



Factors affecting the performance of microbial-induced carbonate precipitation (MICP) treated soil: a review

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Abstract

Soil stabilization technology based on microbial-induced carbonate precipitation (MICP) has gained widespread interest in geotechnical engineering. MICP has been found to be able to improve soil strength, stiffness, liquefaction resistance, erosion resistance, while maintaining a good permeability simultaneously. MICP processes involves a series of biochemical reactions that are affected by many factors, both intrinsically and externally. This paper reviews various influential factors for MICP process, including bacterial species, concentration of bacteria, temperature, pH, composition and concentration of cementation solution, grouting strategies, and soil properties. Through this comprehensive review, we find that: (1) the species and strains of bacteria, concentration of bacteria solution, temperature, pH value, and the cementation solution properties all affect the characteristics of formed calcium carbonate, such as crystal type, appearance and size, which consequently affect the cementation degree and distribution in geomaterials; (2) the condition with temperature between 20 and 40 °C, pH between 7 and 9.5, the concentration of the cementation solution within 1 mol/L, and high bacteria concentration is optimal for applying MICP in soil. Under the optimal condition, relatively low temperature, high pH value, and low concentration of cementation solution could help retain permeability and vice versa; (3) the effective grain size ranging from 10 to 1000 μm. MICP treatment works most effectively for larger size, well-graded sand; (4) the multi-phase, multi-concentration or electroosmotic grouting method can improve the MICP treatment efficiency. The grouting velocity below 0.042 mol/L/h is beneficial for improving the utilization ratio of cementation solution. The recommended grouting pressure is generally between 0.1 and 0.3 bar for MICP applications in sand and should not exceed 1.1 bar for silty and clayey soils.

Keywords MICP · Influencing factors · Microbial-induced carbonate precipitation · Calcium carbonate · Temperature · pH · Mechanical behavior · Microstructure

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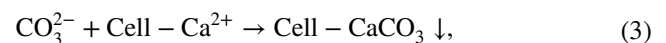
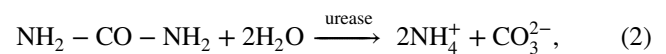
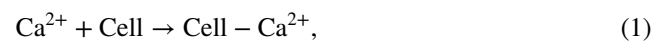
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Introduction

With the growth of the global population and urbanization, more and more infrastructure needs to be built. In this process, some soils with poor engineering properties are often encountered and must be improved to meet construction requirements. Commonly, there are two categories of soil improvement technologies. One is physical technology, such as increasing soil density by compaction, enhancing the confining pressure by reinforcement (Tang et al. 2007, 2016; Chen et al. 2019). The other is chemical technology, such as adding cement, lime, fly ash or organic adhesive materials to chemically stabilize soil so as to improve soil hydro-mechanical behavior (Amini and Ghasemi 2019; Ikeagwuani et al. 2019).

In recent years, a biological technology based on microbial induced carbonate precipitation (MICP) for soil improvement has attracted wide attention in geotechnical engineering. The MICP technology utilizes some bacterial strains in nature which could deposit calcium carbonate to fill and repair cracks in stone and concrete materials, prevent building leakage, avoid the liquefaction of sandy soil, and prevent soil erosion, slope failure and other types of disasters (Chu et al. 2013; DeJong et al. 2013; Liu et al. 2019). MICP-based soil modification technology is simpler than traditional chemical soil modification technology and has less impact on environment. Compared with the traditional chemical grouting, the bacteria solution and cementation solution used in this method have lower viscosity and are easy to infiltrate into geotechnical materials (Chu et al. 2011, 2012; An et al. 2019), which makes it suitable for dealing with the deeper and thicker geomaterials (Ivanov and Chu 2008). In addition, the mechanical/hydraulic properties of rock and soil improved by MICP can also maintain superior durability (Mitchell and Santamarina 2005). Common mineralization

microbes include denitrifying bacteria, sulfate reducing bacteria, oxidizing bacteria, and urease-producing bacteria (Li et al. 2011). Urease-producing bacteria have attracted wide attention in geotechnical engineering because of their low cost, high efficiency in triggering cementation, controllable reaction process, and straightforward isolation and harvest process. The mechanism of ureolysis-based MICP is that urease-producing bacteria can absorb Ca^{2+} on the cell surface from surrounding environment, and at the same time, urea can be decomposed into CO_3^{2-} , HCO_3^- , NH_4^+ by urease secreted from the cell. When Ca^{2+} binds to CO_3^{2-} , a large number of calcium carbonate crystals can be formed on the cell surface (Fig. 1) which can bond the granular particles and fill the internal pores and cracks of geomaterials (Chu et al. 2012; Muynck et al. 2010). The main reaction equations are as follows (DeJong and Fritzes 2006):



Bacillus and *Sporosarcina* are the most typical strains of urease-producing bacteria. Compared with other bacteria, these two species have better adaptability to the environment, higher specific surface, and less aggregation between cells (DeJong et al. 2010; Jiang et al. 2019). In addition to the urea hydrolysis process, they can also use urea as energy and nitrogen source through respiration, in which CO_2 is obtained and converted into CO_3^{2-} in alkaline environment, subsequently metabolizing and depositing calcium carbonate (Muynck et al. 2010; DeJong and Fritzes 2006; Montoya et al. 2013; Qian et al. 2015). In addition, *Bacillus*

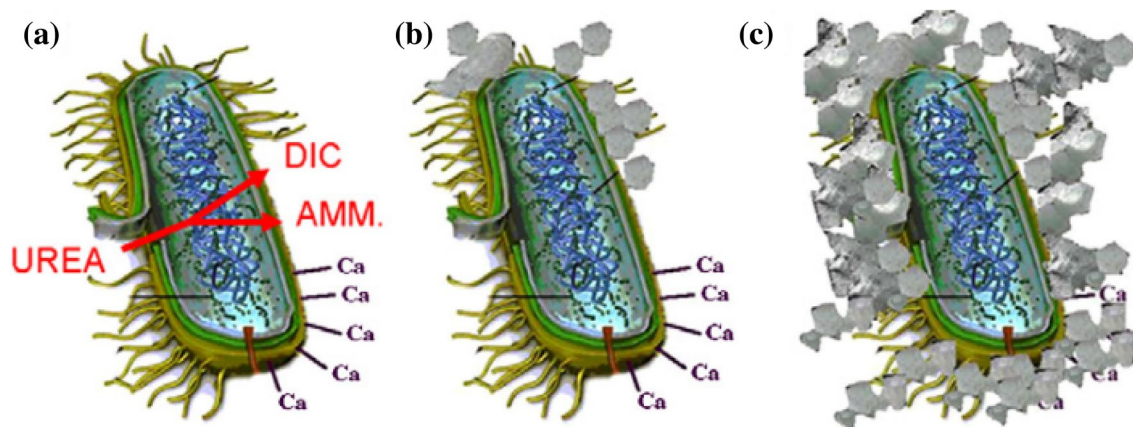


Fig. 1 Schematic diagram of calcium carbonate precipitation induced by urease producing bacteria (DeJong and Fritzes 2006)

and *Sporosarcina* could produce calcium carbonate more quickly and with high yield. Therefore, they are mainly used to trigger MICP for the stabilization/improvement of weak geomaterials.

This paper reviews various physical, chemical, biological, and environmental factors that affect the performance of ureolysis-based MICP-treated geomaterials, highlights existing studies regarding the optimization of MICP technology based on these factors, and gives an insight into how to achieve MICP optimization in various applications. The structure of the paper is organized as follows: “The species and strains of microbes”, “Bacterial solution concentration”, “Temperature”, “pH”, “The composition and concentration of cementation solution”, “Soil properties”, and “Grouting technology” discuss the dependence of MICP cementation on the species and strains of microbes, bacterial solution concentration, temperature, pH, the composition and concentration of cementation solution, soil properties, and grouting technology, respectively. “Future opportunities and challenges” discusses the future opportunities and challenges on MICP research and application. “Summary” summarizes the main conclusions obtained from this review work.

The species and strains of microbes

The diverse microbe species and strains would affect the characteristics of calcium carbonate crystal such as crystal precipitation rate and morphology including crystal phases, size, structure, and appearance, which can largely determine the cementation effect. Therefore, it is essential to understand the relationship between microbe species/strains and the characteristics of calcium carbonate crystals.

Generally, calcium carbonate produced by different ureolytic bacteria differs in crystal type and morphology. This is primarily attributed to the extracellular polymeric substances (EPS) (composed of proteins and polysaccharides), which are deposited on the surface of bacterial cells. Different ureolytic bacteria species produce EPS that vary greatly in their biochemical properties, which consequently results in diverse crystal type, morphology during MICP process. Tournay and Ngwenya (2009) found an organic carbon function from the EPS on the surface of *Bacillus licheniformis*,

which could reduce the saturation of the calcium carbonate surrounding the bacteria cells and precipitate crystals in the form of vaterite. Ercole et al. (2012) extracted an EPS that could induce calcite from a strain of *Bacillus sphaericus*. Calcite is a preferred phase of calcium carbonate precipitation for MICP applications, as it has the best stability and a good binding effect macroscopically (Meldrum and Cölfen 2009). Vaterite is a metastable crystal form and easily transforms into calcite (Kralj et al. 1994). However, the cementation effect after transformation has not been reported in literature. Sporadic studies also reported that aragonite has a good cementation effect (Gorospe et al. 2013), but more studies, in particular microscopic research, are needed for validation.

In terms of crystal size, large-size crystals are conducive to the cementation of coarse-grained soil and also contribute to the improvement of the mechanical properties of geomaterials. On the other hand, small-size crystals are more conducive to the cementation of fine-grained soil and the improvement of soil impermeability (Al Qabany et al. 2012; Al Thawadi 2008; Zeng et al. 2019). In terms of crystal morphology, the spherical, ellipsoidal, and irregular cubic-like calcium carbonate contributes to better cementation (Meldrum and Cölfen 2009; Rong et al. 2013; Wang et al. 2019).

By studying the characteristics of calcium carbonate crystals produced by various ureolytic bacteria strains, the optimal strain can be selected to maximize MICP efficiency. Dhami et al. (2013) compared the crystal characteristics of five strains of *Bacillus* and found the different types, sizes, and appearances of crystals secreted by different strains under the same culture. Table 1 (modified from Dhami et al. 2013), summarizes the characteristics of calcium carbonate crystals secreted by different bacteria.

Fundamentally, the activity of urease secreted by different bacterial strains varies. McCoy et al. (1992) found that the activity of urease secreted by *Ureaplasma urealyticum* was ten times that of *Bacillus Sporosarcina*. This is because that the urease secreted by *U. urealyticum* has more substructural protein units and nickel ions than *Sporosarcina pasteurii*. It is generally believed that the higher the bacterial urease activity, the faster the rate of calcium carbonate formation, and the more calcium carbonate produced in a certain period of time (Whiffin 2004; Al salloum et al. 2017;

Table 1 Characteristics of calcium carbonate crystals secreted by different bacteria (collected from Dhami et al. 2013)

Species	Crystal-type	Size (μm)	Appearance
<i>Bacillus megaterium</i>	Mainly calcite, little vaterite	30–50	Spherical, ellipsoidal, rhombic, triangular, with smooth surface
<i>Bacillus subtilis</i>	Mainly calcite, trace vaterite	10–50	Spherical, ellipsoid, with smooth surface
<i>Bacillus thuringiensis</i>	Mainly vaterite, little calcite	2–15	Small size round, circular, rectangular
<i>Bacillus cereus</i>	Mainly calcite, trace vaterite	15–40	Needle shape, lamella shape, irregular square shape
<i>Fusobacterium</i>	Vaterite	2–10	Mesh-like lamella, with rough surface

Okwadha and Li 2010; Stocks-fischer et al. 1999; Huang et al. 2009). Higher calcium carbonate content in geomaterials significantly contributes to their mechanical and hydraulic properties, and erosion resistance. Zhang et al. (2014) used two groups of *S. pasteurii* solution with urease activity of 12.2 mmol/L·min and 26.1 mmol/L·min to solidify cylindrical sand column at a concentration of 0.5 mol/L cementation solution. The cementation solutions were divided into three types according to different calcium sources, namely urea—calcium chloride, urea—calcium nitrate, urea—calcium acetate, and the molar ratio of urea to calcium source is 1:1. The unconfined compressive strength (UCS) of the high urease activity group reached twice or more of the low urease activity group after three grouting. Therefore, it is considered that the strains with high urease activity would have a better cementation effect on geomaterials.

In recent years, many research works have focused on identifying, isolating, and cultivating urease-microbes that are able to induce calcium carbonate with excellent properties, like high mechanical strength crystal, high CaCO₃ yield, stable crystal phase, and stronger adaptability to the environment. These research efforts are summarized in Table 2. It is noteworthy that Cussac et al. (1992) introduced the urease gene of *Helicobacter pylori* into *E. coli* plasmid to obtain the gene recombinant *E. coli* capable of secreting urease. This attempt provided a new approach for microbe selection in MICP. Whitaker (2016) integrated the high-production urease gene of a urease-producing microbe, *urea bacillus*, into *E. coli* plasmids, and induced Bacillus gene recombinant *E. coli* (pET28(b)-UreAG) with high urease solubility and productivity by genes targeted mutagenesis. The urease activity of strain SDM2 could reach 15.56 mmol/L·min, which was similar to that of *S. pasteurii*, making it viable for potential MICP applications. Referring to these examples, it is promising to obtain strains using gene recombination technology, which are highly efficient in inducing calcium carbonate precipitation, with better cementation property and superior adaptability to complex environment.

Bacterial solution concentration

In the MICP process, bacteria cells have two major roles in the formation of calcium carbonate crystals. First, bacteria act primarily as nucleation sites in the formation of calcium carbonate crystals. By decomposing the urea in the solution, bacteria release a large amount of HCO₃⁻, CO₃²⁻, and OH⁻, providing the required ionic components and alkaline environment for the formation of calcium carbonate (Ferris et al. 1987). Second, the EPS and negative ion groups on the surface of bacteria cells could also act as nucleation sites for calcium carbonate crystals and also regulate the type and morphology of crystals (Cheng and Qian 2006; Dhama

et al. 2013; Al salloum et al. 2017). Microscopically, the amount of microorganisms would greatly affect the concentration and supersaturation of H⁺, Ca²⁺, and CO₃²⁻. The aggregation and flocculation of bacteria cells also control the growth pattern of calcium carbonate at the nucleation site. Therefore, the bacterial concentration can apparently affect the crystal morphology, production of calcium carbonate and the cementation effect of geomaterials (Bosak et al. 2004). Whiffin (2004) measured the urease activity of *S. pasteurii* solution at various cell concentrations. It was found that there was no obvious correlation between the concentration of the bacterial solution and the unit urease activity. In particular, the urease activity of some samples could be ten times higher than others with the same bacterial concentration, as shown in Fig. 2. However, Okwadha and Li (2010) found that the urea decomposition rate and calcium carbonate precipitation amount were positively correlated with the bacterial cell concentration. And when the urea as well as calcium ion concentration reached a certain level, the bacterial cell concentration became the main factor. Al-Thawadi and Cord-ruwisch (2012) focused on the effect of bacterial cell concentration on the size of the calcium carbonate crystals. They diluted the original bacterial culture by a sterile saline solution to reach the desired dilution factors (30 g/L, 3 g/L, 0.3 g/L, 0.03 g/L). It was observed that the average size of crystals increased with the increasing bacterial cell concentration, as shown in Fig. 3. Cheng et al. (2007) studied the relationship between cell concentration and the morphology of calcium carbonate crystal. They found that the crystal of calcium carbonate was rhombus and cube, sharing a similar morphology with calcite at low bacterial cell concentration, whereas the crystal of calcium carbonate was spherical and overlapped with agglomeration at high bacterial cell concentration, as shown in Fig. 4. Cheng et al. (2007) proposed that at low cell concentration, the nucleation sites of organic matter on the surface of bacteria were far apart, and the crystals growth did not interfere with each other, so it was easy to form natural cubic calcium carbonate. At high cell concentration, the bacterial cells flocculated with each other, and the polar groups and the organic macromolecule substances on the surface of bacteria were curled and intertwined to form a curved surface structure, finally leading to the spherical calcium carbonate. Finally, they concluded that the bacteria cell size, cell secretion size, the interaction between cells' organic substances and inorganic substances, and the electrostatic charge density on the surface of organic substances were the key factors affecting the morphology of crystal formation.

Based on the review of the characteristics of calcium carbonate formation at the micro-scale, it is feasible to achieve specific cementing effect by changing the bacterial concentration to control the appearance, size, and production of calcium carbonate. At the macro-scale, numerous

Table 2 Characteristics of different types of urease-producing bacteria and areas of application

Strains	Characteristics	Application	Reference
<i>Bacillus megaterium</i>	Isolated from calcareous soil, the urease activity was 690 U/ml (690 mmol/L·min), and the production of EPS and bacterial biofilm was higher (36.4 nmol/ml, 283 CFU/mm ² , respectively). 187 mg/ml	Repairing building materials, soil reinforcement, concrete protection	Dhami et al. (2013)
<i>Bacillus subtilis</i>	Isolated from calcareous soil, the urease activity was 515 U/ml (515 mmol/L·min), and the production of EPS and bacterial biofilm was higher, 26.8 nmol/ml, 194 CFU/mm ² , and calcium carbonate production 178 mg/100 ml		
<i>Sporosarcina pasteurii</i>	Strain B-20, induced by nitrosoguanidine, urease activity is up to 28.4 mmol/L·min, and the amount of calcium carbonate produced by 1 ml of the solution in 9 ml of 1.1 mol/L urea-calcium chloride solution is 8.49 mmol/ml, soy flour can be used as a nitrogen source, and is suitable for large-scale production with high genetic stability	Reinforcement of weak roadbed, reinforcement of liquefied sand, directional reinforcement of tunnels, repairing cracks in masonry	McCoey et al. (1992)
<i>Sporosarcina pasteurii</i>	Strain MTCC 5428, induced by UV, the urease activity was 550 U/ml (550 mmol/L·min), the pH tolerance value could be up to 11, and the production of EPS and bacterial surface biofilm was higher (37 nmol/ml, 352 CFU/mm ² , respectively), the cementation effect on sand is better, and the calcium carbonate content in the surface layer of sand after solidification is 40%	Repairing cracks and surface on building materials, improve the compressive strength of cement mortar	Whiffin (2004)
<i>Bacillus sphaericus</i>	Strain LMG 22,257, obtained from the Belgian Collection of Microorganisms, unit urease activity 0.23–0.85 mmol/L·min·OD (10–37 °C), calcium carbonate crystal-size could be up to 50 µm, better temperature adaptability (10–37 °C), the optimum mineralization temperature is 20–28 °C	Excellent performance in cementation and waterproofing, excellent mineralization effect, high temperature adaptability, suitable for surface repairing of masonry	Al salloum et al. (2017)
<i>Bacillus lentus</i>	Strain GCMCC No.6483, isolated from the nursery of Tsinghua University. The unit urease activity was 12.32 mmol/L·min·OD, the crystal-size could exceed 100 µm, the allowable survival temperature was 4–37 °C, and the allowable survival pH value was 7–9.5, the UCS strength of the sand column after cementation can reach 2.4 MPa, and the calcium carbonate content per gram of sand sample reaches 163.15 mg/g	Wide applicability, can be used to reinforce liquefied sand, weak roadbed, tunnel orientation reinforcement, foundation pit slope protection	Okwadha and Li (2010)

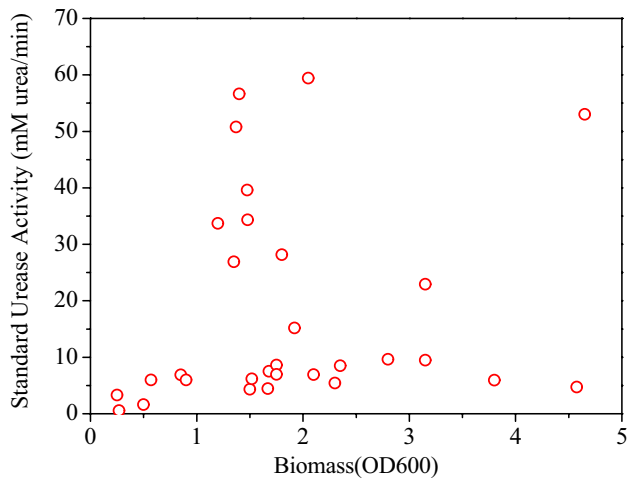


Fig. 2 Relationship between concentration of bacteria solution and urease activity (Modified from Whiffin 2004)

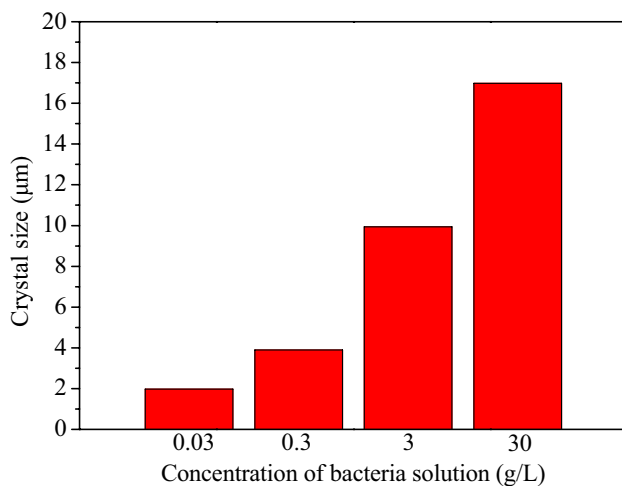


Fig. 3 Calcium carbonate crystal size of different concentration of bacteria solution (Modified from Al Thawadi and Cord-ruwisch 2012)

experimental works have been conducted to investigate the effect of bacterial concentration on the cementation of geomaterials. Soon et al. (2014) used *Bacillus megasporus* to induce MICP in tropical residual soil. They found that increasing the concentration of bacterial solution could significantly increase the content of calcium carbonate in soil, thereby improving the strength and lowering the permeability of soil. Zhao et al. (2014) cemented quartz sand using bacterial solution with OD_{600} of 0.3, 0.6, 0.9, 1.2, and 1.5, respectively. They found that urease activity, unit urease activity, calcium carbonate content, and UCS in cemented samples increased with the increase of bacterial concentration. When OD_{600} was 1.5, calcium carbonate content could reach 14.44%, yielding a high UCS of 2.22 MPa. Sharma and Ramkrishnan (2016) studied the reinforcing effects of *S. pasteurii* cell concentration (10^5 , 10^6 and 10^7 cfu/mL) on low-liquid-limit (47.1% sand, 53.6% fine-grained clay) and high-liquid-limit clay (17.48% sand, 82.28% fine-grained clay), respectively. The results showed that the UCS of both soils increased with elevated bacterial cell concentration. Chou et al. (2011) carried out direct shear tests on quartz sand specimens strengthened with the *S. pasteurii* octopus solution (10^3 cfu/mL and 10^7 cfu/mL). The results showed that the specimens treated with high concentration *S. pasteurii* cells had higher shear strength and lower volumetric strain, as shown in Fig. 5 and Fig. 6. In addition, Al-Salloum et al. (2017) measured the compressive strength of cement mortar specimens cured by the *S. pasteurii* solution. It was found that the strength of cement mortar specimens treated with bacterial solution of high cell concentration (10^9 cfu/mL) was higher than that of low cell concentration (10^8 cfu/mL) and also higher than that of the sterile solution control group. From the review of previous works, it can be seen that the higher concentration of bacterial solution in a certain range is beneficial for improving the engineering properties of a variety of MIC-treated soils.

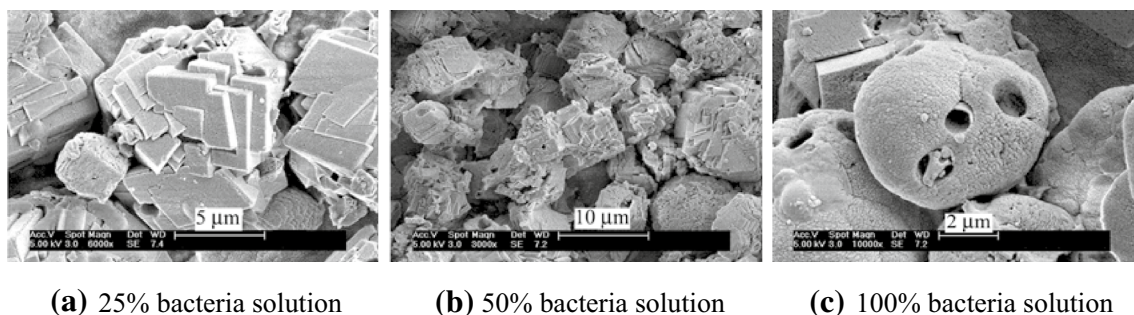


Fig. 4 Calcium carbonate crystal appearance of different concentration of bacteria solution (Cheng et al. 2007)

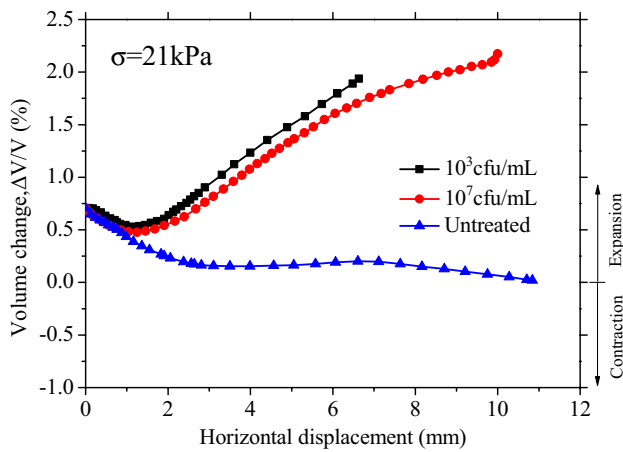


Fig. 5 Characteristics of straight shear test volume strain—horizontal displacement (Modified from Chou et al. 2011)

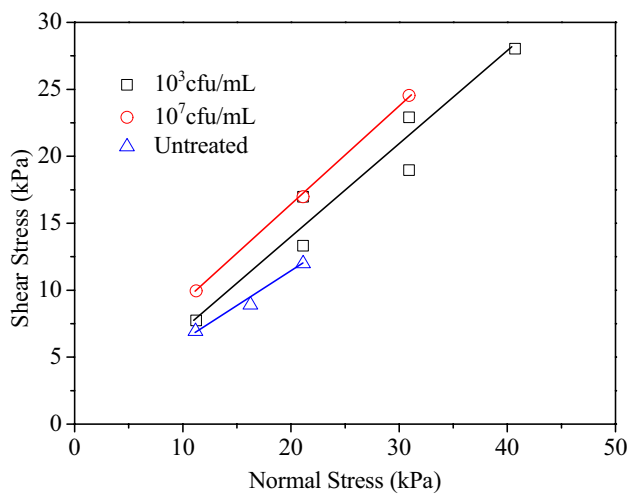


Fig. 6 Characteristics of straight shear test shear stress—normal stress (Modified from Chou et al. 2011)

Temperature

For the MICP process involving urease-producing bacteria, the change of temperature can affect the activity of urease and the degree of urea spontaneous ionization in cementation solution, and consequently the rate of ureolysis. Van Paassen (2009) studied the functional relationship between urease activity and temperature and proposed a fitting formula as shown in Eq. (4). The urease activity factor Q_{10} is 3.4 between 5 and 35 °C. r is the ureolysis rate at a certain time, r_0 is the initial ureolysis rate, and T is the temperature at the same time, as shown in Fig. 7.

$$\frac{r}{r_0} = \exp\left(\left(T - T_0\right) \frac{\ln Q_{10}}{10}\right). \tag{4}$$

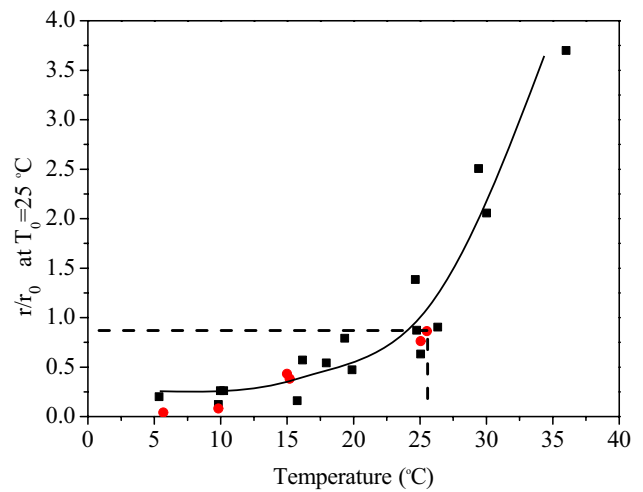


Fig. 7 The relationship between urease activity and temperature, where a constant amount of bacteria was supplied with 0.5 M urea (black quadrate symbol) and 0.5 M urea and CaCl₂ (red circular symbol) (Modified from Van Paassen 2009)

It has been pointed out in some literatures (e.g., Ferris et al. 2004) that the ionization constant of urea in solution increased about ten times when the temperature was elevated from 10 to 20 °C. The decomposition rate of urea also increased with the increased ionization constant of urea solution. Ferris et al. (2004) also studied the effect of temperature on the ureolysis rate of *S. pasteurii*. They mixed equal amounts of bacterial solution ($OD_{600} = 0.07$) with artificial groundwater containing CaCl₂, NaHCO₃, and urea. The bacteria ureolysis was measured at 10 °C, 15 °C, and 20 °C. Experimental results showed that the temperature was positively correlated with the ureolysis rate. Gillman et al. (1995) pointed out that the ureolysis rate of urease-producing bacteria increased by nearly 18 times when the ambient temperature increased from 5 to 20 °C.

Temperature fluctuation can also change the morphology and chemical stability of calcium carbonate crystals. Wang et al. (2005) analyzed the crystallization characteristics of the calcium carbonate produced by *S. pasteurii* using X-ray diffraction (XRD) and scanning electron microscope (SEM). They found that at 5 °C, bacteria-induced CaCO₃ crystal is mainly calcite with amorphous, aggregated poorly crystallized appearance, whereas at 25 °C it induced the uniformly distributed well-crystallized calcite with spherical-shaped appearance. However, at 50 °C, calcite and vaterite were crystallized with spherical, square, and spindle appearance and poor stability, as shown in Table 3 and Fig. 8. It is worth noting that the perfect crystallization degree and uniform distribution crystals at 25 °C demonstrated that the room temperature was in favor of MICP process.

In terms of the effect of temperature on the calcium carbonate crystal type, Kralj et al. (1994) found that the change

Table 3 Crystalline morphology of calcium carbonate induced by *Sporosarcina pasteurii* at different temperature (re-organized from Wang et al. 2005)

Temperature/°C	Crystal type	Crystal appearance
5	Calcite	Amorphous
25	Calcite	Spherical
50	Calcite and vaterite	Spherical and square and spindle

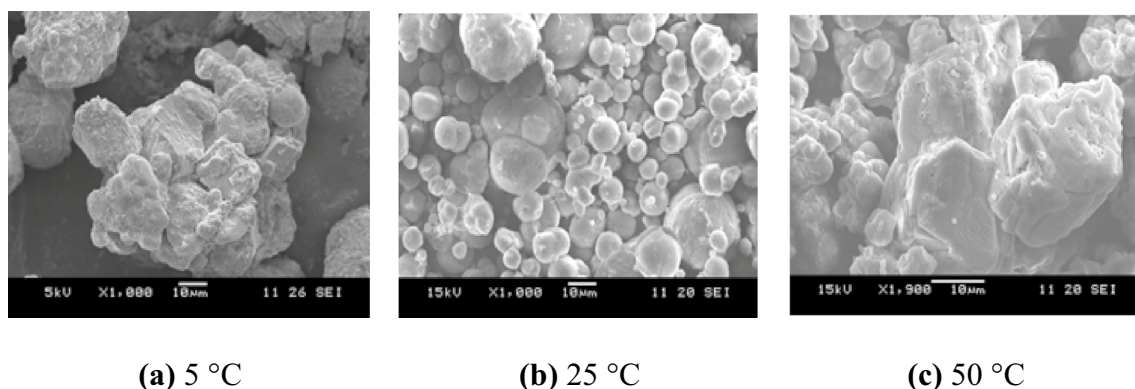
in temperature of an inorganic salt solution only affected the crystallization rate of calcium carbonate, not its crystal type. Somani et al. (2006) suggested that temperature had no significant effect on the crystal type of calcium carbonate during the crystallization process. However, in the process of microbial-induced calcium carbonate crystallization, the effect of temperature involves many factors, such as microbial metabolism, the activity of related enzymes, and the chemical stability of mineral components in solution. The interaction between these factors is complex and current state of the art has not yet thoroughly addressed the complexity. Further research efforts are needed in this area.

Temperature also affects the production of calcium carbonate in MICP process. Huang et al. (2009) treated sand–bacteria mixture with urea–CaCl₂ cementation solution (pH=8, $c[\text{Ca}^{2+}] = 25.2 \text{ mM}$) at 10 °C, 20 °C, 30 °C, and 40 °C, respectively. Calcite production was calculated after 10 days of mixing. The results showed that the calcite production was the highest at 30 °C, about five times higher than that at 40 °C or 20 °C. Meanwhile, there was almost no calcite precipitated at 10 °C.

The effects of temperature on the crystallization rate, morphology, and calcium carbonate production can consequently alter the engineering properties of MICP treated geomaterials. Cheng et al. (2014) used *B. sphaerius* to perform MICP in pure silica sand column (mean particle

size = 0.425 mm) with 180 mm length and 45 mm diameter at high (50 °C) and low (25 °C) temperatures, respectively. The results showed that the content of calcium carbonate in cemented specimens at 50 °C are three times higher than that at 25 °C. However, the UCS at 50 °C is only 60% of that at 25 °C. Using SEM, they found there are much more calcium carbonate formed at 50 °C, but the crystal diameter was only about 2–5 μm, with fine crystals dispersed on the surface of sand particles, as shown in Fig. 9a and b. At 25 °C, the size of calcium carbonate crystals was 15–20 μm and could well fill the cracks and bond to sand particles, as shown in Fig. 9c and d.

Based on Rodriguez-navarro et al. (2003), higher temperature induced higher urease activity and supersaturation in solution, causing a higher crystallization rate and smaller calcium carbonate crystal, which is not conducive to strength improvement. While at suitable room temperature, the crystallization rate of calcium carbonate is relatively slow, and the calcium carbonate crystals tend to follow a uniform distribution with larger particle size, which results in the enhanced mechanical strength and water resistance of soil. Muynck et al. (2013) applied the MICP process to limestone and tested the performance of *S. pasteurii* under various temperatures. It was found that MICP applied under high temperature (37 °C) exerted poor effects on improving the strength of limestone, but better effects on reducing the water absorption of limestone. Moderate temperature (20 °C and 28 °C) only contributed to the improved strength. At excessively low temperature (10 °C), the strength and water resistance have no obvious improvement. Bang et al. (2011) studied the effect of temperature on the wind erosion resistance of microbial reinforced soil. Blowers were used to simulate wind erosion of soil samples cemented by *B. sphaericus* at 20 °C, 35 °C, and 45 °C. It was found that the wind erosion resistance increased at elevated temperature. Peng et al. (2016) used *S. pasteurii* to study the effect of MICP on

**Fig. 8** Scanning electron microscopy of calcium carbonate produced at different temperature conditions (Wang et al. 2005)

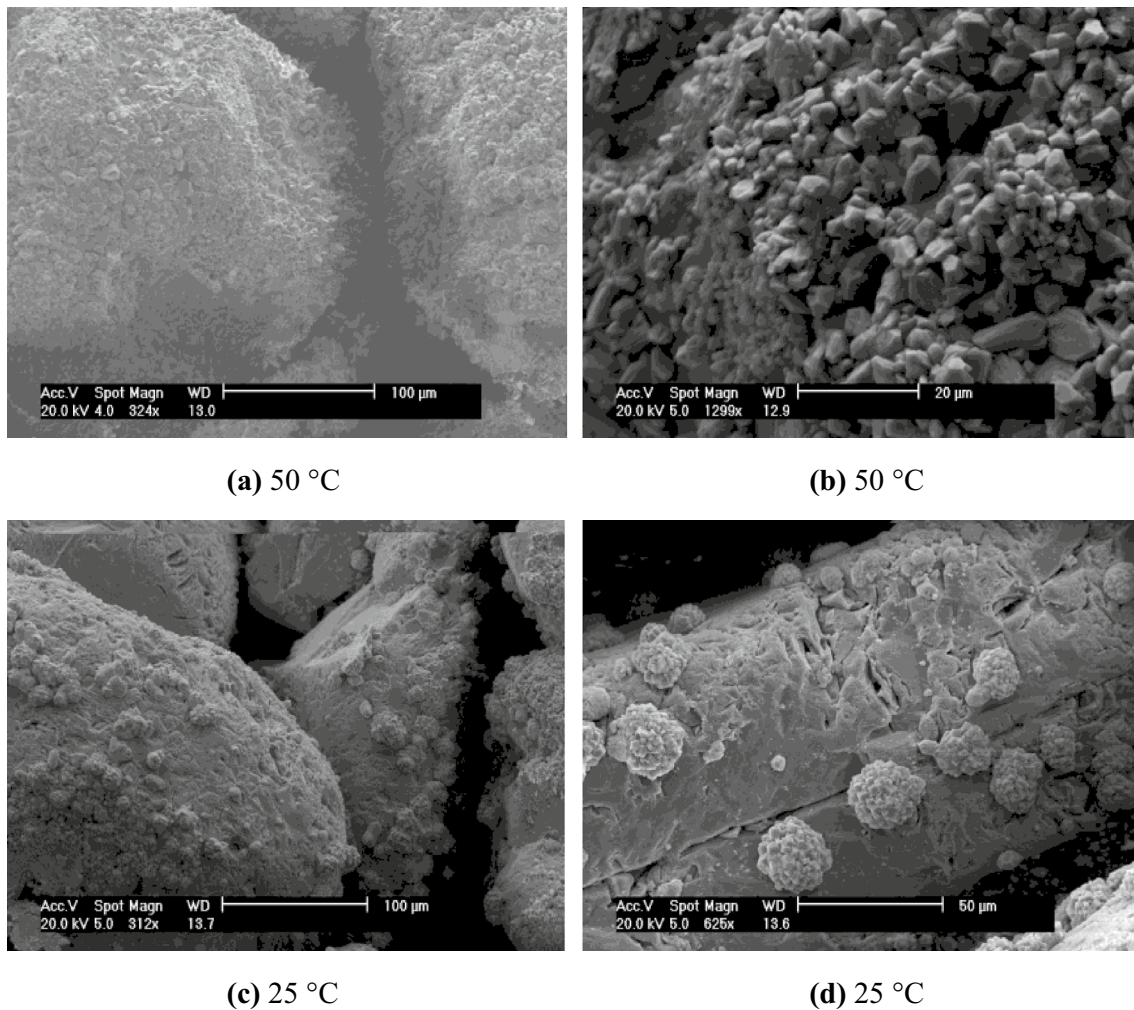


Fig. 9 Calcium carbonate crystals in sand particles under high and low temperature (Cheng et al. 2014)

cementing sand column at low temperature. It was found that the UCS of bio-cemented sand column at 10 °C is one-third of that at 25 °C, but permeability is three orders of magnitude higher.

From the review above, we can see that the optimum temperature of MICP process primarily depends on that of bacterial growth and metabolism, which is about 20–40 °C. This temperature range is very common in a natural environment, which means that optimized MICP process can be achieved for engineering applications in natural environments. However, at relatively low temperature, calcium carbonate crystal size is larger with a more uniform size distribution, resulting in a higher soil strength. At higher temperature, despite of the smaller crystal size and the lower soil strength, the resistance to wind erosion and water absorption are also stronger. Therefore, when using MICP to cement geotechnical materials, appropriate temperature for MICP implementation should be considered based on the specific engineering purpose.

pH

Most of the urease-producing bacteria commonly used in MICP are heterotrophic facultative aerobic ones, which are suitable for growing in alkaline environment. Most of the optimum growth pH of *Bacillus* and *Sporosarcina* is above 9.0. The pH range plays an important role in the urease-producing bacteria-related growth, metabolism, calcium carbonate crystallization, and the improvement of soil engineering properties.

Wiley et al. (1962) found that the optimum growth pH of *S. pasteurii* was about 9.25, and when pH exceeded 10.0, the bacteria stopped growing. Ryznarluty et al. (2015) determined the optimum growth pH values of *B. licheniformis*, *Bacillus cyclobacillus*, *Bacillus lateralis*, and *Bacillus filamentosa*, which were all around 9.5. In the aspect of microbial function metabolism, pH value can affect urease activity through biochemical reactions. Stocks-fischer et al. (1999) studied the effect of pH value on the activity of

urease extracted from *S. pasteurii*. It was found that urease activity peaked at pH=7.5–8, as shown in Fig. 10. pH not only has an effect on the performance of urease, but also substantially changes the bacterial metabolism, consequently changing the ability of bacteria to decompose urea. In order to understand the adaptability of *S. pasteurii* to pH value, Whiffin et al. (2007) inoculated the bacterial solution under different pH conditions using 25 mmol/L urea at 25 °C and measured the urea decomposition rate after incubating for 5 h. It was found that the unit urea decomposition rate of the bacterial solution is the highest when pH was in the range of 7–8, which was slightly lower than the optimum pH value for *S. pasteurii* growth.

In sedimentary mineralogy, first, the pH change alters the concentration of NH_3 , NH_4^+ , CO_3^{2-} , and HCO_3^- in pore solution, thus changing the calcium carbonate precipitation rate and production. Stocks-Fischer (1999) used 25.2 mmol/L CaCl_2 and urea solution (concentration ratio=1:1) inoculated with *S. pasteurii* at different pH levels. When the pH value exceeded 9, the calcium carbonate production reached the maximum and remained stable. Arunachalam et al. (2010) studied the effect of pH on the production of calcium carbonate by *B. sphaericus*. They found the production reached its maximum (about 25 mg/ml) at pH=8. Second, pH also plays an important role in the crystallization of calcium carbonate. As mentioned above, the higher the supersaturation concentration of ions (such as CO_3^{2-} , HCO_3^- , Ca^{2+}) in solution, the faster the mineral crystallizes and the smaller the crystal size, and vice versa (Cheng et al. 2014; Rodriguez-navarro et al. 2003). Whiffin (2004) found that when pH was less than eight, the concentration of CO_3^{2-} supersaturation was lower, and therefore, a lower precipitation rate of the calcium carbonate. Thus, the resulting crystals were larger and the bonding strength

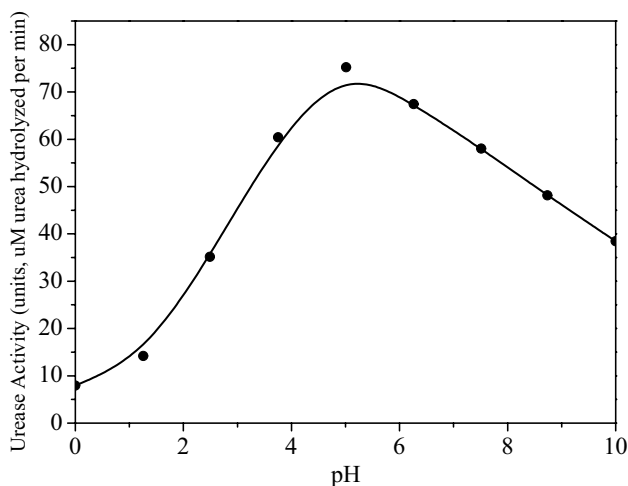


Fig. 10 Effect of pH on the urease activity (Modified from Stocks-Fischer et al. 1999)

between crystalline particles was higher, which was beneficial for improving cemented soil strength. Third, pH affects the morphological characteristics of calcium carbonate crystals. Cheng et al. (2007) analyzed the morphology of calcium carbonate crystallized by *Sporosarcina pasteurii* at pH=8.0 and 9.0. XRD analysis showed that the crystal phase of calcium carbonate was calcite at pH=8.0 and the shape was mainly spherical (Fig. 11a). At pH=9.0, the crystalline phase was still calcite, but the shape was mainly square and petal-shaped (Fig. 11b). Finally, pH could affect bacterial adsorption in soil directly or indirectly, thus controlling the bacteria-induced calcium carbonate spatial distribution in soil (Cheng et al. 2007). However, underlying mechanisms that interpret the pH effects on bacteria/ CaCO_3 distribution remain unclear, and SEM, high-density resistivity measurement, and fluorescent tracer technique should be used in figuring out more details regarding the governing mechanism of pH.

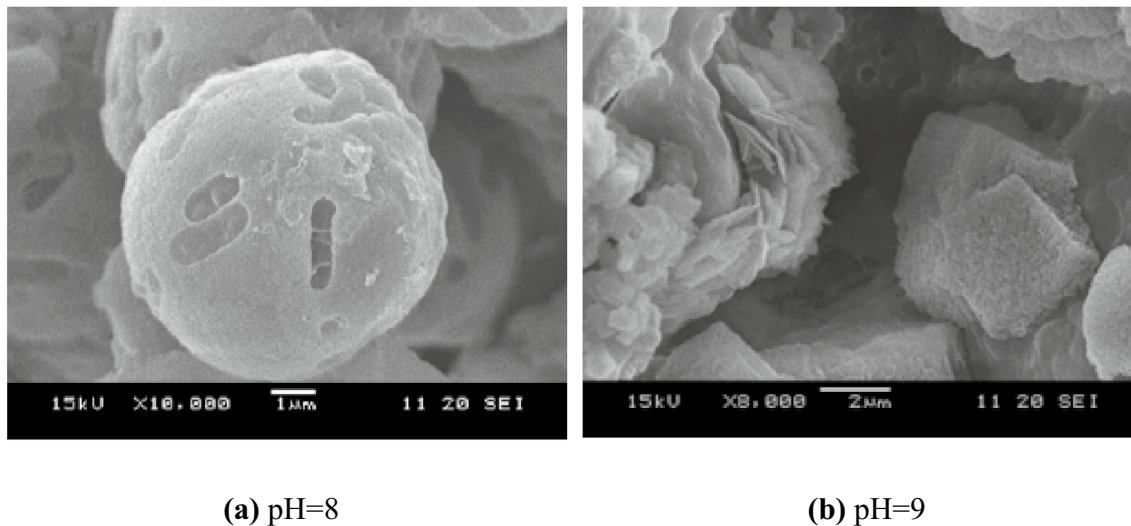
In terms of the effect of pH on the cementing effect of geomaterials, Keykha et al. (2017) used *S. pasteurii* to induce MICP in a silty soil (passing 75 μm sieve) at different pH values of 5, 6, 8, and 9. The results showed that the content of calcium carbonate and UCS of cemented samples increased with the increasing pH value. Cheng et al. (2014) cemented sand columns at an initial pH of neutral, acidic and alkaline conditions, respectively, and compared their UCS values and calcium carbonate contents. It was found that the content of calcium carbonate in the samples increased with pH. But the highest strength of cemented sand columns was obtained after curing under the neutral condition. Both acidic and alkaline conditions were detrimental to the strength of the cemented soil.

The composition and concentration of cementation solution

The cementing effect of MICP depends to a great extent on the composition and concentration of cementation solution, which not only affect the crystal type, appearance, size, composition of pore fluid, and pH value (Mcwhirter et al. 2002; Harkes et al. 2010; Soon et al. 2013), but also the distribution of bacteria and crystals in the pore spaces (Stocks-fischer et al. 1999). Macroscopically, it affects calcium carbonate production and precipitation efficiency (Cussac et al. 1992). It also affects the pore size distribution, compression behavior, shear strength, and stiffness of cemented geomaterials.

Cementation solution composition

The composition of cementation solution controls the growth and metabolism of microorganisms and the precipitation



(a) pH=8

(b) pH=9

Fig. 11 Calcite crystal morphology under different pH value (Modified from Huang et al. 2009)

process of calcium carbonate. More specifically, the composition of cementing cementation affects the activity of bacteria and urease, the type and morphology of calcium carbonate crystal as well as the properties of soils.

Hammes et al. (2003) found that the activity and mineralization ability of some strains of *S. pasteurii* could be enhanced in low concentration calcium solution at 30 mmol/L (urease activity increased by 4–10 times). However, the results obtained by other researchers showed that calcium ions had a negative effect on urease activity. Gorospe et al. (2013) dripped different types of calcium salts (concentration was 50 mmol/L) into the urease extract secreted by *S. pasteurii*. The urease activity in all samples generally decreased by 40–50% compared with the control group (water) without calcium salts, as shown in Fig. 12. These contradictory results implied that the role of calcium ions in the cementation solution for the mineralization of urease-producing bacteria was complex. De Muynck et al. (2010) found that different types of calcium salt affected the calcium carbonate crystal morphology. When CaCl_2 was used as calcium source, most calcium carbonate crystals were rhombohedral. When $\text{Ca}(\text{Ac})_2$ was used as calcium source, calcium carbonate was mainly globular. Moreover, Zhang et al. (2014) used *S. pasteurii* to cement industrial sand (particle size is 200–380 μm) with various calcium salts (0.5 mol/L). When using CaCl_2 , calcium carbonate was in the form of hexahedral calcite with smooth surface. When using $\text{Ca}(\text{Ac})_2$, calcium carbonate was mainly aragonite with needle crystal. When using $\text{Ca}(\text{NO}_3)_2$, calcium carbonate was mainly hexahedral calcite with rough surface, as shown in Fig. 13. In addition, Zhang et al. (2014) found that the UCS of the cemented soil using $\text{Ca}(\text{Ac})_2$ solution was 1.4 times of those using CaCl_2 or $\text{Ca}(\text{NO}_3)_2$. At the same UCS

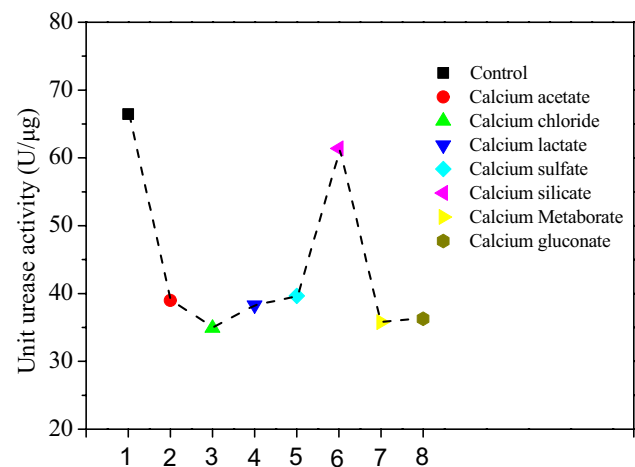


Fig. 12 Inhibition of different kinds of calcium salt solution on urease activity (only water was used in control sample) (Modified from Gorospe et al. 2013)

level, the pore size in the cemented soil followed a more uniform and wider distribution when $\text{Ca}(\text{Ac})_2$ was used as calcium source. Gorospe et al. (2013) studied the crystallization characteristics of MICP by *S. pasteurii* KCTC 3558 using CaCl_2 , $\text{Ca}(\text{Ac})_2$, calcium lactate, and calcium gluconate as calcium source. The results showed that using CaCl_2 led to the formation of calcite with rhombohedral shape. $\text{Ca}(\text{Ac})_2$ induced a lettuce-like or lamellar-shape vaterite. Calcium lactate mainly induced aggregated spherical shaped vaterite, which had the largest crystals, up to tens of microns. Calcium gluconate tended to induce spherical shape vaterite crystals of only about 5 μm . Some researchers claimed that organic/inorganic components and some

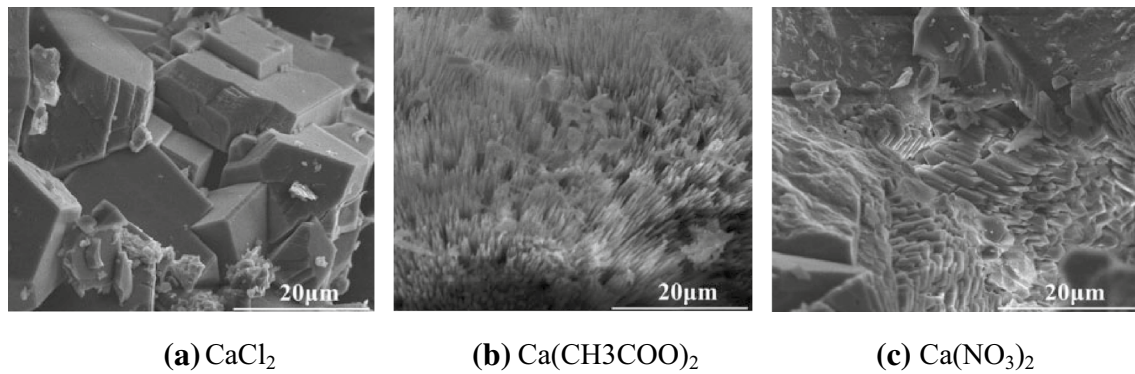


Fig. 13 Crystal morphology of CaCO_3 under different calcium sources (Soon et al. 2013)

proteins in the bacterial culture medium and cementation solution could be adsorbed onto the crystal surface during the formation of calcium carbonate, which could influence the type, size, and appearance of crystals (Muynck et al. 2008; Cheng et al. 2019). Consequently, the difference in crystal properties could largely determine the effectiveness of MICP in engineering applications. Achal and Pan (2014) compared the effects of CaCl_2 , $\text{Ca}(\text{Ac})_2$, $\text{Ca}(\text{NO}_3)_2$, and CaO on bacterial activity and calcium carbonate production in liquid environment using *Bacillus sp. CR2*. It was found that the bacterial growth rate, urease activity secreted, and the total amount of calcium carbonate produced reached the highest when CaCl_2 was used. Mortensen et al. (2011) used artificial seawater as cementation solution to strengthen sand column, which had higher calcium carbonate formation rate and shear wave velocity than those cured with conventional cementing fluid, reflecting the advantages of seawater as the cementation solution. Cheng et al. (2015) carried out more experiments in-depth. Their results showed that the UCS of quartz sand column obtained by seawater curing was higher than 1 mol/L urea- CaCl_2 solution with the same calcium carbonate content. After 100 times of seawater washing, UCS could reach 25 MPa. Meanwhile, adding sufficient urea to the seawater could promote the formation of magnesium carbonate, which could accelerate the overall precipitation rate and reduce the treatment times. Using seawater as cementation solution to reinforce coastal sand can reduce not only the amount of chemical reagents in MICP, but also the cost of treatment process, which provides a promising and viable source of cementation solution for large-scale coastal sand dune and reef reinforcement projects.

Cementation solution concentration

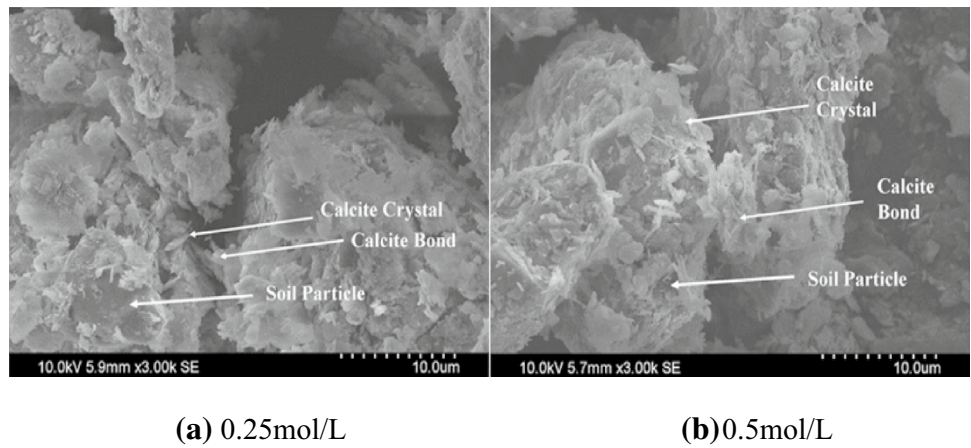
Most of cementation solutions in MICP are a mixture of urea and calcium salt, sometimes with trace nutrients (Stocks-fischer et al. 1999; Whiffin 2004; Qian et al. 2007a; Muynck et al. 2008; Arunachalam et al. 2010; Zhang et al. 2014;

Keykha et al. 2017; Tang et al. 2019). The concentration and proportion of cementation solution have a great influence on the activity of bacterial urease, production of calcium carbonate, mineralization efficiency, crystal structure, spatial distribution, and mechanical and hydraulic properties of cemented geomaterials.

Soon et al. (2013) found that calcium carbonate precipitated mostly at the grain-to-grain contact point of sandy soil at the cementation concentration of 0.5 and 0.25 mol/L when using *B. megaterium*. The calcium carbonate precipitated more densely at the concentration of 0.5 mol/L than at 0.25 mol/L, as shown in Fig. 14. Nemati et al. (2005) studied the effects of the concentration and proportion of cementation solution on MICP cementation effectiveness using *Proteus vulgaris*. It was found that the mineralization efficiency of calcium carbonate in solution was over 80% when the concentration of CaCl_2 was below 15 g/L. The production of calcium carbonate was almost zero when the concentration of CaCl_2 exceeded 20 g/L. The mineralization efficiency of calcium carbonate was 99% when the concentration ratio of CaCl_2 and urea was 2.5 and 3.0 (molar ratio 1 and 1.2). However, low concentration ratio could reduce mineralization efficiency, which consequently lowered cementation effect on soil. Figure 15 shows the effect of different mineralization efficiency on the cementation effect of quartz sand. Zhao et al. (2014) found that increasing urea concentration within a certain range (less than 1.6 mol/L) could increase urease activity of *S. bacillus*. Okwadha and Li (2010) found that, when urea concentration increased from 333 to 666 mmol/L, the initial concentration of calcium ion increased ten times (from 25 to 250 mmol/L) and the yield of calcium carbonate increased 100%.

Al Qabany and Soga (2013) studied the size and distribution of calcite crystals on the surface of quartz sand using *S. Pasteurii*. It was found that in the range of 0.25–1 mol/L, high-concentration cementation solution facilitated larger calcite crystals and more heterogeneous distribution, as shown in Table 4 [collated from (DeJong

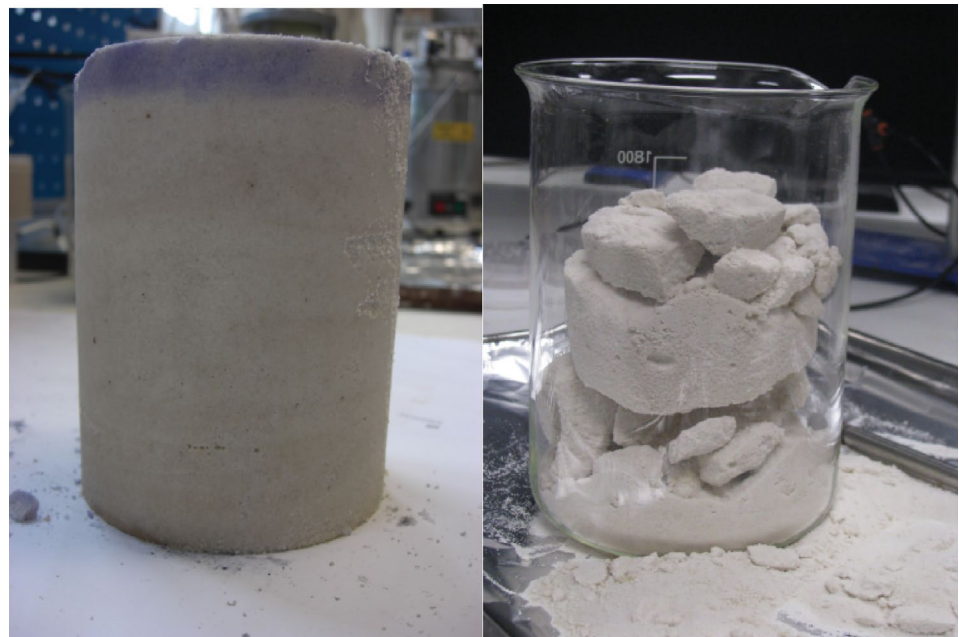
Fig. 14 Distribution of calcium carbonate under different concentration (Soon et al. 2013)



(a) 0.25mol/L

(b) 0.5mol/L

Fig. 15 Effect of mineralization efficiency on sand solidification (Mortensen et al. 2011)



(a) Mineralization Efficiency 70%-100%

(b) Mineralization Efficiency 20%

Table 4 Effect of different concentration of cementation solution on the size and distribution of calcite (re-organized from DeJong et al. 2010)

Concentration (mol/L)	Crystal size	Crystal spatial distribution
0.25	Small, uniform	Uniformly distributed on the surface of quartz sand particles
0.5	Medium, uneven	Not uniform, agglomeration occurs
1.0	Generally large, nonuniform	Extremely uneven, agglomeration occurs

et al. 2010)]. In other studies, it was reported that high-concentration cementation solution could reduce the activity of some carbonate-mineralizing bacteria. This is possibly related to the inhibition of the production of enzymes required for bacterial functional metabolism (Kunst and Rapoport 1995). Rivadeneyra et al. (2000) studied the carbonate mineralization by *Coriobacterium*

neisseriae and found that high-concentration cementation solution not only inhibits the activity of microorganisms, but also reduces the calcite-forming efficiency. Meanwhile, cementation solution concentration also affects calcium carbonate crystal type. In the study of improving the corrosion resistance of cement-based materials using *S. pasteurii*, Qian et al. (2009) found that the calcium carbonate

crystals were mainly calcite. The crystal became larger with the higher concentration of cementation solution (calcium source: calcium nitrate, the maximum concentration in the test: 0.3 mol/L). Meanwhile, they found that the appropriately controlled increase of Ca^{2+} concentration and usage of bacteria in the logarithmic phase during the MICP process could induce the formation of large vaterite crystals, reaching 100 μm at 0.3 mol/L calcium. In summary, concentration and proportion of calcium source and urea in the cementation solution largely determine the type and size of calcium carbonate crystals and consequently affect the efficiency of MICP.

In terms of the effect of cementation solution concentration on the mechanical properties of MICP treated geomaterials, Soon et al. (2014) cemented a low liquid limit residual silt in tropical rain forest of Malaysia with *Bacillus mega* with 0.25, 0.5, and 1.0 mol/L cementation solution with equal molar ratio of urea to calcium. The strength of the cemented soil increased by 26–57% at 0.25 mol/L, 25–69% at 0.5 mol/L, and no significant change at 1 mol/L, respectively. Some literatures have pointed out that the growth and metabolism of *B. megaterium* in urea-Ca cementation solution exceeding 1 mol/L could be inhibited (Nekolny and Chaloupka 2000; Li et al. 2019). It was also reported that the produced calcium carbonate crystals at high saturation and high concentration of cementation solution were smaller (Muynck et al. 2010). In addition, Ng et al. (2014) researched the crystal morphology and distribution of calcite in samples treated by different cementation solution concentrations was observed by SEM; it was found that calcite mainly grew in the contact area between soil particles, and calcite was most densely distributed at 0.5 mol/L. DeJong et al. (2010) studied the density of calcite in the process of cementing soil with *S. pasteurii*. By measuring the shear wave velocity of soil samples cured with different concentrations of CaCl_2 cementation solution in the range of 12–200 mmol/L, it was found that higher CaCl_2 concentration resulted in denser distribution of calcite and greater soil stiffness. In terms of improving the hydraulic properties of geomaterials, Van Paassen (2009) used *S. pasteurii* to solidify sandy soil under 1 mol/L Ca^{2+} condition, and the permeability could be reduced by 60%. Nemati and Vordouw (2003) explored the effect of concentration of urea-Ca cementation solution on improving the permeability of cylindrical sand-glass beads porous media (average diameter of sand = 225 μm , average diameter of glass beads = 75 μm , mass ratio 7:3) with a diameter of 2.5 cm and a height of 30 cm using *Proteus vulgaris*. It was found that the permeability was significantly reduced after perfusion with bacterial and cementation solution. The decrease of permeability was mainly caused by bacteria blocking the soil pores, whereas calcium carbonate did not show apparent clogging effect, as shown in Table 5.

Table 5 The decrease of the permeability of the sample under the different concentration of cementation solution (Harkes et al. 2010)

Cementation solution	Totally permeability reduce (%)	CaCO_3 caused permeability reduce (%)
5 g/L urea, 12.5 g/L CaCl_2	72	7
10 g/L urea, 25 g/L CaCl_2	75	20
15 g/L urea, 37.5 g/L CaCl_2	60	-0.04

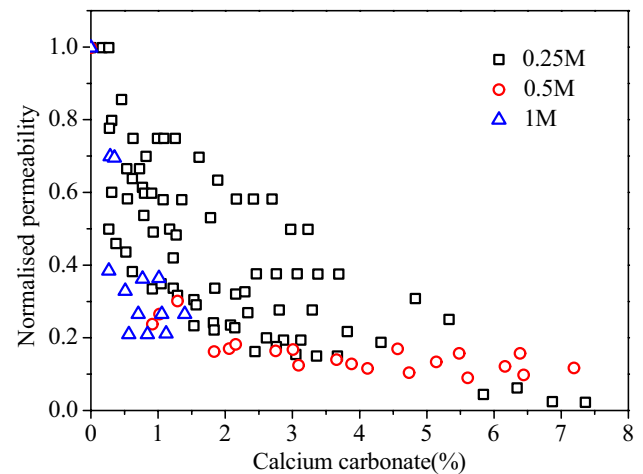
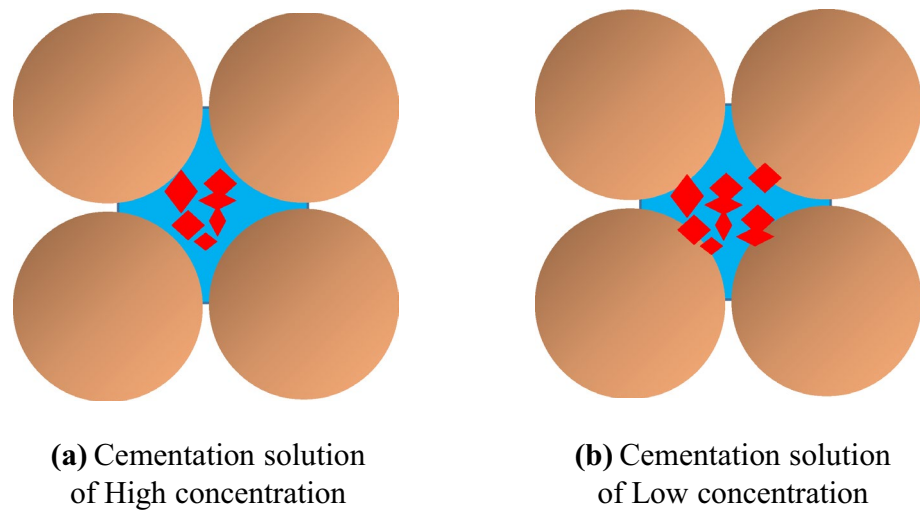


Fig. 16 Decrease in permeability with calcium carbonate precipitation for three chemical concentrations (Modified from Al Qabany and Soga 2013)

Al Qabany and Soga (2013) were positive about the clogging effect of bacterial calcium carbonate on soil pore. They explored the effect of cementation solution concentration on the impermeability of soil. As shown in Fig. 16, when the cementation solution was 1 mol/L, the permeability decreased by 30% with the majority increase of calcium carbonate content occurring at the initial stage of MICP, whereas the impermeability of soil reached its plateau with a longer curing time. When the cementation solution was 0.25 mol/L, the permeability of soil decreased slowly with the increase of calcium carbonate content.

After investigating the effect of cementing solution concentration on permeability reduction, Al Qabany (2011) provided an explanation to the underlying mechanism: high cementation solution concentration contributes to the formation of large calcite crystals and their non-uniform distributions, which in combination with the aggregation effects of bacteria results in the blockage of soil pores (Fig. 17a). Therefore, in the presence of highly concentrated cementation solution, soil permeability exhibited a strong spatial variation and decreased at a faster rate at the initial stage. Comparatively, at low cementation solution concentration, calcite precipitated at the contact points between soil

Fig. 17 Schematic of pore clogging as result of MICP using a high chemical concentration (left), and low chemical concentration (right) (Modified from Al Qabany and Soga 2013)



particles and distributed uniformly in soil matrix without local agglomeration effect (Fig. 17b). The seepage channel disappeared gradually with the increase of calcite content. Therefore, cemented soil's permeability showed a more gradual decreasing trend and had less spatial variabilities.

From the review of existing experimental studies, it can be seen that the cementation solution plays an important role in the MICP process. Variations in cementation solution composition/concentration can cause different cementing effects. Appropriate cementation solution type and concentration ratio need to be carefully selected for implementing MICP in various engineering applications.

Soil properties

Among a large variety of geomaterials that could be potentially treated by MICP, soil is particularly challenging, considering the complex soil properties such as mineral composition, particle size, gradation, and relative density of soil.

Mineral composition in soil could change the chemical and thermodynamic properties of the pore fluid, thus providing more nucleation sites for calcium carbonate precipitation. Therefore, soils with diverse mineral compositions are better hosts for MICP. Montoya (2012) cemented five different sands (which were rich in quartz, calcite, feldspar and iron oxide respectively) using *S. Pasteurii* and measured the shear wave velocity of cemented sands. It was found that different mineral compositions had significant effects on the precipitation rate of calcium carbonate, with the fastest precipitation rate obtained in the sand rich in calcite, as shown in Fig. 18.

Soil particle size is another important soil property that controls MICP efficiency. Soil particle size is directly related to the pore throat size in soil matrix, which controls whether bacteria can flow freely and distribute uniformly

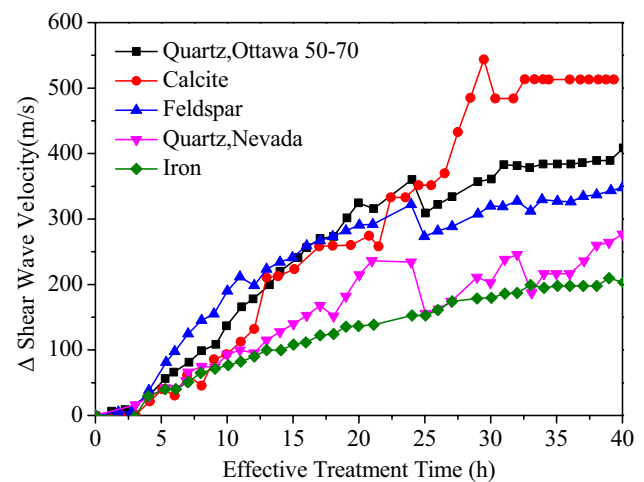


Fig. 18 Normalized shear wave versus effective time for varying soil mineralogy. Note that the 1:1:1 recipe was used for all soil types (Modified from Montoya 2012)

in soil matrix. In general, the size of *Bacillus* and *Sporosarcina* is mostly about 1–5 μm . Therefore, soils containing particles smaller than bacterial size (e.g., clay) could prevent the free flow of bacteria in soil matrix, leading to limited and heterogeneous calcium carbonate precipitation (Mitchell and Santamarina 2005). Moreover, larger particles (such as coarse sand, gravel, etc.) have less intergranular contacts and larger inter-granular distance. Thus, the majority of calcium carbonate coats on the surface of coarse particles rather than the contact points, which could also weaken the overall cementation efficiency. Many studies have been conducted to investigate the effect of soil particle size on MICP cementation efficiency. Rebata-Landa (2007) carried out a soil column cementation experiment using MICP technique (Fig. 19). Eleven soils with different particle sizes including kaolin, silt, fine sand, coarse

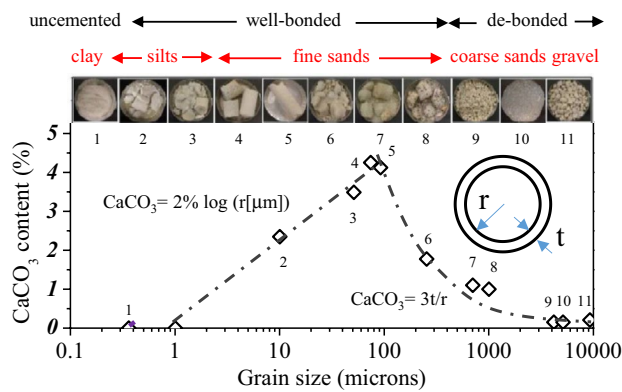


Fig. 19 CaCO₃ content and models as a function of the grain size (Modified from Rebata-Landa 2007)

sand and gravel (D_{10} ranges from 0.36 to 11,500 μm) were used for the experiment. The results showed that most tested soils had good cementation effect except the samples #1 (kaolinite, $d_{10} = 0.36 \mu\text{m}$, $S_a = 33\text{m}^2/\text{g}$), #9 (fine sand, $d_{10} = 5000 \mu\text{m}$, $d_{50} = 5000 \mu\text{m}$, $\text{Cu} = 1$), #10 (coarse sand, $d_{10} = 4000 \mu\text{m}$, $d_{50} = 5300 \mu\text{m}$, $\text{Cu} = 1.48$), and #11 (fine gravel, $d_{10} = 9000 \mu\text{m}$, $d_{50} = 11,500 \mu\text{m}$, $\text{Cu} = 1.67$), indicating that the soils with particle size between 10 and 1000 μm could be most effectively cemented. A formula was also proposed to calculate the content of calcium carbonate in two soils with different particle sizes (Fig. 19).

Regarding the relationship between particle size and the soil strength after MICP treatment, Amarakoon (2016) compared the UCS of two silica sands (Mikawa sand: $d_{50} = 600 \mu\text{m}$; Toyoura sand: $d_{50} = 200 \mu\text{m}$) after MICP treatment. It was found that Mikawa sand had better cementation effect and UCS could reach 3 MPa. Meanwhile, Toyoura sand showed relatively poor cementation effect with only about 1 MPa UCS. Similarly, Zhao et al. (2014) found that the mechanical strength of standard sand with $d_{50} = 460 \mu\text{m}$ was twice stronger than that of Mississippi sand with $d_{50} = 330 \mu\text{m}$ under UC tests. However, it is noteworthy that Cui et al. (2016) applied MICP and tested UCS on three Xiamen standard sands with particle sizes ranging from 50 to 125 μm , 125 to 250 μm , and 40 to 500 μm , respectively. The measured UCS of the three soils were 969 kPa, 754 kPa, and 152.7 kPa, respectively, which showed a negative correlation between particle size and soil strength, contrary to the result of previous researchers. Cui et al. (2016) explained that smaller soil particle was conducive to calcium carbonate accumulation and stack, which could promote the cementation effect. On the other hand, in larger soil particles, calcium carbonate tended to attach to the surface of single soil particle, which has less contribution to the inter-granular bond enhancement. From the review of different experimental study results, it can be seen that the mechanism of soil particle size on MICP

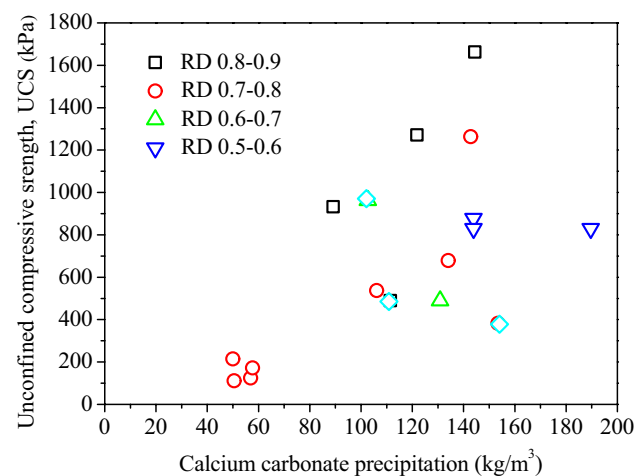


Fig. 20 The relationship between relative density and unconfined compressive strength of quartz sand (Modified from Al-Qabany 2011)

cementation effectiveness is still not clear and further studies are needed.

The particle size distribution and relative density of soil are also important factors to determine the MICP cementation effect. Mortensen et al. (2011) measured the shear wave velocity of MICP treated specimens and found that the cementation in well-graded coarse-grained sand was better than that of poorly-graded fine-grained sand. Al Qabany (2011) studied the relationship between the relative density and strength of cemented British standard quartz sand. It was found that there was a positive correlation between strength and the relative density of quartz sand (Fig. 20). Kim et al. (2013) applied the MICP treatment to three silty soils with 60%, 75% and 90% relative compaction (64.3% of the soil particles passed through 200 # sieve). It was found that the content of calcium carbonate in the soil with 90% relative compaction was the highest, which was 1.5–2 times higher than that with 60% and 75% relative compaction, respectively. Some researchers reported that calcium carbonate was preferably deposited at the contact points between soil particles in the MICP process and there was a positive correlation between the number of grain-to-grain contact points in soil and the cementation efficiency in soil (Joer et al. 2002). The sandy soils with well size grading and greater relative compaction have more grain-to-grain contact points, and thus have better cementation, as validated by Rowshanbakht et al. (2016). They applied MICP to cylindrical specimens of siliceous sand ($d_{50} = 200 \mu\text{m}$) at relative density of 40%, 70%, and 85% and tested the UCS of samples extracted from the top and bottom of the cylinder. Results showed that the strength increased with the increase of relative density (Fig. 21). Soon et al. (2013) carried out direct shear tests on medium-sized sand columns with relative density of 0.85, 0.90, and 0.95, respectively. It was found that the shear

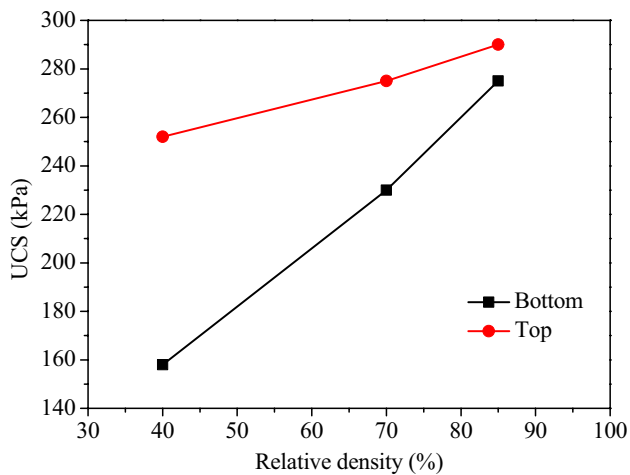


Fig. 21 The relationship between the relative density of siliceous sand and UCS (Modified from Kim et al. 2013)

strength of sand after MICP treatment was also positively correlated with the relative density.

Through the summarized review of existing experimental works, it can be seen that higher relative density and well-graded particle size distribution lead to enhanced cementation and binding in soils. In engineering practice, increasing the relative density by soil compaction or improving soil gradation levels are effective ways to improve the cementation effect of MICP.

Grouting technology

Grouting technology (grouting method, grouting speed and grouting pressure) is critical to the in-situ implementation of the MICP cementation method. The inhomogeneity of MICP cemented soil is one of the most challenging factors that restricts the up-scaled use of MICP in field applications. Via using different grouting methods, it is possible to alter the homogeneity and distribution of calcium carbonate precipitation after MICP treatment, and consequently the strength and permeability of MICP treated soil. The simplest grouting method is to inject the mixture of bacterial and cementation solution into the soil. However, this method is likely to cause the clogging of precipitated calcium carbonate crystals around the injection point and their non-uniform distributions over time. Thus, the spatial distribution of soil strength after MICP treatment is also highly non-uniform (Joer et al. 2002).

Whiffin et al. (2007) proposed a two-phase grouting method. First, the bacteria solution was injected and retained inside the soil specimen for a period of time. Then, cementation solution was injected. This grouting method could reduce the clogging of calcium carbonate around injection

point and improve the uniformity of calcium carbonate inside the soil specimen. Compared with the traditional mixing grouting method, the two-phase method could reduce the porosity by 20%. Harkes et al. (2010) improved the two-phase grouting method by injecting 0.05 mol/L CaCl_2 solution at low speed after the injection of bacteria solution but before the injection of cementation solution. This method maximized the flocculation reaction between bacteria and calcium ions so that the previously uniformly distributed bacteria in soil would not be washed out by the subsequent cementation solution (Tobler et al. 2012). With respect to the bacterial adsorption after pretreatment by salt solution, Chu et al. (2014) showed that the bacterial adsorption rate in sand generally increased by 29–37% when the soil was pretreated by calcium, iron, and aluminum salts. Cui et al. (2017) proposed a multi-concentration phase combined grouting method: injecting 0.5 mol/L cementation solution at the early stage and then 1.0 mol/L cementation solution at the later stage. Compared with single concentration grouting method, the multi-concentration phase combined grouting method could obtain higher soil strength with fewer groutings. Xu et al. (2017) used the electroosmotic biological method (EBM) by applying an electric field with a certain voltage gradient in silty soil to carry out MICP cementation experiments. The results showed that, compared with gravity grouting and low-pressure grouting method, EBM could facilitate bacteria diffusion in soil voids and induce a uniform distribution of calcium carbonate. This provided a new possible solution for improving the uniformity of MICP in soils, particularly those containing fines.

Grouting rate determines the utilization efficiency of cementation solution. Al Qabany (2011) injected the same volume of urea- CaCl_2 cementation solution with different concentrations into two graded British standard sands (Grade D: $D_{50} = 165$ μm , $d_{90} = 250$ μm ; Grade E: $d_{50} = 165$ μm , $d_{90} = 250$ μm) to test the utilization efficiency of cementation solution by *S. pasteurii* under different grouting rates. The results showed that for all cementation solution concentrations and sand gradations used in this study, the utilization efficiency of cementation solution could reach 90% at a grouting rate of 0.042 mol/L/h. However, the utilization efficiency was reduced to 50% at the grouting rate of 0.084 mol/L/h, as shown in Fig. 22. Whiffin et al. (2007), DeJong and Fritzges (2006) and Rebata-Landa (2007) had similar conclusions: the utilization rate of cementation solution reached 88%, 92%, and 95% at a grouting rate of 0.0088 mol/L/h (concentration 1.1 mol/L), 0.025 mol/L/h (concentration 0.1 mol/L) and 0.042 mol/L/h (concentration 0.25 mol/L), respectively. From these existing studies, it can be found that the utilization rate of cementation solution maintains at a high level at relatively low grouting rate. However, if the grouting rate is lower than the rate of calcium carbonate

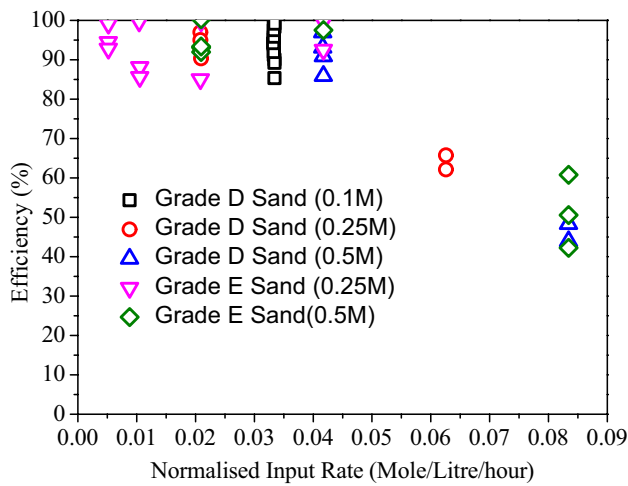


Fig. 22 The relationship between the grouting rate and efficiency of cementation solution (Modified from Al-Qabany 2011)

formation through MICP, the calcium carbonate crystals are likely to accumulate around and block the grouting point, which will prevent the further injection of cementation solution, resulting in non-uniform distribution of cementation in soil.

Grouting pressure is another important grouting parameter that determines mechanical and hydraulic properties of MICP treated soils. For bio-grouting in sandy soils, the grouting pressure is generally controlled between 0.1 and 0.3 bar (Nemati et al. 2005; Rowshanbakht et al. 2016). For fine silt and silty clay, the grouting pressure needs to be increased to ensure sufficient flowability of cementation solution. Soon et al. (2014) studied the effect of grouting pressure of cementation solution (0.2, 1.1 and 2.0 bar) on the evolving calcium carbonate content, UCS, and permeability in MICP-treated tropical residual soil, as shown in Figs. 23 and 24. The results showed that calcium carbonate content in cemented soil under medium pressure (1.1 bar) reached 2.6%, slightly higher than that under low pressure. Meanwhile, the formed calcium carbonate content was only 1.4% under high pressure. Moreover, the permeability of cemented soil was negatively correlated with the increasing calcium carbonate content in soil under various grouting pressures. The cementation effect that higher strength was achieved under lower grouting pressure indicated that low-pressure grouting was more beneficial to the deposition of calcium carbonate at grain-to-grain contacts in soil. The strength of soil at high grouting pressure was 13% lower than that of untreated soil, for the reason that excessive grouting pressure led to elevated pore water pressure and decreased effective stress. Moreover, excessive hydraulic gradient caused internal soil erosion, which impaired the original soil structure and reduced the ultimate strength and impermeability of soil.

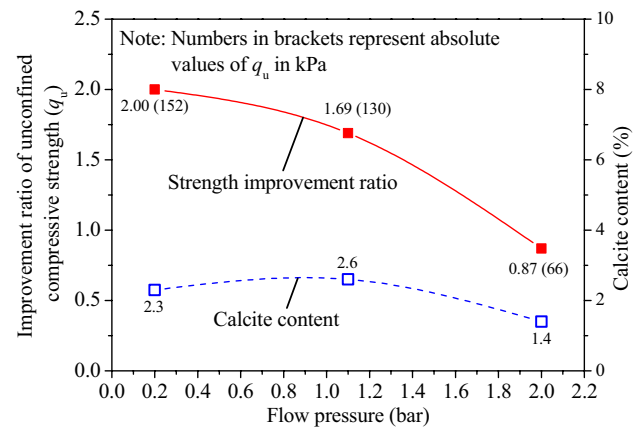


Fig. 23 The relationship between the grouting pressure and improvement ratio unconfined compressive strength and calcite content (Modified from Soon et al. 2014)

Future opportunities and challenges

The improvement of engineering performance of geomaterials through MICP is a complex process involving solid–liquid, organic–inorganic, and biological–abiotic interactions. The cementation extent and efficiency are controlled by many factors, such as bacterial physiological characteristics, concentration of bacterial and cementation solutions, geomaterial properties, and surrounding physical and chemical environment. This paper reviews existing research works on these influential factors and groups them into six major categories including microbial strain selection, composition of bacterial and cementation solutions, temperature, pH, soil properties, and grouting technology. This review has figured out the following opportunities and challenges that necessitate further research efforts in MICP:

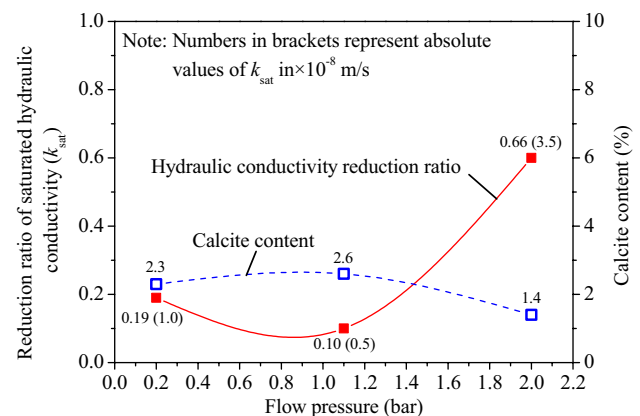


Fig. 24 The relationship between the grouting pressure and the reduction ratio of standard hydraulic conductivity (Modified from Soon et al. 2014)

1. Urease-producing genes have been introduced into *E. coli* plasmids and are successfully expressed, which proves the potential of using transgenic strains for MICP. However, there are still many scientific and practical issues to be addressed before it can be applied in large scale. The cultivation of genetically modified bacteria with high urease-producing capacity, superior environmental adaptability, and better cementation effect is the focus of future research in this field.
2. The mechanism of how pH affects bacterial adsorption, distribution, and calcium carbonate production in soil pore scale is not clear. Future studies can be devoted to the utilization of advanced analytic techniques such as mercury intrusion porosimetry (MIP), multi-electrode resistivity method, and fluorescence tracer display to understand the mechanism at pore scale.
3. The correlation between particle size and strength of MICP-treated soil requires further investigations. Thus, research opportunities exist on the improved fundamental understanding of the micro-scale mechanism that governs this correlation. In addition, the application of MICP to fine-grained soils (e.g., silt, clay), ammonium ion treatment, and in-situ bio-stimulation are also compelling challenges.
4. MICP-treated soils generally exhibit more brittle behaviors. Fibers or other reinforcement materials can be added to soil to improve their ductility and mechanical properties. On the one hand, fibers could increase the number of contact points of soil particles, thus improving mechanical strength. On the other hand, the toughness of the soil treated by MICP can be effectively improved. Further research is needed to systematically develop the fiber-aided MICP technique.
5. The MICP technology has been gradually applied to more complex and extreme environments, which may pose adverse conditions for the MICP process such as high pressure, limited oxygen, and competition with other microbial species. Although sporadic studies have been performed to address the effect of adverse conditions on MICP efficiency (Jiang et al. 2016; Gat et al. 2014), substantial research efforts are still needed to address more adverse conditions and to understand underlying mechanisms. It also becomes more important to explore the possibility of using existing in-situ materials to facilitate the MICP process and lower the operational cost.

Summary

In this work, a total of seven types of factors that influence the performance of MICP-treated soil are reviewed. The obtained main conclusions related to each type of factor are summarized as follows:

1. *The species and strains of microbes* Bacillus and Sporosarcina are the main strains used in MICP-treated geotechnical technology. Different strains of bacteria have different types, sizes, shapes, and yields of calcium carbonate crystals secreted due to the differences in chemical composition of extracellular organic groups and ureases, which makes the cementation effect of geotechnical materials different. Spherical and cubic calcite-like calcium carbonate is good for cementing geotechnical materials. Aragonite crystals have also been reported to have a good cementing effect. Large-sized calcium carbonate crystals are more suitable for cementing coarse-grained soil and suitable for improving the strength of geotechnical materials, whereas small-sized calcite is more suitable for cementing fine-grained soil and reducing the permeability of soil.
2. *Bacterial solution concentration* The concentration of bacteria solution has an effect on the appearance, size, and quantity of calcium carbonate. Generally, the higher concentration of bacterial solution results in a better cementation reinforcement to soils. It is recommended to use higher concentration bacterial solutions for engineering applications.
3. *Temperature* The optimum temperature for MICP is between 20 and 40 °C. In this range, lower temperature would increase the particle size of calcium carbonate crystal and yield a relatively uniform distribution of calcium carbonate among particles, which jointly contributes to the improved strength of the cemented samples. At higher temperatures, the calcium carbonate particles are smaller, but the yield is higher. The strength of the cemented samples is lower, but the resistance to wind erosion and water absorption is stronger.
4. *pH* pH value has no obvious effect on the crystal form of calcium carbonate secreted by bacteria, but has certain effect on the crystal appearance. The most suitable pH value for the growth of Sporosarcina and Bacillus is above nine. Although the reduction of pH value would reduce the concentration of carbonate ion and the rate of calcium carbonate formation, if applied properly, it helps increase the size of calcium carbonate crystal, and thus improve the solidification effect of MICP. According to the results of micro-analysis and macro-test, we believe that the most significant increase of soil strength can be achieved under the pH range of 7–8, while the

enhancement in impermeability and water absorption is optimized under $\text{pH} > 8$.

5. *The composition and concentration of cementation solution* Calcium chloride is the most commonly used calcium source in the experiment. It is compatible with bacterial growth, urease activity, calcium carbonate yield, crystal type, and morphology. Calcium acetate is very effective in improving the strength of soil, which makes it a new possible calcium source for future analyses. The cementation solution that is less than 1 mol/L has a good effect on improving the geotechnical properties. 0.25 mol/L concentration has a good effect on improving permeability and long-term stability, but the reaction time is longer. The content of calcium carbonate and the UCS of soil are relatively higher when cementation solution concentration increases to 0.5 mol/L. Increasing the concentration ratio of urea-calcium ions (e.g., 1.2:1) properly can improve the mineralization efficiency of calcium carbonate and the activity of urease.
6. *Soil properties* Abundant minerals would contribute to calcium carbonate deposited. Calcite-rich soils are most conducive to the microbial deposition of calcium carbonate, with the most effective particle size in MICP ranging from 10 to 1000 μm . The particle size with the highest deposition efficiency of calcium carbonate is about 100 μm , but there is no clear conclusion about the optimum particle size to reach the highest strength. Designated soil properties such as higher density and better particle size distribution generally contribute to the improved cementation effect.
7. *Grouting technology* Multiphase grouting, multi-concentration phase grouting, and electroosmotic grouting could improve the uniformity of bio-cementation bonds in the soil. The grouting speed below 0.042 mol/L/h can improve the utilization ratio of cementation solution. The suggested grouting pressure is generally between 0.1 and 0.3 bar for sand samples and less than 1.1 bar for silty clay samples. Excessive grouting pressure would destroy the soil structure and reduce the solidification effect.

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