave	-	-

2.3. Sand

China standard sand (medium sand) was used in presented study. Weighing 300 grams of standard sand, the test of particle analysis was performed. Figure 2 shows the grading curve of the sand. The sand had a particle diameter (d_{50}) of 0.64mm, d_{60} of 0.80mm, uniformity coefficient (C_u) of 5.33 and curvature coefficient (C_c) of 1.33, the grading of which is good. Before the experiment, the sand was immersed in water and vacuum saturated.

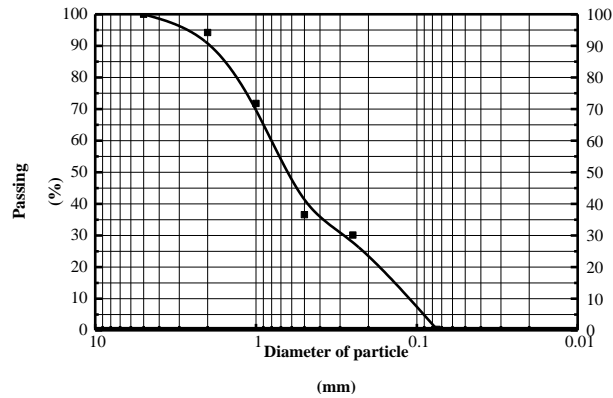


Figure 2. Grading curve

2.4. Model test

The model experiments conducted in the present study included 1D and 3D model chamber experiments. The 1D model tank was a cylindrical (polymethyl methacrylate) (PMMA) tube with an internal diameter of 5cm and a height of 17cm. There was an opening at the top of the tube. The location of the grout injection point is shown in the Figure 3. There was a water outlet at the center of the bottom of the 1D model chamber. The 3D model consisted of a 17cm×30cm×5cm (inner dimensions) model chamber that was made from PMMA. There was an opening at the top of the 3D model chamber. During the grout injection process, there were three grout injection points on the upper surface of the 3D model chamber (Liu 2018), (Okpoli and Iselowo, 2019). The locations of the grout injection points are shown in the Figure 3. The left and right grout injection points were both 10cm from the midline. The third grout injection point was located on the midline. There were three water outlets at the bottom of the 3D model chamber. The locations of the water outlets corresponded to those of the grout injection points. The 1D model chamber was used to study the reinforcing effect of MICP under different temperature conditions. The 3D model chamber was used to study the reinforcing effect of the 3D grout injection method.

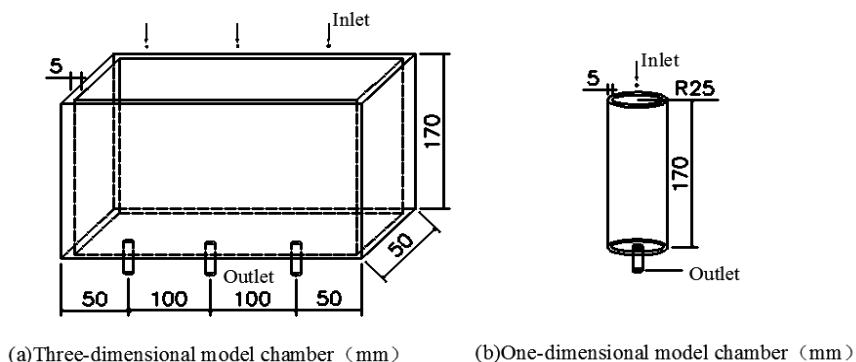


Figure 3. Figure of model chamber device

In the 1D experiment, the pore volume of the sand column was 87.06ml, and the total volume of the bacterial solution injected was 307ml. In the 3D experiment, the pore volume of the sand sample was 633.44ml, and the total volume of the bacterial solution injected was 2290ml. In both the 3D and

1D experiments, the volume of the cementing solution used was the same as that of the bacterial solution used.

3. Measure Methods

3.1. Urease activity

After mixing of 1.0mL bacteria solution and 9.0mL 1.67M urea solution, conductivity was monitored under different temperatures for 5mins using a conductivity meter. The actual conductivity variation rate (mS/min) is the measured conductivity variation rate multiplied by the dilution factor. The dilution factor is defined as the ratio of original bacteria concentration to the bacteria concentration after mixing with urea solution. The actual conductivity variation rate can be converted to urea hydrolysis rate (mM urea hydrolyzed/min), based on a correlation that 1mS/min corresponds to a hydrolysis activity of 1mM urea/min in the measured range of activities. This urea hydrolysis rate is the urease activity (Whiffin 2004). Specific urease activity (mM hydrolyzed urea/min/OD) can be calculated by dividing urease activity by bacteria biomass (OD_{600}), which reflects the urease catalytic ability of urea hydrolysis.

$$\text{specific urease activity (mM urea hydrolyzed/min/OD)} = \frac{\text{urease activity (mM urea hydrolyzed/min)}}{\text{Biomass (OD}_{600}\text{)}} \quad (1)$$

In accordance with Equation (1), due to urea hydrolysis catalyzed by urease, ion concentration rises with the increasing of the electrical conductivity of the solution, which is proportional to the concentration of active urease. The growth of electrical conductivity rate reflects the hydrolysis rate of urea, describing the urease activity of bacteria solution (Whiffin 2004).

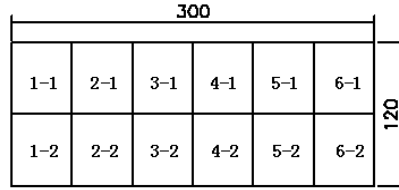
3.2. Unconfined compressive strength

The unconfined compressive strength is an important index that evaluates the reinforcing effect on a specimen. To study the reinforcing effect at different locations, each specimen was divided into several pieces, each of which was then subjected to an unconfined compressive strength test. For the 3D model chamber (Fig. 4), the specimen was divided into 12 small specimens. For the 1D model chamber, the specimen was divided into two small specimens. Each small specimen was then subjected to a compression test on an unconfined compressive strength tester.

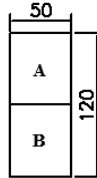
3.3. C_aCO_3 content

In the test tube experiments, the amount of C_aCO_3 that precipitated in each test tube was measured using the drying method. The upper portion of the liquid in each test tube was removed. The lower portion in each test tube was rinsed with distilled water several times, and then, the clear liquid was removed (Liu and Baghban 2017). The precipitates were retained and dried in an oven ($200^\circ C$) to allow the ammonium chloride and urea that existed in the precipitates to decompose and evaporate. The difference in the mass of the test tube before and after the drying process was the mass of C_aCO_3 .

In the 1D MICP sand column experiments, the acid washing method was used to determine the C_aCO_3 content of each reinforced specimen. Specimens were crushed by a mortar and oven-dried. The dry soil was washed in HCl solution (0.1M) to dissolve precipitated carbonates, then rinsed, drained, and oven-dried. The weight of precipitated C_aCO_3 in specimen was calculated as the difference between the two weights mentioned before (Rebata-Landa and Santamarina 2006).



(a) Three-dimensional samples (mm)



(b) One-dimensional samples (mm)

Figure 4. Figure of sample segmentation

3.4. Permeability

The permeability coefficient of each sand specimen was directly measured in the sand column container using a variable head permeability test device.

4. Results and Analysis

4.1. The strength of MICP-reinforced soil and amounts of C_aCO_3

Table 2 lists the unconfined compressive strength of the 1D - and 3D-reinforced specimens. It can be observed from Table 2 that the unconfined compressive strength of the top section of a specimen was greater than that of the bottom section of the specimen regardless of it being a 1D - or 3D-reinforced specimen. In addition, the unconfined compressive strength of the 3D MICP-reinforced specimens were greater than that of the 1D MICP-reinforced specimens (Fig. 5).

Table 2. Unconfined compressive strength of samples

samples	strength (kP_a)	samples	strength (kP_a)
1-1	294.7	4-2	72.9
1-2	82	5-1	275.3
2-1	83.6	5-2	108
2-2	81.2	6-1	122.8
3-1	185.2	6-2	137.4
3-2	61.2	A	165.1
4-1	161.2	B	82.1

For each 3D-reinforced specimen, small portions were removed from the specimen at locations that were 1.5, 4.5, 7.5, and 10.5cm from the bottom of the specimen in the vertical direction to determine the C_aCO_3 content of the specimen. For each 1D-reinforced specimen, small portions were removed from the specimen at locations that were 2, 4, 6, 8, and 10cm from the bottom of the specimen in the vertical direction to determine the C_aCO_3 content of the specimen. Figure 5 and Table 3 show the C_aCO_3 content distributions of the 1D - and 3D-reinforced specimens, respectively.

It can be observed from Figure 6 that for each 1D-reinforced specimen, the C_aCO_3 content in the top section was essentially consistent with that in the bottom section, whereas the C_aCO_3 content in the central section was the highest and close to twice that in the other section. The C_aCO_3 content had a relatively significant uneven distribution in each 1D-reinforced specimen. It can be observed from Table 3 that the C_aCO_3 content was not as unevenly distributed in the 3D-reinforced specimens compared with the uneven distribution in the 1D-reinforced specimens, and there was no sharp change in the C_aCO_3 content in any of the 3D-reinforced specimens (Kang et al. 2018), which indicates that the C_aCO_3 formed in each 3D-reinforced specimen was more evenly distributed than that formed in each 1D-reinforced specimen. Figure 7 shows the isolines of the C_aCO_3 content. It can be observed from Figure 7 that for each specimen, the C_aCO_3 content in the section 6 cm above the bottom was greater than that in the section within 6 cm of the bottom in the vertical direction, and the C_aCO_3 content in the section whose lower boundary was 3 cm above the bottom and upper boundary was 6 cm above the bottom was generally lower than that in other sections. In addition, the C_aCO_3 content of the sand specimens taken from locations near the tank boundaries was generally relatively high.

Table 3. C_aCO_3 content of three-dimensional samples

Position away from the bottom of the specime (cm)	#I	#II	#III	#IV	#V	#VI
10.5	6.88	8.66	7.13	5.23	5.16	7.98
7.5	6.61	5.37	8.83	7.14	6.85	7.19
4.5	4.92	4.99	4.70	3.14	3.39	5.71
1.5	6.87	5.91	7.25	5.75	5.77	9.46
Average	6.32	6.23	6.98	5.35	5.29	7.74

Note: For ease of comparison with one-dimensional C_aCO_3 content, in three-dimensional samples, according to Figure 4, 1-1 and 1-2 named #I sample, and so on. In other words, #I sample is divided into two parts (1-1 and 1-2).

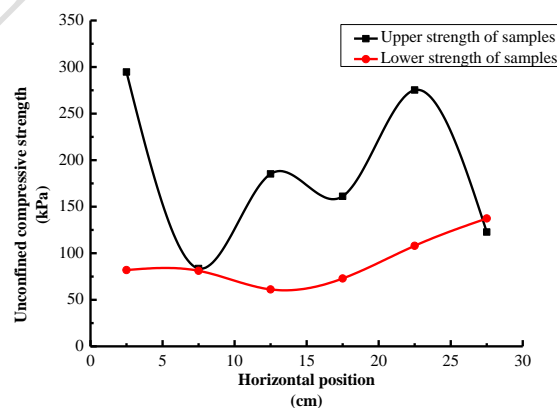


Figure 5. Unconfined compressive strength of three- dimensional samples

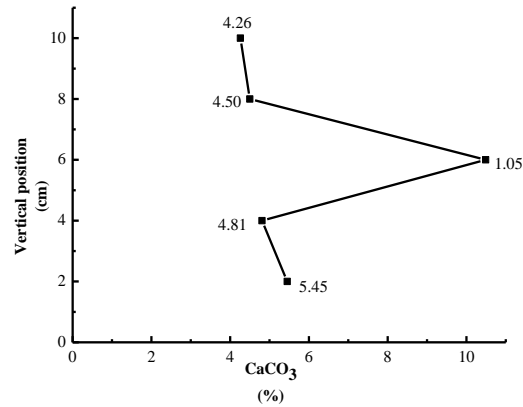


Figure 6. Figure of one-dimensional C_aCO_3 content

To compare the amounts of C_aCO_3 formed in the 1D- and 3D-reinforced sand specimens, the mean C_aCO_3 content of each specimen was calculated (Table 3). The mean C_aCO_3 content of the 1D-reinforced specimens was 4.01% (mass percentage), and the mean C_aCO_3 content of the 3D-reinforced specimens was 5.29% (mass percentage). Therefore, the mean C_aCO_3 content of the 3D-reinforced specimens was greater than that of the 1D-reinforced specimens.

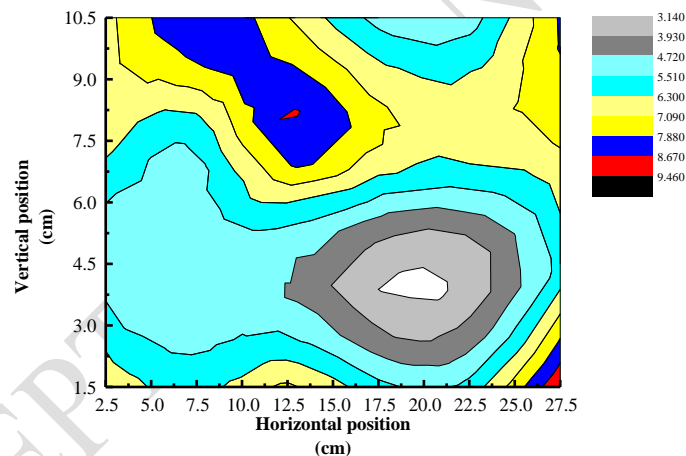


Figure 7. Contour map of two-dimensional C_aCO_3 content. Colors indicate C_aCO_3 content from < 3.14 (white) to > 9.46 (black) [% of dry weight].

When the mean volume of the bacterial solution used was the same, the effect of the 3D reinforcement treatment was superior to that of the 1D reinforcement treatment, which was primarily due to the following reason: The sand specimens were not completely homogeneous. Therefore, local permeability variations occurred in both the 1D and 3D experiments. Consequently, the bacterial solution and the cementing solution injected into each soil specimen were not completely evenly distributed in the soil, resulting in a large difference in the C_aCO_3 content among different sections. However, compared with the 1D-reinforced specimens (Sonogo et al. 2018), the 3D-reinforced specimens had a larger volume and more grout injection points and thus had more possible permeation paths that reached a certain reinforcement point. Therefore, the bacterial solution and the cementing solution could more evenly permeate the soil under the 3D condition. Hence, the C_aCO_3 content was more evenly distributed under the 3D condition than under the 1D condition. In addition, the mean C_aCO_3 content formed under the 3D grout injection condition was also greater than that formed under the 1D grout injection

condition.

4.2. The volumes of the bacterial solution and change in the permeability coefficient

The result of the division of the total volume of the bacterial solution by the volume of the sand specimen was used to evaluate the volume of the bacterial solution used. Table 4 lists the volumes of the bacterial solution used.

Table 4. Microbial amount

Test	Total amount of liquid (ml)	Sample volume (cm ³)	Each volume of the liquid dosage (mL/cm ³)
One-dimension	307	235.62	1.30
Three-dimension	2287	1800	1.27

It can be observed from Table 4 that the volume of the bacterial solution used per unit volume of the sand specimen under the 1D grout injection condition was $1.30\text{mL}/\text{cm}^3$, and the volume of the bacterial solution used per unit volume of the sand specimen under the 3D grout injection condition was $1.27\text{mL}/\text{cm}^3$ (a 3% decrease compared with that under the 1D grout injection condition). The unconfined compressive strength and the C_aCO_3 content of the 3D-reinforced specimens were greater than those of the 1D-reinforced specimens, and the volume of the bacterial solution per unit volume used in the 3D-reinforced specimens was less than that used in the 1D-reinforced specimens, indicating that the effect of the 3D MICP grout injection method was superior to that of the 1D MICP grout injection method.

There was a significant change in the permeability coefficient of both the 3D- and the 1D-reinforced specimens. The 1D sand specimens had an initial permeability coefficient of $2.17 \times 10^{-2}\text{cm}/\text{s}$. After reinforcement treatment, the 1D specimens had a permeability coefficient of $1.65 \times 10^{-4}\text{cm}/\text{s}$, which was two orders of magnitude less than their initial permeability coefficient. The permeability coefficient of the 1D sand specimens significantly decreased after the reinforcement treatment (Reeves et al. 2018). During the bacterial solution injection process, it was found that the rate at which the grout exited became increasingly lower during the late stage, indicating that C_aCO_3 precipitates were formed in the sand pores, which exhibited a certain “blocking” effect; this subsequently affected the permeability coefficient of the sand specimen. The mean permeability coefficient of the 3D-reinforced specimens was $8.65 \times 10^{-4}\text{cm}/\text{s}$. While the permeability coefficient of the 3D specimens decreased after the reinforcement treatment, the permeability coefficient of the 3D-reinforced specimens was greater than that of the 1D-reinforced specimens, indicating that the 3D specimens had more permeation paths, i.e., the C_aCO_3 content of the 3D specimens was greater than that of the 1D specimens.

5. Conclusions

The present study conducted test tube and 1D sand column experiments of MICP under $10-25^\circ\text{C}$ using a ureolytic bacterium (ATCC 11859), investigated the feasibility and effect of this method under different temperature conditions in future practical engineering. The following conclusions were obtained:

The unconfined compressive strength and C_aCO_3 content of the 3D MICP-reinforced specimens were greater than those of the 1D MICP-reinforced specimens under the same condition. In addition, the mean volume of the bacterial solution used per unit volume of the sand specimens under 3D grout injection condition was less than that under 1D grout injection condition. Based on the change in the C_aCO_3 content throughout different sections of each specimen, we know that the 3D-reinforced

specimens had better homogeneity than the 1D-reinforced specimens, indicating that the scale effect of MICP is significant. The grout injection condition in practical engineering is often 3D. Therefore, the limitations of using the results of 1D MICP experiments to guide practical engineering should be considered.

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