Use of microfluidic experiments to investigate the effects of seawater salinity on microbially induced carbonate precipitation

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ABSTRACT: Microbially induced carbonate precipitation (MICP) is a promising soil reinforcement and anti-erosion technology for marine engineering. This study used microfluidic technology to investigate the effects of seawater salinity on the microscopic processes and mechanisms of MICP. Results show that high seawater salinity affects the type, shape, size, growth, and transformation rate of carbonate minerals. Hemispherical vaterite and rod-shaped aragonite were the predominant carbonate crystal polymorphs observed. While bacterial activity was reduced by 10% due to salinity, the average crystal diameter increased by 21.6%, suggesting that conventional MICP protocols can still be successfully used in the marine environment. This study improves our understanding of the microscale mechanisms of MICP and promotes MICP for offshore applications, such as seabed stabilization and scour prevention around offshore structures in the marine environment.

1 Introduction

Microbially induced carbonate precipitation (MICP) is a new soil reinforcement and anti-erosion technology. Normally in this technology, microbes with ureolysis activity and cementation solution which mainly contains urea and $CaCl_2$ are injected into the pores of soils via hydraulic gradient, after which the microbes produce urease which catalyze the hydrolysis of urea, producing carbonate which react with calcium forming calcium carbonate crystals. These crystals bind soil particles enhancing soil strength and resisting against erosion.

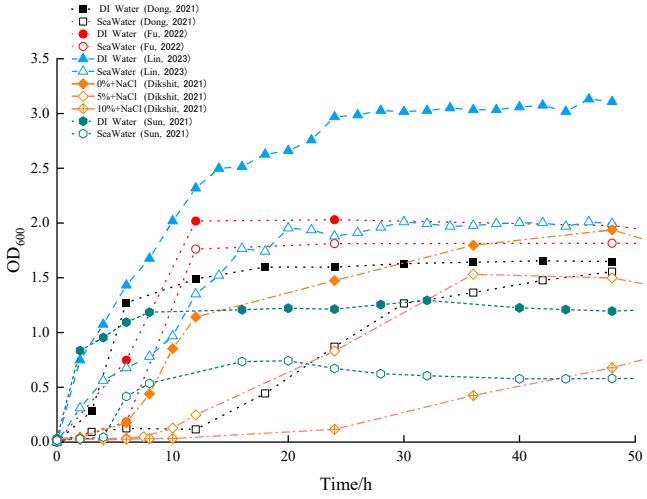
Research on the potential of using MICP in marine environments has increased with its development. This technique has shown potential applications in marine engineering, such as reinforcing island reefs and controlling shoreline erosion. *Sporosarcina pasteurii* (*S. pasteurii*), a type of soil bacteria, is widely used in MICP because of its high urease activity and has been widely explored for stabilizing onshore and offshore soils. Previous studies have demonstrated that *S. pasteurii* can grow in seawater environments and that increased salinity promotes calcium carbonate precipitation (Mortensen et al. 2011). However, the limitation of calcium ion concentration in seawater has been a challenge, requiring samples to be washed numerous times before reaching adequate compressive strength (Cheng et al. 2014). Additionally, experiments using sand columns have revealed that higher salt content may reduce the unconfined compressive and tensile splitting strength of samples by around 30-45% (Peng et al. 2022).

Currently, research on MICP has primarily focused on solution and soil column experiments to investigate the impact of salinity on bacterial growth and the strength of MICP cemented samples. However, there has been a lack of research on the dynamic generation process of MICP in pore conditions under different salinity levels. To address this gap, this paper utilizes a microfluidic chip that simulates real pores to observe and interpret the cementation process of MICP in the marine environment. The aim is to explore the influence of salt on the MICP process at the pore scale and quantitatively analyze the effects of salt on the type, shape, size, and growth and transformation rates of calcium carbonate crystals generated by MICP. This microscopic analysis provides insights into the potential marine applications of MICP.

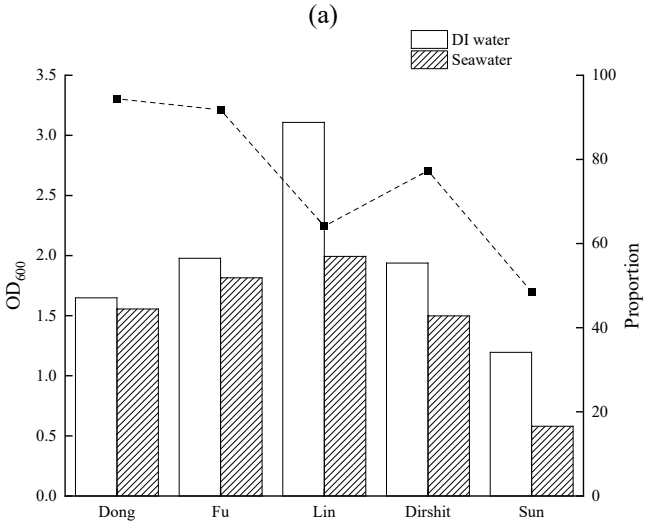
2 Effects of salinity on bacterial reproduction

Previous studies have shown that *S. pasteurii* can grow in seawater, but the salt content in seawater can inhibit the growth rate of *S. pasteurii*, and the inhibitory effect of salt on the growth rate of *S. pasteurii* increases with the increase of salinity (Dikshit et al. 2021, Dong et al. 2021, Sun et al. 2021, Fu et al. 2022, Zhao et al. 2022, Lin et al. 2023). Optical density (OD) measured at a wavelength of 600 nm (OD_{600}) is commonly used as an indirect measure of bacterial density in a liquid culture. OD_{600} is based on the principle that the presence of bacteria in a liquid culture can affect the transmission of light through the medium. The correlations between Optical density (OD) and bacterial cell number per unit ml was quantified by Wang et al. (2021). As shown in Figure 1a, rapid growth of *S. pasteurii* was observed using OD_{600} measurements in both fresh,

deionized (DI) water and seawater environments within 24 hours, and the OD_{600} value tended to stabilize after 48 hours of cultivation. To analyze the impact of salt further quantitatively on the growth of *S. pasteurii*, the OD_{600} values of bacteria after 48 hours of cultivation in various studies were selected as a comparative indicator, and the analysis results are shown in Figure 1b. The results depicted in Figure 1b indicate that the addition of salt leads to varying degrees of reduction in the OD_{600} value of *S. pasteurii* at the same culture time. Therefore, the ratio of the OD_{600} value of artificial seawater introduced for 48 hours to the OD_{600} value of deionized water at the same time is used to characterize the extent of salt's influence on *S. pasteurii*. The addition of salt causes the OD_{600} value of bacteria to change to 48%-94% of deionized water. The large fluctuation in the range of OD_{600} reduction is attributed to differences in bacterial culture and seawater composition.



(a)



(b)

Figure 1 Effects of sea salt contents on growth of *S. pasteurii* (a) growth curve of *S. pasteurii* in pure water, seawater, and sodium chloride solution; (b) ratio of optical density of *S. pasteurii* after being cultivated for 48 hours indeionized (DI) water and seawater.

3 Effect of salinity on urease activity

S. pasteurii has been found to secrete active urease in a seawater environment, but the presence of salt in seawater can inhibit its urease activity compared to a deionized water system, as reported in previous studies (Dong et al. 2021, Fu et al. 2022, Lin et al. 2023, Xiao, et al. 2023). Urea itself does not possess the ability to conduct electricity. However, when urea undergoes hydrolysis catalyzed by the enzyme urease, it is converted into CO_3^{2-} (carbonate) and NH_4^+ (ammonium ions). This conversion results in an increase in the electrical conductivity (EC) of the solution. As a result, by measuring the difference in EC before and after mixing a bacterial suspension with a urea solution, the activity of urease can be estimated. This method has been proposed by Whiffin (2007) and widely adopted. To investigate the effect of salt on urease activity, the ratio of EC in a salt environment to that in a deionized water environment was used as a parameter to quantify the impact of salt on urease activity. The results of this analysis are presented in Figure 2. The findings depicted in Figure 2 demonstrate that the presence of salt in seawater leads to a reduction in the activity of urease that *S. pasteurii* secretes. As the culture time increases, the attenuation of urease activity caused by salt decreases gradually in a logarithmic manner. In the initial stage of bacterial growth (0-10 h), the activity under salt conditions is only 25% of that under deionized water conditions, indicating that salt significantly inhibits urease activity in the early stage of bacterial growth. During the rapid growth phase of bacteria (10-40 h), the activity of the salt group continued to increase, and the activity ratio elevated from 25% to 70%. When the bacterial plateau period was reached (40-50 h), the activity of the salt group remained constant, and the activity ratio stayed around 75%. At this point, salt caused a 25% reduction in urease activity. This could be because salt can impede the reproduction of *S. pasteurii*. Nevertheless, with the increase of culture time, some bacteria gradually adapted to the saline environment and commenced reproducing, resulting in a gradual increase in overall urease activity in the saline system.

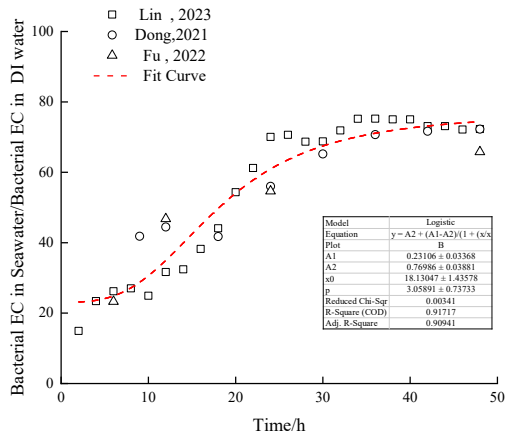


Figure 2 Effects of sea salt contents on activity of *S. pasteurii.*

4 Microfluidic chip experiment

The apparatus employed in this study is depicted in Figure 3 and comprises an injection pump, microscope, syringe, and microfluidic chip. The microfluidic injection pump model used in this research is Lange LSP01-1A, which has a flow control range of 0.001-43.349 ml/min. The microscope utilized in the investigation is the German-made Carl Zeiss Axio Observer7. The microfluidic chip demonstrated in Figure 3 was produced using the microfluidic chip structure and fabrication principles described in Wang's earlier research (Wang et al. 2017, 2019).

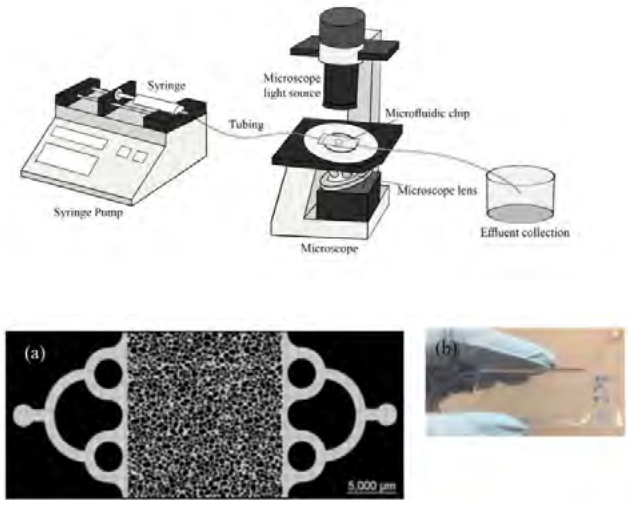


Figure 3 Schematic of experimental setup

The bacterium selected in this study is *S. pasteurii*, and the medium used is (ATCC) 1376 NH4-YE agar medium, which contains 20 g/L yeast extract, 10 g/L ammonium sulfate, 20 g/L agar, and 0.13 M tris(hydroxymethyl)aminomethane. To simulate the salinity conditions in the marine environment, the paper configures artificial seawater using the formula of ASTM D1141-98 (2013). The cementing fluid used is composed of 0.5 M $CaCl_2$, 0.75 M urea, and 3 g/L beef extract, which is dissolved in deionized water and artificial seawater to form salt-containing and salt-free cementing fluids, respectively.

The experiment was carried out using the device shown in Figure 3. *S. pasteurii* was cultivated to OD_{600}=1, and then 1.25 pore volumes (PV) of *S. pasteurii* were injected into the microfluidic chip at a flow rate of 5.6 PV/h, followed by 24 hours of standing. Next, 1.25 PV of the specified cementing solution was injected into the microfluidic chip at a flow rate of 5.6 PV/h and left to stand for 24 hours. Multiple injections of cementing solution were then performed using the same injection parameters until 6 times of cementing solution was injected. After injecting 6 times of cementing solution, the microfluidic chip was washed with deionized water. The experimental temperature was set at 20 degrees Celsius, serving as an illustrative representation of the typical temperature observed in shallow seawater during the summer season. To characterize the effect of salt on the microbially induced calcium carbonate precipitation (MICP) process, the paper introduces the calcium carbonate pore ratio as a measure of the total amount of calcium carbonate generated, which is the ratio of the calcium carbonate volume to the initial pore volume. The paper also introduces the chemical conversion rate to represent the conversion efficiency of calcium carbonate

$$\frac{V_{c100\%}}{V_v}=\frac{0.5\times IN\times 100}{2.71\times 1000}\times 100\% \quad (1)$$

$$CTE=\frac{\frac{V_c}{V_v}}{\frac{V_{c100\%}}{V_v}}\times 100\% \quad (2)$$

where, V_c represents the total volume of precipitated calcium carbonate crystals, $V_{c100\%}$ represents the total amount of calcium carbonate that would be generated if all the Ca^{2+} in the injected cementing fluid had reacted, V_v represents the pore volume, *IN* represents the number of times cementing fluid was injected, and *CTE* represents the chemical conversion efficiency.

Figure 4 presents microscopic images of calcium carbonate crystals 24 hours after 1, 3, and 6 injections of cementing fluid. The results show that both the size and number of calcium carbonate crystals increase with the number of injections in both the artificial seawater and deionized water systems. In the deionized water system, mainly rhombohedral calcium carbonate crystals are formed, while spherical and rod-shaped calcium carbonate crystals are mainly formed in the artificial seawater system. Moreover, the introduction of salt inhibits the transformation of vaterite to calcite, as confirmed by a comparison of the microscope images with SEM images in the literature.

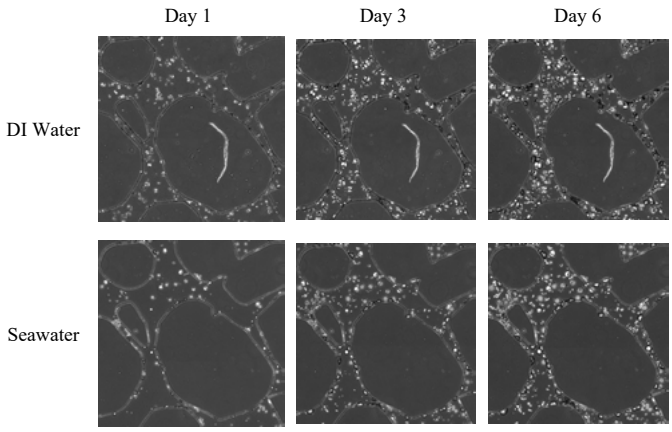


Figure 4 Microscopic images of $CaCO_3$ formed in DI water and seawater in microfluidic chips

To further quantify the effect of salt on the size and quantity of calcium carbonate crystals, this study selected a 200 μm×200 μm area of crystals for statistical analysis. The statistical results, including the diameter and quantity of calcium carbonate crystals, are presented in Figures 5 and 6. Figure 5 shows the statistical area of the microscope after 1, 2, 4, and 6 injections of cementing fluid for 24 hours. The results in Figure 5 suggest that the introduction of salt leads to an increase in the diameter and a decrease in the number of calcium carbonate monomers. Furthermore, combined with the statistical chart of equivalent diameter of calcium carbonate presented in Figure 6a, it can be observed that the equivalent diameter of calcium carbonate in both deionized water and seawater systems increases with the number of cementation times. After six injections of cementing fluid, the average effective diameter increases by up to 21.6% due to the presence of salt (Figure 6b).

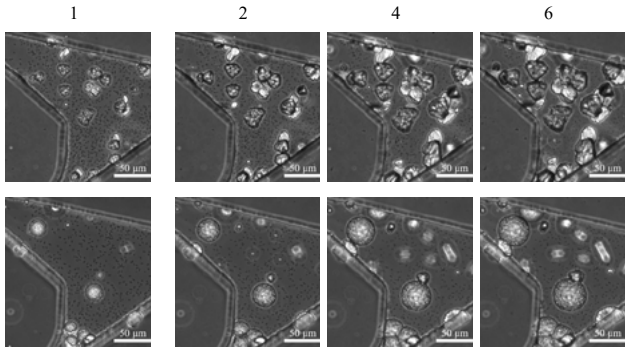
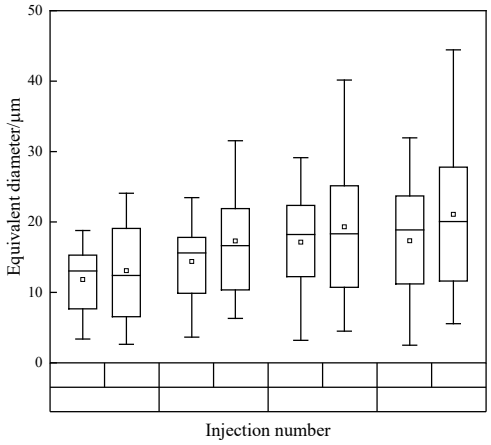
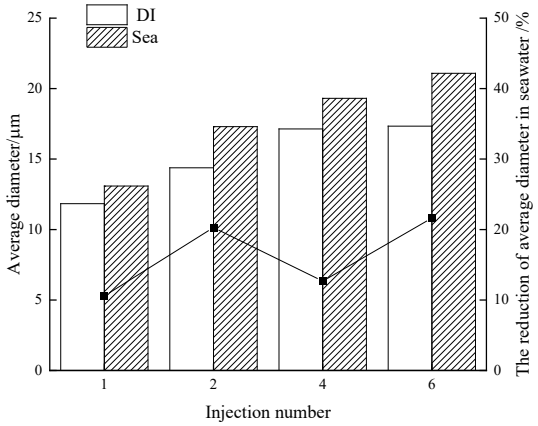


Figure 5 200 μm ×200 μm images of $CaCO_3$ crystals formed in DI water and seawater in microfluidic chips



(a)



(b)

Figure 6 Equivalent diameter of calcium carbonate after different degrees of cementation

Figure 7 displays the number of calcium carbonate crystals in the statistical area after 1, 2, 4, and 6 injections of cementing fluid for 24 hours. The number of calcium carbonate crystals in both deionized water and seawater systems increased with an increase in the number of cementation times. However, after six injections of cementing fluid, the presence of salt caused a decrease of 33.3% in the number of calcium carbonate crystals.

Figures 8 and 9 shows the impact of salinity on the calcium carbonate yield and chemical conversion rate (CTE%) in MICP. As depicted in Figure 8, the calcium carbonate content in both systems increases linearly with an increase in cementation times, but the growth rate of the salt group is slower. Figure 9 suggests that the introduction of salt leads to a reduction in the chemical conversion efficiency of MICP by approximately 10%. However, the chemical conversion rate is still quite considerable. Hence, the utilization of *S. pasteurii* in MICP processes in marine environments holds significant potential for development.

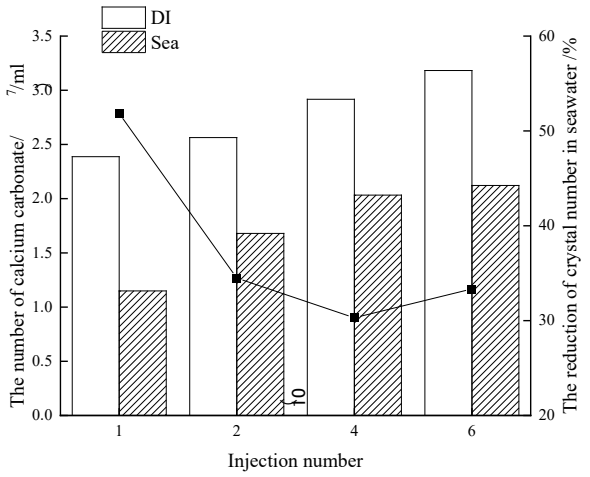


Figure 7 Number of calcium carbonate after different degrees of cementation

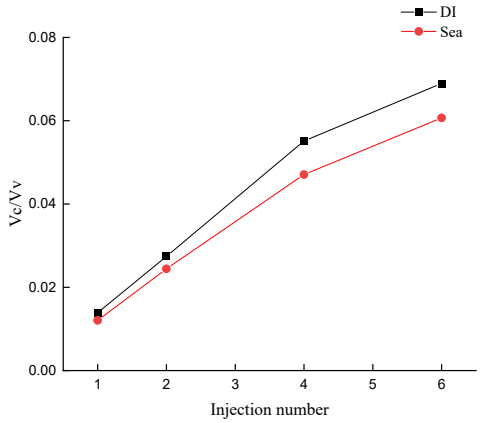


Figure 8 Volume of $CaCO_3$ crystals (CTE%, chemical conversion rate) formed in DI water and seawater in microfluidic chips in relative to volume of pores

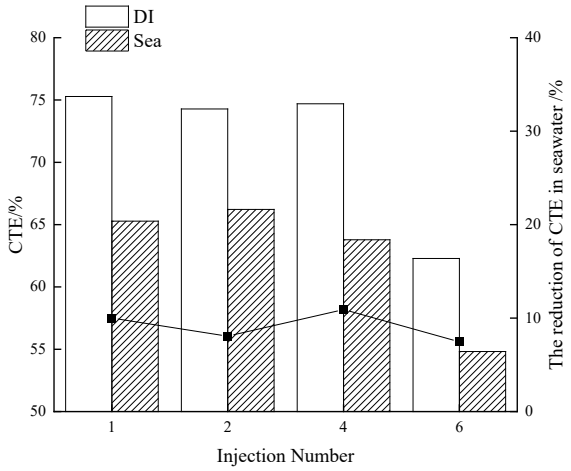


Figure 9 Effect of salt on chemical transform efficiency of $CaCO_3$

5 Conclusions

This paper provides a comprehensive review of the influence of salt on the reproduction of *S. pasteurii*, as well as its impact on urease activity, calcium carbonate crystal diameter, quantity, and chemical conversion rate in the process of MICP, examined through microfluidic experiments. The key findings of the study are as follows: Firstly, the presence of salt hinders the growth rate of *S. pasteurii*, resulting in a reduction of the bacteria's optical density (OD_{600}) to approximately 48%-94% compared to distilled water. Secondly, the introduction of salt, particularly in seawater, decreases the activity of urease produced by *S. pasteurii*, with the attenuation of urease activity logarithmically decreasing as the culture time progresses. Thirdly, the analysis of crystal morphology indicates that in distilled water, the crystals primarily consist of calcite, whereas in artificial seawater, vaterite and aragonite become the predominant forms. The addition of salt impedes the transformation of vaterite into calcite. Fourthly, following six injections of cementing fluid, the presence of salt leads to an average increase of 21.6% in the equivalent diameter of calcium carbonate crystals, while the number of crystals decreases by 33.3%. Lastly, the introduction of salt diminishes the chemical conversion efficiency of MICP by approximately 10%, although the overall chemical conversion rate remains significant.

6 Implications

MICP technology has the potential to significantly impact seabed stabilization and scour prevention around offshore structures in the marine environment. It offers an alternative to traditional methods like rock dumping, providing enhanced stability and reduced erosion risks. MICP can be applied for pre or post-foundation installation, promoting load-bearing capacity and reinforcing sediments. This eco-friendly approach utilizes microorganisms and naturally occurring minerals to induce calcium carbonate precipitation, minimizing environmental impact. While further development is needed, MICP shows promise for sustainable offshore engineering practices.

Acknowledgements

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