

Research Article

Biom mineralization Performance of *Bacillus sphaericus* under the Action of *Bacillus mucilaginosus*

Yingying Hu,^{1,2} Weitao Liu ,¹ Wenjing Wang,³ Xinlei Jia ,² Lanjuan Xu,² Qing Cao,² Jianjun Shen,² and Xiangming Hu ¹

¹College of Safety and Environmental Engineering, Shandong University of Science and Technology, Qingdao 266590, China

²College of Chemical Engineering and Safety, Bin Zhou University, Binzhou 256600, China

³Department of Mechanical Engineering, University of Alberta, Edmonton, Alberta, Canada

Correspondence should be addressed to Weitao Liu; sdkdwtl@163.com

Received 21 February 2020; Accepted 25 June 2020; Published 18 July 2020

Academic Editor: Antonio Caggiano

Copyright © 2020 Yingying Hu et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Microbial Induced Calcite Precipitation (MICP) is a biochemical process widely found in nature, also known as microbial mineralization. This paper investigates whether this process can help promote the intelligent reinforcement and repair of underground projects such as mines and tunnels. We selected *Bacillus sphaericus* and *Bacillus mucilaginosus* as the research objects. The former has an outstanding urease production ability, and the latter can secrete carbonic anhydrase in vitro. *Bacillus mucilaginosus* was introduced into the culture solution of *Bacillus sphaericus* in the most suitable culture environment, and the changes of mineralization rate and mineralization yield of *Bacillus sphaericus* were observed and analyzed. The results revealed that, to maintain the highest growth rate of *Bacillus sphaericus*, the optimal pH value was between 7 and 8, the optimal urea concentration was 0.5 mol/L, the optimal Ca²⁺ concentration was 0.6 mol/L, and the optimal Luria-Bertani (LB) culture concentration was 20 g/L. The amount of biom mineralized calcium carbonate precipitated in the double bacteria solution can reach 1.89 times the amount of the precipitation in the *Bacillus sphaericus* solution under the same conditions. It concludes that the introduction of *Bacillus mucilaginosus* can effectively increase the mineralization yield of *Bacillus sphaericus* without affecting the mineralized products.

1. Introduction

Mineralization is a general term for the process of converting organic compounds into inorganic compounds in soil under the action of soil microorganisms. The mineralization studied in this paper is the ability of *Bacillus sphaericus* to produce calcium carbonate. Specifically, *Bacillus sphaericus* can secrete urease, and the latter is capable of decomposing urea to form carbonate. The generated carbonate is then combined with calcium ions in the environment to form calcium carbonate.

Mineralization has a wide range of applications in engineering practice [1–6]. For instance, the use of microbial mineralization can achieve self-repair of cracks in cement materials such as concrete. Currently, the feasibility of microbial remediation technology has been verified in

engineering and other engineering fields. Scholars such as Belie of Ghent University in Belgium and Jonkers of Delft University in the Netherlands have successfully implemented microbial cemented sand to repair structural surface cracks [7, 8]. Bang and Ramakrishnan [9] found that microbial enzymatic action can promote the precipitation of calcium carbonate, and they also studied how to use microbes to repair cracks on the surface of cement-based materials. Carlos et al. [10] studied the microbial repair of cracks in decorative limestone surfaces. Okwadha and Li [11] explored the microbial repair of cracks on the surface of tunnels. However, it remains a challenge to apply the microbial mineralization and remediation technology to the repair of rock mass fractures under complex geological conditions such as mining [12–15]. One of the challenges is to improve the timeliness and effectiveness of microbial

induced calcium carbonate deposition. The current research has been dramatically restricted in the crack repair width. For example, according to the experiments of Wang et al. [16], the upper limit of the concrete crack that can be repaired by *Bacillus sphaericus* is 0.17 mm. Studies have shown that *Bacillus mucilaginosus* can secrete carbonic anhydrase (CA), and CA has the property of accelerating the hydration of CO_2 to form HCO_3^{3-} [17]. As such, it is possible that *Bacillus mucilaginosus* will exert an influence on the deposition of calcium carbonate under natural conditions. Zhou [18] confirmed this conjecture by studying the role of *Bacillus mucilaginosus* and carbonic anhydrase in the formation and growth of calcium carbonate crystals. That being said, studies on the effects of carbonic anhydrase on microbial induced calcium carbonate deposition have not been reported.

Priya et al. [19] evaluated the mechanical and durability properties of high strength concrete specimens by incorporating an alternative for cement and bacteria for healing cracks. Reddy and Revathi [20] developed a durable cement concrete by combining varying proportions of *Bacillus sphaericus* bacteria in crack filling and strength enhancement through biomineralization. To create environment-friendly biocement materials, Choi et al. [21, 22] studied the effect of PVA fibers and biocement in improving the engineering properties of the silica sand. Yu et al. [23–26] presented a series of experiments to select the optimal formulation of a new biocement that can convert ammonia into cementitious materials. They think that loose quartz sand grains can well be cemented by microbially induced barium phosphate precipitation into a sand column. However, there are few research on the application of biotechnology in the mine leakage stoppage technology.

To sum up, this paper studies the biomineralization performance of *Bacillus sphaericus* under the action of carbonic anhydrase, based on the biochemical theory of microbial mineralization and diagenesis. This work provides a theoretical basis for the engineering application of microbial natural mineralization and water control. It also offers a new technology for the prevention and control of water inrush. Meanwhile, due to the advantages of microbial remediation technology, such as good remediation effect, green environmental protection than other traditional remediation materials, the use of microbial induced deposition of carbonate for seepage control and leakage stoppage has gradually become an important research direction of microbial remediation technology. However, it is necessary to apply the microbial mineralization remediation technology to the rock fracture seepage resistance under the adverse conditions such as mining and study the mechanism of microbial impermeability. Therefore, the optimization research of calcium carbonate deposition becomes more and more urgent.

With the intelligent development trend of concrete materials, the application value of microbial mineralization self-repair technology has become increasingly prominent. Through the sensitive and automatic sensing of cracks by microorganisms, automatic detection and intelligent repair of cracks can be realized. Accordingly, the structural damage

caused by crack propagation can be avoided [27–35]. Furthermore, the natural dormancy and reproduction of microorganisms can promote the sustainability of this intelligent repair. At present, the urgent problem to be solved in microbial intelligent repair research is how to speed up the formation of microbial mineralization products and how to increase the production of calcium carbonate. Given this, this paper selected *Bacillus sphaericus* as the research object and explored the effects of carbonic anhydrase, pH, Ca^{2+} , and urea concentration on its mineralization performance.

Additionally, we adopted the SEM, XRD, FTIR, and TG-DSC analysis methods to investigate the crystal structure and microstructure of microbial minerals rapidly formed after the introduction of carbonic anhydrase. We hope to increase the yield and rate of calcium carbonate precipitation without affecting the crystal structure of calcium carbonate and ultimately improve the efficiency of microbial repair of cracks. On the other hand, in order to further study the mineralization performance, we tried to add *Bacillus mucilaginosus* based on the above experiments to study the mineralization under the action of double bacteria. In the experiment, we obtained the urease activity by testing the conductivity and acquired the amount of bacterial growth by testing the optical density (OD) value.

2. Experiments and Materials

2.1. *Bacillus sphaericus* Inoculation. As the start of our experiments, we performed the *Bacillus sphaericus* inoculation as follows:

- (1) Weigh 5 g of LB medium and 3 g of agar powder, pour it into an Erlenmeyer flask containing 200 ml of distilled water, stir well, and seal it.
- (2) Sterilize the conical flask in a high-temperature sterilizer for 30 minutes (with a temperature of 121°C). After one and a half hours, remove the sterilized conical flask and place it in the ultraclean workbench. Turn on the ultraviolet (UV) lamp and fan to cool down.
- (3) After cooling, turn off the UV lamp and fan, and then turn on the light. After disinfecting the hands with alcohol, ignite the alcohol lamp, and open the conical flask. Burn the bottle mouth and the stopper over the flame of the alcohol lamp, pick a small amount of the bacteria from the solid medium colony of the plate by the inoculating loop, and place it in a conical flask liquid medium. Slightly swing the inoculating loop to disperse the cells in the liquid medium. As such, the inoculation was completed and cultured in a constant temperature shaking incubator.

2.2. Factors Affecting the Growth of *Bacillus sphaericus*. In this section, to find the most suitable culture environment for *Bacillus sphaericus*, we will explore the effects of five

factors on *Bacillus sphaericus*, namely, pH value, urea concentration, Ca^{2+} concentration, calcium source, and LB nutrient solution concentration.

2.2.1. Effect of pH Value. We prepared 1300 mL liquid medium, among which 1200 mL was evenly distributed in 6 Erlenmeyer flasks with the pH values sequentially adjusted to 7, 8, 9, 10, 11, and 12, respectively. After sterilization, they were inoculated on a clean bench, and the inoculum was 1% of the volume of the medium. We then put them in an incubator for shake culture. After 24 hours, the OD value at different pH was measured by a microplate reader. The experimental results are shown in Figure 1.

From Figure 1, the optimal pH value of the growth environment of *Bacillus sphaericus* is 7, and the growth activity is also high when the pH value is 8.

2.2.2. Effects of Urea Concentration. We prepared 1500 mL liquid medium, among which 1400 mL was evenly distributed in 7 Erlenmeyer flasks, and the pH was adjusted to 8. After sterilization, we added the urea solution prepared by filtration and sterilization method to the seven flasks of the medium, respectively. The concentrations were 0 mol/L, 0.25 mol/L, 0.5 mol/L, 0.75 mol/L, 1.0 mol/L, 1.25 mol/L, and 1.5 mol/L, respectively. We then inoculated on a clean bench, with the medium volume of 1%. The culture was shaken at a temperature of 28°C, and the OD value at different urea concentrations was measured after 24 h. Figure 2 demonstrates the experimental results.

As shown in Figure 2, although 0 mol/L urea concentration had the highest OD value, considering the necessity of urea for the bacterium's optimum biomineralization condition, the optimal urea concentration should be adjusted to 0.5 mol/L.

2.2.3. Effects of Ca^{2+} Concentration. We prepared 1100 mL of liquid medium and evenly distributed 1000 mL in 5 Erlenmeyer flasks. Next, we added anhydrous calcium chloride of 0 g (0 mol/L), 22.2 g (0.20 mol/L), 44.4 g (0.40 mol/L), 66.6 g (0.60 mol/L), 88.8 g (0.80 mol/L), and 111 g (1.00 mol/L) to the Erlenmeyer flask. The pH value was adjusted to 8. After sterilization, we inoculated the medium on a clean bench, and the inoculum was 1% of the volume of the medium. The mediums were shaken at a temperature of 28°C, and the OD values at different Ca^{2+} concentrations were measured after 24 h. The experimental results are revealed in Figure 3.

As illustrated in Figure 3, the best concentration of Ca^{2+} is 0.6 mol/L.

2.2.4. Effects of LB Nutrient Solution Concentration. LB medium was prepared at a concentration of 5 g/L, 10 g/L, 15 g/L, 20 g/L, 25 g/L, and 30 g/L, respectively, and the pH was adjusted to 8. After sterilization, the medium was inoculated on a clean bench at an amount of 1% of the medium volume. After inoculation, the inoculated medium was

cultured by shaking at a temperature of 28°C. The OD value at different LB concentrations was measured after 24 h, with the results shown in Figure 4.

As shown in Figure 4, the optimal LB nutrient solution concentration is 20 g/L.

2.3. Effect of pH on Enzyme Activity of Double Bacteria Solution. After we completed the bacterial culture, we measured the activity of urease in the bacterial liquid to determine whether the bacterial liquid meets the test requirements. In this paper, the conductivity of the enzyme was measured by a conductivity meter. We first prepared four bottles of *Bacillus sphaericus* with pH values of 7, 8, 9, and 10, respectively. Then, 25 ml of *Bacillus mucilaginosus* solution was added to each bottle of the bacterial solution and cultured for 24 hours. Meanwhile, as a comparison, we prepared four bottles of *Bacillus sphaericus* liquid with pH values of 7, 8, 9, and 10, respectively. Before the measurement, we prepared 90 mL of urea solution with a concentration of 1.1 mol/L. We pipetted 10 mL of the tested bacteria into the urea solution and mixed them evenly afterward. After immersing the electrode in the urea solution for calibration, we tested the change in the conductivity of the solution with conductivity meter within 5 min (the urease activity of the bacterial solution is the average change in conductivity multiplied by the dilution factor of the bacterial solution).

The experimental results showed that when the pH value increases from 7 to 10, the conductivity change values of *Bacillus sphaericus* added with *Bacillus mucilaginosus* were 0.20 ms/cm, 0.21 ms/cm, 0.19 ms/cm, 0.17 ms/cm, and 0.17 ms/cm, respectively. In the comparison group without added *Bacillus*, the conductivity changes were 0.18 ms/cm, 0.25 ms/cm, 0.19 ms/cm, and 0.20 ms/cm, respectively. The above data confirmed that *Bacillus sphaericus* produced the highest enzyme activity when pH value equals 8 in both cases.

2.4. Mineralization under the Action of *Bacillus mucilaginosus*. In order to study the effect of mineralization on the introduction of *Bacillus mucilaginosus*, we first inoculated *Bacillus sphaericus* in six bottles of a liquid medium with 100 ml per bottle. At the same time, we inoculated *Bacillus mucilaginosus* in 100 ml of liquid medium for culture. After the inoculated medium was placed in a constant temperature shaking incubator at 31°C for 24 h, we took out the *Bacillus mucilaginosus* solution and added them into the previously prepared 5 bottles of *Bacillus sphaericus* with the percentage of 10:1, 8:1, 6:1, 4:1, and 2:1, respectively. After that, they were further incubated with the blank comparison group for 24 hours. Figure 5 illustrates the experimental process.

After 24 hours of mixed culture, we took out six bottles of bacteria and centrifuged them by a centrifuge. In the centrifugation process, both anhydrous ethanol and distilled water were added three times. After that, we took out the lower layer of the centrifuge tube and dried it in a glass Petri dish. The experimental results show that

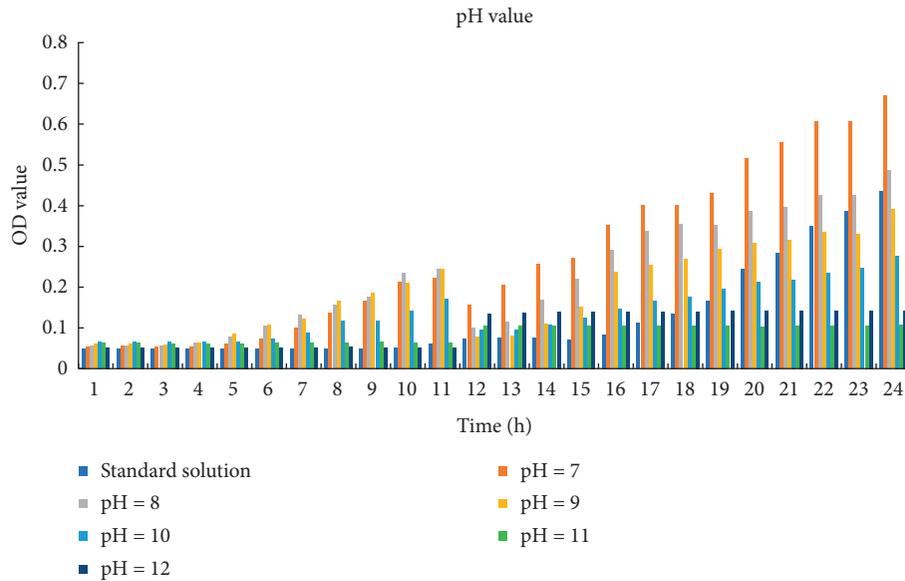


FIGURE 1: Effects of pH value on *Bacillus sphaericus*.

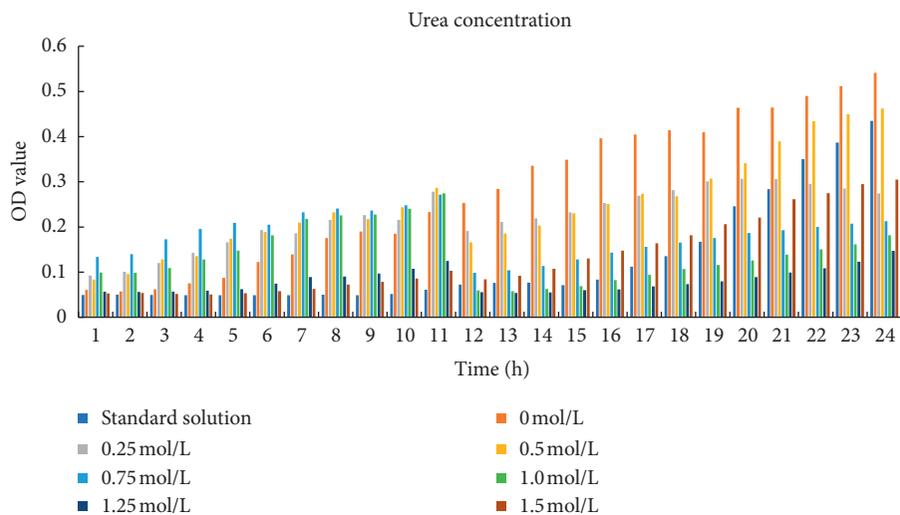


FIGURE 2: Effects of urea concentration on *Bacillus sphaericus*.

when the ratio of *Bacillus mucilaginosus* and *Bacillus sphaericus* is 1 : 6, the deposition of calcium carbonate is the highest, as shown in Figure 6. The amount of calcium carbonate formed when *Bacillus mucilaginosus* was added in a ratio of 1 : 6 was 0.852 g; in contrast, under the same conditions, the amount of calcium carbonate precipitated by *Bacillus globosa* was only 0.451 g. Experiments reveal that *Bacillus mucilaginosus* can coexist with *Bacillus sphaericus* and continuously secrete carbonic anhydrase in vitro. Carbonic anhydrase can accelerate the combination of carbonate ions and calcium ions to promote the formation of calcium carbonate and effectively increase the yield of *Bacillus sphaericus* mineralization products. In other words, *Bacillus mucilaginosus* can significantly improve the rate of crack repair together with *Bacillus sphaericus*.

3. Experimental Results Analysis

We selected two samples for the analysis of the experimental results: the mineralization products formed by the *Bacillus mucilaginosus* experimental group (sample 1) and that formed only by *Bacillus sphaericus* (sample 2).

3.1. XRD Analysis. Figure 7 shows the XRD patterns of the two samples.

It can be seen from Figure 7 that the characteristic diffraction peaks of the two samples are approximately coincident, and all appear at 2θ values of 25° , 27° , 33° , 44° , and 50° . That is, both samples comply with the characteristics of the characteristic diffraction peaks of vaterite-type calcium carbonate. Besides, the two samples exhibited weak

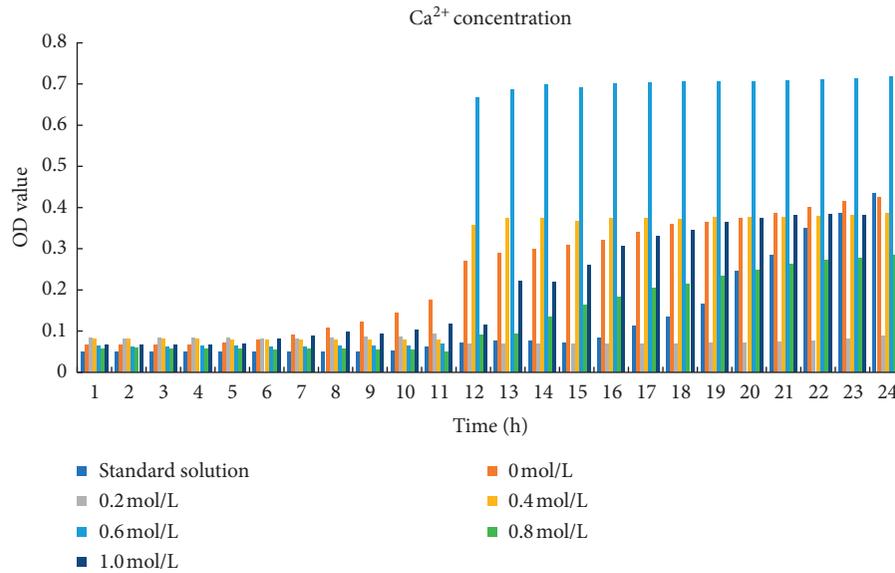


FIGURE 3: Effects of Ca²⁺ concentration on *Bacillus sphaericus*.

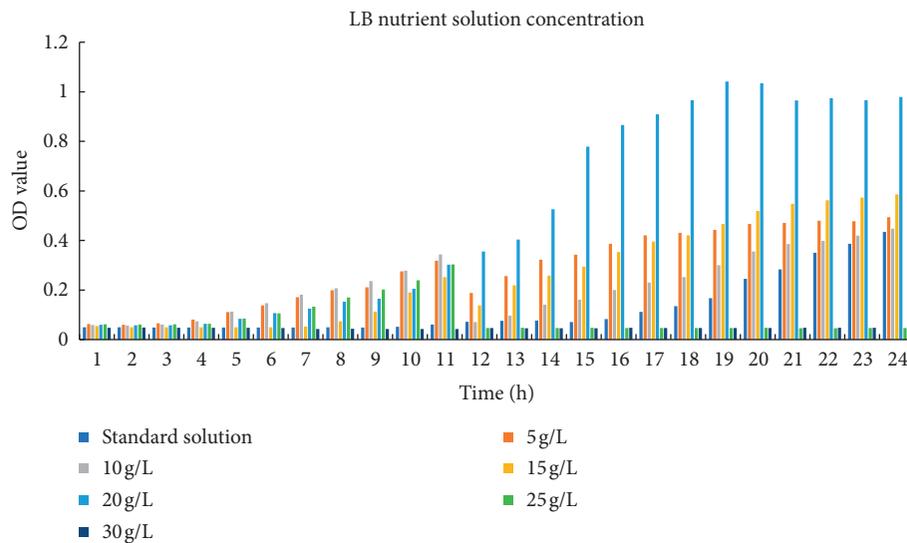


FIGURE 4: Effects of LB nutrient solution concentration on *Bacillus sphaericus*.

diffraction peak signals at 23°, 29°, 36°, 39°, 43°, 47.5°, and 48.5°; i.e., they both follow the characteristics of the characteristic diffraction peaks of calcite-type calcium carbonate. As a consequence, the main components of the two minerals are vaterite and contain a small amount of calcite as well.

3.2. SEM Analysis. The SEM analysis of the two samples is demonstrated in Figure 8.

It can be seen from Figure 8 that the crystals of the two samples are spherical calcium carbonate particles, and the calcium carbonate particle of sample 2 is slightly larger than that of sample 1. This is because in the double bacterial liquid mineralization, due to the action of carbonic anhydrase secreted by *Bacillus mucilaginosus* in vitro, the double bacteria liquid accelerates the formation of small-sized calcium

carbonate particles, and the spherical calcium carbonate particles formed have a diameter of about one μm . In addition, the morphology of the two mineral crystals is not much different, and the surface has prominent ridges. This is because bacteria and enzymes are involved in the crystallization process, resulting in incomplete smoothness of the crystal surface.

3.3. FTIR Analysis. Figure 9 shows the FTIR spectra of the two samples.

The peak values of sample 1 appeared at 745.71 cm^{-1} , 876 cm^{-1} , 1088.22 cm^{-1} , and 1490.6 cm^{-1} , respectively. Among them, 876 cm^{-1} corresponds to the CO_3^{2-} out-of-plane deformation vibration of the calcite crystal form, 1088.22 cm^{-1} corresponds to the CO_3^{2-} symmetric expansion of the calcite crystal form, and 1490.6 cm^{-1} corresponds

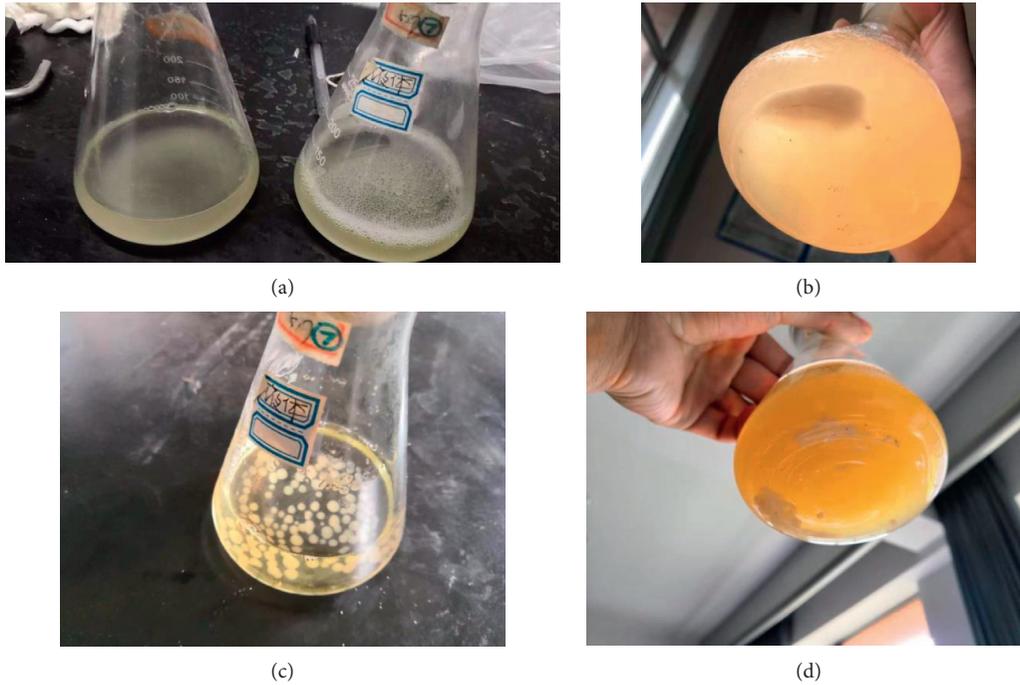


FIGURE 5: Mineralization process with *Bacillus mucilaginosus* added.

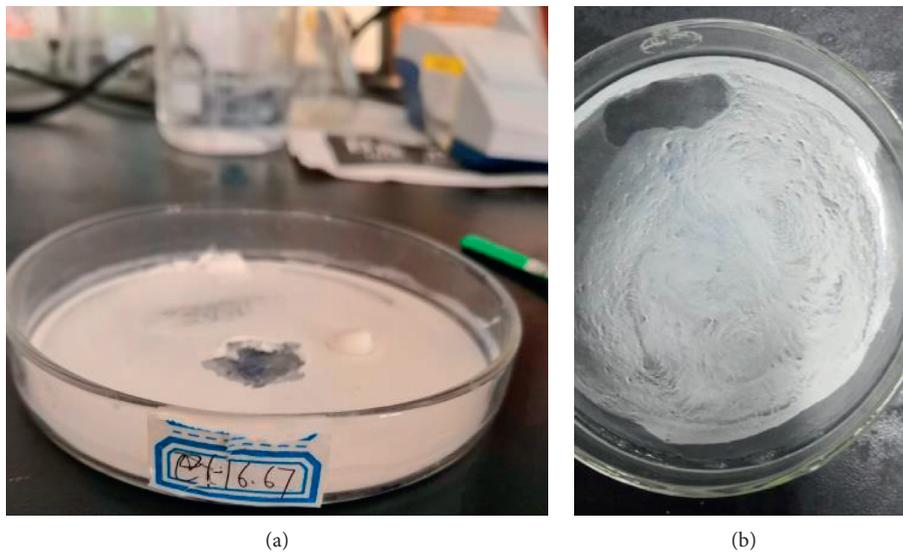


FIGURE 6: Comparison of the amount of calcium carbonate with (a) and without (b) *Bacillus mucilaginosus*.

to the C-O antisymmetric stretching vibration of the calcium carbonate. However, the absorption peak at 1490.6 cm^{-1} showed a redshift of 69.6 cm^{-1} , indicating that *Bacillus mucilaginosus*, carbonic anhydrase, and organic matter were embedded in the crystal during mineralization in the process of mineralization, which affected the morphology of calcium carbonate crystals. This is consistent with the SEM analysis. The absorption peak at 745.71 cm^{-1} corresponds to the characteristic peak of in-plane bending of the gangue crystal

form CO_3^{2-} , revealing that the calcium carbonate formed during the biomineralization of *Bacillus sphaericus* is mainly composed of vaterite, and some calcite crystal is formed as well. The peaks of sample 2 appear at 745.67 cm^{-1} , 876 cm^{-1} , 1075.99 cm^{-1} , and 1498.21 cm^{-1} , respectively, corresponding to the CO_3^{2-} in-plane deformation of the smectite crystal, the CO_3^{2-} out-of-plane deformation vibration, the CO_3^{2-} symmetric expansion, and the C-O antisymmetric stretching vibration of the calcite crystal. Infrared comparative analysis

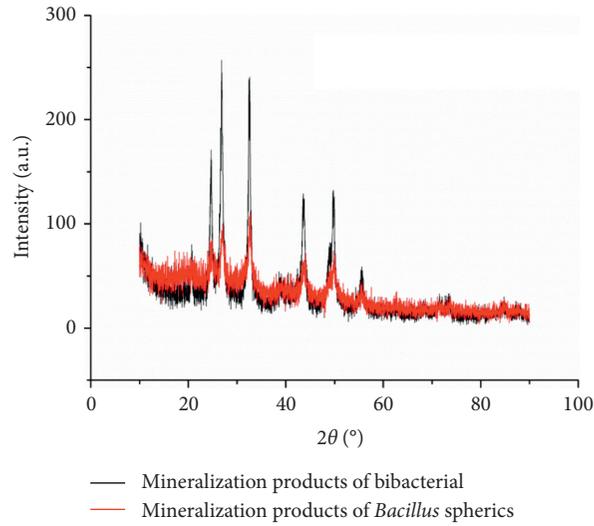


FIGURE 7: XRD patterns of the two samples.

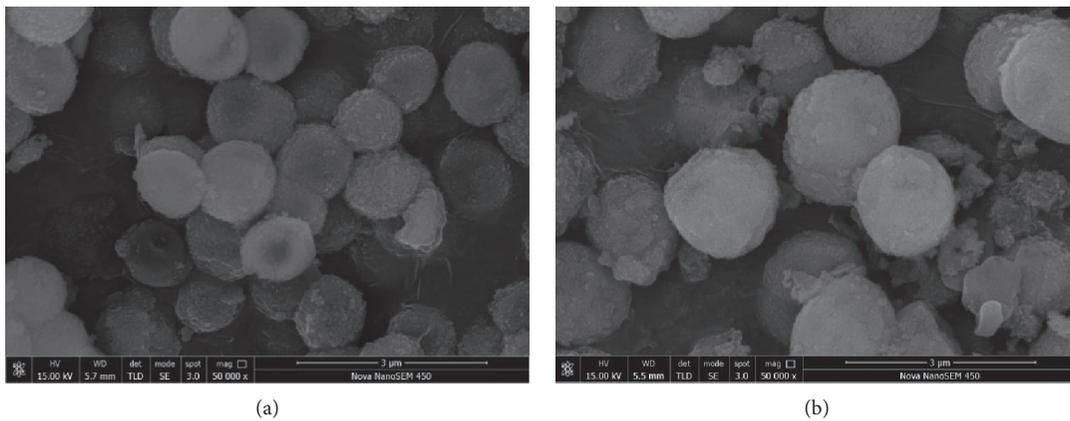


FIGURE 8: SEM spectra of the two samples. (a) Mineralization materials of sample 1. (b) Mineralization materials of sample 2.

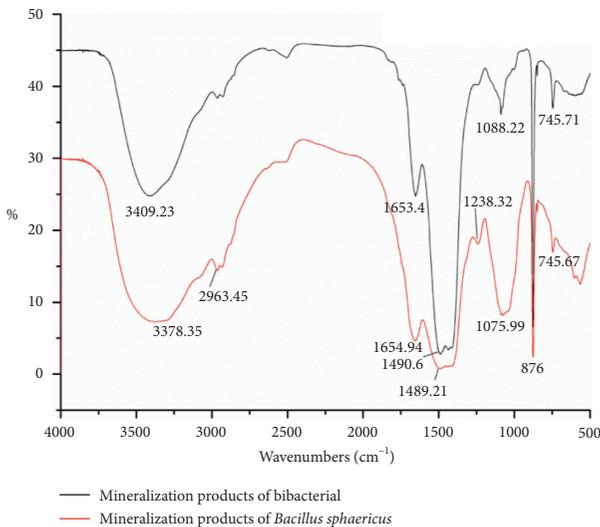


FIGURE 9: FTIR spectra of the two samples.

of the two groups of samples revealed that the addition of *Bacillus mucilaginosus* did not alter the crystal morphology of the *Bacillus sphaericus* mineralization product.

3.4. TG-DSC Analysis. Figures 10 and 11 depict the TG-DSC spectra of the two samples, respectively.

When Sample 1 and Sample 2 were decomposed by 5% (corresponding TG was 95%), the initial decomposition temperatures were 102.5°C and 95.5°C, respectively. This indicates that the thermal stability of sample 1 is slightly higher than that of sample 2 and the addition of *Bacillus mucilaginosus* does not affect the thermal stability of *Bacillus sphaericus* mineralization. At the same time, the peak temperature of the maximum decomposition rate of sample 1 appears at around 650°C; and the peak temperature of the maximum decomposition rate appears at around 300°C and around 680°C. In addition, Sample 1 processes three absorption peaks between 580°C and 720°C, representing three consecutive endothermic decomposition processes. That is,

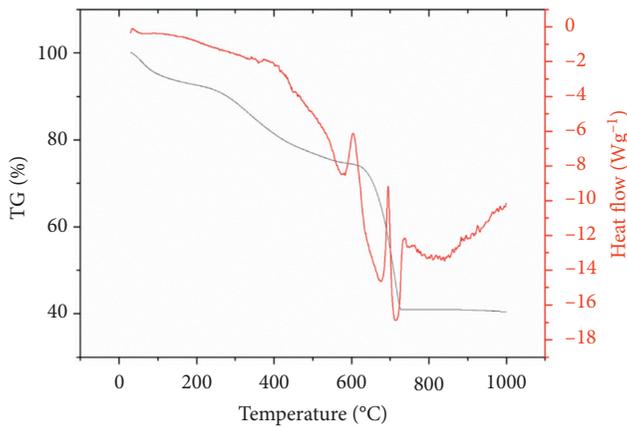


FIGURE 10: TG-DSC spectra of sample 1.

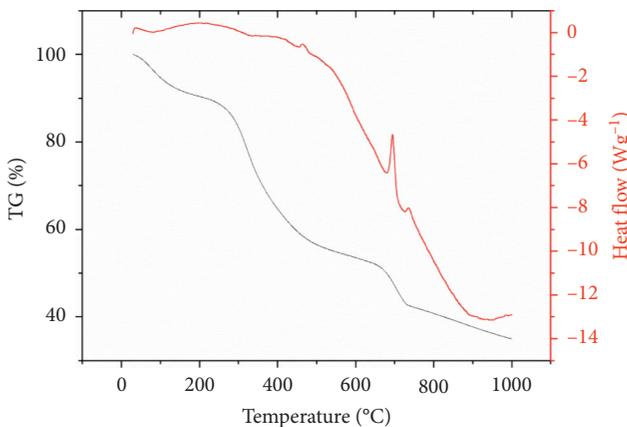


FIGURE 11: TG-DSC spectra of sample 2.

Sample 1 showed rapid decomposition at this stage. Sample 2 had two absorption peaks between 680°C and 720°C, indicating that Sample 2 showed rapid decomposition at this stage.

4. Conclusions

This paper analyzed the influencing factors of growth of *Bacillus sphaericus* and the change trend of urease activity with pH value. On this basis, we discussed the crystal type and microstructure of mineralized products before and after introduction of *Bacillus mucilaginosus* and explored the effect of *Bacillus mucilaginosus* on the biomineralization ability of *Bacillus sphaericus*. The paper concludes the following:

- (1) The optimum pH value for the growth of *Bacillus sphaericus* is 7, and the environment with a pH value of 8 can maintain the growth activity of *Bacillus sphaericus* as well. The optimal amount of urea concentration, Ca^{2+} concentration, and LB medium concentration for growth of *Bacillus sphaericus* are 0.5 mol/L, 0.6 mol/L, and 20 g/L, respectively.
- (2) Both *Bacillus sphaericus* and *Bacillus oxysporum* introduced the highest enzyme activity at a pH of 8.

- (3) When the volume ratio of *Bacillus mucilaginosus* to *Bacillus sphaericus* was 1 : 6, the amount of calcium carbonate precipitated was the highest, which was 1.89 times of the amount of calcium carbonate precipitated by *Bacillus sphaericus* under the same conditions.
- (4) The main component of both single and double bacterial mineral products is vaterite. They contain a small amount of calcite as well. The crystals of the two mineralized samples are spherical calcium carbonate particles, and the particle size of the single bacterial mineral products is slightly larger. The thermal stability of the double bacterial mineralized product is slightly higher than that of the single bacterial bacteria.

In our future research, we plan to prepare bio-mineralization promoter out of carbonic anhydrase by extracting the supernatant of *Bacillus mucilaginosus*, and we will attempt to investigate the direct influence of carbonic anhydrase on biomineralization.

Data Availability

All data included in this study are available upon request by contacting the corresponding author.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

This work was funded by the National Natural Science Foundation of China (Grant No.51904032), the National Natural Science Foundation of China (Grant no. 51874192), the Research Fund of Binzhou University (Grant no. BZXYL1914), and the National College Students Innovation and Entrepreneurship Training Program (Grant no. 201910449002).

References

- [1] S. Joshi, S. Goyal, A. Mukherjee, and M. S. Reddy, "Microbial healing of cracks in concrete: a review," *Journal of Industrial Microbiology & Biotechnology*, vol. 44, no. 11, pp. 1–15, 2017.
- [2] M. Seifan, A. K. Samani, and A. Berenjian, "Bioconcrete: next generation of self-healing concrete," *Applied Microbiology and Biotechnology*, vol. 100, no. 6, pp. 2591–2602, 2016.
- [3] M. O. Cuthbert, L. A. Mcmillan, S. Handley-Sidhu, M. S. Riley, D. J. Tobler, and V. R. Phoenix, "A field and modeling study of fractured rock permeability reduction using microbially induced calcite precipitation," *Environmental Science & Technology*, vol. 47, no. 23, pp. 13637–13643, 2013.
- [4] H. Johannes, L. Ellen, P. Adrienne et al., "A revised model for microbially induced calcite precipitation: improvements and new insights based on recent experiments," *Water Resources Research*, vol. 51, no. 5, pp. 3695–3715, 2015.
- [5] J. Y. Wang, A. Mignon, D. Snoeck et al., "Application of modified-alginate encapsulated carbonate producing bacteria

- in concrete: a promising strategy for crack self-healing," *Frontiers in Microbiology*, vol. 6, p. 1088, 2015.
- [6] S. Bhaskar, K. M. Anwar Hossain, M. Lachemi, G. Wolfaardt, and M. Otini Kroukamp, "Effect of self-healing on strength and durability of zeolite-immobilized bacterial cementitious mortar composites," *Cement and Concrete Composites*, vol. 82, pp. 23–33, 2017.
 - [7] K. V. Tittelboom, N. D. Belie, W. D. Muynck, and W. Verstraete, "Use of bacteria to repair cracks in concrete," *Cement and Concrete Research*, vol. 40, no. 1, pp. 157–166, 2010.
 - [8] J. Wang, K. Van Tittelboom, N. De Belie, and W. Verstraete, "Use of silica gel or polyurethane immobilized bacteria for self-healing concrete," *Construction and Building Materials*, vol. 26, no. 1, pp. 532–540, 2012.
 - [9] S. S. Bang and V. Ramakrishnan, "Microbiologically-enhanced crack remediation (MECR)," *Proceedings of the International Symposium on Industrial Application of Microbial Genomes*, vol. 1, pp. 3–13, 2001.
 - [10] R. N. Carlos, R. G. Manuel, B. C. Koutar, and G. M. Maria Teresa, "Conservation of ornamental stone by myxococcus xanthus-induced carbonate biomineralization," *Applied and Environmental Microbiology*, vol. 69, no. 4, pp. 2182–2193, 2003.
 - [11] G. D. O. Okwadha and J. Li, "Optimum conditions for microbial carbonate precipitation," *Chemosphere*, vol. 81, no. 9, pp. 1143–1148, 2010.
 - [12] W. Cheng, X. Hu, J. Xie, and Y. Zhao, "An intelligent gel designed to control the spontaneous combustion of coal: fire prevention and extinguishing properties," *Fuel*, vol. 210, pp. 826–835, 2017.
 - [13] J. L. Zhang, C. G. Wang, Q. L. Wang et al., "A binary concrete crack self-healing system containing oxygen-releasing tablet and bacteria and its Ca²⁺-precipitation performance," *Applied Microbiology and Biotechnology*, vol. 100, no. 24, pp. 10295–10306, 2016.
 - [14] G. Zhou, Q. Zhang, R. Bai, T. Fan, and G. Wang, "The diffusion behavior law of respirable dust at fully mechanized caving face in coal mine: CFD numerical simulation and engineering application," *Process Safety and Environmental Protection*, vol. 106, pp. 117–128, 2017.
 - [15] Q. Zhang, G. Zhou, X. Qian, M. Yuan, Y. Sun, and D. Wang, "Diffuse pollution characteristics of respirable dust in fully-mechanized mining face under various velocities based on CFD investigation," *Journal of Cleaner Production*, vol. 184, pp. 239–250, 2018.
 - [16] J. Y. Wang, N. De Belie, and W. Verstraete, "Diatomaceous earth as a protective vehicle for bacteria applied for self-healing concrete," *Journal of Industrial Microbiology & Biotechnology*, vol. 39, no. 4, pp. 567–577, 2012.
 - [17] N. Favre, M. Lorraine Christ, and A. C. Pierre, "Biocatalytic capture of CO₂ with carbonic anhydrase and its transformation to solid carbonate," *Journal of Molecular Catalysis B: Enzymatic*, vol. 60, no. 4, pp. 163–170, 2009.
 - [18] X. Zhou, "Effect of different culture conditions on carbonic anhydrase from *Bacillus mucilaginosus* inducing calcium carbonate crystal formation," *Acta Microbiologica Sinica*, vol. 50, no. 7, pp. 955–961, 2010.
 - [19] T. S. Priya, N. Ramesh, A. Agarwal, S. Bhusnur, and K. Chaudhary, "Strength and durability characteristics of concrete made by micronized biomass silica and bacteria-*Bacillus sphaericus*," *Construction and Building Materials*, vol. 226, pp. 827–838, 2019.
 - [20] B. M. S. Reddy and D. Revathi, "An experimental study on effect of *Bacillus sphaericus* bacteria in crack filling and strength enhancement of concrete," *Materials Today: Proceedings*, vol. 19, pp. 803–809, 2019.
 - [21] S. G. Choi, S. F. Wu, and J. Chu, "Biocementation for sand using an eggshell as calcium source," *Journal of Geotechnical and Geoenvironmental*, vol. 142, no. 10, Article ID 06016010, 2016.
 - [22] S.-G. Choi, K. Wang, and J. Chu, "Properties of biocemented, fiber reinforced sand," *Construction and Building Materials*, vol. 120, pp. 623–629, 2016.
 - [23] X. Yu, C. Qian, and L. Sun, "The influence of the number of injections of bio-composite cement on the properties of bio-sandstone cemented by bio-composite cement," *Construction and Building Materials*, vol. 164, pp. 682–687, 2018.
 - [24] X. Yu, C. Qian, and J. Jiang, "The influence of curing time on properties of the sand columns cemented by bio-barium phosphate," *Construction and Building Materials*, vol. 188, pp. 255–261, 2018.
 - [25] C. Qian, X. Yu, and X. Wang, "A study on the cementation interface of bio-cement," *Materials Characterization*, vol. 136, pp. 122–127, 2018.
 - [26] X. Yu, Q. Zhan, C. Qian, J. Ma, and Y. Liang, "The optimal formulation of bio-carbonate and bio-magnesium phosphate cement to reduce ammonia emission," *Journal of Cleaner Production*, vol. 240, Article ID 118156, 2019.
 - [27] M. Seifan, A. K. Samani, and A. Berenjian, "Induced calcium carbonate precipitation using bacillus species," *Applied Microbiology and Biotechnology*, vol. 100, no. 23, pp. 9895–9906, 2016.
 - [28] J. Wang, H. M. Jonkers, N. Boon, and N. De Belie, "*Bacillus sphaericus* LMG 22257 is physiologically suitable for self-healing concrete," *Applied Microbiology and Biotechnology*, vol. 101, no. 12, pp. 5101–5114, 2017.
 - [29] Y. Ç. Erşan, E. Hernandez-Sanabria, N. Boon, and N. de Belie, "Enhanced crack closure performance of microbial mortar through nitrate reduction," *Cement & Concrete Composites*, vol. 70, pp. 159–170, 2016.
 - [30] J. Y. Wang, D. Snoeck, S. Van Vlierberghe, W. Verstraete, and N. De Belie, "Application of hydrogel encapsulated carbonate precipitating bacteria for approaching a realistic self-healing in concrete," *Construction and Building Materials*, vol. 68, pp. 110–119, 2014.
 - [31] M. Wu, X. Hu, Q. Zhang, D. Xue, and Y. Zhao, "Growth environment optimization for inducing bacterial mineralization and its application in concrete healing," *Construction and Building Materials*, vol. 209, pp. 631–643, 2019.
 - [32] M. Y. Wu, X. M. Hu, Q. Zhang, W. Lu, Y. Y. Zhao, and Z. L. He, "Study on preparation and properties of environmentally-friendly dust suppressant with semi-interpenetrating network structure," *Journal of Cleaner Production*, vol. 259, Article ID 120870, 2020.
 - [33] Y. Li, X. Hu, W. Cheng et al., "A novel high-toughness, organic/inorganic double-network fire-retardant gel for coal-seam with high ground temperature," *Fuel*, vol. 263, Article ID 116779, 2020.
 - [34] D. Xue, X. Hu, W. Cheng, J. Wei, Y. Zhao, and L. Shen, "Fire prevention and control using gel-stabilization foam to inhibit spontaneous combustion of coal: characteristics and engineering applications," *Fuel*, vol. 264, Article ID 116903, 2020.
 - [35] Q. Zhang, G. Zhou, Y. Hu et al., "Risk evaluation and analysis of a gas tank explosion based on a vapor cloud explosion model: a case study," *Engineering Failure Analysis*, vol. 101, pp. 22–35, 2019.