

# Experimental study on Microbially Induced Calcite Precipitation for expansive soil stabilization

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**Abstract.** Microbially induced carbonate precipitation (MICP) is extensively discussed as a promising topic for ground stabilization. The practical effect of stabilizing the expansive soil is presented in this paper with a logical process from the bacterial activity to the treatment technology. Temperature, pH, shaking frequency, and inoculation amount are discussed to evaluate the bacterial activity. The physic-mechanic properties are also evaluated to discuss the effect of the MICP process on expansive soil. Results indicate that the MICP method achieves the mitigation of expansion. The treated soil has a low proportion of fine particles (< 5 μm), the plasticity index significantly decreases, and strength values improve much. MICP process has a significant cementation effect on the soil matrix. Moreover, the infiltration model test presents the coating effect on the topsoil. According to the relation between the CaCO<sub>3</sub> content and the treatment effect, the topsoil has better treatment than the deeper soil.

**Keywords:** cementation effect; coating effect expansion; expansive soil; Microbially induced carbonate precipitation (MICP); the treated soil

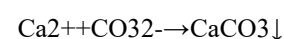
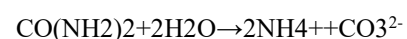
## 1. Introduction

Due to the high susceptibility to water, natural expansive soil is characterized by wetting swelling, and drying shrinkage and therefore has a significant adverse impact on engineering construction (Nowamooz *et al.* 2013). The deformation and fracture of the foundation caused by repeated swelling-shrinking behaviors along with seasonal moisture changes in the expansive soil area destroy the construction (Ikeagwuani and Nwonu 2019, Gibbs 1977). Thus, the natural expansive soil is regarded as unsuitable foundation material. Worldwide, mitigating the foundation disease brought by expansive soil is inevitable in soil science.

Cement, lime, or other pozzolanic additives mixing methods have been used for many years to improve the engineering properties to meet different conditions (Fazal *et al.* 2020, Chen *et al.* 2020). However, commonly accepted the above material in engineering has brought severe environmental problems. For instance, Carbon dioxide emissions can result in a worldwide greenhouse effect, and the increased pH in the soil causes the loss of soil fertility (DeJong *et al.* 2011). Significantly, the treatment effect can not be guaranteed due to the common premature failures in expansive soil areas (Chittoori *et al.* 2018). The

complicated construction technology, unsatisfactory effect, and serious environmental problems prove the necessity of seeking suitable methods to solve soil problems. Based on the demand to dispose of such waste products, many waste by-product has been used to treat the expansive soil. For example, lignosulfonate (LS), rubber, coffee husk ash, etc., have been used to mitigate the volume deformation of expansive soil (Atahu *et al.* 2019, Soltani *et al.* 2019, Zhang *et al.* 2020, Mohanty *et al.* 2017). These by-products generally mix with expansive soil to achieve the purpose with a uniform blend. On the other hand, bio-geotechnical technology is a meaningful attempt to solve the expansive soil problems based on the need for a green environment (Chuo *et al.* 2020, Castro-Alonso *et al.* 2019, Kumar and Sujatha 2020).

Microbial-induced carbonate precipitation (MICP) is a bio-based mineralization process (Jiang *et al.* 2021, Al Qabany and Soga 2013, Hang *et al.* 2019). Generally, the urease promotes the hydrolyzation of urea in nutrition solutions. The pore liquid condition is rich in NH<sub>4</sub><sup>+</sup> and CO<sub>3</sub><sup>2-</sup> by the hydrolyzation process. The negatively charged bacteria surface has adsorption to the Ca<sup>2+</sup>. As the Ca<sup>2+</sup> concentration increases, the calcium carbonate is effectively generated around the bacteria in an alkaline environment (De Muijnck *et al.* 2010, Castro-Alonso *et al.* 2019).



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Table 1 Physical/ Mineralogical properties of the expansive soil

Properties	Values
Geotechnical properties	
Specific Gravity, $G_s$	2.70
Liquid Limit, LL	63.0%
Plastic Limit, PL	24.9%
Plasticity Index, PI	38.1
Free swell ratio (GB/T50123, 2019)	67.5%
Maximum dry density ( $g/cm^3$ )	1.837
Optimum moisture content	15.0%
Mineral composition (wt%)	
Quartz	43.8
Feldspar	2.0
Kaolinite	25.7
Calcite	2.8
Illite-montmorillonite mixed layer	22.1
Illite	3.6

The generated calcium carbonate is commonly regarded as biological cement, which has significant cementation and pore-filling effect in the soil matrix. Microorganism technology, a much-discussed topic to treat soil problems, has achieved shift development in recent years (Cheng *et al.* 2019, Jiang *et al.* 2021). Low energy consumption, environment-friendly mechanism, and unique effect broaden the application in soil science. For example, MICP has been applied to soil stabilization, sand liquefaction treatment, crack repair, and treatment of contaminated soil, etc. (Li *et al.* 2013, Anbu *et al.* 2016, Achal *et al.* 2012). Coarse-grained soil (i.g., sandy soil) is early considered to be treated by MICP technology due to the proper permeability (Wu *et al.* 2021, Pan *et al.* 2020). The method is always that MICP promotes the calcium carbonate generation in the pore by injecting the bacteria solution and nutrition solution with specific concentration into the soil, rich in the pore structure (Whiffin *et al.* 2007). The generated calcium carbonate has a significant cementation effect on repairing the crack and preventing penetration in the soil structure (Liu *et al.* 2020, Jongvivatsakul *et al.* 2019, Choi *et al.* 2017).

Meanwhile, reactivity, time effect, injection method, and nutrient solution development have been discussed in recent years to acquire the optimal treatment conditions (Al Qabany *et al.* 2012, Jiang *et al.* 2021, Cheng *et al.* 2013). Biological cement is always discussed in coarse-grained soil. Also, many studies reported the laboratory test of MICP. However, it is unavoidable to involve the clayey soil area in the field application. In comparison, the research about fine-grained soil, which is difficult to penetrate by the bacteria solution, is hardly reported. Thus, the research about MICP on clayey soils is significant to put forward the MICP method to application. The expansive soil commonly has high-proportioned clay content and low permeability with water as pore liquid. Also, expansive soil generally is the typical clayey soil. the negatively charged sites, which cause the hydration swelling, are more universal in the fined particles of expansive soil. Thus, the effect of MICP on expansive soil is a significant topic. However, the practical effect of mitigating the expansion of expansive soil is

hardly described in recent research. Due to the environment-friendly perspective of the MICP process. expansive soil (a typical clayey soil) is worthy of study. The influence degree, the variation of engineer properties, and the feasibility of the infiltration method are also hardly discussed. In this paper, the engineer properties are evaluated to judge the effect of MICP on expansive soil. Besides, the spray infiltration test is conducted to discuss the treatment technology.

## 2. Materials and methods

### 2.1 Materials

The urease active *Sporosarcina pasteurii* was provided by Guangdong Microbial Culture Collection Center (GDMCC), China. The lyophilized microorganism was saved in vacuum ampoules. The process of bacterial activation needs to cultivate in the aerobic growth medium with a super clean aseptic environment. Activated bacteria are inoculated into the solid medium plate and stored at 6°C. The recipe of nutrient mediums included 20 g/L yeast extract, ammonium sulfate 20 g/L, and 0.13 M *Tris buffer*, and the solid medium needs another 20 g/L agar compared with the liquid culture medium. The colony plate and bacterial solution are shown in Fig. 1. Expansive soil was obtained from Guangxi Province, China, located at low latitudes, with abundant precipitation and frequent high-temperature weather. X-ray diffraction patterns are shown in Fig. 2. Basic geotechnical properties and mineral composition were summarized in Table 1.

### 2.2 Optimal test conditions

#### 2.2.1 Bacterial activity

Bacterial density is usually expressed by the turbidimetric method, and the number of bacteria is calculated by measuring the absorbance of the bacterial liquid. This article uses a spectrophotometer at 600 nm wavelength absorbance ( $OD_{600}$ ) to measure the absorbance

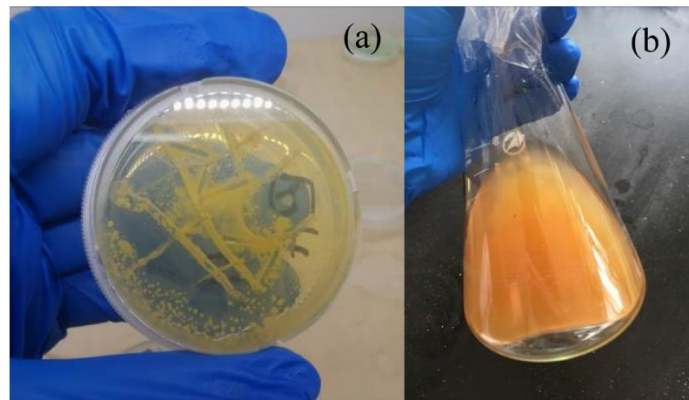


Fig. 1(a) Plate for colony formation and (b) Cultured bacteria

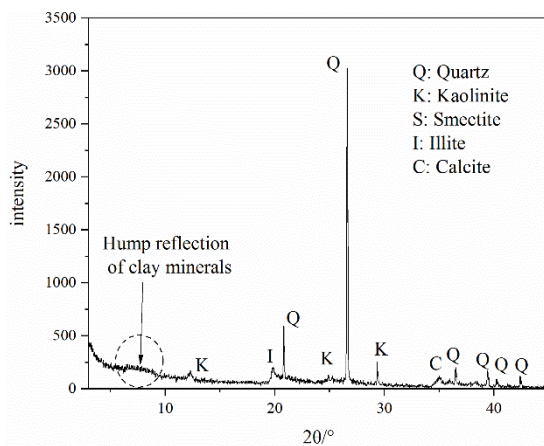


Fig. 2 X-ray diffraction patterns of expansive soil

of the *Sporosarcina pasteurii* suspension and uses the  $OD_{600}$  value to characterize the concentration of bacteria. When  $OD_{600}$  is between 0.2 and 0.8, the following conversion relationship is satisfied (Santhosh *et al.* 2001).

$$Y = 8.59 \times 10^7 Z^{1.3627} \quad (1)$$

Where  $Z$  is the  $OD_{600}$  value, and  $Y$  is the number of cells per milliliter of bacterial solution. When the  $OD_{600}$  value exceeds the range of 0.2 ~ 0.8, it is necessary to dilute the bacterial solution so that the absorbance is within this range and then use Formula (1) to calculate the actual concentration of the bacterial solution.

*Sporosarcina pasteurii* can continuously produce urease during its metabolism. Under the action of urease, the urea is hydrolyzed into ammonium and carbonate ions. As the hydrolysis process progresses, electrolyte concentration gradually increases in the solution. The amount of urea hydrolyzed is directly proportional to the conductivity of the solution. The change in the conductivity of the solution per minute was used to measure the urease activity. The urea hydrolyzed per minute (mM/min) of the bacterial solution was used to express the urease activity (Whiffin *et al.* 2007).

### 2.2.2 Experimental conditions

Experiments were carried out to study the optimal growth conditions of *Sporosarcina pasteurii*. Absorbance

and urease activity was the measurement standards to evaluate the conditions, of which the bacterial culture temperature, pH value, inoculum volume, and shaker vibration frequency.

The urea and calcium chloride solution was set as 1 M, respectively, and the bacteria were cultured for 24 hours for determination.

The test conditions are set as follows:

(1) The bacterial culture temperature was 20°C, 25°C, 30°C, and 35°C. The inoculation amount was 1 vol%, the pH was 8, and the shaking frequency was 200 r/min.

(2) The pH of the solution is set to 5, 6, 7, 8, and 9. The temperature was 32°C, the inoculation amount was 1 vol%, and the shaking frequency was 200 r/min.

(3) The inoculation amount is set to 1, 2, 3, 4, and 5 vol%. The temperature was 32°C, pH was 8, and the shaking frequency was 200 r/min.

(4) The shaking frequency of the shaker is set to 160 r/min, 180 r/min, 200 r/min, 220 r/min, and 240 r/min. The temperature was 32°C, the pH was 8, and the inoculation amount was 1 vol%.

### 2.3 Samples preparation

First, the expansive experimental soil was dried at 105°C and then passed the 0.5 mm sieve to obtain uniform soil particles with soil particle size below 0.5 mm. Second, the expansive soil was poured into a cylinder (diameter = 40 mm, height = 100 mm, thickness = 1.5 mm) made by porous geotextiles without compaction. The advanced experiment has proved that the precipitate has a little blocking impact on the geotextile. Third, a bracket within a square container and an oxygen pump were prepared to provide a treatment environment. The bacterial suspension was cultured at a temperature of 30°C, a pH of 7, and a shaker speed of 200 r/min. The 1 M  $CaCl_2$ -Urea solution and bacterial suspension, mixed with a volume ratio of 1:1, were poured into the container to submerge the cylinder. As shown in Fig. 3, the rotation speed was set at 600 rpm/min to accelerate the solution flow and increase oxygen content in the treatment process. Above procedures aimed to provide an ideal experimental condition for optimal test results.

The different treatment periods were set, namely 0, 3, 5,

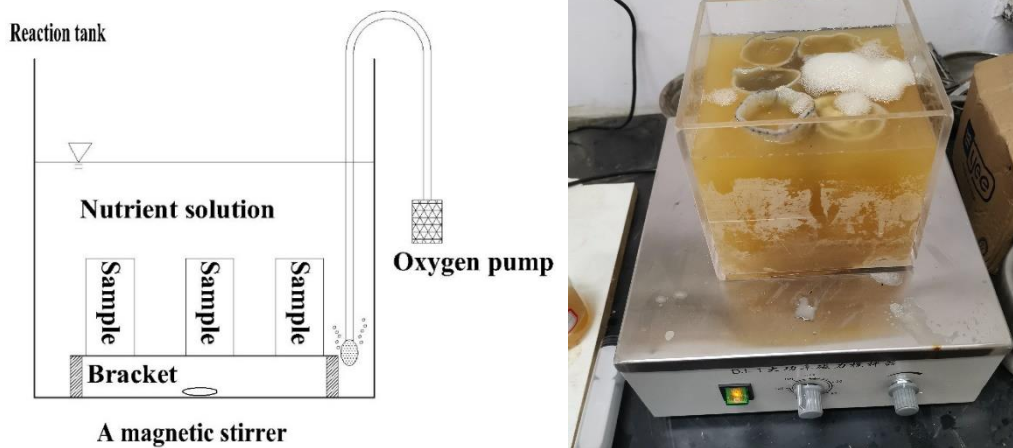


Fig. 3 Sketch and picture of the test device of expansive soil treated by MICP

7, and 14 days at 30°C, and the mixed solution of 1000 ml needed to be replaced every day. Different treated soils corresponding to different treatment periods were dried to constant weight at 105°C and passed the 0.5 mm sieve after ground thoroughly. Moreover, The soaked soil (14 days) was prepared by the same method except for not adding the bacterial suspension

#### 2.4 Test program

Atterberg limits and particle size distribution test were according to ASTM D 422-1963. Moreover, free swelling ratio test, one-dimensional swell test, swelling pressure test, unconfined compression strength tests (UCS), and direct shear test are also conducted to present the physical and mechanical properties difference between the expansive soil and treated soils (GB/T50123, 2019). In brief, a 10 ml dried soil sample (free sedimentation) was poured into the measuring cylinder (50 mL). The free sedimentation volume was acquired to evaluate the expansion degree. One-dimensional swell and swelling pressure tests are conducted on a specimen size of 20 mm in height and 61.8 mm in diameter. Meanwhile, the deformation and pressure were only evaluated in the axial direction (one dimension). UCS specimens (height = 76 mm, diameter = 38 mm) are conducted to analyze the strength properties based on expansive soil's initial maximum particle size.

#### 2.5 Laboratory model test

As shown in Fig. 4, a cubic model tank (30×30×30 cm<sup>3</sup>) was made with acrylic plates. The condition of the one-dimensional spray-soaked test to evaluate the MICP method. The procedures are described as follows. Prepared expansive soil needs to mix with distilled water as a uniform mixture (optimum moisture content 15%). The above expansive soil was compacted in the tank with a 70% compaction degree, and the compaction process needed a process of multilayer compaction to ensure the expansive soil's homogeneity. Calibration stickers were stuck on the ekstexine of the acrylic plates to improve the test accuracy, and the upper surface of expansive soil needs to be smooth.

Every model tank contained 3472 g of expansive soil (Height = 30 mm). A series of nutrient solutions were tested, corresponding to five tanks, namely 0, 0.25 M, 0.5 M, 0.75 M, and 1 M. The nutrient solution ratio was summarized in Table 2. Every tank needed a process of spraying 100 ml bacteria liquid and corresponding 100 ml nutrition solution every 24 hours for 21 days. Moreover, in-situ samples (diameter = 61.8 mm, height = 20 mm) were collected in every model tank on the 7th day, 14th day, and 21st day. At the end of the process, the in-situ samples were obtained by cutting rings within the height range of 0-10 mm and 10-20 mm from the topsoil. An acid-washing process was conducted to measure the calcium carbonate content. Multiple water washing processes for soil samples were necessary before mixing the sample and the hydrochloric acid. The weight change was regarded as an index to present the calcium carbonate content.

$$c = \frac{m_1 - m_2}{m_1} \quad (2)$$

where  $c$  (%) is the calcium carbonate content;  $m_1$  (g) is the dried soil weight before a pickling process;  $m_2$  (g) is the dried soil weight after a pickling process.

Meanwhile, the UCS samples were acquired from the uniform mixing of different depths in the vertical direction, and the over-dried treated soil was mixed to remolded soil samples.



Fig. 4 Sketch of the model tank with the condition of one-dimensional infiltration

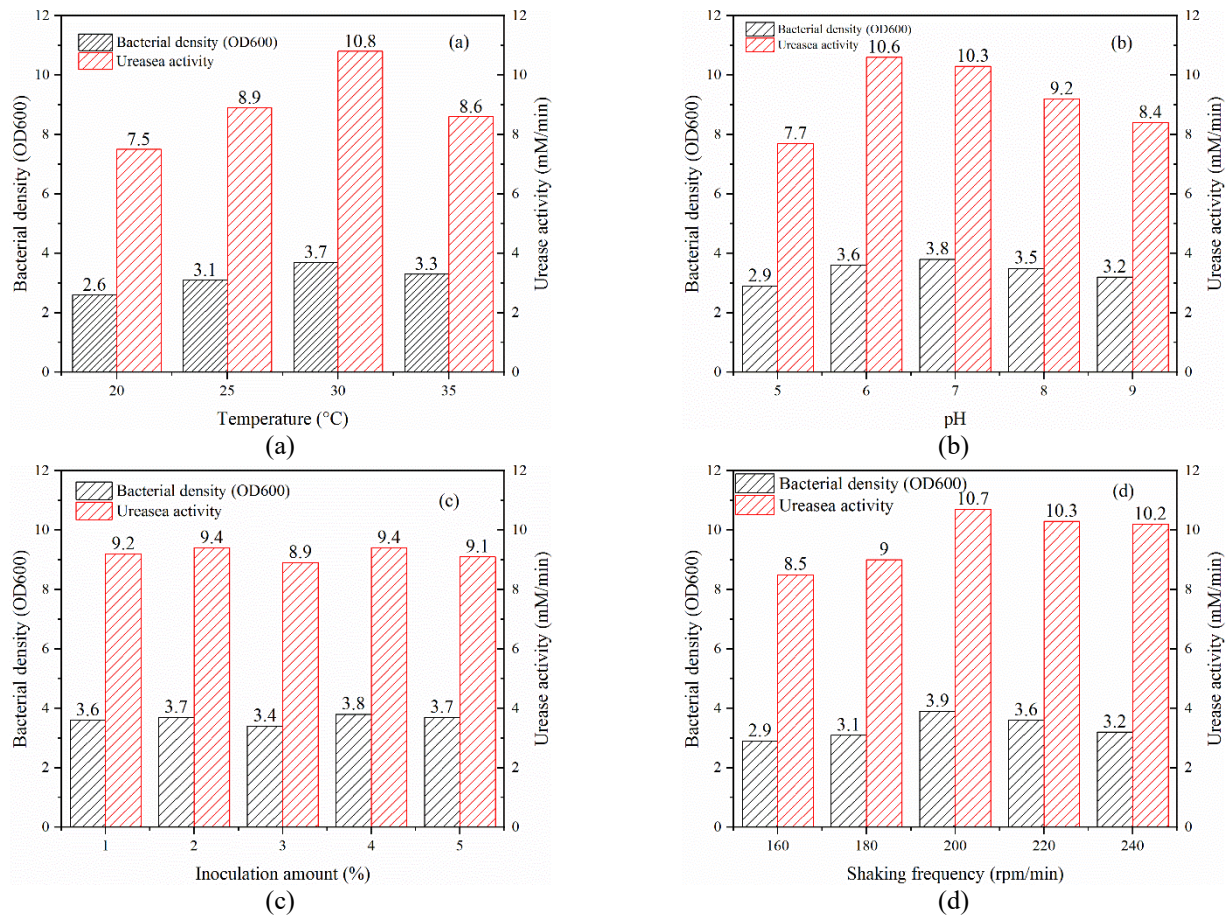


Fig. 5 The effect to bacterial density and urease activity: (a) Temperature, (b) pH, (c) Inoculation amount and (d) Shaking frequency

Table 2 Nutrient solution ratio

Number	1	2	3	4	5
Urea (M)	0	0.25	0.5	0.75	1.0
CaCl <sub>2</sub> (M)	0	0.25	0.5	0.75	1.0

### 3. Results and discussion

#### 3.1 Optimal incubation conditions

The effect of temperature on bacterial incubation is shown in Fig. 5(a). As the temperature increased, the bacterial density and urease activity initially increased and then decreased. The maximum OD<sub>600</sub> and the urease activity emerged at 30°C. The value was 3.7 and 10.8 mM/min, respectively. Meanwhile, the bacterial density and urease activity trend decreased after 30°C. Therefore, setting the culture temperature to 30°C was most conducive to the growth of *Sporosarcina pasteurii*. The activity of microorganisms in the MICP process could be divided into two stages: the first stage was the growth process of bacteria. During the initial culture, the rapid reproduction of bacteria increased the concentration of bacteria. The second stage was the metabolic process of bacteria, and the production of urease hydrolyzed urea. The results showed that 30°C was the suitable environment temperature for the

growth and the urease activity when the inoculation amount was 1 vol%, pH was 8, and the shaking frequency was 200 r/min.

Compared with the urease activity, the change of pH value had little effect on the bacterial density. However, the urease activity was more sensitive to the change in pH. Fig. 5(b) showed that the bacterial density OD<sub>600</sub> and urease activity are 2.9 and 7.8 mM/min, respectively, when the pH of the environment is 5. Results indicated that *Sporosarcina pasteurii* was very intolerant in an acidic environment. In the pH range of 6-8, the growth and reproduction status of *Sporosarcina pasteurii* was relatively stable. Due to the hydrolysis of urea in the nutrient solution, the pH value of the solution gradually increased. Thus, it is suitable to set the pH value to 6 or 7.

Fig. 5(c) showed that the OD<sub>600</sub> value of the bacterial density fluctuated between 3.4 and 3.8 as the inoculation amount varied between 1 vol% and 5 vol%. Meanwhile, the urease activity fluctuated between 9.1 and 9.4 mM/min. Results indicated that inoculation changes had little impact on the amount of bacterial density and urease activity. Nutrients might limit the growth, metabolism, and proliferation scale of bacteria in the culture solution and the growth space of the bacteria. From the perspective of the final bacterial density and urease activity, the initial inoculum concentration has a limited effect on the culture

of bacteria. Thus, 2% of the inoculum was used in this paper.

As shown in Fig. 5(d), the bacterial density and urease activity gradually increased with the increased shaking frequency of the shaker. The shaking process increased the oxygen content in the solution, needed by *Sporosarcina pasteurii* (an aerobic gram-positive bacteria). Increasing the oxygen content in the culture fluid was conducive to the growth and reproduction of bacteria. However, when the frequency reached a certain level, the reproduction of bacteria also reached a stable period. The shaker reached the frequency of 200 r/min, and the bacterial density of OD<sub>600</sub> value and urease activity were 3.9 and 10.7 mM/min, respectively. Therefore, the shaking frequency was set as 200 r/min, suitable for bacterial growth and reproduction.

In all, The bacteria suspension was suggested to be cultured at a temperature of 30°C, pH range of 6 to 8, and a shaker speed of 200 r/min.

### 3.2 Atterberg limits

Fig. 6 showed the Atterberg limits of the variation of the treated soil with different treatment periods. The curve indicated that the variations were mainly reflected in the liquid limit. The plastic limit had low variation fluctuation after the treatment, and the liquid limit significantly decreased within the first 5 days. After 7 days, the variation of the liquid and plastic limits tended to be gentle. Meanwhile, the plastic index had a trend of decrease similar to the liquid limit. Most variation was located in the early treatment process. The decrease of the plastic index indicated that the variation range of moisture content decreased in the plastic state, which meant a reduced sensitivity to water. The loosely treated soil particles had weak adsorption to water. However, the plastic index was still high, and the treated soils were high plasticity soils. The reasons could be explained as follows. The ion exchange effect of calcium ions, a rapid process in the solution, was not a persistent effect on the expansive soils. Thus, the cementation effect could also slightly decrease the plastic index. On the other hand, calcium carbonate precipitation had not changed the negatively charged site quantity in the mineral structures based on the mechanism of MICP. In conclusion, the cementation and ion exchange effects had a synergetic impact on Atterberg Limits. However, according to the results, the treated soils were still regarded as high plasticity soils.

### 3.3 Particle size distribution (PSD)

Sodium Hexametaphosphate (SHMP), classified as a strong dispersant, was used in this test. The treated soil (14 days), the soaked soil (14 days), and the expansive soil were dispersed to present the MICP effect on PSD. Two comparative tests were conducted to analyze the particle distribution curve. The soil particles with a particle size of less than 0.002 mm or 0.005 mm were regarded as clay particles. As shown in Fig. 7, the content of the fine particle was reduced to 3% in the curve of treated soil. Meanwhile, the content of the fine particle was 36% in the curve of the

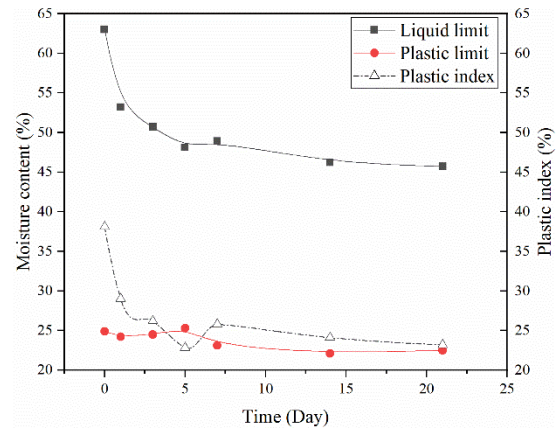


Fig. 6 Variation of plasticity index against treatment period

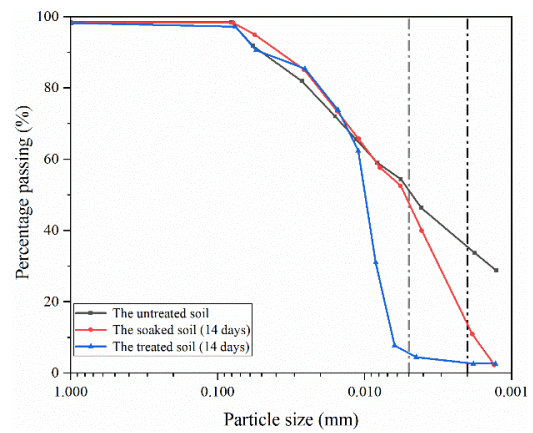


Fig. 7 Three PSD curves of expansive soil; treated soil (without bacteria solution); the treated soil

expansive soil. The significant results indicated that the bacteria had a prominent effect on the fine particle content of the treated soil. The fine particle content of the treated soil (without the bacteria solution) was 16%. The control group added urea and calcium chloride nutrient solution without the bacterial solution. Calcium ions and urea would exchange with the exchangeable cations in the expansive soil and stably existed in the soil particle structure. Thus, the exchange and adsorption behavior rebuilt the mineral unit structure in soil particles. According to the treated soil and treated soil (without bacteria solution) curve, the MICP process had an extra cement effect on the mineral units. Fined particles were aggregated to form large undispersed particles.

### 3.4 The swelling behavior

The volume change of soils can be intuitively presented by free sedimentation behavior. The free swelling ratio (FSR) evaluates the swelling behavior of 10 ml soil with a free setting in a 50 ml measuring cylinder. As shown in Fig. 8, different treatment periods significantly impacted FSR values.

The treated soil (14 days) could be regarded as low expansive soil with an FSR value of 27.7. Compared with expansive soil (FSR = 67.5), the FSR value of the treated

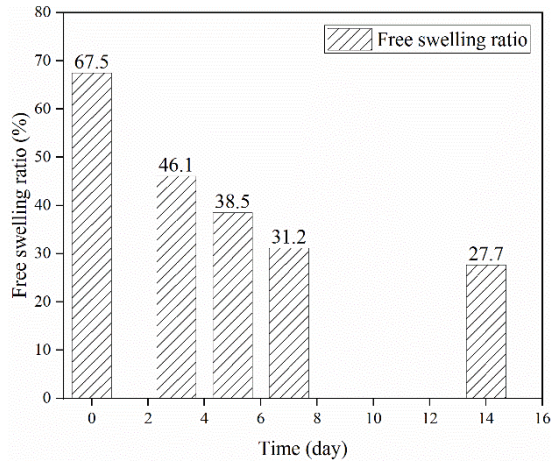


Fig. 8 Variations of the free swelling ratio against time (day)

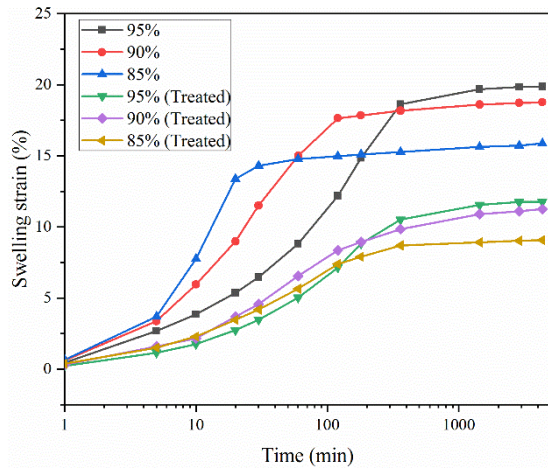


Fig. 9 Swelling strain-time curve with different compaction degrees of expansive soils and treated soils

soil (14 days) decreased by 59.25%. The FSR curve had a sharp downtrend within the first seven days. As discussed in 3.2, the proportion of clay (< 5 μm) part decreased. Fined particles' dispersion and the hydration of negative clay colloidal particles had been inhibited. Hence, the sedimentation volume achieved a significant decrease.

Moreover, the calcium chloride and urea might have a dominant impact on the expansion based on the infiltration process of ion exchange. As the treatment time increased, the calcium carbonate combined the fined particles with a specific cementation effect. The cementation effect could further decrease the expansion of soil. The FSR test of treated soils included a dehydration and rehydration process. Thus, the cementation effect could be regarded as an irreversible process in the water environment.

Another test (one-dimensional) was considered an evaluation method for soils' expansion with different compaction degrees. Consistent with the FSR test, the treated soil (21 days) presented a low expansion with water infiltration from the bottom of the specimens. Swelling pressure ( $P_s$ ) was also measured to evaluate the expansion.  $P_s$  was the pressure without the one-dimensional deformation of the one-dimensional swelling test. The

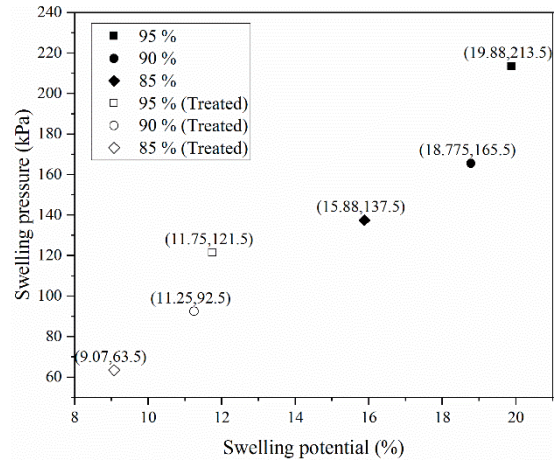


Fig. 10 Variations of swelling pressure against swelling potential for different compaction degrees of expansive soils and treated soil

swelling strain-time curve in Fig. 9 indicated that MICP achieved excellent inhibition on the expansion of one-dimensional. The expansive soil exhibited relatively high swelling potential with different compaction degrees. The swelling potentials of expansive soil were 19.88%, 18.78%, and 15.88% at compaction degrees of 95%, 90%, and 85%, respectively. Differently, the values of treated soil were 11.75%, 11.25%, and 9.07%, respectively. Moreover, variations of  $P_s$  followed a trend similar to that of swelling potential. As shown in Fig. 10, regarding different compaction degrees, i.e., 95%, 90%, and 85%,  $P_s$  of expansive soil were 213.5 kPa, 165.5 kPa, and 137.5 kPa, respectively. Significantly, the  $P_s$  of treated soil decreased to 121.5 kPa, 92.5 kPa, and 63.5 kPa, respectively.

### 3.5 Direct shear test

Significant differences between the expansive soil and the treated soil were presented in Figs. 11 and 12. The shear strength achieved great improvement with the same vertical stress. Moreover, the shear strength of the treated soil (compaction degree: 85%) had also been improved compared with the results of expansive soil (compaction degree: 95%). According to the Mohr-Coulomb criterion, the cohesive strength and friction angle variations were presented in Fig. 12. The cohesive strength increased from 26.652 kPa to 51.739 kPa (85%), 31.949 kPa to 66.804 kPa (90%), and 38.53 kPa to 74.39 kPa (95%), respectively. The friction angle increased from 8.45° to 13.14° (85%), 10.73° to 13.4° (90%), and 11.85° to 15.52° (95%), respectively. The results showed the cementation of the MICP process.

### 3.6 SEM

In Fig. 13, hydrated expansive soil particles presented lamellar and crimped surface morphologies. The mineral's morphology was consistent with montmorillonite and illite. Obviously, the treated soil has compact and smooth surface morphologies and many spherical structures of calcium carbonate, which confirmed the cementation of MICP.

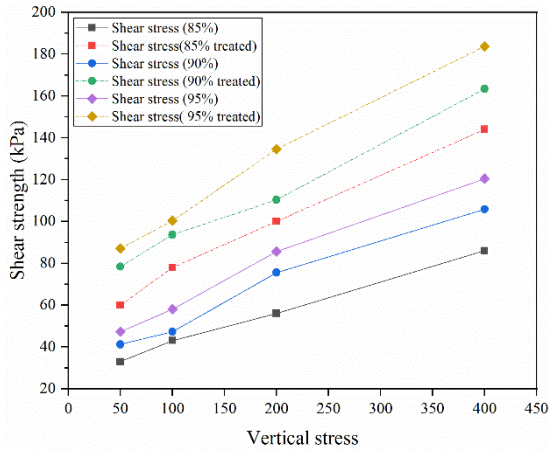


Fig. 11 Variations of shear strength against vertical stress with different compaction degrees of expansive soils (saturated) and treated soils (saturated)

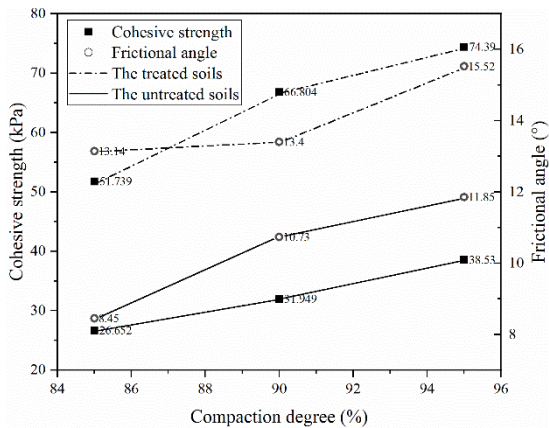


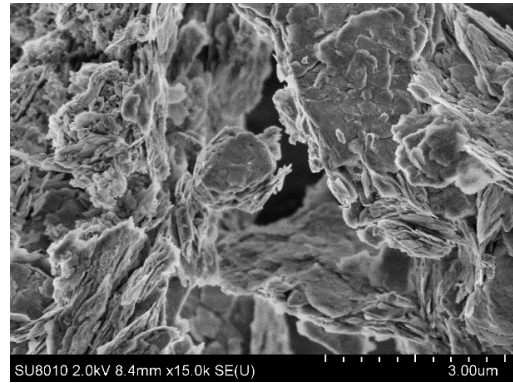
Fig. 12 Variations of cohesive strength and friction angle against compaction degree for expansive soils (saturated) and treated soils (saturated)

Besides, the curled edge and crimped surface had been improved, which indicated the inhibition of hydration of clay minerals. Meanwhile, the fined particles of soil were aggregated by the cementation process of MICP. Apparent calcium carbonate structures were observed on the soil's surface with high magnification. The SEM images supported the phenomenon of inhibiting soil expansion and intuitively presented the anti-swelling effect.

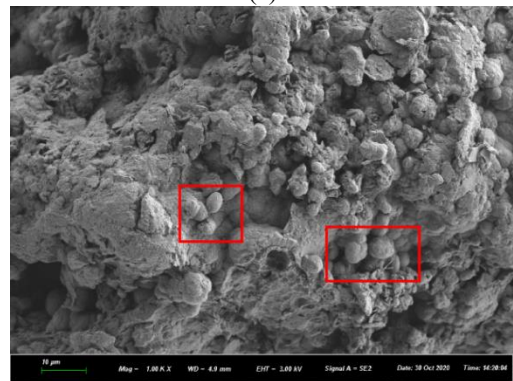
### 3.7 Laboratory model test

#### 3.7.1 Calcium carbonate content

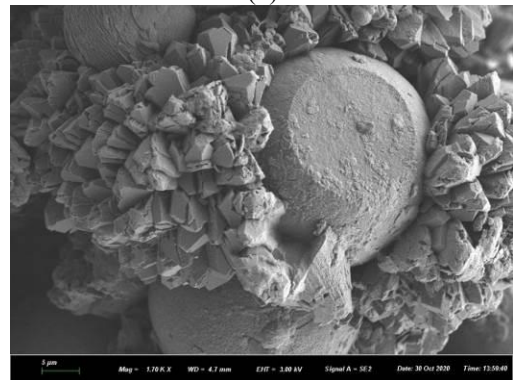
As shown in Fig. 14, the calcium carbonate content proportionally increases with the nutrient solution concentration in the depth range of 0-10 mm and 10-20 mm. When the nutrient solution concentration is 0.25 M, The internal calcium carbonate content is low, 3.79% and 0.82%, respectively, in the two depth ranges. When the nutrient solution concentration is 1 M, the calcium carbonate content is 8.24% and 2.67%, respectively. It can be seen that the calcium carbonate content in the 10-20 mm depth range was significantly lower than that in the 10 mm range. The permeability was reduced because of the hard



(a)



(b)



(c)

Fig. 13 SEM images:(a) expansive soil, (b) the treated soil and (c) crystal structure of calcium carbonate in the treated soil

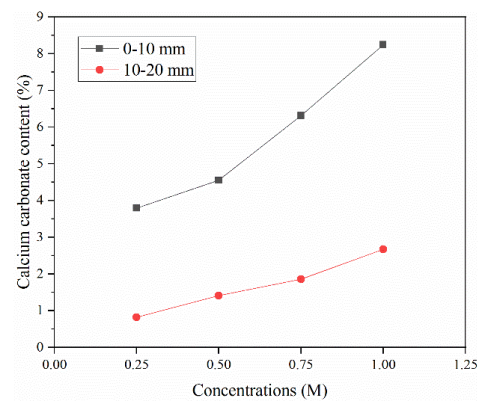


Fig. 14 Calcium carbonate content-concentration of solution curve of different depths of 10 mm and 10-20 mm

calcium carbonate crust on the soil's surface, preventing the infiltration of bacterial and nutrient solutions later. Further, the crust makes the calcium carbonate content lower within the 20 mm range. Results indicated that the MICP technology could effectively reduce the permeability of the topsoil's surface, retarding solution infiltration.

**3.7.2 Direct shear test**

After 21 days treatment period, samples obtained from five model tanks were prepared for a shear test. As shown in Fig. 15, the higher the concentration was, the better the MICP modification effect and the higher the corresponding strength was. When the concentration is lower than 0.5 M, the intensity index increases slowly, and when the concentration is greater than 0.5 M, the intensity index increases faster. Also, observation showed that the increase in cohesive strength was much more significant than the increase in the angle of internal friction. The phenomenon indicated that the cementation of the MICP process directly affected the topsoil, and the solution concentration significantly impacted the treatment results.

**3.7.3 Unconfined compressive strength (UCS)**

It could be seen from Fig. 16 that when the concentration was 0, 0.25 M, 0.5 M, 0.75 M, and 1 M, the corresponding unconfined compressive strength was 255, 265, 376, 413, and 587 kPa, respectively. The UCS and calcium carbonate content significantly increased with nutrition concentration increased. Moreover, the UCS samples were acquired from the uniform mixing of different depths in the vertical direction. Every sample obtained from the corresponding model tank had the same trend of calcium carbonate content. According to the consistency of nutrition concentration and calcium carbonate content in Fig. 14, the correlation of concentration-UCS and 0-10 mm calcium carbonate content ( $\text{CaCO}_3$ )-UCS were presented in Fig. 17. Thus, calcium carbonate content had an active effect on the strength improvement. Due to different calcium carbonate contents, the MICP effect in the model test caused a significant difference in the strength of different depths. The topsoil's strength improvement might be more significant to the depth with lower Calcium carbonate content.

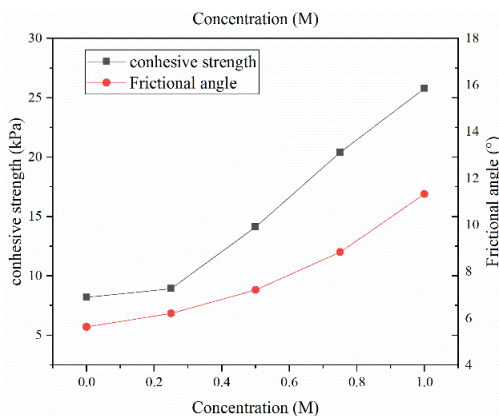


Fig. 15 Variations of cohesive strength and friction angle against the solution concentration

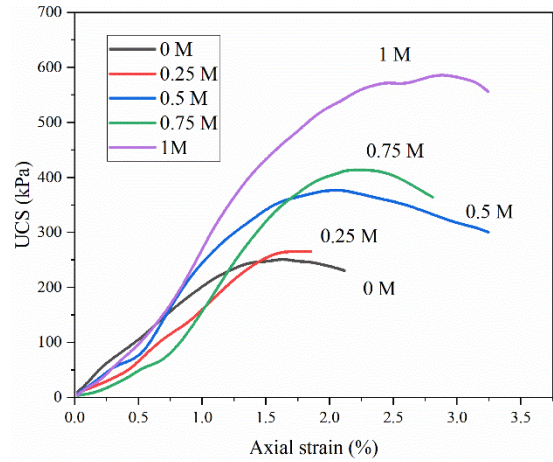


Fig. 16 UCS-axial strain curve for treated soils with different nutrient solution concentrations

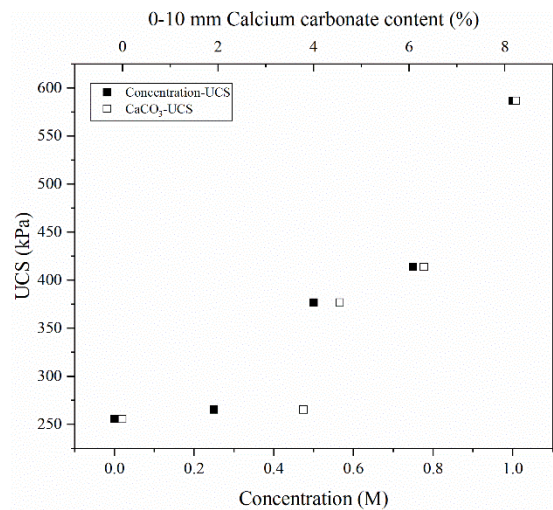


Fig. 17 variations of unconfined compressive strength against calcium carbonate content (0-10 mm) and solution concentrations

**3.7.4 The discussion of coated effect**

Fig. 18 showed the expansive soil's apparent state at different treatment ages with a nutrient solution concentration of 1 M. Initially, the bacterial and nutrient solution caused the expansive soil's crack due to typical water sensitivity, which was evident on the surface. On the second day, a small amount of white calcium carbonate appeared on the soil's surface with visual observation. As the output of calcium carbonate gradually increased, an evident change has happened since the 7th day. The continuous MICP process in the soil pores caused the healing of cracks on the surface. Cracks were hardly observed on the 14th day, and the coated effect was produced due to the apparent white calcium carbonate precipitate. When the coated effect occurred, the nutrient and bacterial solution were challenging to penetrate the soil's surface. On the 21st day, it could be seen that the surface of the soil had formed a hard calcium carbonate shell, and the thickness of the calcium carbonate shell was roughly estimated at 5 mm.

The soil samples' micrograms were analyzed to observe the coating effect after the whole treatment process. As

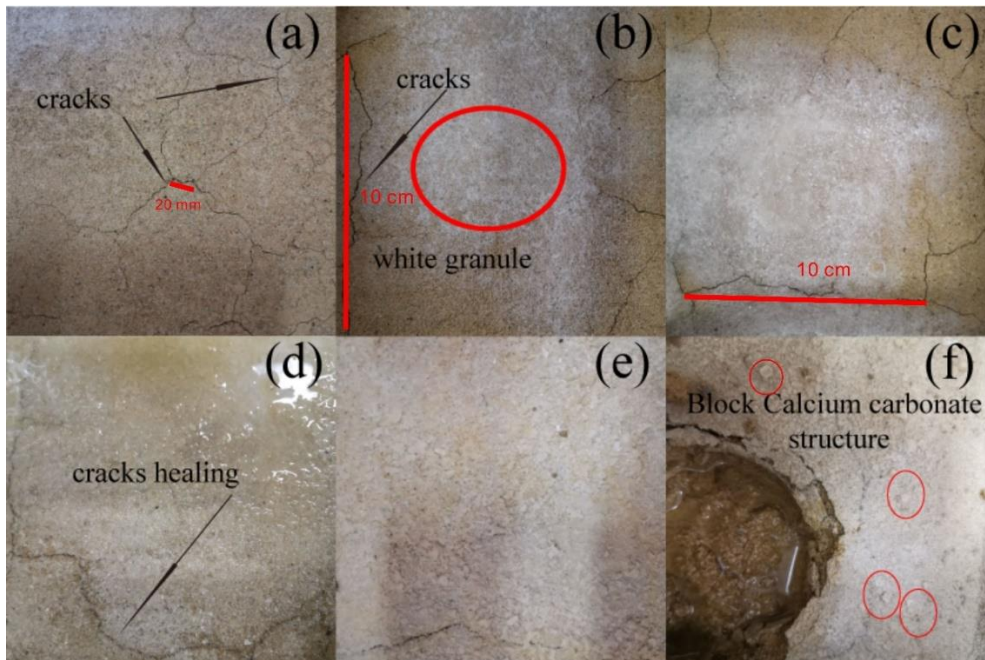


Fig. 18 Different states of the soil in the tank: (a) Day 1: cracks on the soil's surface, (b) Day 2: A small amount of calcium carbonate, (c) Day 3: apparent calcium carbonate generation, (d) Day 7: cracks healing, (e) Day 14: coating effect and (f) Day 21: about 5 mm depth of calcium carbonate coating

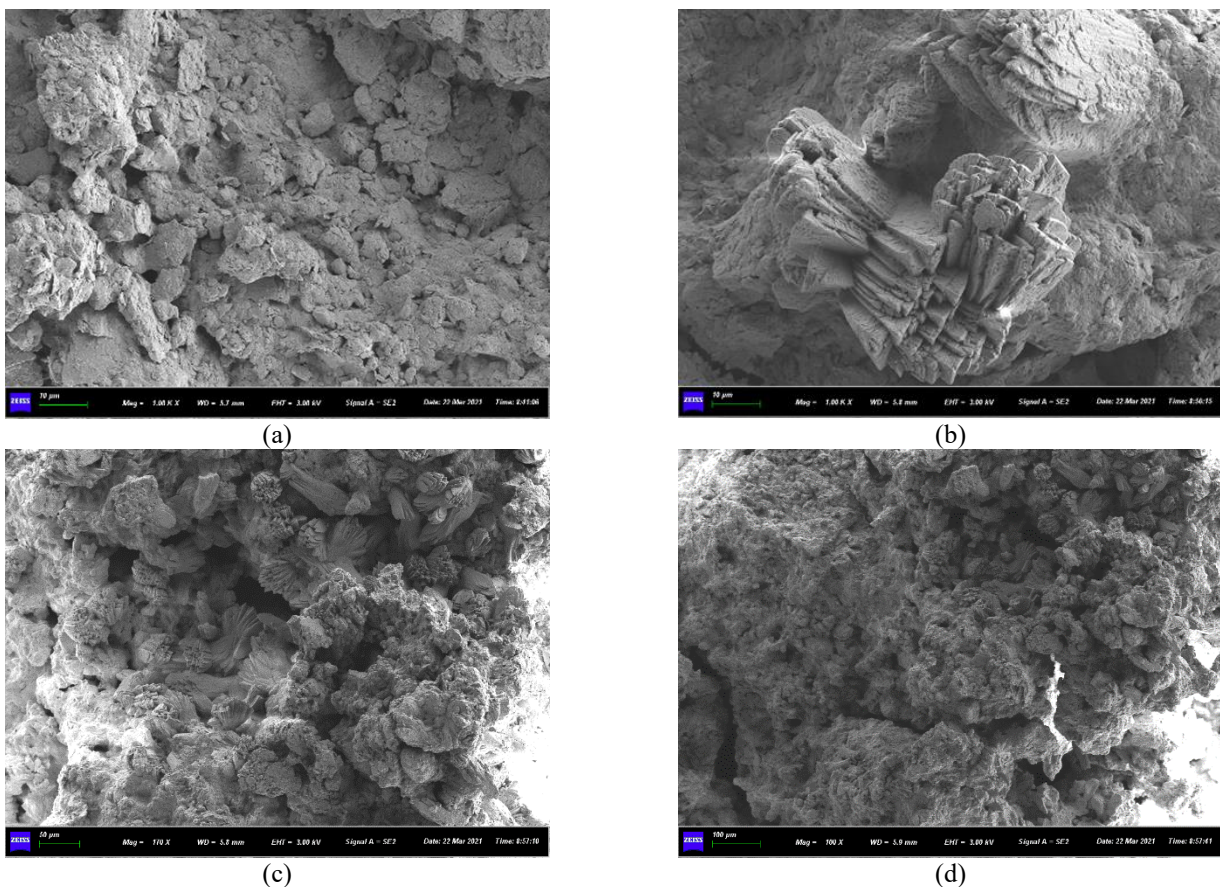


Fig. 19 The SEM images of expansive soil's intersecting surface with 70% compaction degree: (a); the intersecting surface of the topsoil of the model tank (1 M) with 70% compaction degree, (b) 10  $\mu\text{m}$ , (c) 50  $\mu\text{m}$  and (d) 100  $\mu\text{m}$

shown in Fig. 19, the intersecting surface of expansive soil presented a flat and lamellar particle structure.

Distinctively, the lamellar and curl structure had been hardly observed in the treated soil, and the spherical particle

structure was commonly observed. Furthermore, the apparent calcium carbonate crystal structure existed in the soil matrix, and the dense tiny pores due to the bacterial death were diffusely distributed in the soil matrix. According to the above results in this paper, the calcium carbonate had a cementation effect on the soil particles, and SEM images supported the experimental results. The widespread calcium carbonate cemented the soil matrix to improve its strength and inhibited the dispersion of fine particles.

#### 4. Conclusions

The paper systematically studies the MICP method, which is environment-friendly, to stabilize expansive soil for geotechnical engineering properties. According to the present results, significant conclusions are drawn as follows:

- (1) According to the absorbancy and urease activity results, bacteria activity is mainly sensitive to pH and temperature variation. Shaking frequency and inoculation amount are secondary factors. The actual results have instructional meaning to the conduction of treatment technology.
- (2) MICP process on the expansive soil has a significant impact on the physic-mechanical properties, and the engineering properties of the treated soil were improved after the treatment.
- (3) Notably, the MICP process inhibits the expansion of the soil.
- (4) The cementation of calcium carbonate can effectively cement the soil matrix and promote the aggregation of fine particles.
- (5) Besides, MICP technology is a geoenvironmental engineering topic in soil science, worthy of further development. The model tank test has proved the effect of the infiltration method by spraying the bacteria and nutrition solution. Significant calcium carbonate coating existed on the soil surface. As the variation of concentration, the engineering properties have apparent differences.
- (6) According to the above results, the improvement of engineering properties relates to calcium carbonate content. Meanwhile, the calcium carbonate content decreased as the depth increased. The coating effect has a practical stabilization effect on the topsoil. However, as the depth increases, the limitation of solution infiltration decreases the calcium carbonate generation and the treatment area of the MICP process to expansive soil.

Thus, MICP technology can achieve the inhibition of expansion of the soil and the improvement of engineering properties. Limited by the treatment method, MICP technology needs more discussion according to different engineering conditions.

#### Data availability statement

Some or all data, models, or code generated or used during the study are available from the corresponding author by request.

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#### Declaration of interest statement

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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