Effects of environmental temperature on the effectiveness of microbially induced carbonate precipitation

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Abstract

Microbial-induced calcium carbonate precipitation (MICP) is an innovative technique used for soil improvement, for controlled reduction of permeability in porous media or immobilization of soil contaminants. The application of MICP in the field is influenced by the environmental factors. In the present study, the main purpose is to explore the effectiveness of MICP in treating porous media at different environmental temperatures and reveal the underlying mechanisms. The microstructure characteristics were investigated via SEM imaging, EDS and XRD analyses and consolidated drained triaxial compression tests were performed to examine the performance of MICP-treated samples. Results indicate that the shear strength depends heavily on the treatment temperature, which was mainly due to the different content, size and distribution of $CaCO_3$ in samples at different conditions. The observations of pore-scale characteristics revealed that low temperature (4℃) and high temperature (50℃) produced less $CaCO_3$ precipitation, resulted in smaller carbonate crystals precipitation and thus lower strength. In contrast, samples treated at room temperature and 35 ℃ show more $CaCO_3$ precipitation and greater strength. The crystal forms, though, were not influenced by the temperature. The climate conditions are a very important parameter that needs to be tuned specifically for the purposes of each MICP application (whether controlled alteration of permeability or for soil stabilization). However, in most MICP field applications, temperature is nearly impossible to control, and in such conditions where bacterial activity is reduced, the alteration of the MICP recipe is required, and specifically the number of bacterial solution injections are worth to be considered.

Plain Language Summary

We studied the effects of MICP treatment of granular material under various temperatures, that are representative of the cold, tropical, semi-arid (35℃) and arid regions. Results show that the strength enhancement is affected by the

climate conditions since different precipitation patterns of calcium carbonate crystals were observed. Temperature is a very important parameter that needs to be taken into account when designing MICP protocols for field applications whether these are soil permeability control, or soil stabilization.

Keywords: environmental temperature; climate conditions; urease activity; MICP performance; shear strength

Key points:

- Microbially induced carbonate precipitation was applied in granular material under different temperature conditions.
- Microscale analysis showed that the precipitation patterns vary significantly depending on the temperature.
- The strength of the resulting products follows the same trend as the precipitation patterns.

1 Introduction

Microbial-induced calcium carbonate precipitation (MICP) is a biochemical process in which microorganisms induce an enzyme-catalyzed process that increases the supersaturation ratio of calcium carbonate and induces its precipitation in an aqueous environment (Mitchell and Santamarina 2005; DeJong et al. 2006). When the process is applied to soil pore water, precipitated calcium carbonate minerals form at the pore throats of the soil matrix and on the surface of soil particles, increasing soil binding and soil surface roughness, thereby increasing soil strength and stiffness. Therefore, MICP has been widely studied for applications including soil stabilisation (DeJong et al., 2010, 2011, 2013; van Paassen et al. 2010; Umar et al. 2016; Gowthaman et al. 2019; Naveed et al. 2019) and the generation of artificially cemented sandstones from sand for hydraulic fracturing experiments (Konstantinou et al. 2021). In addition, the amount of precipitated calcium carbonate minerals in the soil pores can be controlled to not fully occupy the whole soil pores, thereby maintaining a relatively high soil permeability or providing more control on permeability alteration compared to other cementation methods. Therefore, MICP can also be potentially used for liquefaction (Montoya et al. 2013; Xiao et al. 2018; Darby et al. 2019) and erosion control (Jiang et al. 2017; Chek et al. 2021). Moreover, MICP can also seal cracks of soil or geo-structures for applications such as CO_2 storage, and concrete remediation (Minto et al., 2016; Tobler et al. 2018; Weinhardt et al. 2021). Furthermore, carbonate can co-precipitate with heavy metal ions, so it can solidify heavy metals in soil and water (Warren et al. 2001; Fujita et al. 2004; Li et al. 2013; Mugwar and Harbottle 2016; Torres-Aravena et al. 2018; Jiang et al. 2019; Mwandira et al. 2017).

Ureolysis-driven MICP is the most widely studied MICP process for soil stabilization, among the others such as denitrification and sulfate reduction, due to

its ease of control, high chemical conversion efficiency and short amount of time required (Dhami et al. 2013, Castro-Alonso et al. 2019). In this process, the ureolysis bacteria cells were introduced into soil matrix by either bio-augmentation (Gomez et al. 2017, 2019) or bio-stimulation (Wang et al. 2020; Graddy et al. 2021) followed with the injections of multiple times cementation solution which include $CaCl_2$, urea and nutrient broth. Ureolysis bacteria express a urease enzyme which catalyses the hydrolysis of urea (*Equation 1*); the addition of calcium (Ca^{2+}) to this system induces the precipitation of calcium carbonate ($CaCO_3$) as the ${CO_3}^{2-}$ ions produced by the hydrolysis of urea react with the supplied Ca^{2+} (*Equation 2*) (van Paassen et al., 2010; Al Qabany and Soga, 2013); the addition of nutrient broth to this system maintains the bacterial activity over the precipitation period (Al Qabany and Soga, 2013):

(1)

(2)

MICP rate and efficiency are directly affected by parameters such as bacterial activity (Wang et al. 2021; Konstantinou et al. 2021; Omoregie et al. 2017), concentration of cementation solution (De Muynck et al. 2010; Al Qabany and Soga 2013), urea to calcium chloride ratio (Okwadha and Li 2010; Mahawish et al. 2018a), bacterial and chemical injection methods (Cheng and Cord-Ruwisch 2012, 2014; Cheng and Shahin 2016; Martinez et al. 2013; Wu et al. 2019) and the frequency of injections or retention period (Al Qabany et al. 2013; Qin et al. 2016; Lee Lee et al. 2013). In addition, soil particles properties including grain size and size (Mahawish et al. 2018b; Zamani et al. 2019; Cheshomi & Mansouri 2020; Kim et al. 2020), relative density (Gao et al. 2019) and particle size distribution (Xiao et al. 2021) were shown to affect the effective of MICP for soil strengthening. Further, environmental factors, including temperature (Peng et al. 2019; Sun et al. 2019) salinity and aqueous conditions (Mortensen et al. 2011; Soon et al. 2014) have also arisen research attention.

For MICP to be able to be used in large scale field applications, the considerations of environmental factors on MICP efficiency and optimization are essential. Among these environmental factors, climate is a crucial one. The subsurface temperature in different areas normally falls within the range between 10 and 30℃ (Hu and Feng 2003; Chow et al. 2011; Kumarathunge et al. 2013), whilst it could even become lower than 10 ℃ or higher than 50℃ for some cold or arid regions, respectively (Gao et al. 2007; Huang et al. 2020). Such extreme drought events are much more frequent in the recent years because of climate change which introduces wormer summers and colder winters. Any real-field MICP implementation strategy (for controlling permeability, soil stabilization or contaminant transport control) needs to consider the soil temperature. Studies have shown that temperature influences the $CaCO_3$ precipitation rate, crystal properties, thus soil permeability (Cheng et al. 2017; Peng et al. 2019) and unconfined compressive strength (UCS) (Cheng et al. 2017), Recently, the effects of temperature on the MICP processes and properties were studied by Wang et al. (2022). Despite the studies conducted, the effect of temperature

on the properties of soils treated by MICP at different temperatures were studied in a systematic way. Since triaxial shear testing parameters are important parameters used to define yielding and failure, the evaluation of soil strength enhancement obtained at field temperature properties by triaxial shear testing is essential for all listed MICP field applications.

This research investigates the microstructure characteristics at the pore scale (amount, size, distribution and form of carbonate crystals) and the mechanical response of the bio-cemented specimens generated at different temperatures simulating the field environment including cold (4℃), tropical (20℃), semi-arid (35℃), and arid regions (50℃). The aim is to investigate the effect of treatment temperature on microstructure and mechanical behavior of MICP-treated soils, especially the shear behavior, with the main objective to explore the feasibility of field application of MICP-treated soil. A series of triaxial compression tests were conducted accompanied with SEM imaging, EDX and XRD analyses to estimate fulfill the objectives of this study. Triaxial testing was used to derive cohesion and friction angle which are the ultimate parameters used to define yielding and failure. The mechanical response was correlated with the microstructure characteristics since the calcium carbonate precipitation rate, crystals size and location within the grains affect the strength of the resulting MICP products.

2 Materials and methods

2.1 Sand properties and specimen preparation

Fine CHINA ISO standard silica sand was used in this study, and its main properties are summarized in Table 1. The particle size distribution of the sand is demonstrated in Fig. 1. The mean grain size, D_{10}, and coefficient of uniformity, C_{u} (=$\mathrm{D}_{60}/\mathrm{D}_{10}$), are 0.125 mm and 5.6, respectively, as shown in Fig. 1. The sand is classified as poorly graded according to the Unified Soil Classification System (ASTM, 2017).

A split acrylic cylindrical mold with an inner diameter of 38 mm and a height of 80 mm was used to prepare the specimens (Fig. 2**)**. The weight of dry sand was calculated based on a targeted relative density (RD) of 50%. Sand was poured into the columns in 3 portions via dry pluviation method. After the sand column was prepared, deionized water was flushed in the column to expel air from the columns. This step was repeated several times until the flow rate was stabilized and the specimen became fully saturated. Then, the drain valve was shut such that the sand column remained saturated until the biotreatment process began.

2.2 Biological and chemical treatment procedure

S. pasteurii (CGMCC1.3687) was used in this study as it is a urease-active strain and its urease activity is well demonstrated over many other alternative

ureolytic bacteria. The bacterial strain was cultivated in a NH_4-YE liquid media (ATCC 1366) for about 24 h at 30°C in a shaking incubator at 200 rpm/min to obtain an optical density at 600 nm (OD_{600}) of 1.0. The tested bacterial activity of the bacterial suspension at (OD_{600}) of 1.0 was approximately 40 mM/h. The cementation solution consisted of 0.75 M of urea, 0.5 M of calcium chloride, and 3 g/L of nutrient broth. The grade level of all chemical reagents was analytical grade.

A gravity filtration method was used for MICP treatment process (injection from top to bottom via gravity). Before MICP treatment, the pore volume (PV) of sand columns was calculated using the targeted relative density, so that the injected volume of bacterial suspension (BS) and cementation solution (CS) during each cycle could be determined. The prepared saturated sand column specimen was treated by the two-phase injection method, where certain volumes of BS were injected into the soil matrix, followed by a bacterial settling period to allow bacterial cells to attach to soil particles (Phase 1). Then, the next step was the injections of CS at a designed injection interval (Phase II) to allow MICP to occur. The parameters of MICP treatment are shown in Table 2.

In total, six tests were conducted to assess the effects of temperature on the resulting microstructure characteristics and strength of the MICP products. In tests 1-4, the bacterial settling time and the time interval across the cementation solution injections was 24 hours. Since it was found that the bacterial urease activity decreases rapidly at 50°C (Wang et al. 2022), the number of BS injections in tests 5 and 6 for 50°C case was increased to 3, and 6, respectively, and the time between BS and CS injection was shortened at 2 hours instead of 24 hours in these two tests. In all tests, in either BS or CS injections, 1.0 PV of BS or CS was injected each time, and the total number of CS injections was 6. Before MICP treatment, the pore volume (PV) of sand columns was calculated using the targeted relative density, so that the injected volume of bacterial suspension (BS) and cementation solution (CS) during each cycle could be determined. The samples were placed at the designed temperature during all retention times of the experiment. The four temperatures, 4°C, 20°C, 35°C and 50°C were achieved by placing the setup in a refrigerator, at room temperature, and in two ovens.

2.3 Triaxial tests

After completing the biological treatment process the specimens were carefully extracted from the molds for conducting consolidated drained (CD) triaxial tests (ASTM 2020) to determine of strength and stress-strain relationships of a cylindrical specimen of MICP-treated specimens at these temperatures. Specimens were firstly saturated with vacuum saturation for about 24 h and then placed in the triaxial apparatus. Prior to consolidation, specimens were saturated by hydraulic saturation for 12 h at a confining pressure of 20 kPa and then back-pressure saturation was sequentially applied until the B-value exceeded 0.95. For each experimental condition, four samples were prepared, and the triaxial spec-

imens were consolidated at an effective confining pressure of 100, 200, 300, and 400 kPa, respectively. Following consolidation, specimens were sheared under drained conditions with a constant displacement loading rate of 0.1 mm/min.

2.4 $CaCO_3$ content measurement and chemical efficiency calculation

The ASTM Method (ASTM 2014) was adopted for the determination of the $CaCO_3$ content of biotreated sand. A calibration curve was first developed by adding various amounts of calcite ($CaCO_3$) with hydrochloric acid and measuring the CO_2 pressure. As shown in Eq. (3), a linear relationship between CO_2 pressure and $CaCO_3$ content was developed. After the triaxial tests were completed, the specimens were carefully removed from the membrane and 15 to 25 g of specimens were collected for every 15 mm along the sand column height. The samples were oven-dried at 105℃ for at least 24 h (ASTM 2014). Once removed from the oven, the specimens were grounded, weighed, and placed into the $CaCO_3$ measurement chamber. 30 ml of 3 M hydrochloric acid was added to a container and then placed into the chamber without contacting the soil specimens. The $CaCO_3$ measurement camber was completely sealed and shaken gently to facilitate the reaction between $CaCO_3$ and hydrochloric acid. The generatured CO_2 from the reaction between $CaCO_3$ and hydrochloric acid increase the pressure of the camber and was measrued by a pressure gauge inserted on the top of the camber. When the pressure value indictated by the gauge no longer changed, the reading was recorded and the amount of $CaCO_3$ was calculated utilising Eq. (3).

(3)

The chemical conversion efficiency of MICP was then calculated, which is defined as the ratio of the precipitated mass of $CaCO_3$ in the sand to the calculated mass of $CaCO_3$ from cementation solutions (Al Qabany et al. 2012; Wang 2018):

(4)

where,is the measured $CaCO_3$ content, is the concentration of $CaCl_2$ in the cementation solutions V is total the volume of cementation solution injected into samples is the molar mass of $CaCO_3$ (100 g/mol); is the dry mass of sand used to prepare sample columns. It should be noted that the calculated chemical conversion efficiency may be lower than the actual value, because the $CaCO_3$ precipitated on the mold surface and in the filter layers at the top and bottom of the sample was not taken into account when calculated according to Eq (4).

2.5 Scanning electron microscopy (SEM) imaging, EDS and XRD analyses

Samples of biocemented sand treated at different temperatures were oven-dried at 105℃ and prepared for Scanning electron microscopy (SEM) imaging to observe the microscale properties of the $CaCO_3$ crystals once formed after the

MICP treatment. Scanning electron microscopy (SEM) images and energy dispersive X-ray spectra (EDS) analysis were performed using a PHENOM XL Scanning Electron Microscope. X-ray diffraction (XRD) analyses were also conducted on the samples treated at different temperatures using an X-ray diffractometer (Regiku Miniflex 600).

3 Results

3.1 Distribution of $CaCO_3$ and chemical efficiency

The average $CaCO_3$ content and its uniformity along a soil specimen affecting the strength of MICP-treated soil specimen, and therefore the distribution of $CaCO_3$ content along the height of the sand column were measured and reasults are shown in Fig. 3.

$CaCO_3$ content decreases gradually along the injection direction of BS and CS in the sand column (Fig. 3 a and b). In general, the heterogeneity in the distribution of $CaCO_3$ precipitation along the height of the sand column increased with the average $CaCO_3$ content (Fig. 3a and b). Temperature 4℃ and 50℃ resulted in relativley low average $CaCO_3$ contents, and the highest average $CaCO_3$ contents were obtained when temperature was 35℃, shown by the comparison between the tests where BS was injected once (Tests 1-4, Fig. 3a). Increasing BS injection numbers, increases the aveage $CaCO_3$.

Chemical conversion efficiency is calculated by utilizing the average $CaCO_3$ and thereby these two parameters are consistent. The chemical conversion efficiency of the sample at 35℃ was 67%, which is significantly higher than those samples treated at other temperatures, followed by 46.4% at 20℃, and decreased significantly to 19.6% and 17.3% at 4℃ and 50℃, respectively. The fitted line presents a nonlinear polynomial relationship having a strong correlation coefficient of 0.998 (Fig. 3c). At 50℃, the chemical conversion efficiency has a good logarithmic fit with the injection number of BS, and the correlation coefficient is 0.997. As the BS injection number increased from 1 to 6, the chemical conversion efficiency of the sample increased from 17.3% to 56.5% (Fig. 3d).

3.2 Stress-strain and volumetric-strain responses

Fig. 4 shows the stress-strain behavior of samples treated at temperatures 4, 20, 35 and 50℃ and those at 50℃ treated with different BS injection numbers. All samples showed strain softening behavior, which is consistent with the results of Feng and Montoya (2016), Xiao et al. (2019), and Gao et al. (2019) obtained at temperatures of 25 ℃. The strain softening characteristics of samples treated at 20℃ (Fig. 4b) and 35℃ (Fig. 4c) were more pronounced since a clearer peak was observed. Brittle behavior is observed when the axial strain is in the range of 0.5%-2.2% (Fig. 4 b and c), where the sample reached its peak state. In contrast, the specimens at 4℃ and 50℃ showed gradual softening after reaching

the peak strength, as shown in Fig. 4 a and d. The peak strength of samples at different temperatures increased with the increase of confining pressure as expected, but the brittleness level seemed not to be affected (Fig.4 a-d).

The stress-strain curves of samples under different BS injection numbers (N) at 50℃ are shown in Fig. 4 d-f. All samples exhibited strain softening with the BS injection number having a significant effect on the peak strength and brittle behavior of the sample. When the BS injection number was 3 and 6, the samples showed similar stress-strain characteristics as those treated at 20℃ and 35℃, including the increase of peak stress with the increase of confining pressure and the brittle behavior when the strain was small.

Fig. 5 presents the variation of volumetric strain with axial strain behavior of samples under different confining pressures (representing various depths of the soil) at different experimental conditions. All specimens showed volumetric expansion at small strain while exhibited dilative response under large strain. The results show that the treatment temperature has a significant effect on both the volumetric behavior of the specimens at small strain and the dilatant behaviour at large strain. For example, samples treated at 4 and 50℃ showed less contraction at small strain than that of 20℃ and 35℃ (Fig. 5 a-d). The dilatancy of the sample treated at 35℃ was the largest when the strain was 15% for a given confining pressure (for example at 100 kPa), followed by 20℃, 4℃ and 50℃, as shown in Fig.5 a-d. The volumetric behavior of samples is related to the treatment temperature, which directly affects the $CaCO_3$ content. In other words, the higher the carbonate content is, the higher dilatancy at large strain occurs at a given confining pressure. Similar results were also reported by several published studies (e.g., Lin et al. 2016; Xiao et al. 2019; Wu et al. 2021) obtained at room temperatures. Fig. 5d-f shows the relationship between volumetric strain and axial strain of samples treated at 50℃ with varied injection numbers of BS. The samples treated at 50℃ showed significant dilatancy improvement with the increase of BS injection numbers, and contraction at small strain can be observed for all samples.

3.3 Peak strength and residual strength

Peak strength (q_{p}) and residual strength (q_{R}) can be obtained from the stress-strain curves. The variation of q_{p} and q_{R} with temperature (T) and confining pressure ($_3$) is presented herein. Fig.6 a and c shows the measured q_{p}-T- $_3$ and q_{R}-T- $_3$ relationship for the MICP-treated samples at different temperatures with BS injected only once (Tests 1-4). It can be observed that the peak strength and residual strength increased with the increase of confining pressure. For example, the peak strength of the sample treated at 20℃ increased from 623.6 kPa to 1576.8 kPa with the increase of confining pressure from 100 kPa to 400 kPa, while the residual strength increased from 271.7 kPa to 812.2 kPa. For the specimen treated at the same temperature, the peak strength and residual strength increased first and then decreased with the temperature falling in the range between 4 and 50℃. For instance, the maximum peak strength and

residual strength was 322.8 kPa and 220.7 kPa at 35℃, respectively, followed by 20℃ and 4℃, and the minimum was obtained at 50℃.

Fig. 6b and d presents the q_p-N- $_3$ and q_R-N- $_3$ relationships for the sample treated at 50℃ with BS injection numbers varying from 1 to 6 (Tests 4-6). It can be seen from Fig. 6b that there is an increase of peak strength with confining pressure and BS injection number. The residual strength showed a similar increasing trend with the variation of confining pressure and BS injection number, as shown in Fig. 6d. Obviously, increasing the BS injection number has a significant improvement in the peak strength and residual strength.

Recalling the results shown in Fig. 3, it can be observed that the effect of temperature and BS injection number on the peak strength and residual strength can be attributed to the variation of $CaCO_3$ content. This is illustrated in Fig. 7, which presents the q_p-T- $CaCO_3$ content and q_R-T- $CaCO_3$ content relationship at various confining pressures ($_3$). As the average carbonate content increases the strength of the resulting specimen is higher giving a higher peak and residual deviatoric stress in the triaxial tests. The middle temperatures (20 and 35°C) seem to be optimum for strength enhancement while injecting BS multiple times seems to also facilitate strength enhancement.

3.4 Effective strength parameters

The Mohr-Coulomb failure criterion is widely used to estimate the strength parameters in geomaterials (Wood 1990). Based on the Mohr-Coulomb failure envelope, the limit equilibrium state of soil at CD triaxial tests can be expressed as follows:

(5)

This gives

(6)

where,is the deviatoric stress at limit equilibrium state, i.e., the peak deviatoric stress; c is the effective cohesion; is the effective friction angle. As illustrated in Fig. 8, the relationship between peak deviatoric stress and confining pressure of biocemented sand at different temperatures can be obtained by the following formula:

(7)

and substituting this equation to Eq. (4) gives

(8)

hence, the Mohr-Coulomb effective parameters (c and) can be derived from Eq. (8). Table 3 summarizes the fitting parameters in Eq. (7) and the effective strength parameters calculated according to Eq (8).

Fig. 9 shows the relationship between effective strength parameters and treatment temperatures. The effective cohesion and friction angle increased first and then decreased as the temperature rose from 4 to 50℃ (Fig. 9a). The lower and higher temperatures led to smaller strength parameters. For example, the effective cohesion of the samples at 4℃ and 50℃ was as low as 4.6 kPa and 3.1 kPa, respectively. The maximum effective cohesion value was obtained at 35℃ which was 80.9 kPa. In contrast, the effect of temperature on the effective friction angle is not obvious (Fig. 9a). In the range of 4 to 50℃, the effective friction angle of the sample varied from 26.8° to 36.6°, which was significantly lower than that of cohesion. Fig. 9b shows the variation of the effective strength parameters with the BS injection number of the samples treated at 50℃. There is a linear increase between the effective cohesion and internal friction angle and the injection number of BS, with high correlation coefficients of 0.99 and 0.98 respectively.

To discuss the effect of temperature on strength of MICP-treated sand, the strength parameters (i.e., c and) derived from CD tests are plotted with $CaCO_3$ content, as shown in Fig. 10. For the samples with BS injected once time, the effective cohesion increased exponentially with $CaCO_3$ content (R^2=0.99), while a linear relationship between effective friction angle and $CaCO_3$ content was observed, with a strong correlation as the R-squared value was 0.91 (Fig. 10 a). This is consistent with the results of several previous studies (e.g., Cui et al. 2017, 2021; Choi et al. 2020) conducted at temperatures of 25℃. Strong similar correlation was also found between the two strength parameters and $CaCO_3$ content for the samples treated at 50℃ and at various BS injection numbers (Fig. 10b).

3.5 Microstructure characterization

The variations in strength with respect to the treatment temperatures arise mainly because of the differences in carbonate precipitation rates and size and distribution of the crystals within the granular network. Fig. 11 a-d shows SEM images of MICP-treated sand at different temperatures with BS injected only once. More and more $CaCO_3$ crystals precipitated on the surface of sand particles or at the particle contacts as the temperature increased from 4 to 35℃, while they decreased dramatically for the sample treated at 50℃. This is consistent with the macro-scale measurement of $CaCO_3$ content (Fig. 3). The $CaCO_3$ precipitation in samples treated at 4℃ was mainly small crystals scattered on the sand particle surface and at particle contacts. For the samples treated at 20℃ and 35℃, mainly larger crystal clusters precipitated at the contact of sand particles. Although the $CaCO_3$ in samples treated at 50℃ existed as crystal clusters, the size was obviously smaller than that of 20℃ and 35℃.

Fig. 11 d-f show the SEM images of samples treated at 50℃ with different BS injection number. In samples treated at high temperatures mainly crystal clusters precipitated. When the BS injection number was lower (e.g., BS=1),

the crystal clusters were randomly distributed on the sand surface and among sand particle contacts, whereas the crystals were similar in size to those observed at 4℃. The size of the crystal cluster increased significantly with the increase of BS injection number. For example, the average diameter of crystal clusters can reach 25 and 40 m for the sample treated with BS injected 3 times and 6 times, respectively.

The EDS and XRD results revealed that the main elements in $CaCO_3$ precipitation formed at different temperatures were Ca, C, O, and Si, as shown in Fig. 12a and the mineral content were calcite, vaterite and quartz (Fig. 12b). It can be seen from the XRD diffraction images that at different temperatures, rhombohedral calcite is predominant in the calcium carbonate precipitation, and vaterite is sporadically visible (Fig. 12b).

4 Discussion

4.1 Effect of temperature on urease hydrolysis rate and $CaCO_3$ precipitation

The influence of temperature on urease hydrolysis rate (ureolysis rate) results from combined effects of temperature on bacterial density, bacterial urease producing rate and pure urease activity (Wang et al. 2022). Bachmeier et al. (2002) investigated the effects of temperature on pure free enzyme activity and found that urease activity decreases with time at different temperatures, with higher temperatures reducing the activity more quickly than lower temperatures. Khan et al. (2019) found the urease production ability of bacterial strains increase with temperature until the temperature reached 35°C, after which urease activity started decreasing until 50°C. Wang et al. (2022) found that bacterial density decreases with time at different temperatures, with higher temperatures reducing the bacterial density more abruptly than lower temperatures.

The influence of temperature on $CaCO_3$ precipitation results from combined effects of temperature on urease hydrolysis rate (ureolysis rate), which affects the concentration of CO_3^{2-} in the reaction system and consequently affect the supersaturation ratio of reaction system (Wang et al. 2022). In addition, studies have shown that when the supersaturation ratio is the same, $CaCO_3$ precipitation rate is higher at higher temperatures (Kralj et al. 1990, 1994 and 1997). Therefore, combining the effects of temperature on urease hydrolysis rate, the effects of temperature on $CaCO_3$ precipitation rate is complex.

In Wang et al. (2022), the highest $CaCO_3$ content was obtained when temperature was 20℃, which however in the current study was obtained when temperature was 35℃. The difference might be due to the difference in the injection interval of CS in these two studies. In Wang et al. (2022), the injection interval was 48 hours, but in this study the injection interval was reduced to 24 hours. The 24 hours was sufficient enough for 0.5 M of CS to complete precipitation at either 20 or 35℃. Because bacterial activity reduced with time at 35℃, the

shorter injection interval maintained a relatively higher bacterial activity and higher $CaCO_3$ transform efficiency.

In most of the published studies, MICP treatment is performed at room temperature and the injection interval is 12 or 24 h between BS and CS (Cheng et al. 2013; Mahawish et al. 2018b; Wang et al. 2021). However, the interval between BS and CS needs to be carefully considered at higher temperatures (e.g., 50℃) since the urease activity and density of bacteria exhibit dramatic decay with time. The results of this study indicate that 24 h injection interval is suitable for treatment temperatures lower than 35℃, while a small injection interval should be adopted at high-temperature conditions so that guarantees the expected treatment effect. In addition, increasing the BS injection number acted as a 'compensation' for bacteria decay caused by high temperature, maintaining a supersaturation state in solution, resulting in higher $CaCO_3$ content produced in samples compared to that injected BS only once (Fig. 5b). These results were consistent with the results of Wang et al. 2022.

In addition, temperature does not only affect $CaCO_3$ precipitation rate, but also affects $CaCO_3$ precipitation-dissolution-reprecipitation processes. At low temperatures (i.e., 4℃), bacteria were attached randomly either on the particle surface or at particle contacts which acted as a nucleation site for crystals to precipitate. Small and uniform crystal sizes were observed at low temperatures due to the low supersaturation, resulting in low nucleation rates and slow crystal growth rates (Sahrawat 1984; Kralj et al. 1997). For the samples treated at 20℃ and 35℃, more crystals precipitated at contact points and less crystals at particle surfaces since bacterial aggregations were observed and the crystals accumulated around the bacterial aggregations. Bacterial aggregations are much better candidates for nucleation sites for carbonate crystals to generate comparted to single bacterial cells because they have higher urease activity as shown by Wang et al. (2022). Also, when the crystals are formed around bacteria aggregations, these newly formed crystals serve as new nucleation sites (Mitchell & Ferris 2006; Lioliou et al. 2007). This explains the findings shown in Fig10b-c, where crystals accumulated at particle contacts and grown into larger crystal clusters.

Although higher temperatures (50℃ for example) reduced the supersaturation and more nucleation took place (Mullin 2001), the rapid deactivation of bacteria led to the termination of the MICP process and as a result the crystals' growth was inhibited to then stop completely. The high temperature also resulted in the precipitation of small crystal clusters preferentially around bacterial aggregations against single bacterial cells because the former tends to decay slower. In addition, in the case of multiple bacterial injections, the injected bacteria preferred to attach to calcite rather than quartz, while calcite is easier to serve as the nucleation site than quartz for crystals (Lioliou et al. 2007). As a result, the size of crystal clusters at particle contacts increased significantly as the BS injection number increased from 1 to 6 (Fig. 11d-f).

Since MICP is a complex process with many bio-chemical and medium factors

affecting its effectiveness and the $CaCO_3$ precipitation mechanism, in this study only the temperature varied and all other factors remained fixed in order to isolate the effects. The effect of temperature on $CaCO_3$ precipitation is explained by the variations in urease activity, which is the key factor that affects the ureolysis rate, the crystals nucleation and the growth rate.

4.2 Effect of temperature on the strength of bio cemented sand

The effect of temperature on the strength of bio cemented sand essentially depends on the content of $CaCO_3$ in the specimen and on the uniformity of the MICP products (Feng and Montoya 2016; Cui et al. 2017; Nafisi et al. 2020; Konstantinou et al. 2021). $CaCO_3$ precipitation has a bonding effect on sand cementation, leading to a substantial increase of cohesion (DeJong et al. 2010). Accordingly, the peak strength of the sample increased with the rising temperature due to the increase of $CaCO_3$ content (Fig. 9), which then decreased at the highest temperature of 50°C. For the samples with BS injected once time, the effective cohesion increased exponentially with $CaCO_3$ content (R^2=0.99), while a linear relationship between effective friction angle and $CaCO_3$ content was observed, with a strong correlation as the R-squared value was 0.91. This is consistent with the results of several previous studies (e.g., Cui et al. 2017, 2020; Chou et al. 2020).

The cohesion increases dramatically because of the presence of carbonate crystals at the contact points between the particles since the term defines the non-frictional part of the shear resistance of the soil. The trend can be clearly explained through the microstructure properties. At 20 and 35 °C more carbonate crystal clusters are observed at the contact points between the particles which contribute to strength enhancement. At lower temperatures these clusters are smaller in number while at 50 °C, the crystal clusters seem to decrease in size resulting to a smaller strength gain compared to the previous experiments.

The relationship between strength parameters and $CaCO_3$ content reveals that the effect of temperature on effective cohesion seems to be more significant than that of friction angle. The friction angle depends primarily on grain size distribution, angularity, and particle interlocking. The 'added' calcium carbonate crystals increase the particle interlocking (due to roughness) which in turn increase the friction angle. However, the effect of strength gain when cementation is added at the contact points (cohesion) is much more pronounced in MICP-treated specimens.

The residual state, the strength of the sample is essentially attributed to the non-broken calcium carbonate between the particles and the surface roughness of soil particles caused by $CaCO_3$ precipitation (Montoya and DeJong 2015; Gao et al. 2019). The cementation degraded dramatically after the peak state was reached especially in the stronger specimens, but the non-broken calcite still plays the role of cementation, and the roughness of sand particles may also

contribute to the residual strength (Fig. 10).

The peak and residual strengths also increased when multiple BS were injected because the calcite crystal clusters increased in size as a result of this. However, still the specimen that exhibited the higher strength was the one with one injection of BS at 35°C. This shows that the temperature is indeed a very important parameter that needs to be tuned specifically for the purposes of each MICP application. In cases where temperature cannot be controlled, a proposed solution is the alteration of the MICP recipe, and specifically the number of bacterial solution injections.

5 Conclusions

In this study, a series of consolidated drained triaxial compression tests were conducted to explore the effectiveness of MICP in treating granular media at different environmental temperatures. Very high and very low temperatures such as 50 and 4 ℃ were also considered in this experimental work to capture extreme climate conditions. Micro-scale investigation was also conducted to explore the pore-scale characteristics of the precipitated minerals (amount, size, distribution and form of carbonate crystals) which are responsible for the resulting engineering properties of the treated soils (strength, stiffness and permeability). Based on the test results and analysis presented in this paper, the main conclusions are drawn as follows:

1. $CaCO_3$ content of samples increased as the treatment temperature rose from 4 to 35℃, while it decreased dramatically at 50℃. During triaxial tests, all MICP-treated samples exhibited strain softening and volumetric dilatancy, whereas the value of peak and residual strength showed significant variations with treatment temperature. The maximum peak and residual strength were observed from the specimens treated at 35℃, followed by 20℃. Samples treated at lower temperatures (4℃) and higher temperatures (50℃) resulted in lower peak and residual strength. The peak and residual strength of the samples treated at 50℃ were significantly improved by increasing the BS injection number.

2. The variation of effective strength parameters (i.e., c and) with treatment temperature was similar to that of peak strength and residual strength. With the increase of temperature, the effective cohesion and friction angle increased first and then decreased. The relationship between effective strength parameters and $CaCO_3$ content evidenced the difference in strength enhancement of MICP-treated sand at different temperatures can be attributed to the amount of $CaCO_3$ precipitation.

3. SEM images, EDS and XRD results demonstrated that the $CaCO_3$ crystals produced at different temperatures had the form of rhombohedral calcite. Most calcite crystals precipitated as clusters on particle surfaces or at particle contacts. As the temperature rose from 4 to 35℃, the crystal

clusters grew larger while they 'preferred' to precipitate at particle. At 50℃, the cluster sizes were small, and no preference in location against the sand particle surfaces over the particle contacts.

4. The mechanical behavior of MICP-treated sand is closely related to the treatment temperature that needs to be tuned specifically for the purposes of each MICP application. In cases where temperature cannot be controlled, a proposed solution is the alteration of the MICP recipe, and specifically the number of bacterial solution injections.

Acknowledgements

Y.W. acknowledges the financial support of National Natural Science Foundation of China (Grant No. 52171262, 42141003), Science and Technology Innovation Committee of Shenzhen (Grant No. JCYJ20210324103812033) and Southern Marine Science and Engineering Guangdong Laboratory (Guangzhou, Grant No. K19313901) for conducting this study.

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