Mechanism of bacteria controlling gradient mineralization at surface layer of cement-based materials

Abstract
The development of Steel bar reinforced cement-based composite is rapidly increasing worldwide. As the primary material for the structure of infrastructures, the durability against corrosive ions in the service environment thus becomes matters. The application of bacteria for ecological engineering purpose is becoming increasingly popular as bio-mineralization technology has been successfully applied for the improvement of the properties of the cement-based. Therefore, from a mechanism-researching viewpoint, in this study we investigated the effects of factors on the gradient mineralization at surface layer of cement-based materials. The results indicated that the bacteria could regulate the gradient mineralization at surface of cement-based materials. In order to march further on the action mechanism of bacteria, four specific parts were selected: absorption of CO₂ from air, transformation of CO₂ to HCO₃⁻, adsorption of Ca²⁺ from pore solution of cement-based materials and formation of CaCO₃ in pores, which resulting in the decrease of porosity. In addition to the use of simulated pore solutions of cement-based materials for the former three parts, numerical calculations and experimental measures were added for the analysis on the diffusion of CO₂ in pores with existence of bacteria, the amount CaCO₃ and the porosity of cement-based materials.

Keywords: bio-mineralization, gradient structure, cement-based material

Introduction
Microbial mineralization refers to the formation of inorganic minerals by action of microorganisms. The earliest study in this field could be traced back to the 19th century, where early aquatic and soil microbiologists carried out pioneering studies of non-medical microbial processes in soils, sediments and waters, opening the door to the empire of environmental microbiology, microbial ecology, geological microbiology and microbial geochemistry. In 1838, for the first time Ehrenberg explored the close relationship between ochre iron deposits in swamps and Gallionella ferruginea, investigating the important role of these bacteria in the formation of such sediments. In 1890, Muentz started to probe into the action process of bacteria in rock weathering and soil formation, which initiated the study of microbial weathering. Afterwards, Beijerinck discovered that microbes were involved in the oxidation and deposition of Mn in nature in 1931, who didn’t come singly but in pairs when Woksman discovered that the microbial metabolites participated in geological processes, which initiated the study of microbial weathering. In 1932, in the pores of cement-based materials and its effects on the pore structure were identified by the help of a numerical simulation of gradient mineralization.

Experimental
Strain preparation
Bacillus bacteria C and G powder was prepared to be dissolved in deionized water, activated in the oscillating incubator for 24 hours, and under a centrifuge (6000r/m) for 10minutes. The components of the microbial liquid medium are shown in Table 1.

Cement
The chemical composition of P·O 42.5 cement is shown in Table 2 (according to Chinese Standard GB 175-2007).
Table 2 Chemical composition of cement

| Composition | Al₂O₃ | SiO₂ | MgO | SO₃ | CaO | K₂O | Na₂O | Fe₂O₃ | Total |
|-------------|-------|------|-----|-----|-----|-----|------|-------|-------|
| Content/%   | 7.95  | 22.47| 2   | 4.35| 51.87| 0.93| 0.19 | 3.22  | 92.98 |

Preparation of simulated pore Solutions of cement-based materials and analysis of ions and CaCO₃ in solutions

Methods for reaction of bacteria with CO₂: NaOH solution (200mL, pH 12) was added into the reactor which communicated with the outside through the pore. The culture medium of bacteria (103 cells/mL) was added into the reactor while the flow rate of CO₂ was 100mL/min. Keep the temperature constant in the reactor by circulating water through the constant temperature water bath. The pH values were recorded per minute. In contrast to the aforementioned scheme, only NaOH solution (200mL, pH 12) was added into the reactor and the flow rate of CO₂ was kept the same as that of the microbial group in the reference group. The experimental device for bacterial absorption of CO₂ is shown in Figure 1, which is mainly composed of CO₂ gas supply system, constant temperature water circulation system (composition: the constant temperature circulation bath DFY-5/20, stainless steel high temperature magnetic pump and PID precise digital display temperature control instrument; temperature range: -80~350°C; temperature precision: ±0.5°C), and reaction vessel system. The reactor is 1L double-layer glass reactor with the inner and outer diameters of 15mm and 18mm respectively.

Measurement of Ca²⁺ concentration: The concentration of Ca²⁺ in the simulated pore solution was titrated with EDTA-2Na solution. The principle was that when the solution pH is 12-13, the indicator calcium carboxylic acid will form a red complex with Ca²⁺. In titration, free Ca²⁺ reacted with EDTA-2Na first, and Ca²⁺ complexed with indicator then reacted with EDTA-2Na. In the end, the solution changed from crimson to bright blue. 50mL sample was taken into the conical bottle, NaOH solution (2mol/L) was added to adjust pH to 12~13, and 0.2g calcium carboxylic acid indicator (10mol/L) was injected. Conical bottle was gently shook to mix the solution and titrate immediately. During the process of adding EDTA-2Na solution, the solution oscillates from crimson to bright blue. Record the volume of the added EDTA-2Na solution. The Ca²⁺ concentration was calculated according to the following reaction:

\[
C_{Ca^{2+}} = \frac{C_{\text{EDTA-2Na}} \cdot V}{V_{Ca^{2+}}} \tag{1}
\]

In which, \(C_{Ca^{2+}}\) is Ca²⁺ concentration to be measured; \(C_{\text{EDTA-2Na}}\) is EDTA-2Na solution concentration; \(V\) is volume of EDTA-2Na solution; \(V_{Ca^{2+}}\) is volume of solution to be measured.

Measurement of pH: During the experiment, the pH value was monitored and measured by the temperature probe on the S2206 Maityl pH meter in real time from 0.000 to 14.000 at 0.001 resolution.

Indirect measurement method of HCO₃⁻ concentration

The concentration of HCO₃⁻ in solution was determined by double electrode method. The linear relationship between the potential of the ion selective electrode and the logarithm of the activity of the given ion in the solution can be described by Nernst equation as follows (mercury electrodes):

\[
\Phi_{m_i} = \Phi_{m_{i0}} + S \cdot \log a_i \tag{2}
\]

In which \(\Phi_{m_i}, \Phi_{m_{i0}},\) are the electrode potentials, \(S\) is the slope of the electrode, and \(a_i\) is the measured ionic activity. The activity of ions is proportional to the concentration. In a dilute solution, the activity is approximately equal to the concentration, and is replaced by the concentration. From Eq. (1), the ion concentration can be obtained by the potential of the electrode. Hydrogen ion concentration and CO₂ concentration can be measured by pH electrode and CO₂ electrode respectively.

The dissociation equilibrium of free CO₃⁻, CO₂⁻ and HCO₃⁻ in the solution was set. The first order dissociation constant \(K_1\) and the secondary \(K_2\) at 25°C are as follows:

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In Eq. (3),
\[ H_2CO_3 = H_2CO_3 + CO_2 \]

While
\[ HCO_3^- = [All_{- CO}_3] - [HCO_3^-] \]

It can be deduced from Eq. (3), (4), (5), (6) that:
\[ [HCO_3^-] = K_h [All_{- CO}_3] / [H^+] \]

\[ [CO_3^{2-}] = K_h [HCO_3^-] / [H^+] \]

The concentration of \( CO_3^{2-} \) and \( HCO_3^- \) can be obtained by measuring the total concentration of \( CO_3 \) and the concentration of \( H^+ \) in the solution according to Eq. (7) and Eq. (8).

The electrode was calibrated by \( NaHCO_3 \) standard solution with different concentrations: the electrode was inserted sequentially in a incremental order of concentration, and the potential was measured in turn. The electrode was washed with a large amount of deionized water after each measurement. The pHmV-lgaCO_3 standard curve was drawn on the semi-logarithmic coordinate paper. The preparation method of the solution for test was as follows: 10mL bacterial solution was mixed with \( NaHCO_3 \) solution (10mL, 0.11mol/kg) to prepare the standard solution of \( 10^{-5} \) mol/L, \( 10^{-6} \) mol/L, \( 10^{-7} \) mol/L, \( 10^{-8} \) mol/L with 0.1mol/L sodium citrate which was adjusted to pH 4 with HCl solution of 10mol/L. The HCl solution was added in order to transform all the \( CO_3 \), \( HCO_3^- \), and \( H_2CO_3 \) into \( CO_2 \).

Preparation of biological \( CaCO_3 \) and determination of morphology and size of \( CaCO_3 \): Different kinds of bacterial solution (20mL, 0.1mol/L) were removed into the beaker by pipette. \( CaCl_2 \) solution (100mL, 0.2mol/L) was added, then the beaker was centrifuged (2000r/min for 10min) after it had been placed in natural environment for 7 days. The sediment was obtained by a series of filtering and rinse and desiccated in a vacuum drying oven at 40°C for 24h. The morphology and size of precipitated material was visualized by SEM.

Cement paste preparation and determination of porosity and \( CaCO_3 \) content of hardened cement paste

The cement paste specimen (40mm×40mm×160mm with a water-cement weight ratio of 0.5) mixed with the bacteria was prepared. The upper surface sealing film was removed and the specimens were placed in a carbonization kettle for 10 days after a 3 days of curing under standard curing conditions (temperature 20°C±2°C, relative humidity above 95%) resulted in a microbial mineralization process which was gradually carried out from the surface of the sample to the interior (Figure 2).

The porosity of specimens were determined by AutoPore IV 9510 automatic mercury porosimeter (measurable aperture range: 3.6nm~400μm; maximum pressure: 60000psia (414MPa)). The pore size distribution of specimens was analyzed by computer so that the porosity and the most probable aperture were obtained. The specimens were marked every 1mm by the Vernier caliper and the upper surface was cut by a high precision cutting machine for a deep powderization and desiccation. The content of \( CaCO_3 \) in different depth of cement-based materials was recognized by Thermo gravimetric Analysis (TG). The thermo gravimetric analyzer used in this experiment was STA 449F3 synchronous thermal analyzer which combined TG with DTA or DSC.

**Results and discussion**

Effect of bacteria on \( CO_2 \) absorption in simulated pore solutions of cement-based materials

The pH of the solution without bacteria (Reference) decreased slightly, while the pH of the solution with the bacteria C, G or C&G decreased dramatically at first and then stabilized with the time lapse of \( CO_2 \) absorption (Figure 3), revealing that the bacteria increase the absorption of \( CO_2 \).

![Figure 2 Schematic diagram of \( CO_2 \) curing.](image)

![Figure 3 Effect of bacteria on absorption of \( CO_2 \) in simulated pore solutions.](image)

30mL \( CaCl_2 \) solution (1mol/L) was added into the 10mL compound bacteria (1mol/L) for a further investigation on the absorption of \( CO_2 \) by bacteria under different \( CO_2 \) content. With the increase of \( CO_2 \) concentration in the environment, the amount of \( CO_2 \) absorbed by bacteria increased. Its growth rate approximately met the first-order function relationship (Figure 4) and the absorption coefficient \( k=2.9×10^{-5}/d \) can be simultaneously obtained.
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Effect of bacteria on CO_2 transformation to HCO_3^- in simulated pore solutions

Researchers have proposed a surface-deposited CaCO_3 biochemistry hypothesis. After CO_2 is absorbed by bacteria from air, it reacts with OH^- in solution to form HCO_3^-, and combines with calcium ion to form CaCO_3 precipitation as follows:

\[ \text{CO}_2 + \text{OH}^- \rightarrow \text{HCO}_3^- \]

\[ \text{Cell} + \text{Ca}^{2+} + \text{HCO}_3^- \rightarrow \text{Cell} + \text{CaCO}_3 + \text{H}^+ \]

In order to verify this hypothesis, HCO_3^- concentrations in the solutions were measured.

Figure 5 shows the relationship between the HCO_3^- content and the potential. According to Eq. (7) and Eq. (8), the concentration of HCO_3^- and CO_3^2- can be calculated to be 670mg/L and 682.3mg/L without addition of bacteria in the solution. In bacteria C solution, the concentration of HCO_3^- was 1334.27mg/L and CO_3^2- was 0.33mg/L, while in bacteria G solution, the concentration of HCO_3^- was 1854.37mg/L and CO_3^2- was 0.24mg/L. In compound bacteria of C and G solution, the concentration of HCO_3^- was 2135.13mg/L and CO_3^2- was 0.29mg/L. The results show that when there was no bacteria in the solution, the product of CO_2 reaction with OH^- in solution was HCO_3^- and CO_3^2-, and in bacteria C, bacteria G and compound bacteria solutions, CO_2 was absorbed by bacteria and reacts with OH^- in solution to form HCO_3^- and the formation of CO_3^2- was feeble.

Effect of bacteria on Ca^{2+} dissociation and adsorption in solutions

The mineralization of bacteria C and G was due to the production of enzyme. As the enzymes of these two kinds of bacteria was metal-ion centered which would cause a robust affinity to metal ions as well as the adsorption capacity of external CO_2, thus the mineral precipitation was formed. In this section, the mechanism of whether these two kinds of bacteria can absorb Ca^{2+} in solution was explored. The electrical conductivity of Ca^{2+} solution was measured by electrochemical measurement system in an air-isolated environment and the change of Ca^{2+} concentration in the solution was calculated.

The decreasing degree of the solution conductivity in the order from large to small in bacteria group was as follows: adding bacteria C, adding compound bacteria and adding bacteria G (Figure 6), this indicated that the adsorption ability of bacteria C to Ca^{2+} is stronger than that of bacteria G. The adsorption capacity of Ca^{2+} by bacteria is closely related to the effects of bacterial polysaccharide capsule, mucus, proteins, lipids and their complexes secreted to the extracellular membrane. These extracellular substances have a certain spatial structure because of their charge, so they can absorb and capture metal ions by the force of ionic bond and hydrogen bond and are finally condensed and flocculated, forming precipitation of a structure of three-dimensional network.
mineralization is mainly due to the difference of enzymes secreted by bacteria C and G. Bacteria C were cultured without nitrogen which were characterized by large capsule, low number of bacteria and low activity. Bacteria G is nitrogen-cultured with rapid reproduction rate, high protein synthesis and enzyme activity. Therefore, the growth orientation and size of calcite grains which were greatly affected by their exo-polysaccharides and proteins thus varied a lot.

**Figure 7** Morphology of CaCO$_3$ induced by bacteria C.

**Figure 8** Morphology of CaCO$_3$ induced by bacteria G.

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Effect of bacteria on porosity and CaCO$_3$ content of hardened cement paste

The CaCO$_3$ content of hardened cement paste showed a decline gradient trend as the depth deepened (Figure 10 & Figure 12) while the value of porosity represented a trend of gradient increasing influenced by different concentrations of bacteria (Figures 11 & Figure 13). In addition, compound bacteria of C&G performed better effectiveness than bacteria C alone by comparing Figure 10 with Figure 12, Figure 11 with Figure 13.

Numerical simulation of gradient mineralization by bacteria in cement-based materials

The schematic diagram of mineralization depth is shown in Figure 14. Based on the results of bacteria’s adsorption of CO$_2$, transformation of CO$_2$ to HCO$_3^-$, adsorption of Ca$^{2+}$ and influence on CaCO$_3$ in the simulated pore solutions, a mathematical model of gradient bio-mineralization of cement-based materials was established which listed the diffusion and absorption of CO$_2$ as the exclusive controlling factor, presuming the content of Ca$^{2+}$ and OH$^{-}$ in pores of cement-based materials were sufficient. The distribution of CO$_2$ concentration in cement-based material was analyzed by Comsol Multiphysics simulation, and the gradient mineralization of cement-based materials was characterized by the distribution of CO$_2$ concentration.

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Figure 13 Porosity of hardened cement paste with different amount of bacteria.

Figure 14 Schematic diagram of mineralization depth.

Diffusion coefficient of CO₂ in pore structure of cement-based materials: The cement-based material is supposed to be homogeneous porous. The diffusion and transportation of CO₂ in cement-based material which obey the second law of Fick were assumed to only occur in the pores of cement-based material and all converted into calcium carbonate, regardless of the dissolved part in the pore solution.

In the premise of assuring the internal relative humidity of cement-based material unchanged during the entire process, the one-dimensional diffusion of CO₂ in cement-based materials can be expressed by Eq. (9):

\[ \frac{\partial c}{\partial t} = D \frac{\partial^2 c}{\partial x^2} \]  

(9)

In which \( c \) is the CO₂ concentration, \( t \) is the time, \( D \) is the diffusion coefficient of CO₂ in the cement-based material, \( x \) is the mineralization depth.

Because cement-based material is a kind of porous heterogeneous material and the distribution of internal pores is complicated, it is difficult to determine the diffusion coefficient of CO₂ in cement-based material, which has become a key problem in the study of gas diffusion in cement-based material. Many scholars have done a lot of research on the diffusion coefficient of gas in concrete. Greek scholar Papadakis17 has summed up the effect of porosity and relative humidity on the diffusion coefficient through experiments, as shown in Eq. (10):

\[ D = 1.64 \times 10^6 \phi^{0.6}(1 - RH)^{2.2} \]  

(10)

In which, \( \phi \) is the porosity of cement paste, RH is the relative humidity.

On the basis of the formula given by Papadakis, Chinese scholar Zhang Yu18 summed up the relationship between diffusion coefficient and water cement ratio and relative humidity.

\[ D = 8 \times 10^5 (w/c - 0.34)(1 - RH)^{2.2} \]  

(11)

In which, \( w/c \) is the ratio of water to cement, RH is the relative humidity.

Liu Zhiyong19 gave the following empirical formula for calculating the diffusion coefficient:

\[ D = 5.35 \times 10^6 \varepsilon^{2.08}(1 - S)^{2.2} \]  

(12)

In which, \( \varepsilon \) is the connecting porosity of uncarbonized concrete, \( S \) is the water saturation of concrete.

At present, the method of determining gas diffusion coefficient in cement-based material remains not clear. The calculation formula proposed by Papadakis was chosen in this study.

Consideration of effect of bacteria on CO₂ diffusion coefficient from point of porosity: The addition of bacteria greatly sped up the mineralization process, and the porosity of cement-based materials also decreased accelerated, which in turn affected the diffusion process of CO₂ in cement-based materials. The effects of bacteria on cement-based materials were investigated from two aspects: porosity and CO₂ absorption coefficient, which provided the parameters for the establishment of CO₂ diffusion model. Under the influence of bacteria, CO₂ reacted with Ca(OH)₂ in cement-based material to form insoluble CaCO₃ precipitation, which was irreversible and accumulated gradually as time went by. In order to analyze the influence of the insoluble product on the pore structure, it was assumed that the insoluble product was continuously adsorbed on the inner wall of the pore and gradually reduced the pore volume. As shown in Figure 15, the depth of adsorption layer is \( \delta(x,t) \), \( R₀ \) is the initial average pore size of cement-based material.

\[ k_c = 4 \rho \pi \delta^2 \frac{d\delta}{dt} \]  

(13)

CO₂ diffused into the cement-based material and reacted to form calcium carbonate precipitate, which resulted in the increase of the adsorption layer depth \( \delta(x,t) \). The Eq. (13) can be used to describe the change of the adsorption layer depth.
In which k is the absorption coefficient which is $2.9 \times 10^{-4}$ d according to Figure 4, c is the CO$_2$ concentration, so that kc is the amount of CO$_2$ reaction and ρ is the density of calcium carbonate.

With the increase of the depth of the adsorption layer, the internal porosity of cement-based material was constantly changing. The porosity can be expressed as Eq. (14):

$$\phi = \frac{F(x,t)}{F_0}$$  \hspace{1cm} (14)

$$F(x,t) = \pi R (x,t)^2 = \pi [R_0 - \delta(x,t)]^2$$  \hspace{1cm} (15)

In which, ϕ is the porosity, $F_0$ is the initial mean cross-sectional area of pores, $F(x,t)$ is the cross-section area of the pore at the depth of x at t moment.

According to Eq. (13), (14), (15), considering the effect of initial porosity on porosity variation, porosity can be expressed as:

$$\phi = \frac{F(x,t)}{F_0} = \left[1 - \frac{\delta(x,t)}{R_0} \right]^2$$  \hspace{1cm} (16)

$\frac{\delta(x,t)}{R_0}$ - The ratio of initial pore depth to size

When the depth of the filling layer is δ at the depth of x at t time, the internal porosity of the specimen can be calculated. The absorption coefficient of CO$_2$ (k) was $2.9 \times 10^{-4}$ d according to Figure 4. The filling effect of pore depth in the process of mineralization is expressed as follows:

$$\frac{\delta(x,t)}{R_0} = \int_0^t kC(x,t) dt$$  \hspace{1cm} (17)

**Consideration of effect of bacteria on absorption on CO$_2$:** In addition to diffusion, CO$_2$ can be absorbed by microorganisms in cement-based material. In this section, the CO$_2$ absorption coefficient k is added to the diffusion equation of Papadakis as shown in Eq. (18):

$$\frac{\partial c}{\partial t} = D(x,t) \frac{\partial^2 c}{\partial x^2} + kc$$  \hspace{1cm} (18)

In which c is CO$_2$ concentration, k is CO$_2$ absorption coefficient, which is $2.9 \times 10^{-4}$ d according to Figure 4, D(x,t) is diffusion coefficient related to distance x and time t.

Only the one-dimensional diffusion of CO$_2$ in cement-based materials was considered, and the curing condition of specimens was natural environment, so the CO$_2$ concentration in the model boundary was CO$_2$ concentration in the atmosphere. Considering the volume ratio of CO$_2$ in the atmosphere and the equation of state of ideal gas, the boundary CO$_2$ concentration is:

$$c = \frac{P}{RT} = \frac{\mu P_0}{RT} = 0.016 \text{ mol} / \text{L}$$  \hspace{1cm} (19)

In which μ is the volume ratio of CO$_2$ in air, take it as 0.038%, Pa is standard atmospheric pressure, take it as 1.013 MPa, T is Kelvin temperature and taken as 298.15 K, R is ideal gas constant and taken as 8.314 J/(mol \cdot K).

**Numerical simulation results:** According to Eq. (10), (16), (17), (18), for the same mineralization time, the concentration of CO$_2$ decreased with the increase of depth, and with the diffusion went by, the concentration of CO$_2$ decreased sharply from outside to inside (Figure 16). At the range of about 11 mm depth from the surface of specimens, the concentration of CO$_2$ was relatively high. While deepening the depth of the specimens, the concentration of CO$_2$ was negligible in the further depth, so the maximum mineralization depth of cement-based material controlled by bacteria can be estimated to be about 11 mm under the conditions of this study.

![Figure 16 Distribution of CO2 concentration with specimen depth](image)

For the same depth, the concentration of CO$_2$ decreased gradually with the extension of time, and the distance from the surface was 0.01 m, 0.02 m, 0.03 m, 0.04 m. The concentration of CO$_2$ decreased gradually at different depth, but the extent of decrease was obviously different (Figure 17). The closer to the surface of the specimens, the smaller the reduction range of CO$_2$ concentration was; the further away from the surface of the specimens, the greater the decrease of CO$_2$ concentration was.

![Figure 17 Distribution of CO2 concentration with mineralization time at different depth](image)
The calculated parameters A and B are as Table 3.

### Table 3 Values of simulated parameters A and B at different depth

| Depth/m | A/(×10^{-4}) | B/(×10^{-4}) |
|---------|--------------|--------------|
| 0.005   | 1.43         | 134.72       |
| 0.01    | 2.46         | 113.41       |
| 0.02    | 3.69         | 78.23        |
| 0.03    | 4.12         | 54.23        |
| 0.04    | 4.14         | 37.12        |
| 0.05    | 3.93         | 25.17        |
| 0.06    | 3.64         | 17.22        |
| 0.07    | 3.35         | 11.9         |
| 0.08    | 3.1          | 8.45         |
| 0.09    | 2.95         | 6.72         |
| 0.1     | 2.9          | 6.14         |

According to the distribution of CO₂ concentration with mineralization time, the amount of CaCO₃ produced at different depth of the specimens can be expressed by Eq. (22):

\[
C = \int_{0}^{t} k_{cd} dt
\]  

(22)

In which c is the concentration of CO₂ and C is the amount of CaCO₃ generated.

When the mineralization time was the same, the amount of CaCO₃ decreased with the increase of the depth and had obvious gradient variation (Figure 18). When the depth range of the specimen was 0–0.04m, the amount of CaCO₃ decreased greatly with the increase of depth, and when the depth of the specimen was greater than 0.04m, the amount of CaCO₃ tended to be stable.

According to the numerical simulation results from above, the variation of porosity with time and mineralization depth and the experimental results are shown in Figure 19. The initial porosity determined from previous experimental data was 0.45. The numerical simulation results were basically consistent with the experimental result, indicating that the numerical simulation was in agreement with the actual situation.

![Figure 18 Distribution of CaCO₃ products with different depth.](image)

![Figure 19 Variation of porosity with time and mineralization depth.](image)

### Conclusions

In this project research, the effects of bacteria on the absorption of CO₂, the transformation of CO₂, the adsorption of Ca²⁺ and the formation of CaCO₃ were investigated to depict the mechanism of bacteria in gradient mineralization at surface layer of cement-based materials by means of establishing a numerical model of bacterial regulation on gradient mineralization of cement-based materials. In conclusion, the behaviour of bacteria in gradient mineralization at surface layer of cement-based materials can be summarized as follows:

a) Bacteria accelerates the absorption of CO₂ from atmosphere, forming CaCO₃ deposits and reducing porosity in cement-based materials, thus promoting the formation of a gradient change in porosity. The ability of compound bacteria to absorb CO₂ is stronger than that of single bacteria.

b) When CO₂ is absorbed by bacteria in alkaline environment, it is mainly transformed into HCO₃⁻. Then it reacts with the Ca²⁺ adsorbed by bacteria to form CaCO₃. The morphology of CaCO₃ induced by bacteria C and bacteria G are different.

c) A numerical model of bacterial-regulated gradient mineralization was established, which can calculate and predict the production of CaCO₃ and porosity. The porosity of specimens decreased gradually with the prolong of mineralization time. The farther away from the surface, the greater the decrease of CO₂ concentration. The maximum mineralization depth of microbial-controlled cement-based specimens was about 11mm.

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### Conflicts of interests

Authors declare that there is no conflict of interest.

### References

1. Chen Jun, Yao Su Ping, Ji Jun Feng, et al. Microbial geochemistry and its research progress. Geological Review. 2004;50(6):620–632.

2. Achal V, Mukherjee A, Basu PC, et al. Strain improvement of Sporosarcina pasteurii, for enhanced urease and calcite production. Journal of Industrial Microbiology & Biotechnology. 2009;36(7):981–988.

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3. Wiktor V, Jonkers HM. Field performance of bacteria-based repair system: Pilot study in a parking garage. Case Studies in Construction Materials. 2015;2:11–17.

4. Ramachandran, Santhosh K, Ramakrishnan V, et al. Remediation of concrete using micro-organisms. ACI Materials Journal-American Concrete Institute. 2001;98(1):3–9.

5. Wang J, Tittelboom KV, Belie ND, et al. Use of silica gel or polyurethane immobilized bacteria for self-healing concrete. Construction & Building Materials. 2012;26(1):532–540.

6. Wiktor V, Jonkers HM. Quantification of crack-healing in novel bacteria-based self-healing concrete. Cement & Concrete Composites. 2011;33(7):763–770.

7. Burbank MB, Weaver TJ, Green TL, et al. Precipitation of Calcite by Indigenous Microorganisms to Strengthen Liqueifiable Soils. Geomicrobiology Journal. 2011;28(4):301–312.

8. Wang G, Ren T, Qi Q, et al. Determining the diffusion coefficient of gas diffusion in coal: Development of numerical solution. Fuel. 2017;196:47–58.

9. Ronghui, Qian Chunxiang, Li Longzhi. Mechanism of microbial cement cementation. Journal of Silicate. 2013;41(3):314–319.

10. Whiffin VS. Microbial CaCO3 precipitation for the production of biocement. Australia: Murdoch University; 2004. 162 p.

11. Dick J, De W, De G B, et al. Bio-deposition of a calcium carbonate layer on degraded limestone by Bacillus species. Biodegradation. 2006;17(4):357–367.

12. Muynck WD, Cox K, Belie ND, et al. Bacterial carbonate precipitation as an alternative surface treatment for concrete. Construction & Building Materials. 2008;22(5):875–885.

13. Zhan Nan, Huang Yi, Jao Zhu, et al. In situ rapid determination of bicarbonate and carbonate in groundwater and lake water by dual electrode method. Analytical Chemistry. 2016;44(3):355–360.

14. Majumdar S, Sarkar M, Trinath C, et al. Use of Bacterial Protein Powder in Commercial Fly Ash Pozzolana Cements for High Performance Construction Materials. Open Journal of Civil Engineering. 2012;2(4):218–228.

15. Wang R, Qian C, Wang J. Study on microbiological precipitation of CaCO3. Journal of Southeast University (Natural Science Edition); 2005.

16. Jing R, Kjellerup BV. Biogeochemical cycling of metals impacting by microbial mobilization and immobilization. Journal of Environmental Sciences. 2018;66(4):146–154.

17. Papadakis VG. Effect of supplementary cementing materials on concrete resistance against carbonation and chloride ingress. Cement & Concrete Research. 2000;30(2):291–299.

18. Zhang Y, Jiang LX. Practical mathematical model of concrete carbonation depth based on carbonation mechanism. Industrial Construction. 1998;28(1):16–19.

19. Liu Zhiyong. Study on durability Test and Life Prediction method of Marine concrete based on Environment. Southeast University; 2006.