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Study on domestication of Sporosarcina pasteurii and cementation effect of calcareous sand in seawater environment

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Abstract: This study aims to improve the cementation effect of microbially induced carbonate precipitation (MICP) technology on calcareous sand in the marine environment. On the basis of previous studies, the multi-gradient artificial domestication and culture test of Sporosarcina pasteurii in the artificial seawater environment was carried out. Combined with the mechanical test and microstructural analysis of MICP-cemented calcareous sand column, the domestication effect of Sporosarcina pasteurii was evaluated. The results showed that: (1) The bacterial concentration after five-gradient domestication in the seawater environment can reach more than 97% of that in the freshwater environment, and after interaction with cementing fluid, the carbonate production was increased to a certain extent compared with that in the freshwater environment. (2) The domesticated Sporosarcina pasteurii had a strong temperature adaptability, and it had a good MICP performance at 10 ℃ to 30 ℃. (3) Both carbonate production and unconfined compressive strength of calcareous sand columns cemented in the seawater environment were higher than those before domestication, especially for the bacteria after five-gradient domestication. The bacteria after domestication became smaller, thus the carbonate crystals (calcium carbonate and magnesium carbonate) generated in the seawater environment became smaller and denser. They can better fill the pores between calcareous sand particles and cement the adjacent calcareous sand particles, indicating that the Sporosarcina pasteurii after domestication had an excellent MICP performance. The relevant ideas and methods can provide reference for the research and application of MICP technology in the reinforcement of calcareous sand foundation in the seawater environment.

Keywords: cementation in seawater environment; gradient domestication; calcareous sand; Sporosarcina pasteurii; microbially induced carbonate precipitation (MICP)

1 Introduction

As a type of special geomaterial with high calcium carbonate content, calcareous sand is an important component of islands and reefs in the South China Sea. During the construction of islands and reefs, the reinforcement of calcareous sand foundation is a challenging task[1–2], which directly affects the long-term safety of the project. At present, the treatment methods of foundation soil in the islands and reefs mainly include grouting method, pile foundation treatment method and vibroflotation compaction method. These methods can improve the performance of foundation soil to a certain extent. However, the islands and reefs are quite far from the land, thus the material transportation cost is huge[3]. If the foundation soil can be reinforced using the local materials on or around the islands and reefs, the cost will be saved greatly, and a good environmental compatibility will be achieved.

In recent years, microbially induced carbonate precipitation (MICP) technology has been developed rapidly, and it has been studied and applied widely in the field of geotechnical engineering, such as sand foundation reinforcement[4–6], foundation liquefaction prevention[7–8], contaminated soil treatment[9–10], and anti-wind erosion of sand in the desert area[11–12]. Among these studies, the research on calcareous sand cemented using MICP technology has always been a hot spot. It mainly focused on the freshwater environment[13–16]. The physico-mechanical properties of the biocemented calcareous sand column were greatly improved, and its unconfined compressive strength (UCS) can reach the MPa level. In order to apply MICP technology to calcareous sand projects, some studies about the cementation of calcareous sand have been carried out in the seawater environment according to the actual occurrence environment of calcareous sand. For example, Li et al.[17] studied the cementation of calcareous sand in a simulated seawater environment, and the results showed that the UCS of the samples in the seawater environment was 2.66 times that of the samples in the freshwater environment. Peng et al.[18] also studied the MICP-cemented calcareous sand in the seawater environment. They concluded that the seawater would inhibit the final generation of calcium carbonate in the MICP process, so that the UCS of the cemented calcareous sand column in the seawater environment was lower than that in the freshwater environment. In addition, Yu et al.[19] and Dong et al.[20] used natural seawater to conduct microbial culture and cement calcareous sand, and they found that the natural seawater caused a lag period for microbial growth. The

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above observations on the cementation of calcareous sand in the seawater environment showed different cementation effects. It is mainly caused by different adaptabilities of the bacteria used to the seawater environment. In the available literature, Sporosarcina pasteurii and other mineralized bacteria are usually used to cement calcareous sand in the seawater environment, which requires bacteria to survive and reproduce well in the seawater environment and have good urease production capacity. The essence is that the mineralized bacteria can better adapt to the seawater environment, but rare studies on this topic are reported.

Use of seawater during cementation of calcareous sand can greatly reduce the cost of seawater desalination, and make mineralized bacteria better adapt to seawater environment to achieve the MICP cementation of calcareous sand. Therefore, the focus of this study is how to make mineralized bacteria grow and reproduce normally in the seawater environment and make their cementation effect reach or even exceed that in the freshwater environment. In this study, we introduce a biological method called microbial domestication to adapt microbes to the seawater environment. During microbial domestication, the materials or substrates obtained from the target environment are added into the bacterial culture medium. In this way, bacteria can adapt to and rely on the materials or substrates of the target environment, so that they can also show good growth trends and working characteristics in the target environment[21–24]. In order to make Sporosarcina pasteurii better perform the MICP process in the seawater environment, artificial domestication was adopted in this paper. The effects of different acclimation schemes on the bacterial concentration, the production of calcium carbonate and the mechanical properties of the cemented sand column were studied. By comparing different acclimation schemes, the optimum acclimation scheme and the acclimated Sporosarcina pasteurii species were obtained, which provide a reference for the application of MICP technology to calcareous sand cementation in the seawater environment.

2 Domestication of Sporosarcina pasteurii in seawater environment

2.1 Domestication schemes

Sporosarcina pasteurii (No. ATCC 11859) was selected in this study for artificial domestication and culture in the seawater environment.

According to the chemical composition and substance content of substitute seawater listed in ASTM D1141-98 (2021)[25], the top 10 compounds that make up artificial seawater are NaCl, $MgCl_2$, Na_2SO_4, $CaCl_2$, KCl, $NaHCO_3$, KBr, H_3BO_3, $SrCl_2$ and NaF. The contents of the above compounds are shown in Table 1. According to the data in Table 1, the artificial seawater is prepared with a salinity of 35‰ and a pH value of about 8.2.

Table 1 Main components and contents of artificial seawater

Component	Content /(g • L^{-1})	Component	Content /(g • L^{-1})
NaCl	24.530	$NaHCO_3$	0.201
$MgCl_2$	5.200	KBr	0.101
Na_2SO_4	4.090	H_3BO_3	0.027
$CaCl_2$	1.160	$SrCl_2$	0.025
KCl	0.695	NaF	0.003

Following the methods and ideas in relevant microbial domestication studies[21–24], three domestication test schemes including direct domestication, three-gradient domestication and five-gradient domestication were designed in this study to domesticate the Sporosarcina pasteurii in the seawater environment. The culture medium is prepared as follows: Firstly, artificial seawater was prepared according to the material compositions and contents listed in Table 1, and the same volume of deionized water was used to replace seawater to prepare artificial seawater with different concentrations. Then, in the artificial seawater with different concentrations, referring to previous studies[26–28], four types of medium components, including 10 g/L peptone, 3 g/L beef extract, 5 g/L sodium chloride, and 60.06 g/L urea, were added respectively to make the culture medium with different seawater concentrations. The domestication schemes are shown in Table 2, and the domestication process for multi-gradient schemes is shown in Fig.1.

As shown in Table 2 and Fig.1, the domestication process is described as follows:

(1) Direct domestication: 1 mL Sporosarcina pasteurii was added into the conical flask A containing 100 mL artificial seawater and cultured at 30 ℃ with shaking at 180 r/min for 48 h. After that, direct domestication was completed, and the domesticated bacteria were obtained.

(2) Three-gradient domestication: 1 mL Sporosarcina pasteurii was added into the conical flask B containing 100 mL artificial seawater with 1/3 of seawater concentration, and taken out after 48 h of culture. Then, 1 mL Sporosarcina pasteurii in the conical flask B was added into the conical flask C containing 100 mL artificial seawater with 2/3 of seawater concentration, and taken out after 48 h of culture. Finally, 1 mL Sporosarcina pasteurii in the conical flask C was added into the conical flask D containing 100 mL artificial

Table 2 Artificial domestication and culture test schemes

Testing No.	Testing scheme	Conical flask No.	Volume of domestication culture medium /mL	
			Artificial seawater	Deionized water
1	Direct domestication	A	100.0	0.0
2	Three-gradient domestication	B	33.3	66.7
		C	66.7	33.3
		D	100.0	0.0
3	Five-gradient domestication	E	20.0	80.0
		F	40.0	60.0
		G	60.0	40.0
		H	80.0	20.0
		J	100.0	0.0

Note: The total volume of the culture medium in each conical flask is 100 mL.

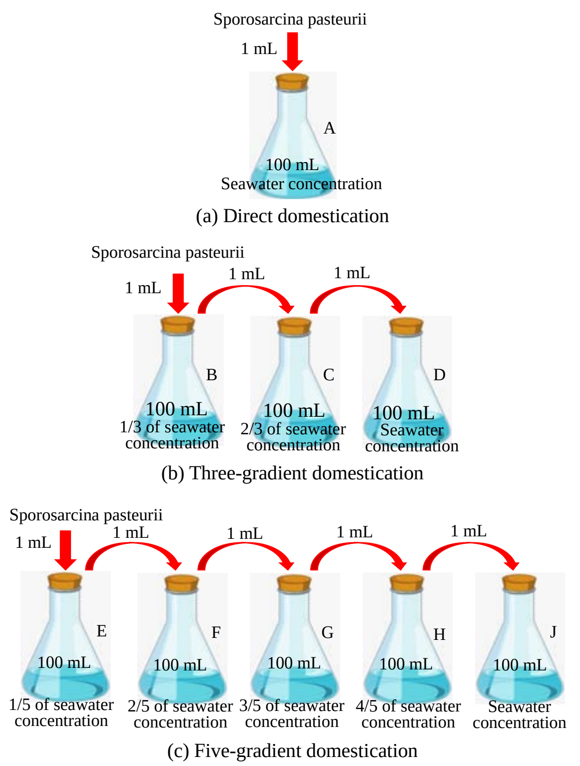


Fig. 1 Schematic diagram of multi-gradient domestication schemes

seawater with the normal seawater concentration, and taken out after 48 h of culture. After that, three-gradient domestication was completed, and the three-gradient domesticated bacteria were obtained.

(3) Five-gradient domestication: 1 mL Sporosarcina pasteurii was added into the conical flask E containing 100 mL artificial seawater with 1/5 of seawater concentration, and taken out after 48 h of culture. Then, 1 mL Sporosarcina pasteurii in the conical flask E was added into the conical flask F containing 100 mL artificial seawater with 2/5 of seawater concentration, and taken out after 48 h of culture. Similarly, 1 mL Sporosarcina pasteurii in the conical flask F was added into the conical flask G containing 100 mL artificial seawater with 3/5 of seawater concentration, and taken out after 48 h of culture. Then, 1 mL Sporosarcina pasteurii in the conical flask G was added into the conical flask H containing 100 mL artificial seawater with 4/5 of seawater concentration, and taken out after 48 h of culture. Finally, 1 mL Sporosarcina pasteurii in the conical flask H was added into the conical flask J containing 100 mL artificial seawater with the normal seawater concentration, and taken out after 48 h of culture. After that, five-gradient domestication was completed, and the five-gradient domesticated bacteria were obtained.

In addition, during the domestication at each level of seawater concentration, the expanded culture of bacteria was performed for many times, and the bacterial concentration was measured. Until the bacterial concentration obtained by the two successive cultures did not change anymore, the next domestication with different seawater concentrations will be carried out. Taking three-gradient domestication for example, the detailed domestication process is shown in Fig.2.

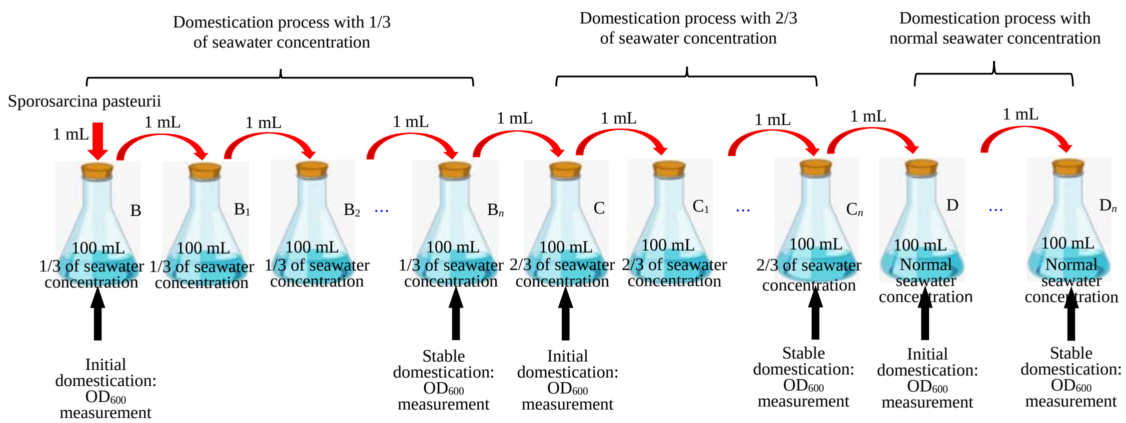


Fig. 2 Detailed process of three-gradient domestication

2.2 Changes in Sporosarcina pasteurii concentration during domestication

To analyze the variation in the concentration of Sporosarcina pasteurii in the process of domestication, for different domestication schemes, the absorbance of bacterial solution was measured using a spectrophotometer (wavelength of 600 nm) during the expanded culture at each level of seawater concentration. The concentration of Sporosarcina pasteurii was expressed based on the OD_{600} value[29–30]. Under three domestication schemes, the bacterial concentrations at the initial and stable stages at each level of seawater concentration are shown in Fig.3.

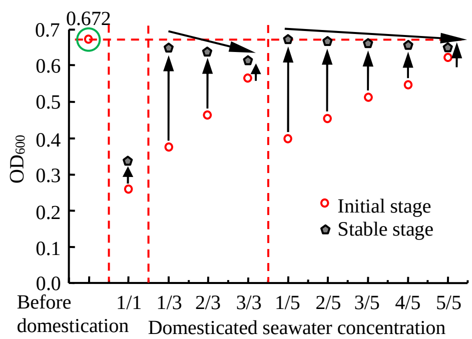


Fig. 3 Bacterial concentration under different domestication schemes

It can be seen from Fig.3 that different domestication schemes had significantly varied domestication effects on Sporosarcina pasteurii, which can be described as follows:

(1) Under direct domestication, the bacterial concentration was low. Even after several times of expanded culture, the increase in bacterial concentration was still limited, indicating that the high concentration of seawater had an obvious inhibitory effect on the growth and reproduction of Sporosarcina pasteurii.

(2) For the three- and five-gradient domestication schemes, the bacterial concentration at the initial stage was relatively low in the domestication process of each level of seawater concentration. After several times of expanded culture under this level of seawater concentration, the bacterial concentration increased rapidly and gradually tended to be stable. The increase in bacterial concentration became more obvious with the decrease in seawater concentration.

(3) In the process of gradient domestication, the concentration of Sporosarcina pasteurii at the stable stage gradually decreased with the increase in seawater concentration. This is because with the increase in seawater concentration, the osmotic pressure gradually increased. In the process of domestication, although Sporosarcina pasteurii had a certain adaptability to the increase of osmotic pressure, high osmotic pressure was bound to affect the growth and reproduction of bacteria. Therefore, after each stage of domestication, the bacterial concentration would be lower than that at the previous level of seawater concentration.

(4) After three- and five-gradient domestication, the bacterial concentrations were 0.612 and 0.652, respectively, equivalent to the values in the freshwater environment. It is indicated that the bacteria could gradually adapt to seawater environment under gradient domestication.

2.3 Variation in carbonate production during domestication

De Muynck et al.[31] and Peng et al.[32] found that the temperature had a great influence on the growth trend and MICP performance of Sporosarcina pasteurii, especially in the range of 10–37 ℃. During the test, in order to explore the effect of temperature on the MICP performance of domesticated Sporosarcina pasteurii, two seawater temperatures of 10 ℃ and 30 ℃ were considered. Under different domestication schemes, the carbonate production after MICP was measured. The specific process is described as follows:

(1) The expanded culture of bacteria under different domestication schemes with different seawater concentrations was conducted and then taken out for use after shaking at 180 r/min for 48 h[33].

(2) The 100 mL of each bacterial solution under different domestication schemes was taken out and added into a conical flask containing 300 mL cementing solution with concentration of 0.5 mol/L (sufficient cementing fluid is an equal volume mixture of 1.0 mol/L $CaCl_2$ solution and 1.0 mol/L urea solution) for full mixing. Then they were placed in an electric thermostatic incubator at 10 ℃ or 30 ℃ for 48 h to make the bacterial solution react fully with the cementing solution.

(3) After 48 h, the conical flask was taken out, and the carbonate generated in the conical flask was poured into the culture dish. After drying, the carbonate production was weighed.

The carbonate production under different domestication schemes is shown in Fig.4, from which it can be seen that:

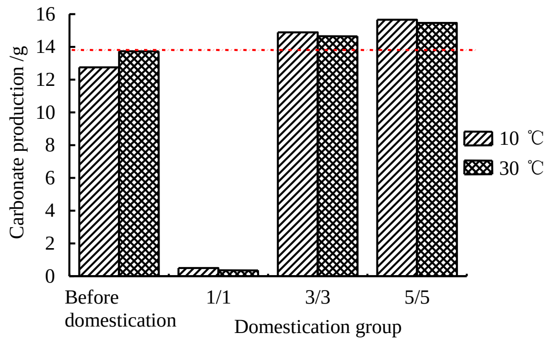


Fig. 4 Carbonate production under different domestication schemes

(1) In the direct domestication scheme, the carbonate production at the two temperatures was significantly lower than that before domestication (i.e. MICP of undomesticated Sporosarcina pasteurii in the freshwater environment, hereafter referred to as “before domestication”), only accounting for 2.62%–3.92% of the carbonate production before domestication. This indicates that Sporosarcina pasteurii had poor adaptability to seawater environment and the MICP process was significantly inhibited, which are consistent with the experimental results obtained by Peng et al.[18]. After three-gradient domestication, the carbonate production increased by 6.68%–16.82%; and after five-gradient domestication, the carbonate production increased by 12.59%–22.79%. This indicates that in the process of gradient domestication, the adaptability of Sporosarcina pasteurii to seawater was gradually enhanced. In addition, the carbonate production in the seawater environment was increased to a certain extent compared to that in the freshwater environment, because there are more calcium and magnesium ions in the seawater environment.

(2) The comparison of carbonate production at the two temperatures shows that before domestication, the difference of carbonate production between 10 ℃ and 30 ℃ was 7.75%, which is consistent with the effect of temperature on MICP obtained in the previous studies[32]. After three- and five-gradient domestication, the difference of carbonate production between 10 ℃ and 30 ℃ gradually decreased to 1.64% and 1.21%, respectively, indicating that the effect of temperature on MICP was significantly weakened after gradient domestication. That is to say, the domesticated bacteria can adapt not only to the seawater environment, but also to the temperature.

3 Cementation effect of domesticated Sporosarcina pasteurii in seawater environment on calcareous sand

3.1 Physical properties of calcareous sand

The density of calcareous sand used in the test is 2.70–2.85 g/cm^3. The calcareous sand before sieving is shown in Fig.5. To obtain the optimum cementation effect, the standard sieve was used in the test. The calcareous sand was prepared according to different particle sizes and corresponding proportions. The particle size distribution curve is shown in Fig.6. Before cementation of calcareous sand, 0.1 mol/L HCl solution and 0.1 mol/L NaOH solution were successively used to soak sand for 12 h[34]. After soaking, the calcareous sand was washed with deionized water and dried for further use.

Fig. 5 Calcareous sand before sieving

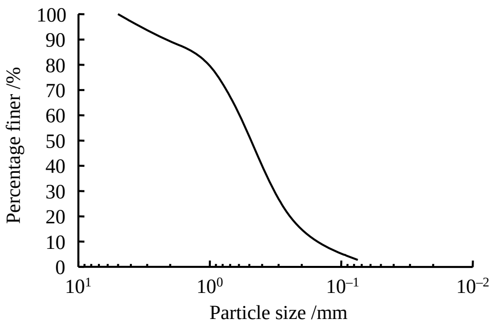


Fig. 6 Particle size distribution curve of calcareous sand

3.2 Test schemes

According to the test results of the domestication of Sporosarcina pasteurii, the bacterial concentration under direct domestication was too low. Therefore, the Sporosarcina pasteurii after three- and five-gradient domestication were merely considered for the cementation test on sand column in this section. For comparison, undomesticated Sporosarcina pasteurii was selected to cement the calcareous sand in the freshwater environment. The specific test schemes are shown in Table 3.

The cementation of calcareous sand was carried out by soaking method[17]. The specific test process is described as follows:

(1) The calcareous sand was poured into the self-developed flexible porous mold in layers, as shown in Fig.7. Each layer was slightly vibrated and compacted, and the roughening treatment was carried out between layers. After sample preparation, deionized water was injected from the top of the sample to eliminate the air between calcareous sand particles.

Table 3 Test schemes of calcareous sand cementation under different domestication schemes

Test group	No.	Bacteria	Water used for culture medium and cementing solution
Before domestication	O	Sporosarcina pasteurii	Deionized water
Three-gradient domestication	D	Three-gradient domesticated Sporosarcina pasteurii	Artificial seawater
Five-gradient domestication	J	Five-gradient domesticated Sporosarcina pasteurii	

Fig. 7 Self-developed flexible porous mold

(2) The 80 mL bacterial solution was injected into the sand sample and stood for 12 h until the bacterial solution was fully adhered to the surface of calcareous sand particles.

(3) The 160 mL cementing solution with concentration of 0.5 mol/L was prepared by mixing 80 mL 1 mol/L $CaCl_2$ solution and 80 mL 1 mol/L urea solution (the cementing solution will be prepared with artificial seawater or deionized water depending on the cementation scheme). It was injected into the sand sample, and the waste solution was allowed to flow out from the bottom of the mold after 3 d.

(4) Steps (2) and (3) belong to single cementation process. The calcareous sand was cemented by repeating cementation process for 10 times.

(5) The fixing sleeve at the bottom of the mold was removed. The sample was inverted, and then Steps (2) and (3) were repeated for 10 times.

(6) The sidewall mold was removed. The samples were placed in the oven and dried at 60 ℃ to constant weight, and then the subsequent macroscopic physico-mechanical tests and microscopic tests were carried out.

The typical calcareous sand column after cementation is shown in Fig.8. Firstly, the carbonate content of the cemented calcareous sand column was determined by measuring the mass difference before and after cementation. Then, the end of the calcareous sand column was polished, and the unconfined compression test was carried out on the sample using the RMT-150C rock mechanics test system at a loading rate of 0.01 mm/s until the sample was failed.

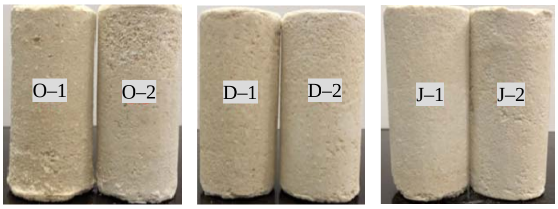


Fig. 8 Typical MICP-cemented calcareous sand column samples

3.3 Effects of domesticated Sporosarcina pasteurii on mechanical properties of cemented calcareous sand columns

3.3.1 Carbonate content in calcareous sand column

The existing studies showed that when the carbonate production was higher than 60 kg/m^3, the strength of the sand column was significantly improved[35], thus the carbonate production can be used to evaluate the cementation effect. The calcium carbonate deposited in common cemented silica sand is usually measured by pickling[36]. However, the calcium carbonate content of calcareous sand itself is high, thus the pickling method is not suitable. Therefore, in this study, the mass difference of the calcareous sand column before and after cementation was used to represent the mass of carbonate production. The carbonate content of the calcareous sand column under different cementation schemes is shown in Fig.9.

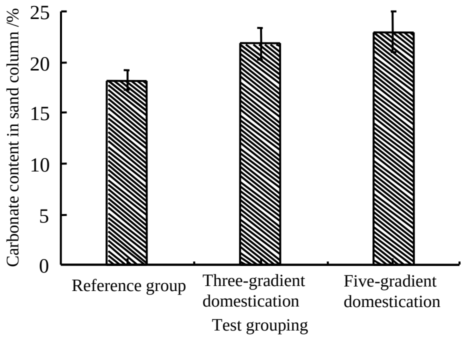


Fig. 9 Carbonate production in sand column under different schemes

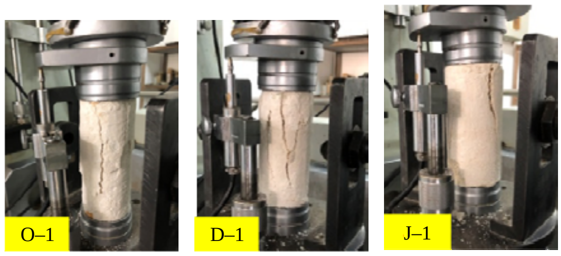
As can be seen from Fig.9, following conclusions can be drawn:

(1) In the seawater environment, the carbonate production in the calcareous sand column cemented by three- or five-gradient domesticated Sporosarcina pasteurii was more than 18% (converted from 60 kg/m^3 by calculation), indicating that the strength of the calcareous sand column was significantly improved.

(2) In the seawater environment, the carbonate content in the cemented calcareous sand column was higher than that in the freshwater environment. The carbonate production of the sand column in the three- and five-gradient domestication groups was 19.67% and 25.86% larger, respectively, than that in the reference group. In comparison, the bacteria produced more carbonate after five-gradient domestication. Compared to previous studies[20], when the concentration of cementing solution in the seawater environment was 0.5 mol/L, the carbonate production in the sand column was up to 14%. In this study, the carbonate content in the same concentration of the cementing solution was more than 20%. It is indicated that the domesticated bacteria can better adapt to the seawater environment, thus more carbonate with cementation can be generated.

3.3.2 UCS of calcareous sand column

Failure modes of sand columns under different schemes are shown in Fig.10. It is shown that the failure modes of sand columns under different schemes are basically the same, with a main crack extending from the top and a number of small cracks, without local failure, indicating good uniformity of soil samples.



(a) Reference group (b) Three-gradient domestication group (c) Five-gradient domestication group

Fig. 10 Failure modes of calcareous sand column under different schemes

Typical UCS curves of calcareous sand columns under different test schemes are shown in Fig.11.

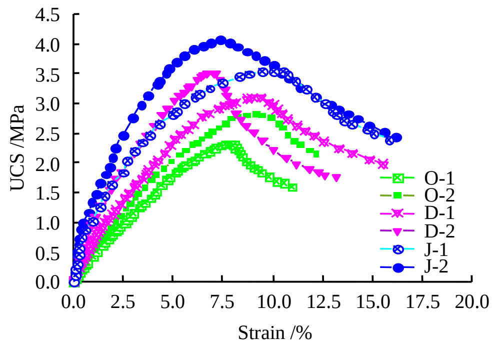


Fig. 11 UCS curves of sand column under different schemes

Figure 11 shows that the axial deformation-UCS curves of calcareous sand columns under different test schemes have basically the same trend. The curve increases linearly before reaching the peak strength and then decreases gradually, showing an obvious yield stage and post-peak softening stage. Before domestication, the average UCS of the calcareous sand column was 2.57 MPa. After three-gradient domestication, the strength increased by 20.86%–36.78%, and after five-gradient domestication, the strength increased by 38.17%–58.19%. The UCS of the calcareous sand column in the five-gradient domestication group reached 4 MPa.

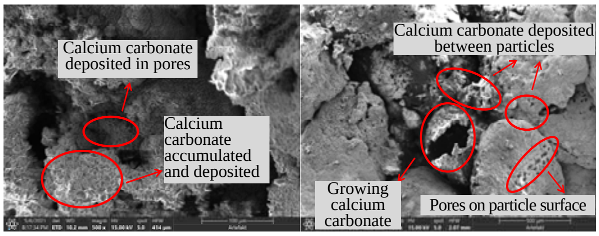
The UCS is consistent with the carbonate production. With the increase in carbonate production, the filling effect of the pores between calcareous sand column particles and the pores in the calcareous sand increases, the cementation between sand particles becomes stronger, the cementation effect becomes more obvious, and the uniformity of the sand column is higher, thus the strength of sand column becomes larger. In the available literature[17], the maximum UCS of the sand column was about 1.75 MPa when the calcareous sand was cemented by Sporosarcina pasteurii in a simulated seawater environment. While the UCS of the sand column tested in this study is greater, indicating that the domesticated Sporosarcina pasteurii has a better cementation effect on calcareous sand.

4 Mechanism of calcareous sand cemented by domesticated Sporosarcina pasteurii in seawater environment

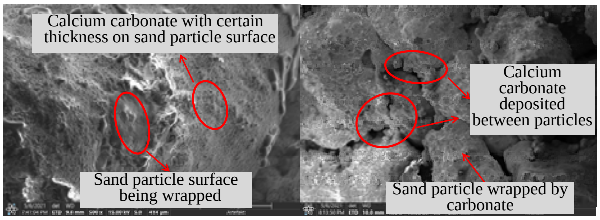
To understand the influence mechanism of the domestication process on the MICP cementation effect, scanning electron microscope (SEM) and energy dispersive spectroscopy (EDS) were used to analyze the microstructure of the calcareous sand column under different cementation schemes. Taking the reference group and the five-gradient domestication group for examples, the samples that failed in the unconfined compression test were selected for SEM analysis. SEM images magnified by 100 and 500 times are shown in Fig.12, and the corresponding EDS spectra are shown in Fig.13.

Combined with Figs.12 and 13, it can be seen that the elemental composition of the product includes Ca, O and C after the cementation of calcareous sand by the undomesticated Sporosarcina pasteurii in the freshwater environment, indicating that the product is mainly calcium carbonate. A large amount of calcium carbonate grows and fills the gap between calcareous sand particles, and the accumulated calcium carbonate crystals are large. Meanwhile, the pores on the surface of calcareous sand particles are gradually filled by the calcium carbonate generated. After the cementation of calcareous sand by five-gradient domesticated Sporosarcina pasteurii in the seawater environment, the elemental composition of the product includes not only Ca, O and C, but also Mg and Cl (the production of Cl may be due to the attachment of seawater to the surface of the product), indicating that the product contains at least two types of carbonate, i.e. calcium carbonate and magnesium carbonate. Sun et al.[37] demonstrated that the strength of the cemented body by magnesium carbonate was higher than that by calcium carbonate. This is one of the reasons why the UCS of the cemented calcareous sand column in the seawater environment is higher than that of freshwater.

Another obvious phenomenon is that when the calcareous sand was cemented with domesticated Sporosarcina pasteurii, smaller carbonate crystals were generated in the particle pores, the cementation between particles was stronger, and the pores on the particle surface were well blocked. An optical microscope was used to observe the morphological changes of Sporosarcina pasteurii before and after domestication, as shown in Fig.14. It is found that the number of bacteria after domestication was significantly reduced compared with that before domestication, which was also the reason for the small and dense carbonate mineral crystals generated.

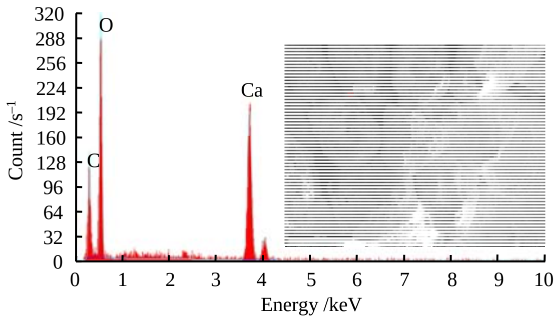


Magnification of 100× Magnification of 500×
(a) Reference group

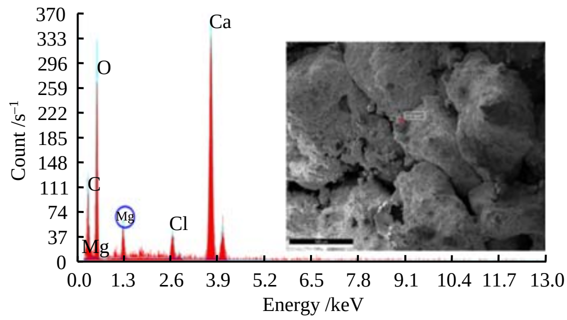


Magnification of 100× Magnification of 500×
(b) Five-gradient domestication group

Fig. 12 SEM results under different schemes

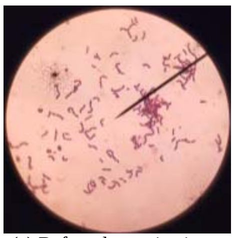


(a) Reference group

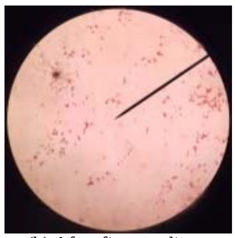


(b) Five-gradient domestication group

Fig. 13 EDS spectrum results under different schemes



(a) Before domestication



(b) After five-gradient domestication

Fig. 14 Comparison of morphological changes of Sporosarcina pasteurii before and after domestication

In general, the influence mechanism of domesticated Sporosarcina pasteurii in artificial seawater environment on the cementation of calcareous sand is mainly shown in the following three aspects: (1) Gradient domestication can make bacteria gradually adapt to seawater environment, maintain good growth and reproduction ability, and secrete urease with high activity for MICP. (2) Due to the presence of Ca^{2+} and Mg^{2+} in the cementing solution, the bacteria induced $CaCO_3$ and $MgCO_3$ precipitation in the process of cementation, and the calcareous sand column was well cemented under the action of these two types of carbonate. (3) After domestication, the morphology of Sporosarcina pasteurii was significantly reduced, resulting in the formation of smaller and denser carbonate mineral crystals during MICP. Under the combined action of the above three aspects, the loose calcareous sand was cemented to form a calcareous sand column, and its UCS was significantly higher than that of the calcareous sand column cemented in the freshwater environment

5 Conclusions

In this paper, Sporosarcina pasteurii was artificially domesticated in the seawater environment, and the cementation effect of domesticated bacteria on calcareous sand was studied by conducting a series of tests. The main conclusions are drawn as follows:

(1) By comparing direct domestication, three-gradient domestication and five-gradient domestication, it was found that the seawater environment can inhibit the formation of bacteria and the synthesis of urease, and the gradient domestication could make bacteria gradually adapt to the seawater environment and temperature. In comparison, the bacteria domesticated by five gradients had more advantages than that domesticated by three gradients. The corresponding bacterial concentration can reach more than 97% of that before domestication, and the carbonate production increased by 12.59%–22.79% at different temperatures compared to that before domestication.

(2) The UCS values of calcareous sand column in three- and five-gradient domestication groups were up to about 3.5 MPa and 4.0 MPa, which increased by 20.86%–36.78% and 38.17%–58.19%, respectively, compared with that in the freshwater environment, indicating that the gradient domestication method for Sporosarcina pasteurii was feasible. The domesticated Sporosarcina pasteurii could effectively improve the cementation effect of calcareous sand, and the effect of gradient domestication was also obvious.

(3) After gradient domestication, the morphology of Sporosarcina pasteurii was significantly reduced, and it showed good adaptability to the seawater environment. Meanwhile, the seawater environment can provide more calcium and magnesium ions for MICP action, which in turn promotes the carbonate production during cementation and improves the strength of the cemented sand column.

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