Factors affecting morphology of microbially induced calcium carbonate

Abstract
Microbially induced calcium carbonate has been adapted to cementing loose sands and repairing cracks of cement-based materials. This article will screen and cultivate four kinds of microbial strains. Through experimental design, calcium carbonate induced by four different microbial strains was prepared. The morphology of the biological calcium carbonate was studied using field emission scanning electron microscopy and atomic force microscopy. The factors affecting the characteristics of calcium carbonate were revealed. And based on the results, the methods of getting higher adhesive capacity by controlling the morphology of calcium carbonate were obtained.

Keywords: morphology, adhesive capacity, microbially induced calcium carbonate

Introduction

Microorganisms are an important part of the Earth’s biosphere, accounting for about 80 percent of the biomass and are likely to be equally important in Earth’s history. Boquet found that some bacteria in the soil could induce the deposition of calcium carbonate crystals, and that the deposition of calcium carbonate in soil microorganisms was a common phenomenon. In 1978, Burne formally put forward the concept of microbialites, which were usually made up of benthic (prokaryotic or eukaryotic) fungi community and cementing plastic particles. The formation of microbialites can be triggered by the biochemical process of carbonate sediments. Microorganisms have the ability of biological mineralization. The biggest difference between microbial mineralization and general mineralization lies in the presence/absence of biological cells, metabolic products, or organic substrate. In the process of biological mineralization, the inorganic minerals (e.g. carbonic acid, phosphoric acid and sulphate) are produced through the regulation of biomolecules. Mineralization process includes nucleation and growth of deposits, which can be controlled by biochemical chemistry, microbial space and microbial structure. The crystal morphology, size, orientation, crystallization and arrangement can be controlled from molecular level to the mesoscopic level. The crystallization of inorganic phase is controlled by the organic matter of biological secretion. The control of the nucleation, morphology and crystallographic orientation of inorganic crystals is unparalleled. Under the condition of normal temperature and normal pressure, the composites with perfect structure and performance are synthesized by a series of energy-saving and non-polluting treatments.

The biological minerals have existed in nature for at least 3.5 billion years. These minerals include organic crystals, inorganic crystals and amorphous minerals, and mainly consist of calcium magnesium phosphate, calcium magnesium carbonite, iron manganese oxide, silica, sulfate and sulfide, halide and organic acid salt. Microbial induced biological minerals are increasingly valued by the researchers because of their special advanced structures, excellent biological functions and environmental friendliness. They can be used for microbial mineralized cementing material, self-repairing concrete, control of heavy metal and radionucleide pollution, cultural relics, building protection and restoration, etc.

Microbes can be used to solidify sandy soils, repair concrete cracks and deal with solid waste, such as steel slag. Mechanisms of bio-mineralization of microbes have been investigated. Microbial mineralization is mainly used in cement-based materials for surface defect repair, passive repair and self-healing of cracks. The application of concrete crack repair was first proposed in 1995 by Bang of the University of South Dakota Mining and Technology University. They mixed the bacteria liquid with the sand and filled it into the concrete crack as a repairing material. The results showed that the bacteria could induce calcium carbonate deposition in the crack and block the crack, so that the strength and rigidity of the concrete were better recovered. The test uses a class of bacteria capable of producing urease, which hydrolyzes urea to carbonate ions and ammonium ions, and then the carbonate ions react with calcium ions in the surrounding solution to form calcium carbonate precipitates. During this process, the bacteria simultaneously provide a nucleation site for the deposition of calcium carbonate.

Dick’s group at the University of Ghent in Belgium used urease bacteria to induce mineralized sedimentation to repair the surface of the cracked limestone. After repairing, the water absorption performance of the limestone surface was greatly reduced. Dick believed that the high zeta potential and the high activity urease were the key to mineralization. Qian Chunxiang selected a carbonate mineralized bacteria, used its enzymatic reaction to decompose the substrate to form CO\textsubscript{3}\textsuperscript{2-}, and introduced Ca\textsuperscript{2+} in time, by immersing the cement stone specimen in the bacterial liquid and nutrient solution environment. Finally, a dense and hard calcium carbonate protective layer is deposited on the surface of the cement stone. In addition, bacterial activity and Ca\textsuperscript{2+} concentration were found to be key factors in mineralization deposition.

Based on the integration of previous research work, Jonkers of Delft University of Technology in the Netherlands first proposed microbial self-repairing concrete design in 2007. The basic idea of this innovative self-healing concept is to pre-bury Bacillus licheniformis and the specific substrate as its food in the concrete when it is mixed. Once the concrete cracks, the moisture and oxygen enter the dormant bacteria. A series of biochemical reactions convert the pre-buried substrate in concrete into calcium carbonate minerals.
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filling the cracks for self-repairing purposes. At the same time, the method proposes a new way to metabolize organic calcium by using the aerobic respiration of bacteria to produce calcium carbonate precipitate and carbon dioxide. In the concrete environment, carbon dioxide continues to react with calcium hydroxide to form additional calcium carbonates precipitate. Qian’s research group\(^{16,17}\) used a kind of facultative aerobic bacteria extracted from soil to study the mechanism of microbial self-repair of concrete cracks, and tested the mineralization reaction of bacteria in solution, which proved that only in aerobic Calcite can be deposited under the environment. They believe that the local CO\(_3^+\) enrichment in cement stone cracks reacts with Ca(OH)\(_2\) to form CaCO\(_3\), which is the key to mineralization. CO\(_2\) is mainly produced by bacteria decomposing substrates, which is compared with the direct formation of CaCO\(_3\) by microbial substrates. The induced mineralization deposition effect is more significant.

A large number of research results have shown that the application of microbial mineralization in cement-based materials is feasible, which can effectively enhance the self-repairing ability of concrete cracks and reduce the water permeability of cracks, thereby improving the durability of concrete structures and reducing maintenance costs. At the same time, the crack repair product is an inorganic calcium carbonate mineral, which has good environmental friendliness. This paper aims to obtain four kinds of carbonate mineralized microorganisms through screening and optimization, and select four kinds of physical and chemical environmental parameters as research variables to compare the characteristics of biological calcium carbonate. And finally bio-induced calcium carbonate with high adhesion force can be obtained.

**Experiment**

**Microbial strain**

Strain A was the earliest prokaryote on earth two billion years ago, and plays an important role in carbon cycle and material transformation in nature. The flora of this group is extremely diverse. It is currently mainly spherical, rod-shaped, spiral and oval. Strain B is a gram-negative bacterium, and the cell wall is composed of lipopolysaccharide, porin and peptidoglycan. Before the available broth can be obtained, it needs to be prepared and activated. Strain C is a facultative aerobic Gram-positive bacterium with a rod-like structure and it is found in soil. It has strong adaptability to high alkaline environments, and has no alkalie damage to humans. It can be added directly to high alkaline cement-based materials. Strain D produces urease, a nickel-containing oligomerase under certain conditions, with absolute specificity that specifically catalyzes the hydrolysis of the substrate to release ammonia and carbon dioxide.

The four microorganisms selected in this article can be inoculated into specific liquid media for subculture. The microorganisms were inoculated into the culture solution and cultured under suitable growth conditions for a period of time without adding bacteria and nutrients. The curve of bacterial concentration change with time is the growth curve of the strain. The growth characteristics of the four microbial strains are shown in Figure 1. According to the results, in order to achieve the highest activity of microorganisms, in this paper, strain A, B, C, D after 28, 36, 25, 30 hours of culture will be used for research, respectively.

In order to analyze the difference in morphology between the chemical method and the calcite formed by the four microbial methods, statistical methods were used for comparison. The chemical method and the four microbial methods induced mineralization of...
calcite should be prepared in three independent experiments. Each sample prepared was randomly selected under scanning electron microscopy, and magnified 2000 times at the same depth of field for shooting. Morphology categories fall into three broad categories: spherical or ellipsoidal, polyhedral, and irregular particles.

**Analysis methods:** The phase composition of the mineralized product was studied by X-ray diffractometer. The

Jade software was used to refine the unit cell parameters of the XRD pattern. The diffraction pattern of the product was calibrated by transmission electron microscopy to determine the crystal form and crystal size. The particle size was analyzed by statistical methods. The adhesive force was measured by atomic force microscopy. By measuring and calculating the characteristic parameters of mineral phase, crystal form, and adhesive force, the microbial mineralization obtained under different microbial strains and different physical and chemical environmental parameters was compared. The phase analysis of this paper uses the D8 Discover type fully automatic X-ray diffraction (XRD) instrument produced by Bruker-AXS, Germany. The detector uses LynXeye (192 sub-detectors): with instrument power 4kW, voltage 40kV, current 40mA, maximum angle range-110°<2θ<169°, minimum step size 0.0001°. The parameters of the test are set to Cu Kα target radiation, the wavelength is 0.154056 nm, the X-ray tube power is 40 kV×30 mA, the step width is 0.02°, the scanning rate is 0.15 s/step, and the 20 angle is from 10° to 90°. The sample is continuously scanned. In this paper, the required powder sample is ground with agate grind, and then passed through a 200 mesh standard sieve. The sieved portion is dried in a vacuum oven for 24 hours and then subjected to X-ray diffraction test.

Atomic force microscopy can detect near-field forces. Firstly, the probe needs to be mounted on the free end of the micro cantilever. Secondly, when the probe is close to or in contact with the surface, the force generated by the probe and the surface causes the micro cantilever to bend. Finally, the force value is obtained by measuring the degree of bending of the micro cantilever. This study requires measurement of the interaction force between the calcium carbonate particles and the probe which indicates the adhesion force. Nuer’s Veeco Dimension ICON atomic force microscope with eight measurement modes was used in this study. Atomic force microscopy test samples are prepared by a tablet press to form a pellet of about 10mm in diameter.

**Results and discussion**

**Influence of microbial species on morphology of bio-calcium carbonate**

A Figure 3 shows the morphology of calcite prepared by 4 strains and chemical method. It can be easily observed that calcites obtained by different methods are totally different in morphology. The calcite crystals formed by strain A (Figure 3a) were mainly polyhedrons and a small amount of spherical particles were present. The calcite crystals formed by strain B (Figure 3b) were mainly polyhedral and spherical particle doping profiles. The calcite crystals formed by strain C (Figure 3c) appeared as various irregularly shaped particles. The morphology of calcite crystals formed by strain D (Figure 3d) were spherical particles or spherical aggregates of different sizes. And the calcite crystals by chemical method (Figure 3e) developed in an oblique hexagonal lattice structure, and the crystal particle size distribution was relatively uniform and the crystal grains were large; sheet or rhombohedral single and twin crystals and their aggregates were observed.

**Figure 3 Morphology of calcite crystals formed by different methods.**

The crystal morphology depends mainly on the relative growth rate of the crystal in all directions. The growth rate, in turn, depends on the surface process and is highly sensitive to the effects of foreign components in the surrounding environment. Since different parts of the crystal lattice are exposed to the external environment, this allows different faces to have different surface structures. Because the number of kink zones, step zones, and screw dislocations are different on different faces, the energy required for surface adhesion and ion bonding is also different, so they grow at different rates. Studies have shown that soluble organic matter (SM) and insoluble organic matter (IM), which have the dual functions of inducing nucleation and inhibiting crystal growth, regulate the crystal morphology. In addition, the results also indicate that microbial secretions are the main factors affecting the crystal morphology of calcium carbonate. SM can be selectively adsorbed on specific crystal faces of calcite crystals, changing the relative growth rate of different crystal faces, resulting in changes in the crystal habit of calcite (compared to chemical method), resulting in different crystal morphology of calcite.

**Figure 4 Calcite morphology diagrams induced by chemical methods and four microbial methods.**

The calcite morphology diagrams induced by chemical methods and four microbial methods are shown in Figure 4. The calcite morphology curves induced by chemical methods and four microbial methods are shown in Figure 5.
Influence of ions in pore solutions of construction materials on size and adhesion

OH- (pH value): According to former researches, most of the pore solutions in construction materials are alkaline, such as geotechnical materials, cement-based materials and steel slag cementing materials. In cement-based materials like concrete, the pH value is around 13. Hence the pH values from 7 to 13 are chosen in this research. The calcite formed by the biomineralization in the simulated solution at different pH values was prepared in three independent experiments.

Figures 6-10 show the morphology and energy spectra of calcite formed by microbial induced mineralization in simulated solutions at different pH values. Comparing the morphology in the simulated solution at different pH values, it can be clearly seen that although the precipitated material obtained is of the same calcite type, the morphology is different.
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When the pH of the simulated solution was 7, the crystal morphology of calcite induced by microbial method was spherical or ellipsoid, polyhedron and a small amount of irregular shape, and the crystal particle size distribution was seriously uneven (Figure 6). When the pH of the simulated solution was 8, the crystal morphology of calcite induced by microbial method was mainly spherical or globular aggregates and polyhedrons, and the crystal particle size distribution was uneven (Figure 7). When the simulated solution pH=10, the crystal morphology of the calcite induced by the microbial method was spherical or spherical aggregates of different sizes, and a small number of irregular shapes. The crystal particle size distribution was relatively uniform (Figure 8). When the simulated solution pH=12, the crystal morphology of calcite induced by the microbial method was only a small amount. Spherical or globular aggregates, partial polyhedrons, and more irregular shapes, the crystal particle size distribution was very uniform (Figure 9). When the simulated solution pH=13, the crystal morphology of calcite induced by microbial method was mainly irregular shape, only a small number of spheres or ellipsoids, partially stacked polyhedrons, and the crystal particle size distribution is relatively uniform (Figure 10). The above-mentioned morphological difference is caused by the different pH environments of the calcite crystals during the growth process.

The phase diagrams and graphs of calcite morphology induced by microbial method in the simulated solution at different pH are shown in Figure 11 and Figure 12. Comparing Figure 11 and Figure 12, it can be seen that as the pH value of the simulated solution increases, the percentage of spherical or ellipsoidal particles in the calcite formed by microbial induction of mineralization increases first and then decreases, at pH=10, the proportion of spherical or ellipsoidal particles reaches the highest; the percentage content of polyhedral particles decreases gradually with the increase of pH value, but when the pH value is greater than or equal to 10, the percentage content of polyhedral particles tends to be constant; Regular particles have a low percentage content when the pH is small. When the pH is greater than or equal to 10, the percentage of irregular particles rapidly increases, and even becomes the highest percentage content. According to the analysis, the pH value in the simulated solution affects the amount of organic-Ca\(^{2+}\) element template, which changes the growth rate of crystals in all directions, resulting in different crystal morphology of calcite.

In order to better observe the surface topography of calcite particles formed by microbial induced mineralization in different pH simulation solutions, high-power scanning electron microscopy images and atomic force microscope images were compared. Results are shown in Figures 13-17. It can be seen from the figure that as the pH value in the simulated solution increases, the surface smoothness of the calcite-induced formation of the calcite begins to increase.

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Figure 14 The calcite surface formed by microbial induction of mineralization at pH=8.

Figure 15 The calcite surface formed by microbial induction of mineralization at pH=10.

Figure 16 The calcite surface formed by microbial induction of mineralization at pH=12.

Figure 17 The calcite surface formed by microbial induction of mineralization at pH=13.

Ca^{2+} concentration: Figures 18-22 show the morphology and energy spectra of calcite formed by microbial induced mineralization at different Ca^{2+} concentrations. Comparing the topographical maps in the simulated solution under different Ca^{2+} concentrations, it is obvious that although the precipitated materials obtained are of the same calcite type, the morphology is different. When the Ca^{2+} concentration in the simulated solution is 0.01mol/L, the crystal morphology of calcite induced by microbial method is more irregular shape particles, a small number of polyhedrons of different sizes, and spheres or ellipsoids. The crystal particle size distribution is relatively uneven (Figure 18). When the Ca^{2+} concentration in the simulated solution is 0.02mol/L, the crystal morphology of calcite induced by microbial method is dominated by irregular particles, a small amount of polyhedron, basic the spherical or ellipsoid is not visible, and the crystal particle size distribution is uneven (Figure 19). The Ca^{2+} concentration in the simulated solution is 0.03mol/L. The crystal morphology of calcite induced by microbial method is more irregular shape and spherical or spherical aggregates of different sizes, and the crystal particle size distribution is relatively uniform (Figure 20). When the concentration of Ca^{2+} in the simulated solution is 0.04mol/L, the crystal morphology of calcite induced by microbial method has a large number of spherical or globular aggregates, a small number of polyhedrons and some irregularities. The shape and crystal particle size distribution are relatively uniform (Figure 21). The calcite concentration induced by the microbial method in the simulated solution is 0.05mol/L. There are many spheres or ellipsoids in the crystal morphology, some irregular shapes, and a small amount of polyhedrons. The crystal particle size distribution is relatively uniform (Figure 22). The above-mentioned morphological difference phenomenon is caused by the different Ca^{2+} concentration environment of the calcite crystal during the growth process.

Figure 18 Morphology and energy spectrum of calcite formed by microbial method in Ca^{2+} =0.01mol/L.

Figure 19 Morphology and energy spectrum of calcite formed by microbial method in Ca^{2+} =0.02mol/L.
The phase diagram and graph of calcite morphology formed by microbial method induced by microbial method in different Ca\(^{2+}\) concentrations are shown in Figure 23 and Figure 24. Comparing Figure 23 and Figure 24, it can be seen that with the increase of Ca\(^{2+}\) concentration in the simulated solution, the percentage of spherical or ellipsoidal particles in the calcite formed by microbial induction of mineralization increases first and then decreases, in Ca\(^{2+}\). When the concentration is 0.04mol/L, the proportion of spherical or ellipsoidal particles reaches the highest; the percentage of polyhedral particles increases gradually with the increase of Ca\(^{2+}\) concentration; the percentage of irregular particles increases with the increase of Ca\(^{2+}\) concentration. After increasing, the maximum is reached when the Ca\(^{2+}\) concentration is 0.02mol/L, and the irregular particle content is rapidly decreased when the Ca\(^{2+}\) concentration is greater than 0.02mol/L. The analysis shows that increasing the Ca\(^{2+}\) concentration in the simulated solution can increase the percentage of spherical or ellipsoidal particles in the calcite morphology.
In order to better observe the surface morphology of calcite particles formed by microbial induced mineralization in different Ca\(^{2+}\) concentration simulation solutions, high-power scanning electron microscopy images and atomic force microscope images are compared, as shown in Figures 25-29. When the Ca\(^{2+}\) concentration of the simulated solution is 0.01mol/L, the surface of the calcite formed by the microbial method is textured, and the grain boundaries and dislocations are many and rough. When the Ca\(^{2+}\) concentration of the simulated solution is 0.02mol/L, the microbial method induces mineralization. The surface of the calcite has various protrusions and small particles. When the Ca\(^{2+}\) concentration of the simulated solution is 0.03mol/L, the surface texture of the calcite formed by the microbial method is more dense and dendritic; the microbial concentration of the simulated solution Ca\(^{2+}\) is 0.04mol/L. The surface of the calcite formed by the method induced mineralization is smooth and flat. When the Ca\(^{2+}\) concentration of the simulated solution is 0.05mol/L, the surface of the calcite formed by the microbial method is obviously stepped and convex, showing a layered structure.

**Figure 25** The surface of calcite formed by microbial induction induced by microbial method when the concentration of Ca\(^{2+}\) in the simulated solution is 0.01mol/L.

**Figure 26** The surface of calcite formed by microbial induction induced by microbial method when the concentration of Ca\(^{2+}\) in the simulated solution is 0.02mol/L.

**Figure 27** The surface of calcite formed by microbial induction induced by microbial method when the concentration of Ca\(^{2+}\) in the simulated solution is 0.03mol/L.

**Figure 28** The surface of calcite formed by microbial induction induced by microbial method when the concentration of Ca\(^{2+}\) in the simulated solution is 0.04mol/L.

**Figure 29** The surface of calcite formed by microbial induction induced by microbial method when the concentration of Ca\(^{2+}\) in the simulated solution is 0.05mol/L.

**Figure 30** Morphology of calcite formed by microbial method induced by simulated solution Ca\(^{2+}\)/Mg\(^{2+}\)=1:1.

**Ca\(^{2+}\)/Mg\(^{2+}\) ratio:** Figure 30-34 show the morphology and energy spectra of calcite formed by microbial induced mineralization in simulated solutions at different Ca\(^{2+}\)/Mg\(^{2+}\) ratios. Comparing the morphology maps in the simulated solution under different Ca\(^{2+}\)/Mg\(^{2+}\) ratios, it can be clearly seen that although the precipitated materials obtained are of the same calcite type, the morphology is different.

**Figure 31** Morphology of calcite formed by microbial method induced by simulated solution Ca\(^{2+}\)/Mg\(^{2+}\)=1:1.

When the Ca\(^{2+}\)/Mg\(^{2+}\) ratio of the simulated solution is 1:1, the crystal morphology of calcite induced by microbial method is most irregular shaped particles, a small number of polyhedrons of different
sizes, and spheres or ellipsoids. The crystal particle size distribution is relatively uneven (Figure 30). When the ratio of Ca\(^{2+}\)/Mg\(^{2+}\) in the simulated solution is 2:1, the crystal morphology of calcite induced by microbial method is dominated by irregular particles, a small amount of polyhedron, basic the spherical or ellipsoid is not visible, and the crystal particle size distribution is uneven (Figure 31). The simulated solution Ca\(^{2+}\)/Mg\(^{2+}\) ratio is 4:1. The crystal morphology of calcite induced by microbial method is more irregular shape, a small number of spherical or spherical aggregates of different sizes, the crystal particle size distribution is relatively uniform (Figure 32). When the Ca\(^{2+}\)/Mg\(^{2+}\) ratio of the simulated solution is 5:1, the crystal morphology of calcite induced by microbial method has more irregular shapes, a small amount of polyhedron, and the crystal particle size distribution is relatively uniform (Figure 33). When the ratio of Ca\(^{2+}\)/Mg\(^{2+}\) in the simulated solution is 9:1, the crystal morphology of calcite induced by microbial method has more spheres or ellipses. The sphere, part of the irregular shape, a small amount of polyhedron, the crystal particle size distribution is relatively uneven (Figure 34). It is caused by the different Ca\(^{2+}\)/Mg\(^{2+}\) ratio environment of calcite crystals during the growth process.

The calcite morphology and curve distribution diagram of the mineralization induced by microbial method in the simulated solution under different Ca\(^{2+}\)/Mg\(^{2+}\) ratios are shown in Figure 35 and Figure 36.

Comparing Figure 35 with Figure 36, it can be seen that the percentage of spherical or ellipsoidal particles in the calcite formed by microbial induction is at a lower level, and the Ca\(^{2+}\)/Mg\(^{2+}\) ratio increases in the simulated solution. The percentage content of large, spherical or ellipsoidal particles also begins to increase; when the Ca\(^{2+}\)/Mg\(^{2+}\) ratio is less than 5/1, the percentage content of polyhedral particles is at a lower level and remains substantially unchanged, but when the Ca\(^{2+}\)/Mg\(^{2+}\) ratio is greater than 5/1, the percentage content of polyhedral particles began to increase significantly; when the Ca\(^{2+}\)/Mg\(^{2+}\) ratio was less than 5/1, the percentage of irregular particles was at a higher level, and as the Ca\(^{2+}\)/Mg\(^{2+}\) ratio increased The percentage content decreases slowly, but when the Ca\(^{2+}\)/Mg\(^{2+}\) ratio is greater than 5/1, the percentage content of irregular particles decreases instantaneously. According to the analysis, Mg\(^{2+}\) replaces part of Ca\(^{2+}\) to induce lattice distortion by entering the CaCO\(_3\) lattice,

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Figure 31 Morphology of calcite formed by microbial method induced by simulated solution Ca\(^{2+}\)/Mg\(^{2+}\)=2:1.

Figure 32 Morphology of calcite formed by microbial method induced by simulated solution Ca\(^{2+}\)/Mg\(^{2+}\)=4:1.

Figure 33 Morphology of calcite formed by microbial method induced by simulated solution Ca\(^{2+}\)/Mg\(^{2+}\)=5:1.

Figure 34 Morphology of calcite formed by microbial method induced by simulated solution Ca\(^{2+}\)/Mg\(^{2+}\)=9:1.
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thereby changing the morphology of calcite crystals and increasing the degree of irregularity.

Figure 35 Morphology of calcite in simulated solution under different Ca\(^{2+}/\)Mg\(^{2+}\) ratios.

Figure 36 Morphology curves of calcite in simulated solution under different Ca\(^{2+}/\)Mg\(^{2+}\) ratios.

Anion species: Figure 37-40 show the morphology and energy spectra of calcite formed by microbial induced mineralization in simulated solutions under different anion species. Comparing the morphology maps in the simulated solution under different anion species, it can be clearly seen that although the precipitated material obtained is of the same calcite type, the morphology is different. When the simulated solution anion is Cl\(^-\), the crystal morphology of calcite induced by microbial method is more irregular shape particles, polyhedrons of different sizes and a small number of spheres or ellipsoids, and the crystal particle size distribution is relatively uneven (Figure 37). When the simulated solution anion is NO\(_3^-\), the crystal morphology of calcite induced by microbial method is mainly polyhedron, a small amount of irregular particles, spheres or ellipsoids, and the crystal particle size distribution is relatively uniform (Figure 38). Chemical simulation solution the crystal morphology of the induced calcite is all polyhedral particles, and there are no spheroids or irregular particles. The crystal particle size distribution is very uniform (Figure 40). The above-mentioned morphological difference is caused by the different types of anions of the calcite crystals during the growth process.

Figure 37 The calcite morphology and spectrum of the mineralization induced by microbial method when the simulated solution anion is Cl\(^-\).

Figure 38 The calcite morphology and spectrum of the mineralization induced by microbial method when the simulated solution anion is NO\(_3^-\).

Figure 39 The calcite morphology and spectrum of the mineralization induced by microbial method when the simulated solution anion is CH\(_3\)COO\(^-\).

The morphology and curve distribution of the calcite morphology induced by microbial method in the simulated solution under different anion species are shown in Figure 41 and Figure 42. Comparing Figure 41 and Figure 42, it can be seen that under the dual regulation of organic macromolecules secreted by
microorganisms, calcite crystals promote crystallization nucleation on the one hand and organic crystal- Ca$^{2+}$ as a template. Development, in simulated solutions of different anion species, the crystals will grow at different rates in all directions, resulting in different morphologies. The morphology of calcium carbonate formed in Cl$^{-}$ biosimulation solution is irregular particles and polyhedron; the morphology of calcium carbonate formed in NO$_3^-$ biosimulation solution is half of polyhedron and irregular particles; CH$_3$COO$^-$ biological simulation solution The morphology of the calcium carbonate formed in the medium is more spherical or ellipsoid; the morphology of the calcium carbonate formed in the chemical simulation solution is all regular polyhedral particles. As can be seen from the analysis, the type of anion can induce the morphology of the calcium carbonate crystals formed in the simulated solution. CH$_3$COO$^-$ can induce calcium carbonate formed in the bio-simulation solution to form more spherical particles; Cl$^-$ and NO$_3^-$ induce the calcium carbonate formed in the bio-simulation solution to exhibit an irregular morphology. Therefore, calcium carbonate crystals of various morphologies can be induced by changing the anion species and quantity during microbial mineralization.

**Figure 39** The calcite morphology and spectrum of the mineralization induced by microbial method when the simulated solution anion is CH$_3$COO$^-$.

**Figure 40** Morphology and spectrum of calcite formed by simulated solution chemistry induced mineralization.

**Figure 41** Morphological phase diagram of calcite in simulated solution under different anion species.

**Figure 42** Morphology curves of calcite in simulated solution under different anion species.

In order to better observe the surface morphology of calcite particles formed by microbial induced mineralization in simulated solution under different anion species, compare the higher-magnitude field emission scanning electron microscope image and atomic force microscope image, as shown in Figure 43-46. When the simulated solution anion is Cl$^-$, the surface of the calcite formed by microbial induction of mineralization exhibits irregular protrusions and irregular scratches on the surface, and the surface also adsorbs fine particles; when the simulated solution anion is NO$_3^-$, the microbial method induces mineralization. The surface of the formed calcite has various pits, the grain boundary is clearly visible, and the surface is very layered. When the simulated solution anion is CH$_3$COO$^-$, the surface of the calcite formed by the microbial method is smooth, the spherical particles are obvious, and the surface is adsorbed with many crumbs.

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The small particles, the spherical particles adsorb to each other; the surface of the calcite formed by the mineralization induced by the chemical simulation solution is very smooth, the steps are obvious, and the shape is regular.

**Figure 43** Surface of the calcite formed by microbial induction of the simulated solution anion to Cl$^-$

**Figure 44** Surface of the calcite formed by microbial induction of the simulated solution anion to NO$_3^-$

**Figure 45** Surface of the calcite formed by microbial induction of the simulated solution anion to CH$_3$COO$^-$

**Figure 46** Chemical simulated solution induced mineralization of calcite surface.

Ellipsoidal particles of calcium carbonate and the decomposition temperature is shown in Figure 47. It can be seen from the figure that as the percentage of the spherical/ellipsoidal particle morphology increases, the decomposition temperature of calcium carbonate also increases. The relationship between the morphology of the spherical/ellipsoidal particles of calcium carbonate and the adhesive capacity is shown in Figure 48. The adhesive capacity between the spherical/ellipsoidal calcium carbonate particles is significantly higher than that of other particles. The analysis shows that the relative contact area between the spherical/ellipsoidal particles is the largest, and the interaction force between the particles is also increased. It can be seen from Figure 49 & Figure 50 that the morphology of the polyhedron and the irregular particle morphology have no effect on the decomposition temperature and adhesion of calcium carbonate.

**Figure 47** Relationship between the morphology of spherical/ellipsoidal particles and the decomposition temperature.

**Figure 48** Relationship between the morphology of spherical/ellipsoidal particles and the adhesive capacity.

**Figure 49**

**Figure 50**
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Conclusion

Four microorganisms suitable for survival and reproduction in typical building materials are screened and cultivated. The pH values, calcium ion concentration, calcium-magnesium ratio and anion species were selected as the research variables, and different microbial induced calcium carbonate was prepared in solutions. The differences between phase and morphology were studied by transmission electron microscopy, scanning electron microscopy, atomic force microscopy and other methods. Following conclusions are obtained:

a. The organic matrix secreted by the microorganisms causes the crystals to have different relative growth rates in all directions, resulting in a difference in the morphology of the calcite.

b. pH can change the ratio of calcite to vaterite crystal form and the crystal plane growth orientation of calcium carbonate; if the pH value is too high or too low, the morphology of calcium carbonate particles will be more irregular due to irregular particles. The contact point between the particles is small and the adhesion between the particles is also reduced.

c. The increase of Ca\(^{2+}\) concentration can increase the percentage of spherical or ellipsoidal particles in calcite morphology.

d. On the one hand, Mg\(^{2+}\) can increase the activity of microbial metallo-enzymes; on the other hand, it can replace the partial Ca\(^{2+}\) induced lattice distortion by entering the CaCO\(_3\) lattice, thereby changing the morphology of calcite crystals and increasing the irregularity.

e. The bio-calcium carbonate induced by Cl\(^-\) and NO\(_3^-\) exhibits more irregular morphology, and the grain size and particle size are relatively larger. Since CH\(_3\)COO relative molecular mass is large, steric hindrance helps to disperse. The organic- Ca\(^{2+}\) motif template can induce the formation of more spherical or spherical aggregate particles of bio-calcium carbonate.

f. As the percentage of the spherical/ellipsoidal particle morphology increases, the relative contact area between the particles increases, the decomposition temperature of the calcium carbonate crystal increases, and the adhesion between the particles also increases.

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Conflicts of interest

Authors declare that there is no conflict of interest.
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