

DOI: 10.11835/j.issn.2096-6717.2025.071



开放科学(资源服务)标识码 OSID:



Salt tolerance domestication of urease bacteria for biocementation

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Abstract: In engineering practice of microbial-induced carbonate precipitation (MICP), urease-producing bacteria (UPB) cannot tolerate high-salt concentrations of cementation solution (CS). Increasing the salt tolerance of UPB to CS can improve the efficiency and the application scope of MICP. In order to enhance the salt tolerance of *Sporosarcina pasteurii*, a gradual salt concentration gradient method was employed for its domestication. Growth characteristics in the domestication medium were evaluated through optical density tests, revealing a slowed growth rate in domesticated bacteria. Furthermore, salt tolerance tests, unconfined compressive strength (UCS) tests, and scanning electron microscopic analyses were conducted to investigate the calcium ion precipitation capacity, strength properties of bio-cemented sand columns, and microstructure of reinforced samples among untamed bacteria (UB), domesticated bacteria (DB), and the control group. The results indicate the following: DB's tolerance to CS salt concentration exhibited an approximate increase of 0.25 mol/L. In the context of high concentration CS conditions, DB-treated samples demonstrated approximately double the UCS in comparison to UB, indicating a more efficient calcium ion precipitation process. Furthermore, DB resulted in a higher proportion of cauliflower-like calcium carbonate crystals under high concentration CS conditions. This crystal morphology is commonly associated with superior mechanical properties. These findings demonstrate that domestication effectively enhances bacterial biomineralization performance in high-salinity environments.

Keywords: microbial-induced carbonate precipitation (MICP); bacteria domestication; salt tolerance of bacteria; calcium utilization rate; unconfined compressive strength (UCS); cementation solution concentration

生物矿化用脲酶细菌的耐盐驯化

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摘要:在微生物诱导碳酸盐沉淀(MICP)的实际应用中,产脲酶细菌(Urease-Producing Bacteria, UPB)难以耐受高盐度的胶结液(Cementation Solution, CS),提高UPB对CS的耐盐性可以提高MICP的效率,并能增加应用范围。为提高巴氏芽孢杆菌(*Sporosarcina pasteurii*)的耐盐性,采用梯度递增盐浓度的驯化方法对其进行驯化,通过光密度试验评价细菌在驯化培养基中的生长特征,发现驯化后细菌的生长速度减慢;通过耐盐测试、无侧限抗压强度试验和扫描电子显微镜试验探究未驯化细菌(Untamed Bacteria, UB)、驯化细菌(Domesticated Bacteria, DB)和对照组沉淀钙离子的能力、生物胶结砂柱的强度特性和加固后样品的微观结构。结果表明:DB可耐受CS的盐浓度相比UB约增加了0.25 mol/L;在高浓度胶结液条件下,DB处理样品的无侧限抗压强度相比UB

Received: 2025-03-21

Foundation item: National Natural Science Foundation of China (No. 52108300)

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约增加了2倍,表明其具有更高效的钙离子沉淀能力;DB在高浓度胶结液条件下产生的菜花状碳酸钙晶体比例升高,该晶型通常带来更高的力学性能;驯化能有效提升细菌在较高盐度环境中的生物矿化性能。

关键词:微生物诱导碳酸盐沉淀;细菌驯化;细菌耐盐性;钙离子利用率;无侧限抗压强度;胶结液浓度

中图分类号:TU411 **文献标志码:**A **文章编号:**2096-6717(2026)03-0001-12

1 Introduction

Microbial-induced calcium carbonate precipitation (MICP) is a fast-evolving technique for soil reinforcement that involves the hydrolysis of urea by urease-producing bacteria (UPB). In this process, carbonate ions can react with calcium ions in solution to form calcium carbonate crystals, with or without bacteria as the nucleation site^[1-3]. Notably, MICP is considered a potential alternative to Portland cement, contributing to sustainable development in the construction industry^[4].

One hallmark of MICP for soil reinforcement is that multiple applications of the cementation solution (CS) are necessary during the cementation stage to achieve the desired strength and calcium carbonate content^[5-9]. However, conducting a high number of MICP treatments can lead to significant costs, particularly in practical engineering applications.

To reduce the number of treatments needed, one approach is to increase the concentration of the CS during each treatment cycle^[8]. Additionally, higher concentrations of urea and calcium ions result in the increased deposition of CaCO₃ for the same bacterial concentration^[10]. Thus, this leads to a more pronounced increase in strength and a decrease in permeability^[11]. In contrast, as the CS concentration increases continuously, calcium carbonate production does not increase proportionally. This may be due to the inhibition microbial and enzyme activities in a high-salt environment^[12-21]. Whiffin^[14] and Zhao^[19] found that for *Sporosarcina pasteurii*, when the concentration of the CS exceeded 1.5 mol/L, the yield of calcium carbonate precipitation began to decrease. Moreover, it is possible to improve the strength of the soil with fewer treatments by using a higher concentration of CS^[22]. Thus, improving bacterial tolerance to the salt in the CS is crucial for the practical application of MICP in engineering.

The productivity of calcium carbonate is affected

by the concentration of the CS. Notably, there is a correlation between the concentration of the CS and the productivity of calcium carbonate. A low concentration of the CS results in low productivity of calcium carbonate. However, the productivity of CaCO₃ is also reduced when the concentration of the CS is too high. This is because high-salt concentrations have an inhibitory effect on bacteria and urease enzymes^[8, 14, 19, 23]. Moreover, the size and distribution of calcium carbonate are affected by the concentration of the CS. Smaller and more homogeneous precipitation is more likely to occur at lower concentrations. At higher concentrations, larger and more heterogeneous precipitates are more likely to occur^[9, 22]. Furthermore, the concentration of the CS affects the chemical reaction rate. As such, the precipitation rate of calcium ions gradually slows down when the concentration of the CS exceeds 1.0 mol/L^[8].

Bacteria adapt to their target environment through a gradual process of adding substances to their medium to ensure that they can thrive in their target environment^[24-26]. This process has been successfully applied in many MICP applications. Xiao et al.^[26] employed domestication techniques to improve the reinforcement of *Sporosarcina pasteurii* in a seawater environment. Sun et al.^[27-28] improved the reinforcement of *Bacillus megaterium* in low-temperature environments through domestication. Furthermore, Shi et al.^[29] successfully tackled the problem of inadequate calcium carbonate production by *Bacillus megaterium* in alkaline environments through domestication.

This study aimed to cultivate a UPB capable of precipitating calcium ions in higher-salt concentrations. Significantly, this would reduce the need for multiple treatments during practical applications of MICP. The study examined calcium ion concentration and UCS to investigate the potential and effectiveness of mineralizing bacteria resistant to high CS concentrations.

2 Materials and methods

2.1 Bacteria, culture medium and CS

Sporosarcina pasteurii was the bacterial strain in this study. The media composition included 15 g/L ammonium chloride, 20 g/L yeast extract, and 0.01 g/L nickel chloride. The pH was adjusted to 9.2-9.3, and the inoculation ratio was 1:100. Before use, all media were sterilized at 121 °C for 20 minutes in an autoclave and then cooled to room temperature in an ultra-clean bench. The bacterial strain was cultured in a thermostatic shaker at a temperature of 35 °C and a speed of 200 r/min. To measure bacterial activity, the conductivity method was used in a water bath at 25 °C for 3 minutes, resulting in an average urease activity of approximately 0.10-0.13 mS/(cm·min) (approximately 10-13 U). The optical density was measured to be approximately 1.0-1.3 using a spectrophotometer, utilizing a wavelength of 600 nm. Furthermore, the CS was prepared using equal molar concentrations of calcium chloride and urea, with concentrations of 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, and 4.5 mol/L.

2.2 Domestication process

This study employed a gradient domestication method utilizing *Sporosarcina pasteurii*. As outlined in Table 1, the process began by inoculating the bacteria in a 2% NaCl medium at a ratio of 1:100. The cultures were incubated in a constant temperature oscillator for 8 hours. This initial culture was then expanded over several generations until domestication was stabilized. Subsequently, the bacteria were transferred to a medium with a 4% NaCl concentration.

Table 1 Domestication schedule

Serial number	NaCl content/%	Number of extensions	Parent bacteria
0%	0	1	
2%-1	2	1	0%
2%-2	2	2	2%-1
...
4%-1	4	1	2%-2
4%-2	4	2	4%-1
4%-3	4	3	4%-1
...

Domestication stabilization refers to maintaining consistent growth rates of bacteria in expanded cultures before and after the transfer^[26]. In this study,

the optical density (OD600) values and urease activity of the bacteria were recorded every two hours.

It is important to note that in this test, sodium chloride was used as the target substance added to the domestication medium, instead of calcium chloride, urea, or a mixture of them (CS). The reason for this is that the microbial medium used in this test has an alkaline pH ranging from 9.2 to 9.3. Calcium chloride generates the slightly soluble compound calcium hydroxide in an alkaline environment. Secondly, urea is not used because it has the potential to promote bacterial growth^[30-33]. Finally, mixtures of calcium chloride and urea are not used to prevent biomineralization during the incubation period^[34].

Although sodium chloride was added at a concentration lower than the target CS salt concentration, we deduced that bacteria capable of growing in media with higher salt concentrations could biomineralize in higher concentrations of CS. When the concentration of calcium ions reaches 0.2 mol/L, the growth rate of bacteria decreases significantly^[30]. However, bacteria can still biocement at calcium ion concentrations as high as 1.0 mol/L. This suggests that although bacteria can normally induce precipitation at this concentration, the culture solution used to cultivate bacteria has a much lower concentration.

2.3 Bacterial salt-tolerability test

Bacterial solution was mixed with CS of varying concentrations (1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, and 4.5 mol/L) in a beaker at a volume ratio of 1:1, and covered with a sealing film. After mixing, the CS concentrations became 0.50, 0.75, 1.00, 1.25, 1.50, 1.75, 2.00, and 2.25 mol/L, respectively. The reaction was allowed to complete, and the production of calcium carbonate was observed. The concentration of calcium ions in solution was determined using EDTA titration^[35]. Subsequently, the utilization of calcium ions was calculated using the following equation:

$$U_{Ca^{2+}} = \frac{c_1 - c_2}{c_1} \times 100\% \quad (1)$$

Where c_1 is the initial concentration of calcium ions; c_2 is the concentration of calcium ions after completion of the reaction.

The following equation calculates calcium ions by precipitation:

$$P_{Ca^{2+}} = n_1 - n_2 \quad (2)$$

where n_1 is the initial amount of substance of calcium ions; n_2 is the amount of substance of calcium ions after reaction completion.

As shown in Table 2, the experiment included three groups. The first group consisted of untamed bacteria (UB) without salt-tolerant domestication

mixed with each concentration of CS. The second group was the control group (CG), which consisted of UB solution containing 4% NaCl mixed with each concentration of CS. The third group consisted of domesticated bacteria (DB) with varying concentrations of CS. All bacterial solutions were prepared on a sterile bench before experimentation.

Table 2 Bacterial salt-tolerability and sand column test schedule

Serial number	CS concentration/(mol/L)	Bacteria	NaCl content of bacterial solution/%	Time of NaCl addition
UB-0.50	0.50	Untamed	0	
UB-0.75	0.75	Untamed	0	
UB-1.00	1.00	Untamed	0	
UB-1.25	1.25	Untamed	0	
UB-1.50	1.50	Untamed	0	
UB-1.75	1.75	Untamed	0	
UB-2.00	2.00	Untamed	0	
UB-2.25	2.25	Untamed	0	
CG-0.50	0.50	Untamed	4	Post-culture
CG-0.75	0.75	Untamed	4	Post-culture
CG-1.00	1.00	Untamed	4	Post-culture
CG-1.25	1.25	Untamed	4	Post-culture
CG-1.50	1.50	Untamed	4	Post-culture
CG-1.75	1.75	Untamed	4	Post-culture
CG-2.00	2.00	Untamed	4	Post-culture
CG-2.25	2.25	Untamed	4	Post-culture
DB-0.50	0.50	Domesticated	4	Pre-culture
DB-0.75	0.75	Domesticated	4	Pre-culture
DB-1.00	1.00	Domesticated	4	Pre-culture
DB-1.25	1.25	Domesticated	4	Pre-culture
DB-1.50	1.50	Domesticated	4	Pre-culture
DB-1.75	1.75	Domesticated	4	Pre-culture
DB-2.00	2.00	Domesticated	4	Pre-culture
DB-2.25	2.25	Domesticated	4	Pre-culture

2.4 MICP treatment

Quartz sand with a specific gravity of 2.64 was used in the test. The particle size distribution, determined by the Sympatec QICPIC/L02-OM, is presented in Fig. 1. The particle size of the sand ranges from approximately 0.019 mm to 1.400 mm. The maximum and minimum pore ratios are 0.81 and 0.55, respectively. Scanning electron microscope (SEM) images of the sand grains are also shown in Fig. 1. In summary, the surfaces of the particles were neat and free of calcium carbonate crystals prior to the MICP treatment.

The sand columns were created using 150 mL syringes as molds. A plastic outlet hose was attached to the bottom of each syringe to control water from entering or draining out. To facilitate the later extrac-

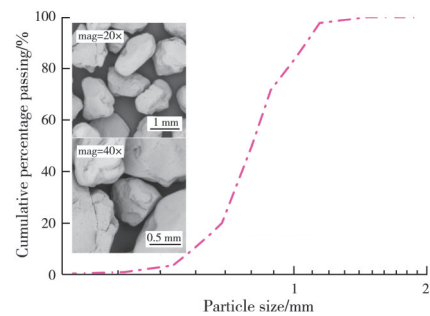


Fig. 1 Particle size distribution curves and SEM images of quartz sand

tion of microbially glued samples, 0.1 mm-thick transparent membranes were inserted in the syringes. A scouring pad was placed at the bottom of each syringe to prevent sand particles from escaping. The initial pore ratio was 0.71, the relative compactness was 40%, and the pore volume measured 41.77 cm³.

To avoid sand particle washout during the grouting process, a scouring pad was also placed over the top of the sand column in the same manner. Each specimen had a diameter of 39.1 mm and a height of 80 mm, following ASTM D2166^[36].

As described in Section 2.3, the sand columns treated with MICP were divided into three groups; the experimental program is also shown in Table 2. A single-phase grouting method was used, with a mixture of bacterial solution and CS at a ratio of 1.5 times the pore volume. Before mixing with the CS, the bacterial solution was adjusted to a pH of 6 using a 1 mol/L HCl solution, following the low-pH method proposed by Cheng et al.^[37]. The mixed solution was poured over the specimen to fill the pores under gravity. To prevent the mixed solution from flowing out, a stopcock was clamped to the plastic outlet hose. After 24 hours, the liquid was drained by opening the stopcock, and the specimens were rinsed with 5 times the pore volume of deionized water to remove residual chemicals^[6, 38]. Three parallel tests were conducted for each group.

2.5 UCS test

The cementation strength of the specimens treated with different bacteria and concentrations of the CS was evaluated using UCS. Following biotreatment, specimens were removed from the molds and placed in a drying oven at 65 °C. The drying process was halted when the difference in mass between the two measurements was less than 0.1%^[39]. UCS tests were conducted on specimens with varying bio-cementation levels resulting from different types of bacteria and CS concentrations. According to ASTM Standards D2166^[36], the axial load was applied at a 0.5 mm/min rate.

3 Results and discussion

3.1 Bacterial growth

Fig. 2(a) shows the growth of 8 hours OD600 and urease activity of *Sporosarcina pasteurii* in media with varying NaCl concentrations (2%, 4%, 6%, 8%, and 10%). The bacterial growth rate decreased gradually with increasing sodium chloride concentration. Additionally, growth was almost non-existent when the concentration reached 6% or higher. This is similar to the result obtained by Zhao et al.^[23]. Thus, this suggests that the addition of NaCl has an

inhibitory effect on the growth of UPB. This is likely because the high osmotic pressure of the external NaCl solution reduces the turgor of the cells^[40]. Fig. 2(b-g) shows the growth curve of bacteria and changes in urease activity. This demonstrates that the optical density of bacteria reached approximately 1.4 within 8 hours in the normal medium without adding NaCl. However, inoculation into the domesticated medium containing a 2% NaCl concentration resulted in a significant decrease in bacterial growth. The optical density value was only about 1.0 at 8 hours, comparable to that of the medium without NaCl after 10 hours of incubation. This indicates that the presence of NaCl inhibits bacterial growth.

To improve the salt tolerance of bacteria, the first generation of bacteria was cultured in a 2% NaCl medium and then inoculated into the same medium for further expansion. As shown in Fig. 2(h), the growth rate of the second generation was generally similar to that of the first, indicating that the bacteria had reached a stable state of domestication^[26]. Subsequent domestication gradients (4% NaCl content) were then initiated. When bacteria that had stabilized in the 2% NaCl medium were first inoculated into a 4% NaCl medium, their growth rate was inhibited. The optical density after 8 and 10 hours of incubation in 4% NaCl medium was equivalent to that after 6 and 8 hours of incubation in 2% NaCl medium, respectively. However, after a second incubation in the 4% NaCl medium, there was a slight increase in the growth rate. The growth rate in the third inoculation culture essentially remained unchanged from the second. Therefore, this indicates that the bacteria gradually adapted to this salinity environment and reached a domesticated steady state under the second inoculation culture.

In summary, it is observed that the growth of bacteria slows down when they are inoculated from media with a lower concentration gradient to media with a higher concentration gradient. This is likely because some bacteria have difficulty tolerating higher salinity and die, resulting in a smaller bacterial population^[41]. The remaining bacteria can tolerate this salinity, but their growth rate is inhibited. Additionally, there was an increase in the growth rate of the bacteria after the expanded culture. This could be due to the bacteria becoming accustomed to

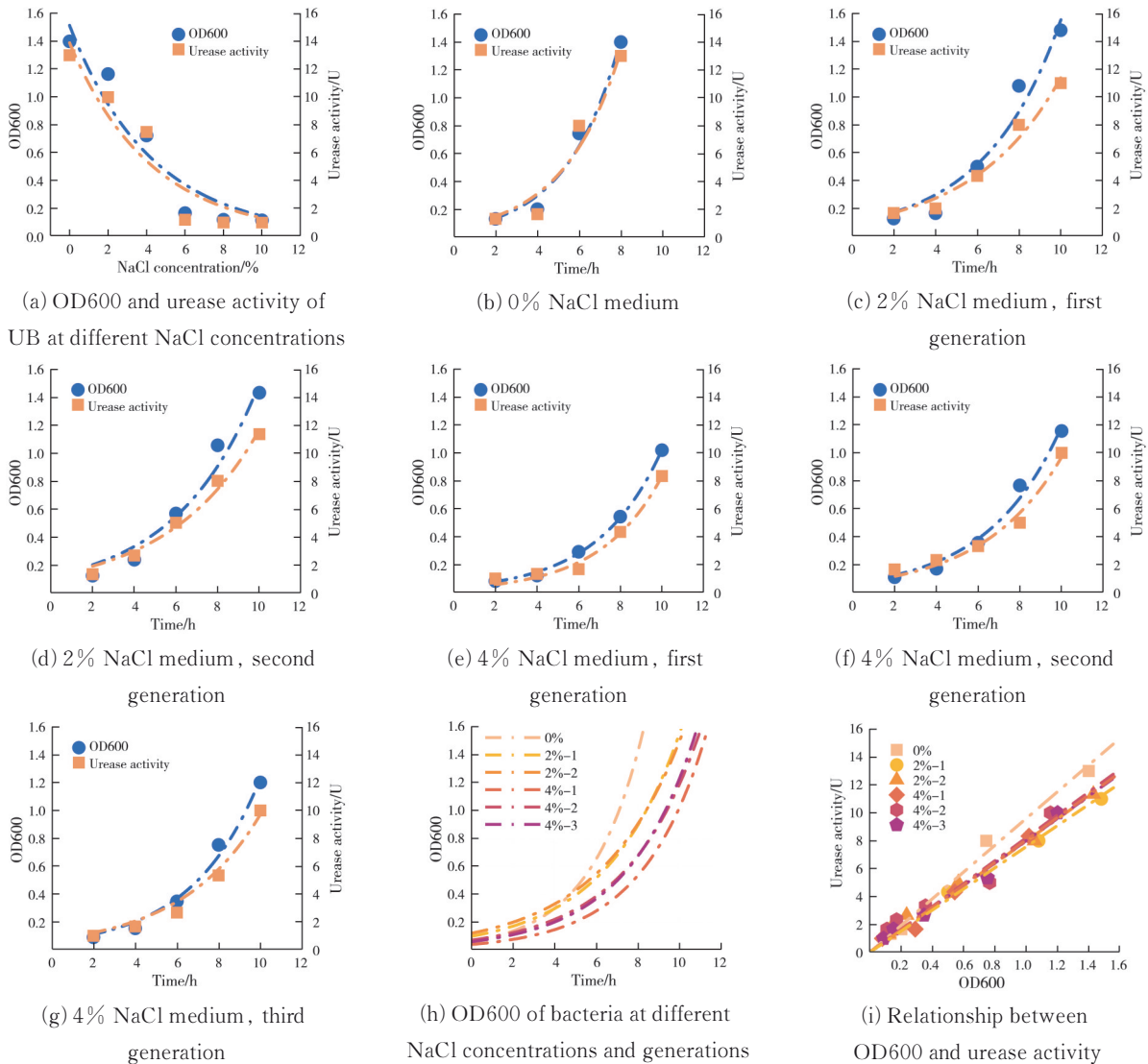


Fig. 2 Bacterial growth characteristics test

the salinity gradient, resulting in fewer bacteria dying from intolerance. Finally, it was discovered that after domesticating and stabilizing the bacteria at a certain salinity level, their optical density in the high-salinity steady state was lower than that in the low-salinity steady state. This is similar to the results of Xiao et al. [26].

The relationship between bacteria concentration and urease activity in media with different sodium chloride contents is shown in Fig. 2(i). The results show a linear correlation between bacterial concentration and urease activity for both UB and DB. This finding is consistent with the results reported by Okwadha and Li [10]. In addition, the increase of urease activity in DB was slower compared to that of UB. Specifically, at the same bacterial concentration, the urease activity of UB was higher than that of DB. In this experiment, the urease activity of UB

was approximately 10-13 U, while the urease activity of DB was approximately 8-10 U, in a bacterial solution with an OD600 of approximately 1.0-1.3. The lower urease activity of the mineralized bacteria trained and selected in the high-salinity medium may be attributed to several factors. Firstly, salt-trained bacteria are not as efficient in urease activity as UB. Secondly, the domestication process involves several expansions of the culture, increasing the likelihood of introducing miscellaneous bacteria [42]. Lastly, high salinity inhibits the activity of urease produced by the bacteria [43-46].

3.2 Bacterial salt-tolerability test

Fig. 3 shows various bacteria reacting with the 1.75, 2.00, and 2.25 mol/L concentrations of CS. These findings demonstrate that the calcium carbonate precipitate generated by the UB and CG began to decrease at a CS of 1.75 mol/L (see Fig. 3(a) and

(d)). Additionally, flocculated insoluble material without cementing power was observed at 2.00 mol/L (see Fig. 3(b) and (e)). At 2.25 mol/L, the material was almost entirely flocculated without cementing ability (see Fig. 3(c) and (f)). In contrast,

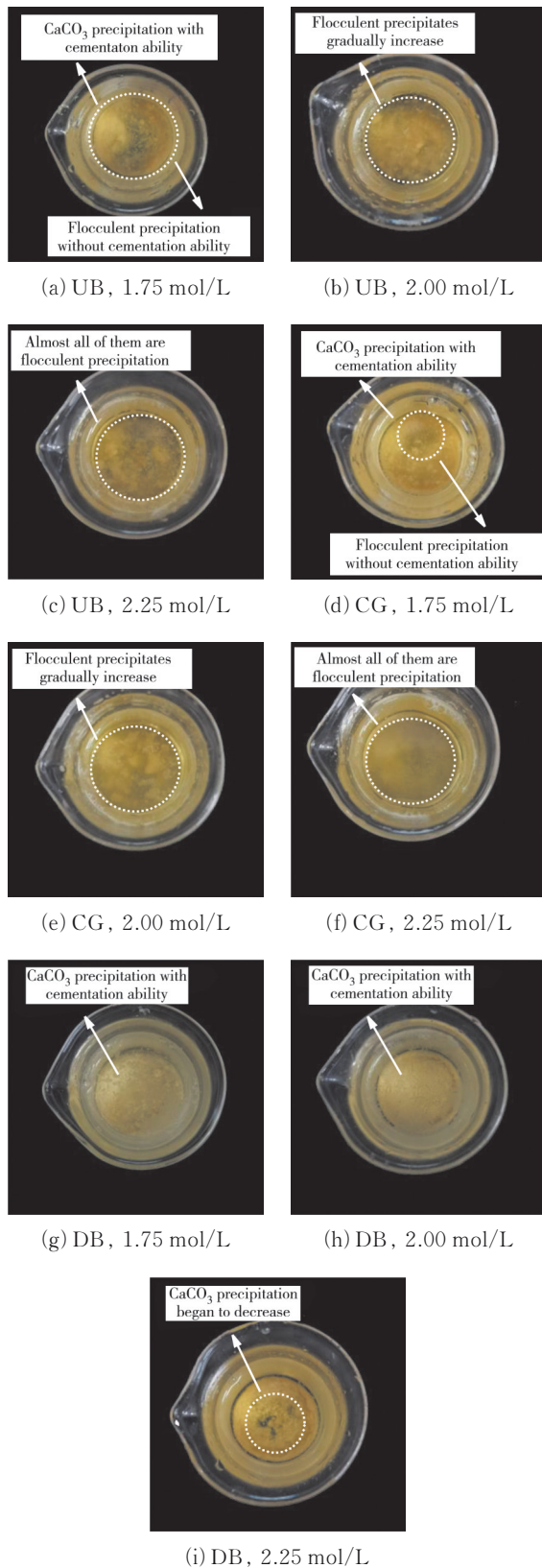


Fig. 3 Reactions of UB, CG, and DB with different CS concentrations

the UPB that were domesticated still showed significantly more precipitation than the other two groups in the reaction with 1.75 mol/L (see Fig. 3(g)). No significant flocculent insoluble material without cementation ability was observed in the reaction with 2.00 mol/L and 2.25 mol/L CSs (see Fig. 3(h) and (i)). Instead, calcium carbonate precipitates had the cementing ability. However, the amount of precipitation was reduced. The above-mentioned insoluble material without cementing ability may form due to divalent calcium that causes bacteria to flocculate^[47]. Calcium ion utilization rate and precipitation were calculated as shown in Fig. 4(a-c). All UBs were over 90% efficient in converting calcium ions into precipitates when reacting with CS of 1.50 mol/L or less. The rate of calcium ion depletion significantly decreased from 1.75 mol/L. This is similar to the findings of Whiffin^[14] and Zhao^[19]. In contrast, DB still converts over 90% of the calcium ions to the precipitate when reacting with a 1.75 mol/L CS. The utilization of calcium ions is about 40% when reacting with a 2.00 mol/L CS. In the CG, calcium ion utilization begins to decrease at 1.50 mol/L. This could be because the inclusion of sodium chloride raises the salt concentration of the CS, reducing the concentration of CS in which bacteria can work properly^[12-21, 47-49]. It also indicates that simply adding NaCl does not enhance bacterial tolerance to CS and decreases it. Instead, salt-tolerant strains should be selected using NaCl. Although the domestication medium was not added to calcium chloride or urea in the target environment, the gradual addition of sodium chloride inhibited or even killed the salt-intolerant UPB. Thus, this allowed the bacteria that could survive and reproduce in the high-salt environment to become the dominant strain over time.

Fig. 4(d) illustrates the correlation between the number of incubation generations and calcium ion utilization and precipitation. The study tested the ability of DB to produce calcium carbonate in a 1.75 mol/L CS across multiple generations of culture. Notably, the rate of calcium ion conversion tended to increase and then decrease with the increase in the number of culture generations. The bacteria of the first generation, which were domesticated and stabilized in a 2% NaCl medium, were inoculated into a 4% NaCl medium. This generation of bacteria did not exhibit a high rate of calcium precipitation. However, the rate

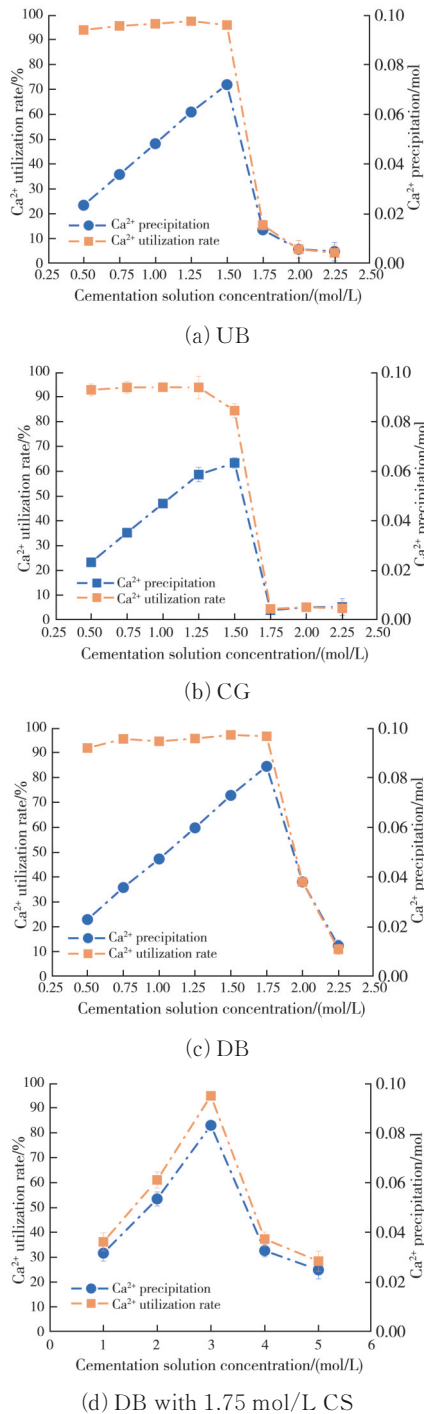


Fig. 4 Ca^{2+} utilization rate and precipitation at different CS concentrations

of precipitation gradually increased as the number of cultural generations increased. Moreover, over 90% of the calcium ions were precipitated in the third generation. Nevertheless, as the number of cultural generations continued to increase, the rate of precipitation began to decrease. The difference could likely be because the inoculation of the previous generation of bacteria from a low-salinity medium caused some bacteria to die due to intolerance. It is important to note that the dead cells were not excluded from the

test, as the bacterial concentrations were tested by optical density. Therefore, the bacterial concentrations were about identical, but the presence of dead cells could have affected the results. After successive domestication, bacteria gradually adapted to the high-salinity environment, resulting in an increase in the rate of calcium ion precipitation with each subsequent generation^[26]. The decrease in the bacteria's ability to transform calcium ions in the higher generations of domestication culture may be due to the introduction of miscellaneous bacteria during the operation after several expansion cultures^[42].

3.3 UCS test and SEM analysis

Fig. 5 illustrates that the UCS generally increases with the rising concentrations of CS before declining. This trend occurs because bacteria struggle to effectively precipitate calcium ions in environments with high CS concentrations^[12-21, 47-49]. The specimens treated with DB and 1.75 mol/L CS exhibited the highest intensity, exceeding 300 kPa. In contrast, the other two groups that used the same concentration of CS were approximately one-third as strong as the DB group. The UCS in the DB decreased significantly at a CS concentration of 2.00 mol/L, to about 150 kPa. The strength of the other two groups decreased significantly when the CS concentration reached 1.75 mol/L, and they lost all strength when the concentration was equal to or greater than 2.00 mol/L.

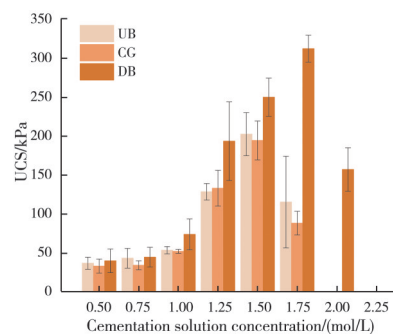


Fig. 5 UCS of the biocemented specimens

The distribution of calcium carbonate on sand particles resulting from MICP treatment of sand columns is classified as either surface coating or bridging. These two forms exist together, but only bridging acts as the cement^[50-51]. Therefore, the cementation ability primarily depends on the formation of adequate bridging forms among grains. There must be enough calcium carbonate to fill the gaps between the soil particles to achieve sufficient bridging forms. In

the MICP treatment process, surface coating and bridging co-exist^[50]. Therefore, when all other factors are equal, an increase in the production of calcium carbonate leads to a corresponding increase in its formation for bridging purposes. Increasing the concentration of the CS results in higher production of calcium carbonate^[8-9, 14, 19-21], which in turn leads to the formation of more bridging structures between

the particles. DBs are more tolerant to CS, allowing them to precipitate more calcium ions at higher concentrations. This results in increased calcium carbonate filling between the particles.

The microscopic images of the specimens treated with UB, CG, and DB groups are depicted in Fig. 6. It is evident that the calcium carbonate crystals increase and then decrease with the concentration

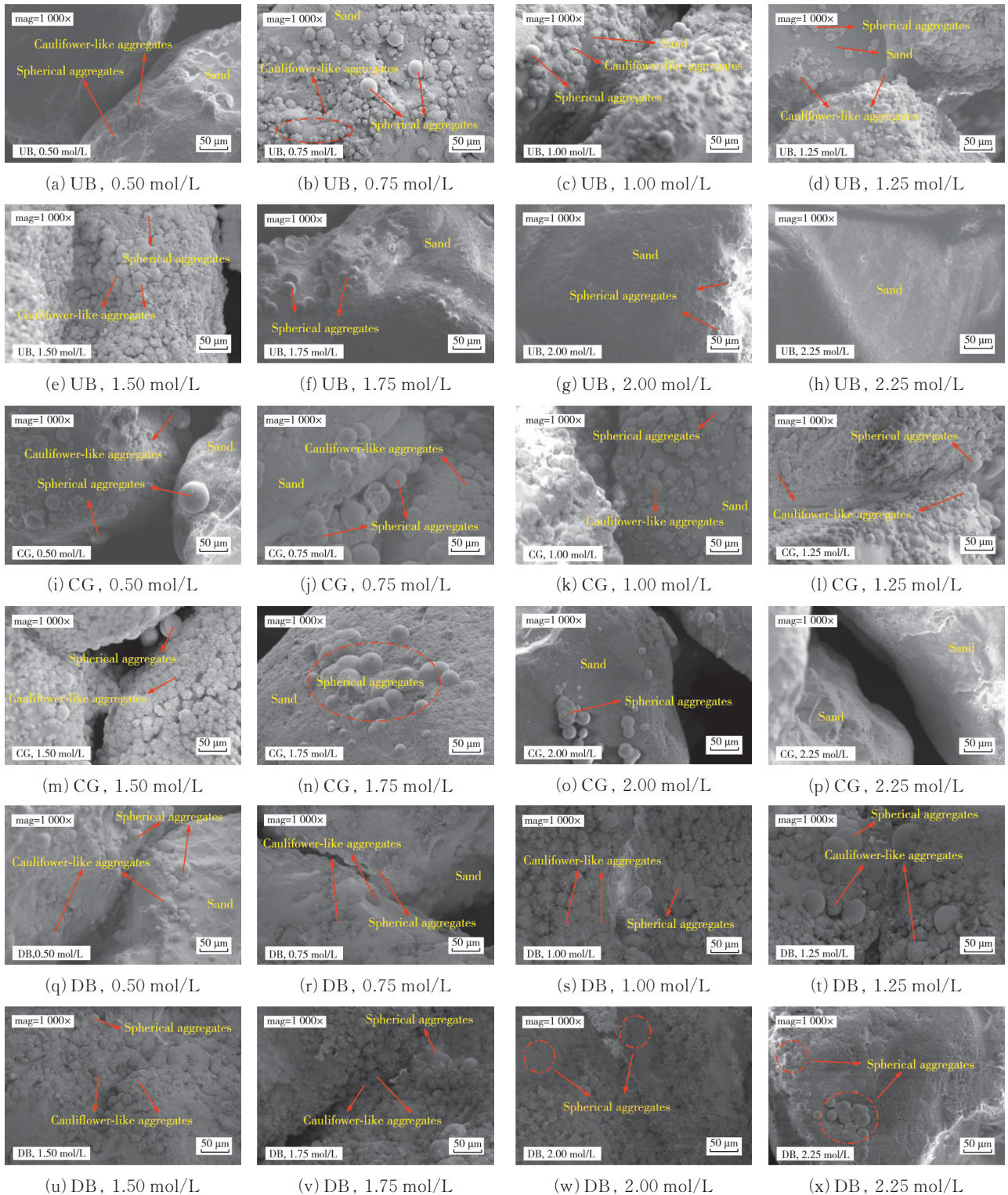


Fig. 6 SEM images of sand columns treated with different strains and CS concentrations

in all three groups. The calcium carbonate began to decline in all three groups at different CS concentrations: 1.75 mol/L for the UB, 1.50 mol/L for CG, and 2.00 mol/L for the DB. Furthermore, sand columns treated with DB showed more calcium carbonate crystals when the CS concentration was 1.75 mol/L or higher. Additionally, the morphology of calcium carbonate crystals was found to be affected by the concentration of the CS. Specifically, the amount of cauliflower-like calcium carbonate increased as the concentration of CS increased. However, as the concentration of the CS continues to grow (1.75 mol/L for the UB and CG, 2.00 mol/L for the DB), the cauliflower-like aggregates become fewer, and the majority of calcium carbonate crystals show spherical shapes. This phenomenon may be attributed to the fact that with the increase in concentration of CS, the active bacteria become fewer, and the precipitate of calcium carbonate is lower. The inadequate quantity of calcium carbonate is unable to facilitate the formation of cauliflower-like aggregations, as crystal growth necessitates a continuous accumulation of newly precipitated crystals^[52]. Due to the one treatment cycle in this study, all observed calcium carbonate crystals were either spherical or cauliflower-shaped, with no rhombic or polyhedral shapes present^[8].

4 Conclusions

This study investigated a method to enhance the tolerance of UPB to CS through salt-tolerant domestication. We compared the growth characteristics of different bacterial strains, their ability to precipitate calcium ions, and their effectiveness in cementing sand particles within CS. A series of growth characterization tests, salt tolerance tests, and UCS tests were conducted. The concentration of CS tolerated by the domesticated UPB was increased from 1.50-1.75 mol/L to 1.75-2.00 mol/L. This resulted in a slight reduction in growth rate and activity compared with UB. However, the ability to precipitate calcium ions in the 1.75 mol/L and 2.00 mol/L CS was significantly enhanced. Additionally, sand column tests indicated that the specimen exhibited a notable enhancement in UCS at these higher concentrations.

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(编辑 胡英奎)