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Living concrete with self-healing function on cracks attributed to inclusion of microorganisms: Theory, technology and engineering applications—A review

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Concrete is the most widely used composite material in civil engineering. Microbial induced calcium carbonate precipitation (MICP) is a green and environmental friendly technology, which has received extensive attention in repair of concrete cracks. This paper introduces the research progress in Southeast University research in past 16 years. In the early stage, MICP technology of urea hydrolyzed by *Bacillus pasteurii* was mainly investigated to repair the surface cracks and to fill large-size cracks with grouting. However, aiming at the hidden cracks that were difficult for human intervention, a new mineralization route of *Bacillus mucilaginosus* was proposed, which could repair faster than *Bacillus alcalophilus*, and the problem of ammonia emission in the repair process of *Bacillus pasteurii* was also solved. In addition, in order to improve the protection of bacteria and the self-healing efficiency of the later age cracks, the methods of fiber immobilization, carrier uniformly immobilization and core-shell structural immobilization had been compared and studied. The results showed that core-shell structural immobilization had good protection ability and strong designability. What's more, the paper also summarized the characteristics of spore germination, cell activity, nucleation and biological calcium carbonate in crack zone, and introduced the application experience of microbial self-healing concrete in water conservancy projects and subway stations.

living concrete, self-healing, crack, microorganisms, engineering application

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1 Introduction

In China, the per capita consumption of concrete in 2019 has exceeded 10 t, which is more than 50% of the global total. Meanwhile, China's future urbanization (the current urbanization rate is about 60%, it will take 10 years to increase to about 70%) and the transportation infrastructure construction still need a lot of concrete. However, due to the inherent defects of high compressive strength and low tensile strength of concrete, it is easy to crack, so the durability and service life of concrete are seriously affected, which is an international problem. So far, most of concrete cracks are attributed to non-loading, such as plastic shrinkage, autogeneous-shrinkage, deformation caused by heat evolution of hydration, drying shrinkage, creep, reactive expansion and explosion at high temperatureand. They happen at different ages of concrete, such as pouring, curing and in-service. After cracking, the mechanical properties, waterproof ability, ion transmission resistance, sound insulation, heat insulation and other functions of concrete will be greatly reduced. In

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fact, a dense and intact concrete has a good waterproof function, but once a penetrating crack occurs, even if the crack width is less than 0.1 mm, water can still instantly penetrate the concrete. Meanwhile, the flow rate is proportional to the third power of the crack width [1,2]. While in presence of chloride ion, it can be enriched at crack tip [3], and the apparent diffusion coefficient of chloride ion has a good linear relationship with the crack width [4]. Similarly, there is a law for the diffusion of sulfate ion [5]. What's more, concrete cracking will also accelerate the transmission of carbon dioxide at the crack and increase the depth of carbonization [6,7]. Furthermore, if there are holes or cracks in the wall, when the size is far greater than the wavelength, the sound wave will pass through the cracks and keep the original waveform to move forward. Therefore, when there are gaps in the wall, the sound insulation will be reduced [8]. In addition, the main heat transfer method in the cracked zone of concrete is transient heat conduction at a higher temperature. However, due to the difference in the thermal properties of air and concrete, the temperature field in the cracked zone will change suddenly. Compared with the uncracked concrete, the temperature of cracked concrete is higher [9]. In summary, cracks have many negative effects on concrete performance. Many scholars have carried out a lot of researches on crack repair technology, including microbial mineralization technology. Compared with other crack repair methods, such as grouting method, epoxy resin method, microcapsule method, shape memory alloy method and mineral admixture method, the microbial mineralization technology has the intelligence advantages of green environmental protection, convenient construction, and the cracks can be repaired multiple times. In addition, the microbial mineralization technology has low repair costs and it can be applied to specific practical engineering.

The development and application of microbial mineralization technology in civil engineering can be traced back to 1992. Ferris et al. [10,11] independently carried out the research on microbial mineralization to improve soil. They used *Bacillus pasteurii* to hydrolyze urea to produce carbonate ions, which could combine with calcium ions provided by calcium chloride to form calcite to regulate the pore structure of soil. In cement concrete, the first report was that Ramachandran et al. [12] used the above mineralization approach to repair the surface cracks of concrete in 1998. Subsequently, in 2004, Southeast University [13] was the first to conduct research on repair of concrete cracks in China, and obtained the invention patent for the preparation of calcium carbonate by the *Bacillus pasteurii* hydrolyzing urea [14]. Generally, this paper mainly introduces the technical route evolution, mineralization mechanism, carrier function, self-healing effect, and engineering application, especially the self-healing concrete in Southeast University (SEU) since 2004.

2 The surface coating of small cracks and the filling repair of large cracks

During 2004–2009, the research group in SEU carried out much work on repair of small cracks by deposition and repair of large cracks by filling and grouting based on microbial bio-mineralization, which mainly used *Bacillus pasteurii* to hydrolyze urea to produce carbonate ion. And the reaction mechanism was shown in eqs. (1)–(4). However, because concrete had strict restrictions on use of chloride ion, calcium nitrate was used instead of calcium chloride to provide calcium ions for mineralization [15].

$$(NH_2)_2CO + 3H_2O \xrightarrow{\text{Urease}} 2NH_4^+ + HCO_3^- + OH^- \quad (1)$$

$$HCO_3^- + OH^- \rightarrow CO_3^{2-} + H_2O \quad (2)$$

$$\text{Cell} + Ca^{2+} \rightarrow \text{Cell} - Ca^{2+} \quad (3)$$

$$\text{Cell} - Ca^{2+} + CO_3^{2-} \rightarrow \text{Cell} - CaCO_3 \downarrow \quad (4)$$

The process of depositing of calcium carbonate on the concrete surface could adopt the immersion method [16], that was, the concrete specimen was immersed in the solution containing bacteria, urea and calcium source. However, it was difficult to apply this method in actual engineering. The more practical methods were the spraying method [17] and the brushing method [18]. In these methods, the agar or sodium alginate was used as the carriers to adjust the consistency, especially for the facade spraying and brushing needed appropriate consistency. The comparison on effects of microbiologically deposited $CaCO_3$ layer with different treatment methods is shown in Table 1 [19]. It can be seen that the mineralized calcium carbonate layer with thickness of 100–300 μm could be formed on the surface of concrete by the three methods, as shown in Figure 1 [15,20]. In addition, the capillary water absorption rate of concrete could be greatly reduced. However, for cracks with a larger width, fly ash, slag powder or quartz sand should be filled first, and then the bacterial fluid, urea and calcium source could be poured to realize the filling and repair of cracks [21,22]. In particular, *Bacillus pasteurii* hydrolyzing urea to deposit calcium carbonate has a high efficiency, at this time, bacteria slowly decomposed the substrate to produce carbonate, and the biomineralization products different from the chemical reaction could be obtained through the mineralization crystallization. Meanwhile, the slow formation of calcium carbonate was accompanied by the change of configuration at the organic-inorganic interface, and finally a dense, hard-textured organic adhesive calcium carbonate film could be formed. In addition, it was well known that the polymorph of calcium carbonate at room temperature was mainly stable calcite, which had a good protective effect on the surface of concrete [20].

Due to the emission of ammonia from urea decomposition,

Table 1 Comparison on effects of microbiologically deposited $CaCO_3$ layer with different treatment methods

Treatment	$CaCO_3$ particle diameter (μm) (morphology)	Thickness of $CaCO_3$ film (μm)	Coefficient of capillary suction		Deposited layer on the side plane
			Absolute value (g m^{-2} $h^{-1/2}$)	Decreased degree (%)	
Immersing	100 (sphere with arris)	280–330	290.2	83	Impossible
Spraying	10 (spherical)	70–100	399.6	76	Impossible
Brushing with sodium alginate	5 (spherical)	100–120	191.4	89	Impossible
Brushing with agar	10 (amorphous)	100–120	174.6	90	Possible

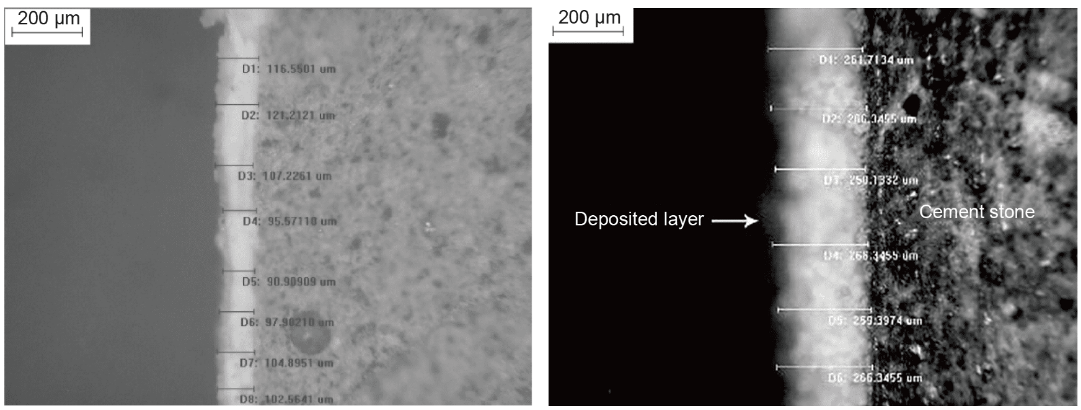

Figure 1 Thickness of the deposited calcite layer on concrete surface [15,20].

the application of this mineralization pathway in concrete and other building materials was strictly restricted, so a new mineralization route was put forward by SEU team. *Bacillus mucilaginosus* could be used to absorb carbon dioxide from atmosphere to provide carbonate, and the biological calcium carbonate mineral could be generated on the surface of concrete. The specific mineralization diagram and reaction equation are shown in Figure 2 and eqs. (5)–(8) [23,24], respectively.

$$CO_2 + H_2O \xrightarrow{\text{Bacteria}} HCO_3^- + H^+ \tag{5}$$

$$HCO_3^- + OH^- \rightarrow CO_3^{2-} + H_2O \tag{6}$$

$$\text{Cell} + Ca^{2+} \rightarrow \text{Cell} - Ca^{2+} \tag{7}$$

$$\text{Cell} - Ca^{2+} + CO_3^{2-} \rightarrow \text{Cell} - CaCO_3 \downarrow \tag{8}$$

It is worth noting that the specific reaction formula of microbial acceleration of CO_2 to HCO_3^- is as follows:

$$E{\cdot}ZnOH_2 \xrightarrow{\text{Bacteria}} E{\cdot}ZnOH^- + H^+ \tag{9}$$

$$E{\cdot}ZnOH^- + CO_2 \Leftrightarrow E{\cdot}ZnHCO_3^- \tag{10}$$

$$E{\cdot}ZnHCO_3^- + H_2O \Leftrightarrow E{\cdot}ZnOH_2 + HCO_3^- \tag{11}$$

3 Microbial self-healing concrete with the addition of bacterial cells or spores

The above surface repair of small cracks and filling repair of large cracks both needed manual intervention after cracking, which were also called passive repair. Sometimes cracks were difficult to be found in time, and sometimes it was difficult to repair in the field. While we could learn from the self-perception and self-healing functions of animals and plants in nature. For example, human skin rupture could be self-healing by self-tissue regeneration [25,26]. Gecko tail fracture could be regenerated by cell proliferation [27,28]. The broken spine of sea urchin would immediately produce mucilaginous material wrapped with calcium carbonate molecules, which could fill the injured area and harden and crystallize for repair [29]. And the roots, stems and leaves of plants could also self-healing after physical damage [30–32] and so on.

3.1 Crack self-healing by *Bacillus alcalophilus* mineralization

Jonkers [33] of Delft University of Technology in the Netherlands started the research on self-sensing and self-healing of concrete cracks in 2007, they used *Bacillus alcalophilus* as the bacteria and calcium lactate was used as the substrate. SEU also began to study this self-healing system in 2010, and obtained the Chinese invention patent in 2012 [34]. In this system, bacteria or spores and different calcium sources were added as admixture during concrete mixing, and the mineralization mechanism could be described by eqs. (12)–(17). At this time, calcium lactate or other calcium source

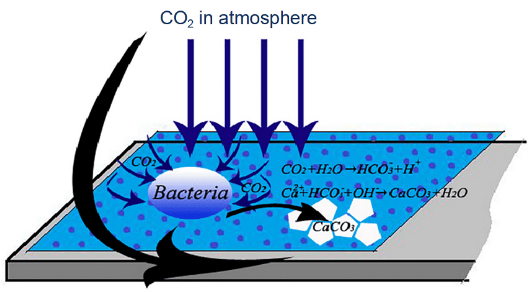

Figure 2 (Color online) The transformation process from CO_2 absorbed and generated calcite on the surface of concrete [23,24].

was used as the carbon source and calcium source. Calcium ions could be released and CO_2 produced under the action of bacteria. But it was not the only carbon source, extra CO_2 could also be released under the respiration of bacteria in the presence of nutrients and oxygen [35].

$$\text{Calcium Lactate} \xrightarrow{\text{Bacteria}+O_2} CO_2 + Ca^{2+} + H_2O \quad (12)$$

$$\text{Bacteria} + \text{Nutriment} + O_2 \xrightarrow{\text{Respiration}} CO_2 \quad (13)$$

$$CO_2 + H_2O \rightarrow HCO_3^- + H^+ \quad (14)$$

$$HCO_3^- + OH^- \rightarrow CO_3^{2-} + H_2O \quad (15)$$

$$\text{Cell} + Ca^{2+} \rightarrow \text{Cell} - Ca^{2+} \quad (16)$$

$$\text{Cell} - Ca^{2+} + CO_3^{2-} \rightarrow \text{Cell} - CaCO_3 \downarrow \quad (17)$$

For the artificial crack with a width of about 1 mm, the repair state of the crack surface in 5 and 40 d was shown in Figure 3 and Table 2 [36]. Due to the need of external oxygen supply, this mineralization route mainly occurred in *X* and *Y* directions of the crack surface as shown in Figure 4. While the *Z* direction along the crack depth was closed on the surface. Consequently, the healing depth was limited [37]. However, due to the closed crack, it still had good resistance to water and corrosive media, which was conducive to the improvement of concrete durability.

Table 2 The width of points A, B and C measured by crack width (mm) [36]

Crack location	0 d	5 d	40 d
A	1.06	0.36	0
B	1.14	0	0
C	1.02	0.98	0

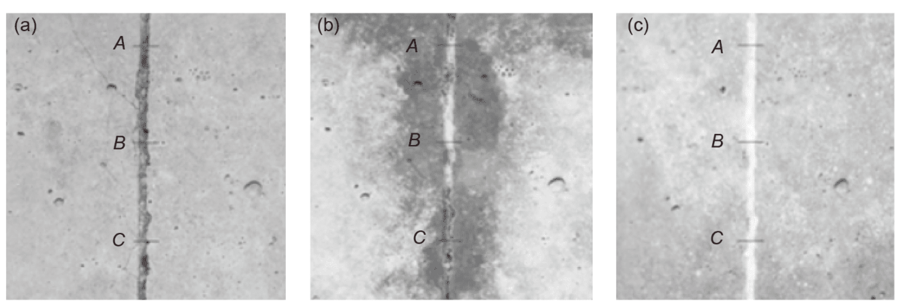

Figure 3 Filling degree of cracks in different healing time [36]. (a) 0 d; (b) 5 d; (c) 40 d.

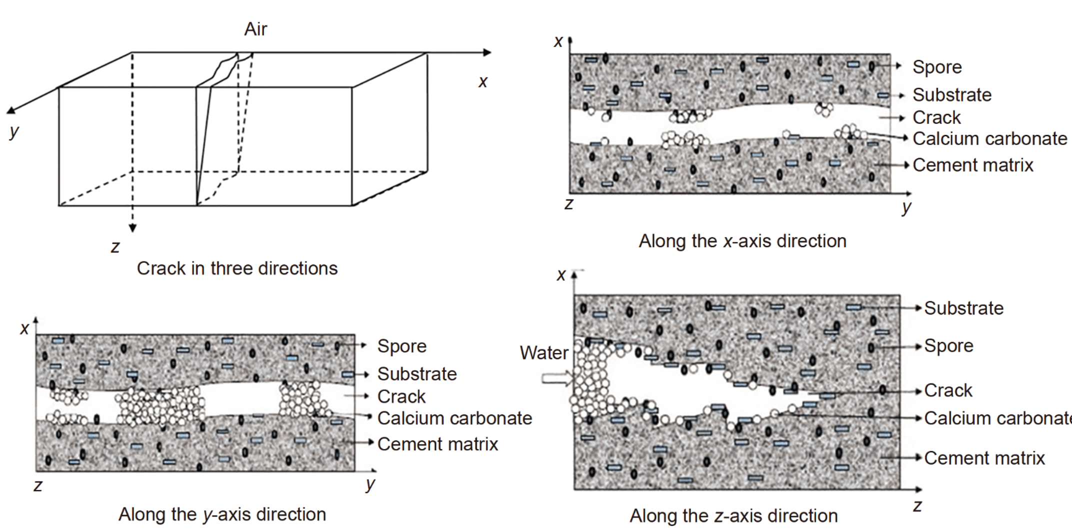

Figure 4 Distribution of healing products in three directions of the crack [37].

However, if spores were directly added into concrete, the production time of crack had a great influence on the self-healing effect (Figure 5) [38]. When the cracks with width of 0.3 mm produced at ages of 7 and 14 d, and then healed for 40 d, the repair rate of water seepage resistance reached 100%. However, with the extension of cracking age, the repair rate of cracks with the width of about 0.3 mm decreased gradually, and the repair rate of cracks occurred at 90 d was only about 20% after healing for 40 d. The main reason could be attributed to the pH of the solution in the crack zone of cement-based materials could reach above 13.0 [39]. At this time, the number of *Bacillus alcalophilus* had been sharply reduced [40] as shown in Figure 6. Even if nutrients were provided, the growth of bacteria was greatly inhibited when pH reached 13 in crack zone solution. However, the alkali resistance could be greatly improved after the cells were transformed into spores (Figures 7 and 8).

3.2 Crack self-healing by *Bacillus mucilaginosus* mineralization

In general, *Bacillus alcalophilus* needed oxygen [35], and its mineralization for repair was slow. The carbon source come from the decomposition of substrate and the respiration of bacteria. Meanwhile, oxygen come from atmosphere, and subsequently entered into the concrete after cracking to activate bacteria. Besides, there was also carbon dioxide in-atmosphere, which could be directly used as the carbon source, and the transformation of carbon dioxide to carbonate could be accelerated by *Bacillus mucilaginosus*. The mineralization reaction was the same as the eqs. (5)–(8). This self-healing system was patented in China in 2013 [41]. It could completely seal the early cracks below 0.5 mm [42], reduce the water permeability [42,43], improve the resistance to chloride ion migration and then protect the reinforcement [44]. By screening low temperature resistant bacteria or carrying out low temperature resistant acclimation, this kind of bacteria could survive, isolate, induce calcium carbon precipitation at 7°C and seal cracks with width of 0.4–0.5 mm after healing for 14 d [45]. The germination state of the spores stored in different pH and cement pastes

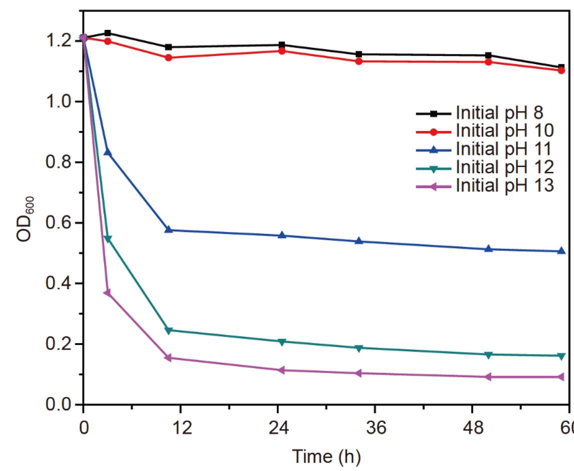

Figure 6 (Color online) Alkali tolerance of *Bacillus alcalophilus* [40].

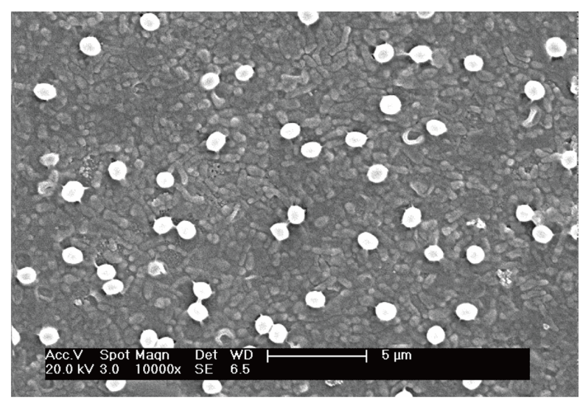

Figure 7 SEM micrograph of spores [40].

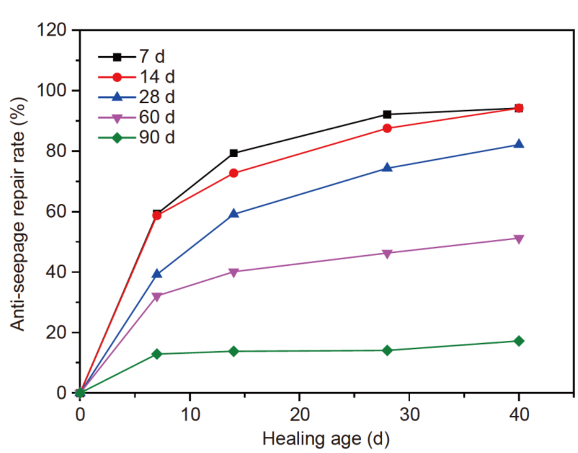

Figure 5 (Color online) Effect of cracking age on healing efficiency of cracks under healing ages [38].

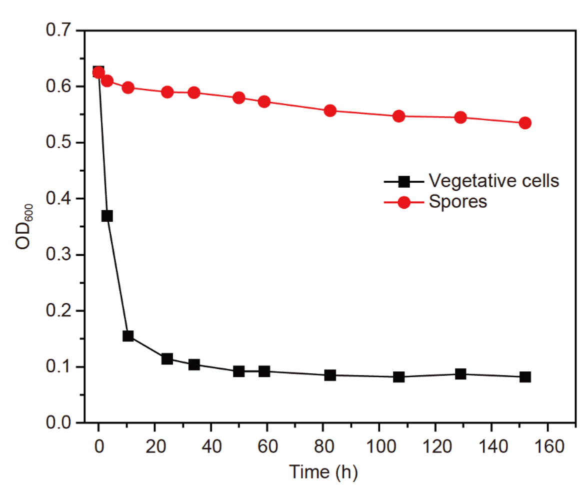

Figure 8 (Color online) Survival capacity of spores in simulated pore solution of cement-based materials [40].

for 3, 7 and 14 d was shown in Figure 9. It could be seen that the spores germinated in pastes with the pH of 12 and at different ages, there was a longer induction time. The scanning electron microscopy (SEM) observation image after incubation for 48 h is shown in Figure 10 [45].

In addition to repair cracks, *Bacillus mucilaginosus* spores directly added into concrete could also alleviate the problem of efflorenscence caused by the precipitation of CH [46,47]. Generally, there was a direct positive correlation between the degree of efflorescence and CH content in concrete [48,49]. What's more, *Bacillus mucilaginosus* could absorb carbon dioxide in the atmosphere, react with CH and transform it into calcite, thus, the content of CH with high solubility and the porosity of concrete could both be reduced, which could also help fixing greenhouse gas of CO_2.

3.3 Comparison of the self-healing systems between *Bacillus alcalophilus* and *Bacillus mucilaginosus*

Compared with the two kinds of self-healing systems of *Bacillus alcalophilus* [36] and *Bacillus mucilaginosus* [43], adding bacteria or spores directly into concrete could better repair cracks, but the common problem was that the healing depth was small. In addition, the self-healing effect of early age cracks was good, but the self-healing effect of later age cracks was greatly reduced.

The self-healing effect of the two systems was compared as Figure 11(a) and (b) [38,42]. It can be seen that with the extension of healing time, the area repair rate of crack increased gradually. When the crack width was 0.3–0.5 mm, the area repair rate of the specimens by *Bacillus mucilaginosus* after healing for 20 d could reach more than 80%, while that of the specimens repaired by *Bacillus alcalophilus* was 50%–70%.

The effect of *Bacillus alcalophilus* and *Bacillus mucilaginosus* on the strength of mortar (40 mm × 40 mm × 160 mm) is shown in Figure 12 [50–54]. It can be seen that *Bacillus alcalophilus* had no obvious effect on the strength, but *Bacillus mucilaginosus* could greatly improve the strength of mortar at 7 and 28 d.

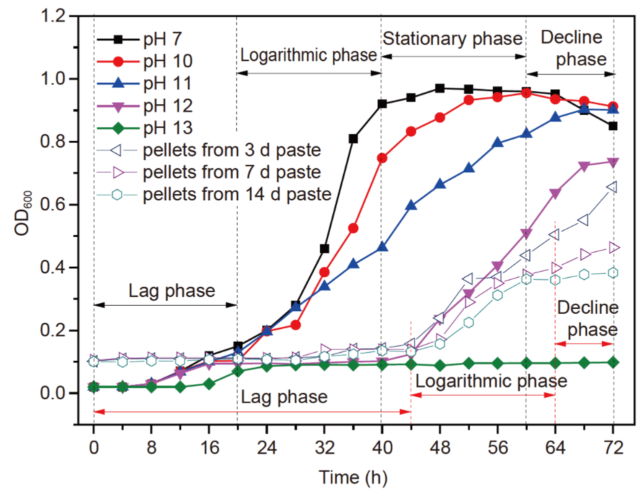

Figure 9 (Color online) Spores germination under alkaline conditions [45].

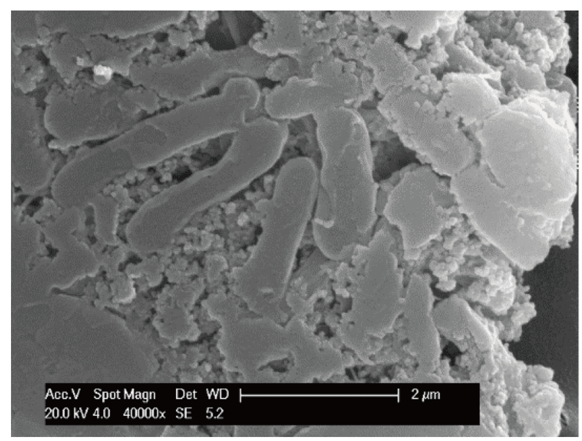

Figure 10 Cells after spores incubation for 48 h [45].

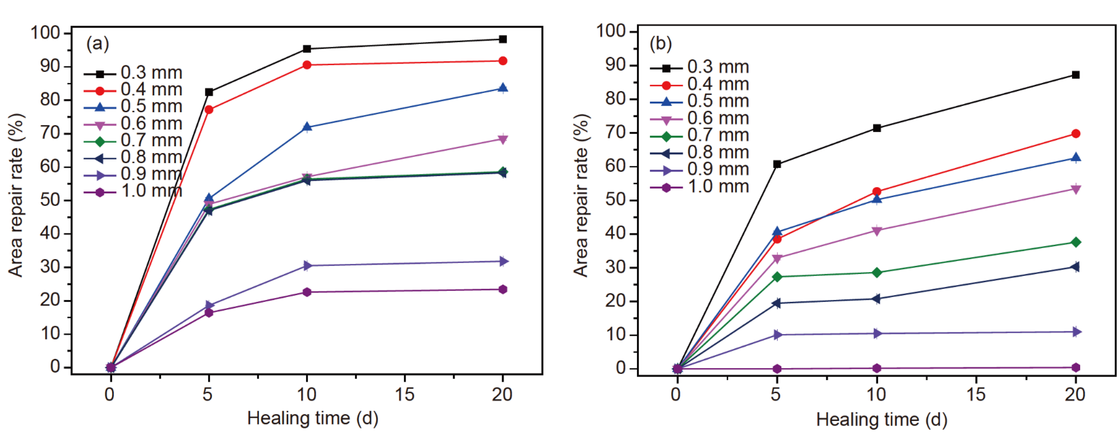

Figure 11 (Color online) The area repair rate of specimens with different crack widths after different healing time. (a) Repair system of *Bacillus alcalophilus* [38]; (b) repair system of *Bacillus mucilaginosus* [42].

On the premise of sufficient calcium ion, the quantity and the formation rate of calcium carbonate depend on the carbonate ion. Theoretically, 1 mol carbonate is needed to form 1 mol mineralized product of calcite. In the *Bacillus alcalophilus* self-healing system, it could be seen from the eqs. (9)–(14) that the formation of carbonate depended on the diffusion of oxygen from air into the crack and the dissolution of CO_2 in the crack solution, the rate of microbial enzyme catalysis and the rate of carbon dioxide conversion to carbonate. The total biochemical reaction equation of mineralization process could be expressed by eq. (18). 1 mol oxygen was needed to form 1 mol product.

$$CaC_6H_{10}O_6 + 5Ca(OH)_2 + 6O_2 \rightarrow 6CaCO_3\downarrow + 10H_2O \qquad (18)$$

According to the literature [55], considering the oxygen consumption of *Bacillus alcalophilus*, when oxygen in atmosphere diffused into the cracks of cement-based materials, the oxygen concentration at different depths of crack with the width of 0.4 mm at different times is shown in Figure 13. It can be seen that the oxygen concentration was about 6.5×10^{-3} kg/m^3 at 10 d and in crack depth of 10 mm, which was equivalent to 2.03×10^{-4} mol/m^3.

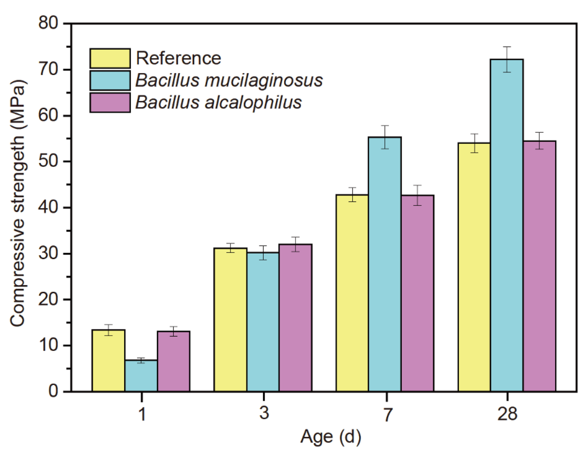

Figure 12 Effect of *Bacillus mucilaginosus* and *Bacillus alcalophilus* on compressive strength of mortar specimens.

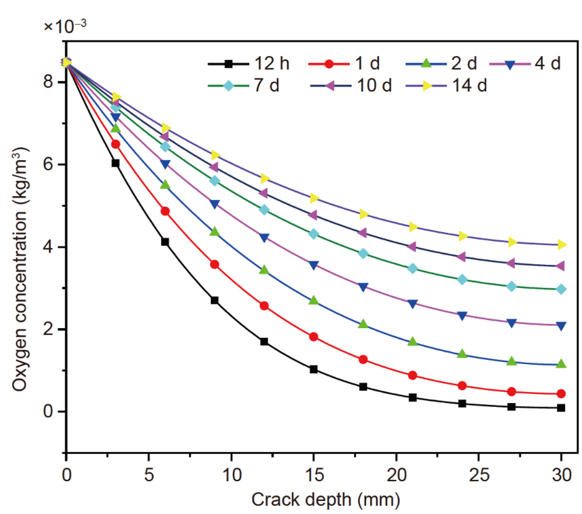

Figure 13 Oxygen concentration at different depths of crack with the width of 0.4 mm at different times [55].

The reference [56] calculated the CO_2 concentration in the cracks of *Bacillus mucilaginosus* self-healing system. Considering the CO_2 could be accelerated absorption by *Bacillus mucilaginosus*, the CO_2 concentration at different depths of crack with the width of 0.4 mm at different times is shown in Figure 14. It can be seen that the CO_2 concentration in the crack at 10 d and in crack depth of 10 mm was about 5.5×10^{-4} kg/m^3, which was equivalent to 1.20×10^{-5} mol/m^3.

Compared with Figures 13 and 14, the concentration of carbon dioxide in the crack of *Bacillus mucilaginosus* system was one order of magnitude lower than that of oxygen in the crack of *Bacillus alcalophilus* system. However, the results (Figure 11) showed that the repair rate of *Bacillus mucilaginosus* system was much higher than that of *Bacillus alcalophilus* system at the same healing time. It can be inferred that oxygen diffusion in *Bacillus alcalophilus* system was not the controlling step for the formation of mineralized products. Theoretically, in *Bacillus alcalophilus* system, bacteria needed to consume 1 mol oxygen to form 1 mol CO_2. In fact, whether 1 mol of oxygen could form 1 mol of carbonate might also depend on the solubility of oxygen in water, the enzyme catalyzed reaction rate of eq. (9), the carbon dioxide released rate by respiration of eq. (10), and the rate of carbon dioxide converted into carbonate by hydration of eqs. (11) and (12). Compared with the solubility of carbon dioxide in water, it was 1.45 g/L at 25°C, while that of oxygen was only 0.00825 g/L at 25°C. In addition, the hydration conversion coefficient of carbon dioxide was 1.3×10^{-1} s^{-1} [57]. Generally speaking, the rate constant of enzyme catalyzed reaction was in the order of 10^{-3} s^{-1}, which was lower than the hydration of carbon dioxide. Therefore, in *Bacillus alcalophilus* system, the control step of mineralization rate was the enzyme catalyzed reaction of SJ bac-

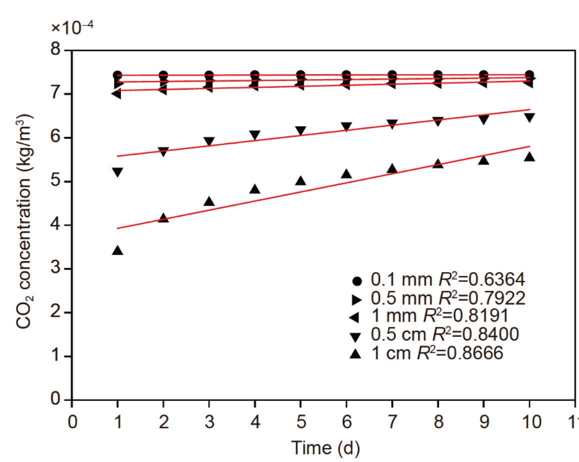

Figure 14 CO_2 concentration at different depths of crack with the width of 0.4 mm at different times [56].

teria on the substrate of calcium lactate. However, in *Bacillus mucilaginosus* system, due to the existence of carbonic anhydrase (CA), the CO_2 hydration conversion coefficient was increased to 1.0×10^6 s^{-1}, which was about 10^7 times than that in nature [57–59]. Hence, the control step of the mineralization rate was the diffusion of CO_2 into the cracks.

4 Self-healing with immobilization

In order to improve the healing depth and the self-healing capacity for the later age cracks, further research on the immobilized bioremediation was carried out. The immobilization methods include fiber pre-immobilization or the interception and fixation during the repair process, uniformly carried, and core-shell structural immobilization with carrier as the shell and bacteria as the core. The ideal carrier should meet the following requirments: (1) high loading rate for bacteria, (2) high release rate, (3) high protection, (4) low cost, (5) no negative impact on concrete performanceand and so on. The immobilized self-healing system posesses flexible design, which can load more components in the carrier according to the needs, without worrying about the adverse effects of the healing agent components on the concrete.

4.1 Fiber immobilization

Figures 15 and 16 [50] demonstrated the mechanism of the fiber bridging in the crack zone and intercepting calcium ions and depositing calcium carbonate to block the fracture channel. However, $CaCO_3$ could only be deposited on the polypropylene (PP) fibers at the surface of crack mouth. While for the polyvinyl alcohol (PVA)fibers, $CaCO_3$ could be deposited on the deep part of the crack. The reason was that PVA had polarity groups, hydrophilic and the crystalline parameter in *b*-axis of polyvinyl alcohol is close to that of calcium, so PVA was easier to adsorb calcium ions and become the nucleation site of calcium carbonate crystal. The experiment further confirmed [60] that it was indeed difficult to “fix” bacteria on the surface of PP fiber (Figure 17(a)), but after the surface of PP fiber was treated with chitosan, the ability to load bacteria was greatly improved (Figure 17(b)). It was obviously that fiber could meet the requirements of (1), (2), and (5) for the carrier, but it was not easy to meet (3) and (4).

Although it was difficult for bacteria to deposit directly on the surface of PP fibers, fluorescence microscope observation showed that the extracellular polymeric substances (EPS) of bacteria can adhere to the surface of PP fibers (Figure 18), and more calcium carbonate was deposited on the fibers under the induction of EPS. The distribution of EPS confirmed that the biological macromolecules covered the PP fibers and crystal products during the repairing process [61].

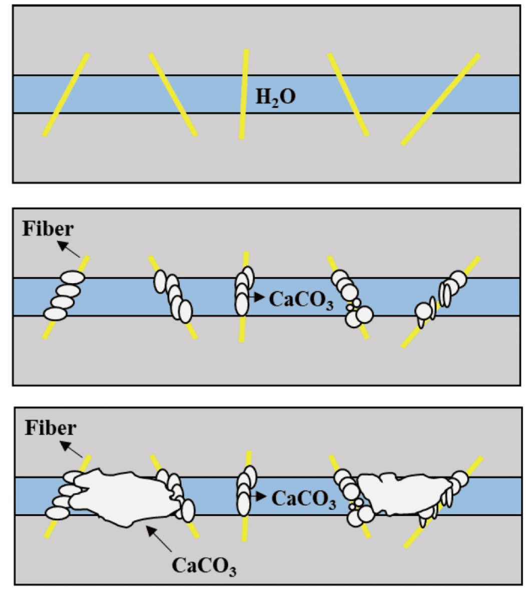

Figure 15 (Color online) Schematic diagram of calcium ion retention and calcium carbonate deposition by fiber bridging cracks [50].

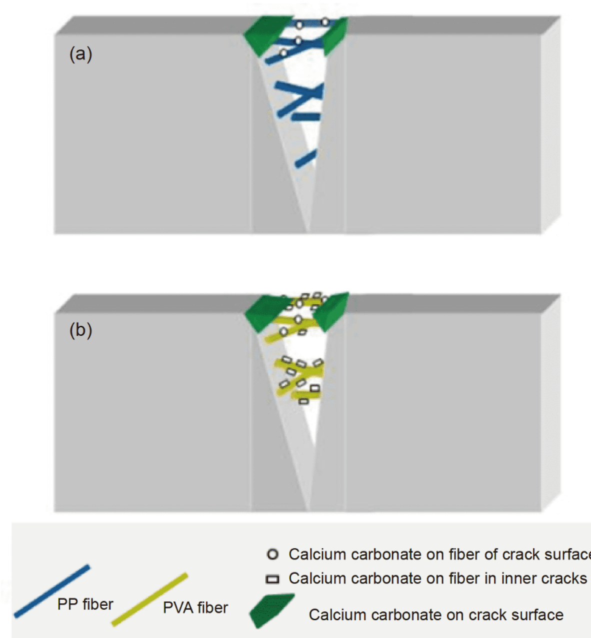

Figure 16 (Color online) Integral schematic of deposition on surfaces of fibers in cracks of (a) specimens with bacteria and PP fiber and (b) specimens with bacteria and PVA fiber [50].

4.2 Uniformly carried spores

In order to improve the ability to protect bacteria, many researchers used porous carriers, but it had a greater negative impact on the strength of concrete. Therefore, we tried to mix the bacteria with magnesium phosphate cement and sulpho-aluminate cement uniformly to make the microbial healing agent (Figure 19). The studies showed that there was no negative effect on the strength and other properties of cement-based materials after addition of spores uniformly carried with small cylinder [62]. In addition, when the concrete cracked, bacteria could be released from the carrier and the mineralization would occur, as shown in Figure 20.

The research [62] showed that the spores immobilized by cylindrical magnesium phosphate cement and sulpho-aluminate cement still had a survival rate more than 80% after 180 d in the pore solution of cement-based materials, and the area repair rate reached 80% after healed for 28 d. While the survival rate of spores directly exposed for 180 d was only about 10%, and the area repair rate of crack was less than 20%. What's more, the healing depth of crack had been improved up to 5 mm, but it was still insufficient.

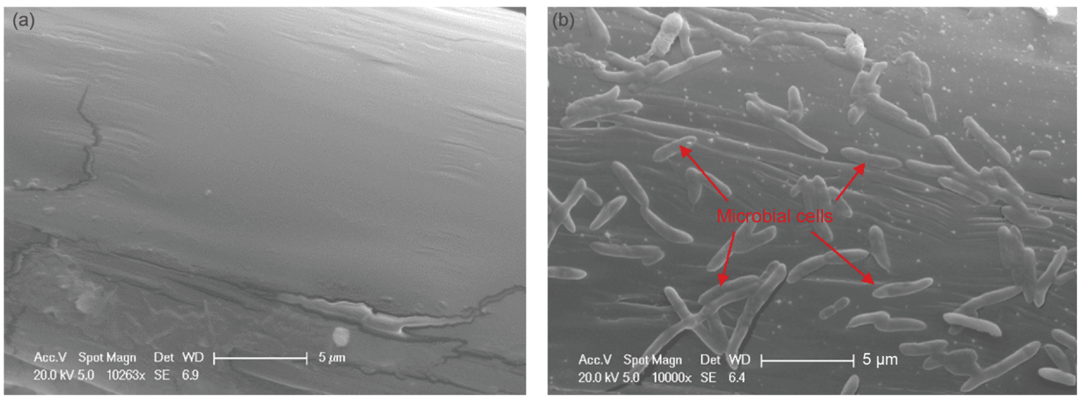

Figure 17 SEM images of surface of PP after adsorption of bacteria (a) and surface of MPP after adsorption of bacteria (b) [60].

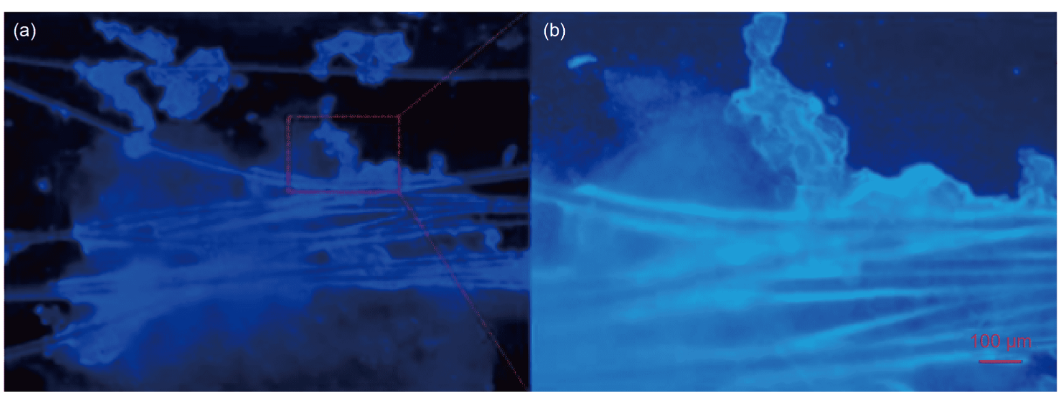

Figure 18 The optical microscope images of deposit on PP fiber. (a) Deposit induced by EPS; (b) biological organic film on PP fiber surface and deposits surface [61].

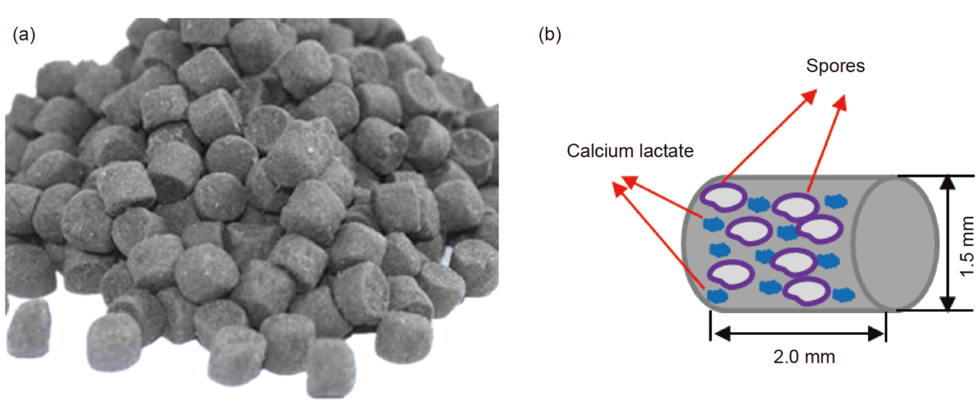

Figure 19 Pictures of microbial self-healing agent. (a) Real samples; (b) schematic diagram [62].

4.3 Core-shell immobilization

The core-shell immobilization method was shown in Figure 21 [63], which had a stronger protection against bacteria. The loading rate, release rate, and the effect on concrete strength and other properties could all be controlled by the design of shell materials. As shown in Figure 22 [63], after the spore-immobilized particles were immersed in the pore solution of cement-based materials for 200 d, the activity of the spores measured by the optical density (OD_{600}) decreased very little. While for the spores without immobilization, the activity was basically lost after 28 d. The above results indicated that the repair for crack must be completed within 28 d, otherwise, the activity of spores would be lost after the concrete and the carrier cracked for more than 28 d. The results (Figure 23) also showed that the concrete containing immobilized healing agent (CH5) (the crack width was about 0.5 mm) cracked at 28 d, the relative permeability coefficient was reduced by about 80% due to the repair effect [63]. While the relative permeability coefficient of concrete directly added the spores (S) was only about 50%, and that of concrete without the healing agent (H0) was less than 20%. Therefore, it could be seen that the immobilization could improve the self-healing effect for concrete cracks.

It was worth noting that another advantage of the core-shell structure was that the composition design of the core was more flexible. As mentioned above, the control step of CA type self-healing system was the transmission of carbon dioxide from atmosphere into the cracks. Therefore, the use of carbon dioxide generated inside the core-shell structure could achieve deep repair of crack [64], which was up to about 20 mm.

5 Mineralization process and repair structure in crack zone

The mineralization process of microorganisms in crack zone and pore solution included spore germination and transformation into cells, cells reproduction, enzyme catalysis to produce ions, nucleation by cell and crystal growth of $CaCO_3$ in the supersaturated solution. Eventually, the mineralization products could be generated to fill the cracks.

When observed directly by inverted fluorescence microscope, due to the refractive index of microbial spores was high, and the refractive index of nutrition body decreased after germination. Therefore, according to the difference of refraction, spores and nutrition body were bright spots and dark spots as shown in Figure 24 under the inverted fluorescence microscope, respectively [65]. The germination rate

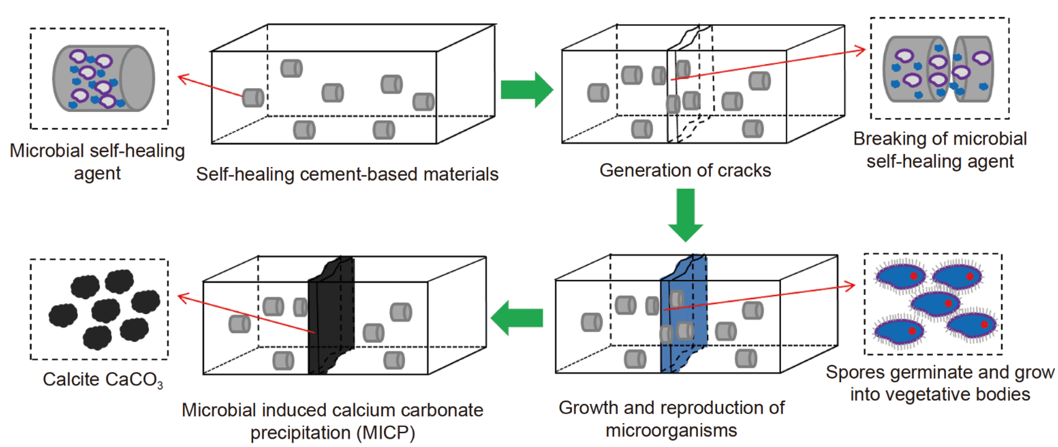

Figure 20 (Color online) Self-healing schematic diagram of cracks in cement-based materials [62].

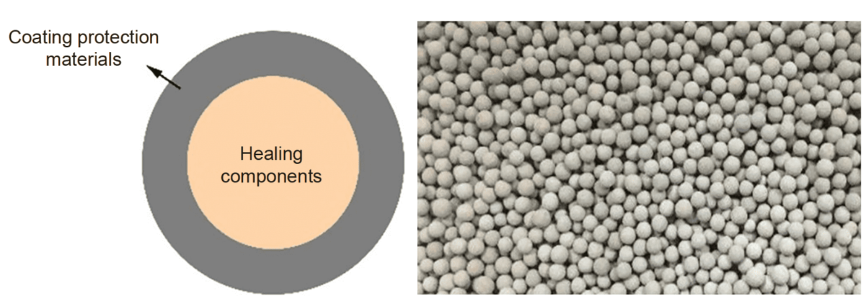

Figure 21 (Color online) Core-shell structure of healing agent and real photo of self-healing agent [63].

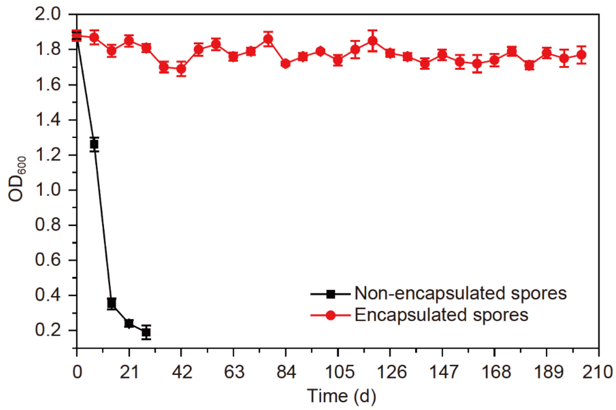

Figure 22 (Color online) Comparison of the resurrection ability of encapsulated and non-encapsulated spores [63].

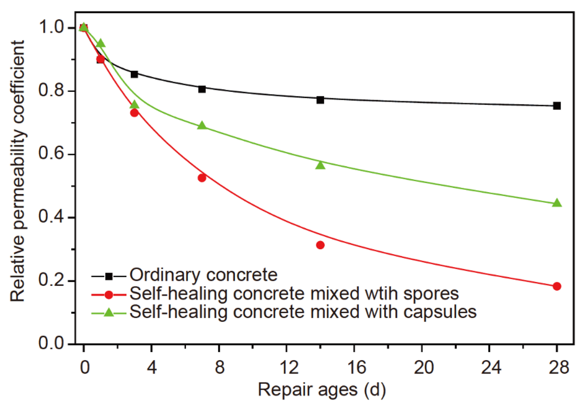

Figure 23 (Color online) Comparison of the relative permeability coefficient between three kinds concrete [63].

of spores could be measured quickly and accurately by counting plate method. Before measurement, the spores could also be dyed. That was, malachite green could dye spores green, and safranine water could dye nutrition bodies red. As shown in Figure 25, most of them were nutrition bodies in the stable germination period, and only a few spores were left. It was noting that in the crack zone of self-healing cement-based materials, pH, calcium ion concentration, temperature, nutrient content and other factors would affect the germination of spores.

The germinated spores were placed on the glass slide and observed with inverted fluorescence microscope, as shown in Figure 26(a). But it was impossible to distinguish living cells from dead cells. Therefore, it should be observed after staining. In general, diacetic acid fluorescence (FDA) and propidium iodide (PI) could be used for staining, in which FDA could label living cells and stimulate green fluorescence, PI could label dead cells and stimulate red fluorescence. After staining, the bacterial liquid after staining was dropped to the slide and observed under the inverted fluorescence microscope. And then switched the filter, selected the green and red fluorescence to observe, respectively. Finally, the distribution of live and dead cells could be obtained as Figure 26(b) and (c).

In addition, the number of cells in the bacterial liquid could also be determined by flow cytometry after fluorescence staining. Trimethylthiazole orange could pass through the living and dead cell membrane of the microorganism, PI dye could only pass through the dead cell membrane of the microorganism, and the number of dead bacteria could be measured by propidium iodide dye after staining. In general, the number of dead cells and living cells could be obtained, as shown in Figure 27.

As shown in Figure 28, with the increase of time, the total number of cells and the proportion of living cells in the hardened cement paste decreased, especially in the early age of 14 d. Afterwards, the subsequent changes were relatively stable.

Only living cells could produce enzyme to catalyze substrates, which could also provide the ions needed for mineralization. *Bacillus pasteurii* [15], *Bacillus alkalophilus* [35], *Bacillus mucilaginosus* [42] and *Bacillus megaterium* [66] had been used to repair cracks by mineralization. However, the enzyme structure produced by bacteria and the corresponding substrates were very different. Just for car-

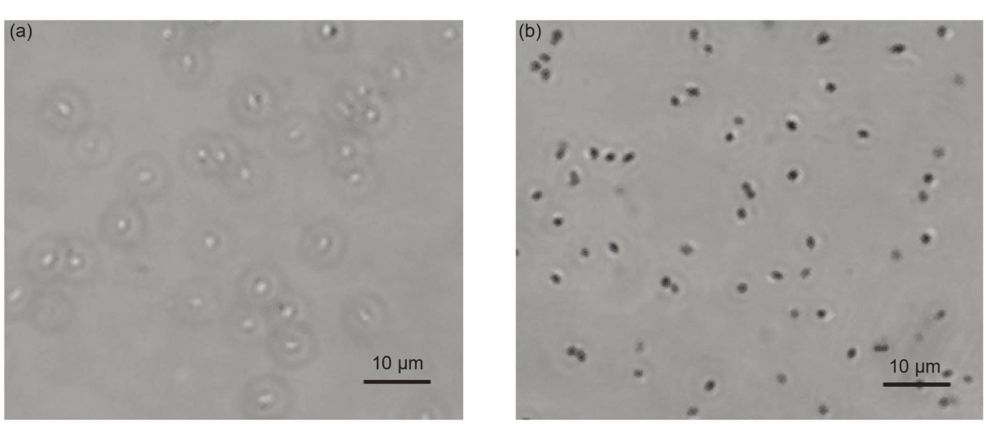

Figure 24 (a) Spore; (b) nutrition body [65].

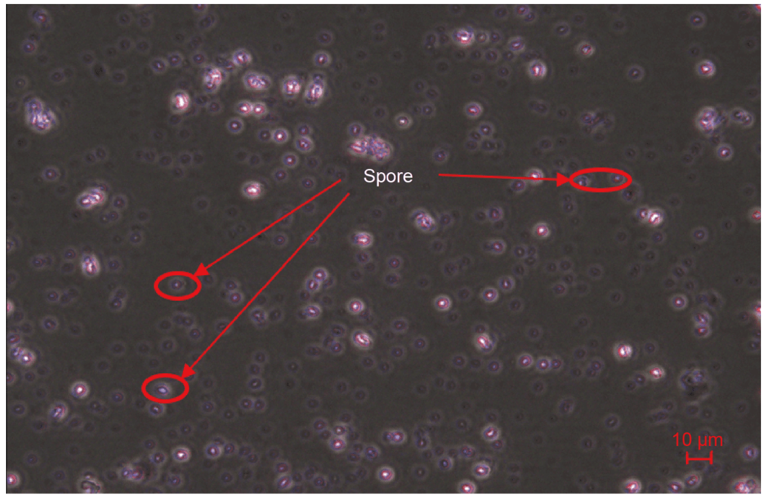

Figure 25 A few spores and most nutrient bodies in the stable germination stage.

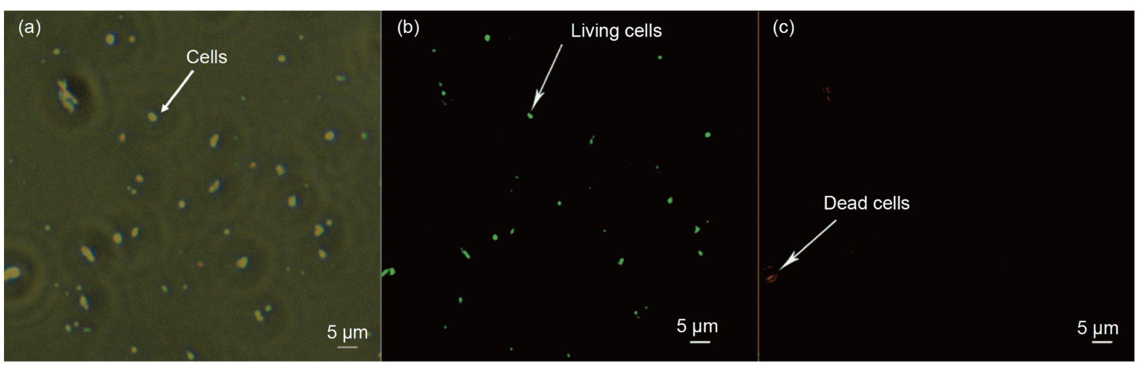

Figure 26 (a) Image without fluorescence; (b) image with green fluorescence; (c) image with red fluorescence.

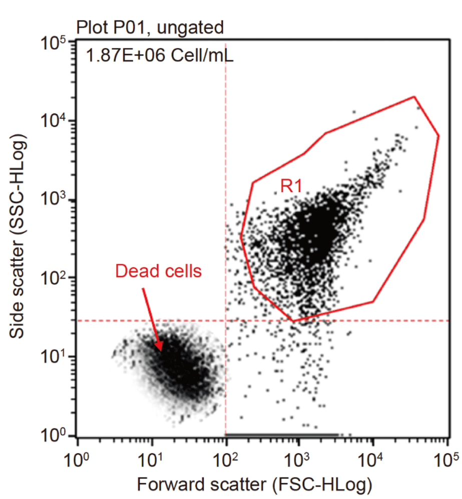

Figure 27 The number of living cells (R1 region) in cement paste after 0.5 d.

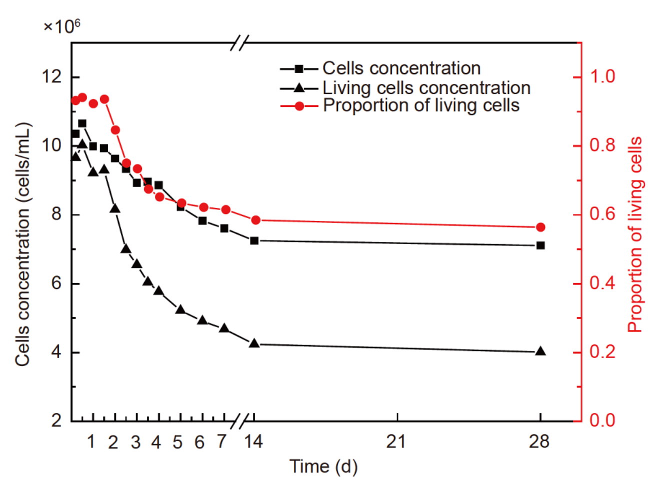

Figure 28 Living cells and total cells in cement paste after 28 d.

bonic anhydrase produced by *Bacillus mucilaginosus*, there were many types. In addition to temperature, pH and calcium ion concentration were also the key factors affecting enzyme activity in cement-based materials [67].

Except for producing enzymes, living cells could also nucleate crystal. As shown in Figure 29(a) and (b), the cal-

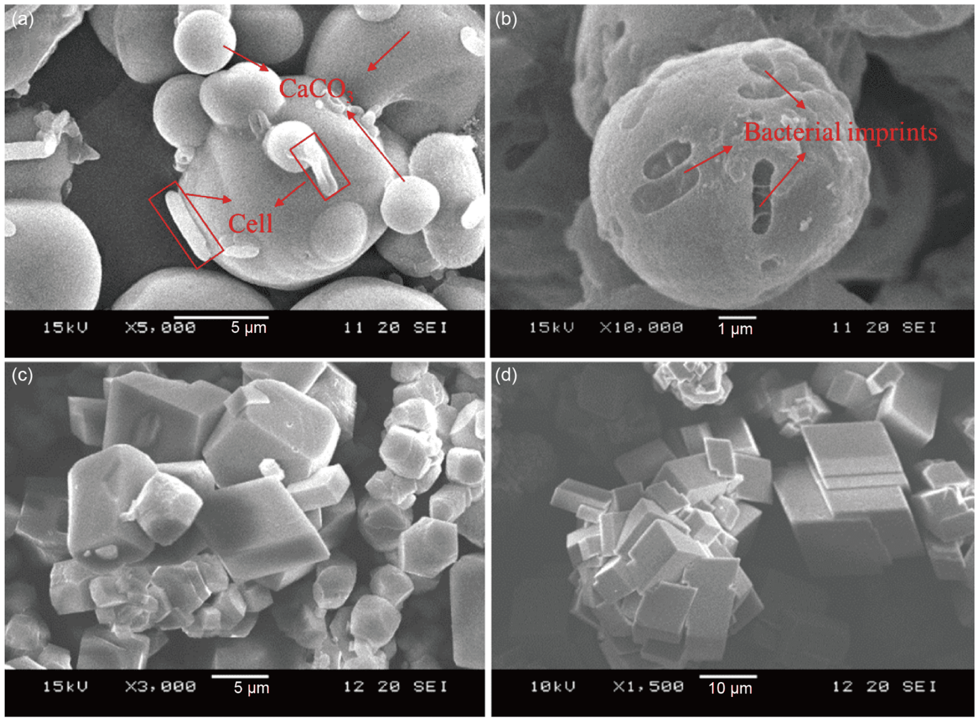

Figure 29 (Color online) Morphology of calcium carbonate formed under different conditions. (a) Cell body on the surface of calcium carbonate; (b) imprint after cell abscission; (c) calcium carbonate induced by plant urease; (d) calcium carbonate formed by chemical method [21].

cium carbonate induced by *Bacillus pasteurii* had obvious bacteria and marks on the surface, which was quite different from the morphology induced by plant enzyme (Figure 29 (c)) and the chemical synthesis calcium carbonate (Figure 29 (d)). Generally speaking, because of the negative charge on the surface of bacterial bodies, which could have electrostatic adsorption on calcium ion, and they could be used as the nucleation sites, but they had no substantial effect on the morphologies of $CaCO_3$. While bacterial secretion could delay nucleation and induce spherical, spindle and other forms of metastable vaterite. Besides, the polar groups (COOH, C=O, etc.) of the molecular chain of bio organic matter and Ca^{2+} could produce a series of electrostatic and coordination effects to regulate the growth of crystals [21].

In the repair process of concrete cracks, the amount of biological calcium carbonate formed in the crack zone directly affected the sealing effect of cracks. However, it was difficult to directly detect the filling degree of calcium carbonate in the crack zone. Fine slice or layer by layer fine grinding sample preparation could be used [68], meanwhile, combined with thermogravimetric analysis, which could detect the distribution of mineralized products in the crack zone. However, this method was complex, which could be feasible for cement paste, but it was difficult for mortar and concrete having aggregates. In addition, it was not easy to detect directly by CT method. It could be attributed to the sample size was large, the accuracy of CT was not enough, moreover, the density of calcium carbonate was too small for CT to distinguish. Consequently, it could be firstly marked, such as Eu^{3+} doping, and then observed by CT, as shown in Figure 30 [69].

In the repair process of concrete cracks, in addition to forming enough mineralized products, the characteristics of products also have a very important impact on the repair effect of cracks. Research [70] showed that the grain size of biological calcium carbonate was generally smaller than that of chemical calcium carbonate (Figure 31). However, in the microbial mineralization system, due to the existence of organic matter, calcium carbonate tended to agglomerate into larger particles (Figure 32), which was beneficial for plugging cracks.

In the microbial mineralized cementation system, due to the existence of organic substance, tiny calcium carbonate

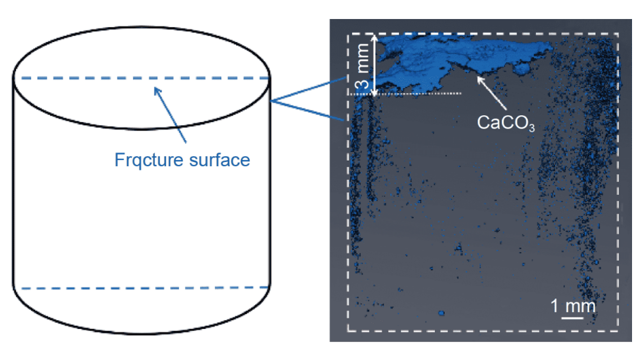

Figure 30 (Color online) Eu^{3+} doped labeled Bio-$CaCO_3$ in crack zone [69].

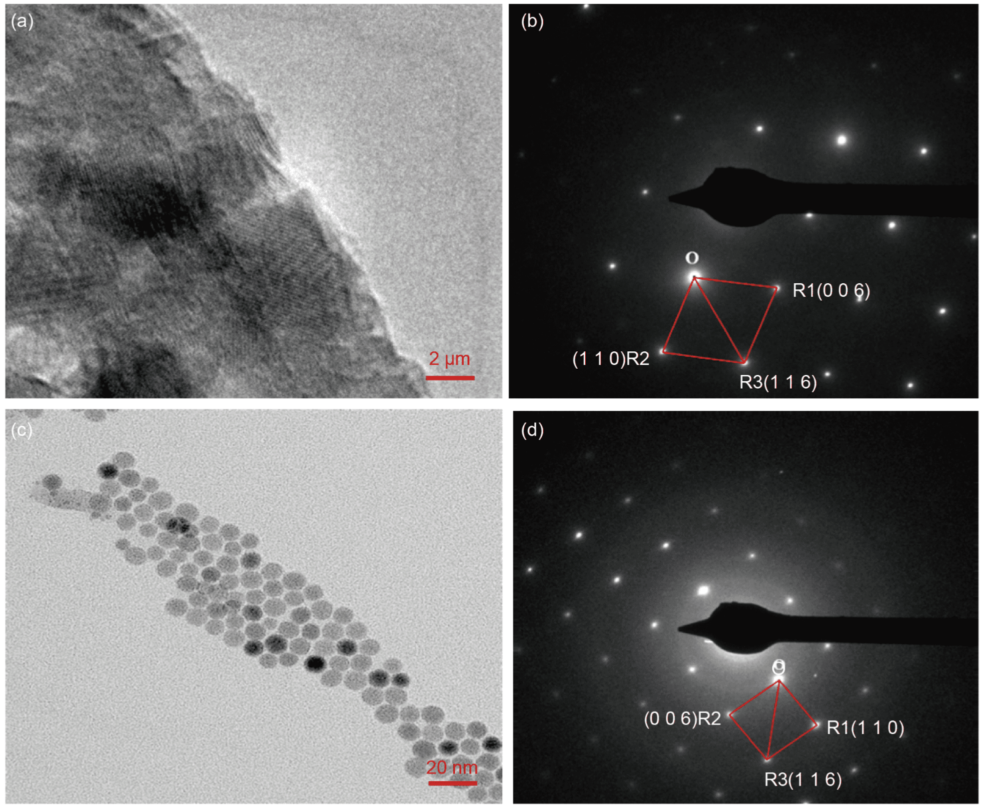

Figure 31 (Color online) (a) Transmission electron microscopy (TEM) image; (b) electron diffraction pattern of chemically synthesized calcite; (c) TEM image; (d) electron diffraction pattern of calcite formed by bacterial-induced mineralization [70].

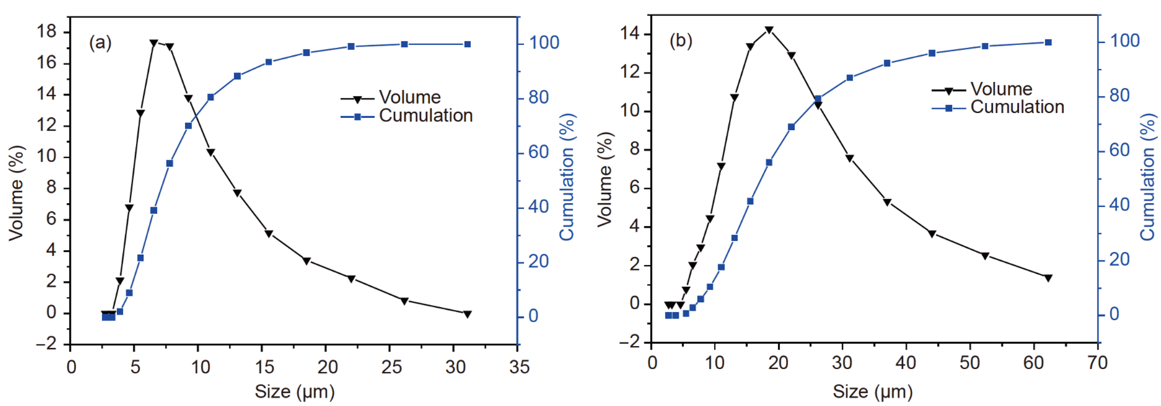

Figure 32 (Color online) Particle size distribution curves for the two types of calcium carbonate. (a) Chemical method; (b) bacterial-induced mineralization [70].

particles would have hydrogen bonding with cemented materials (such as quartz sand particles), resulting in the shift of nuclear magnetic resonance [71] and vibration peaks of photoelectron spectroscopy [72]. Figure 33 showed that the photoelectron spectrum of biological calcite cemented quartz sand was different from that of chemical calcite and pure quartz sand. As a result, the adhesion of biological calcium carbonate from nano scale (AFM) to macro scale (scratch method) was higher than that of chemically synthesized calcium carbonate [72]. What's more, this feature was also beneficial to crack repair.

6 Engineering application of microbial self-healing concrete

Microbial self-healing concrete has been studied for many years, but there are still few reports on large-scale practical engineering applications. The main reason may be that microorganisms are difficult to survive for a long time in high

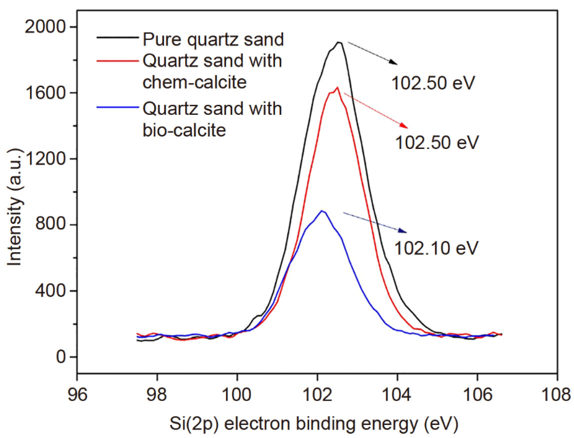

Figure 33 (Color online) The Si 2p electron binding energy affected by the bio-calcite and chem-calcite [71].

alkali environment and continuously dense pore structure, so the self-healing effects of later age cracks are bad, which greatly limits the application of microbial self-healing concrete. In addition, how to add microbial self-healing agent in the process of concrete, there is also a lack of corresponding regulations and standards. As above-mentioned, adding bacteria directly to concrete had good self-healing effect for the early age cracks. In order to improve the self-healing effect of the later age cracks, the carrier technology can be used. While the core-shell structural (Figure 22) microbial self-healing agent had good designability and protection ability for bacteria, and it was easy to be produced on a large scale. In addition, no special equipment was required to produce self-healing concrete with the different self-healing agents, and the pouring process and curing method were similar to that of ordinary concrete. In particular, if water containing nutrients was used for curing after cracking, it could promote the germination of spores, growth and reproduction of bacteria in the crack zone, and then improved the self-healing effect of cracks. Finally, the characteristics of “living concrete” could be brought into full play. Therefore, these two kinds of self-healing agents and self-healing concrete have been successfully applied in the expansion project of ship lock of South-to-North Water Transfer project of China [73], and a metro transit underground station in Nanjing, Jiangsu [74]. Water conservancy projects, underground structures, marine engineering and so on are all suitable application sites for self-healing concrete.

The self-healing performance of concrete can be evaluated by the capillary suction water coefficient before and after repair, apparent water permeability coefficient at a certain water head height, diffusion coefficient of chloride ion, ultrasonic pulse velocity, and the corrosion rate of reinforcement in concrete under accelerated electromigration [44] and so on. It is worth noting that when the self-healing agents are added into the concrete, the workability of concrete mixture, the physical, the mechanical properties and the durability of the uncracked hardened concrete need to be fully tested before engineering application.

Self-healing concrete is a new type of concrete that imitates the self-healing characteristics of natural animals and plants. According to the prediction of American Allied Market Research [75], the global self-healing concrete market will reach 1.375 billion US dollars in 2025, which has a broad development prospect.

7 Summary

This paper summarizes the self-healing effects of different mineralization systems in the the research of SEU team on concrete cracks, and also studies the mineralization mechanism. However, the self-healing effect of cracks still needs to be further improved. Therefore, it is necessary to study the compound of different mineralization systems to achieve the purpose of improving the self-healing effect of concrete cracks. At present, our research team has carried out cooperative reaction of various mineralization modes. In general, based on the research over the past 16 years, the passive repair technology for concrete cracks has gradually developed into a self-healing technology for the early age cracks of concrete. Subsequently, for the later age cracks, several carrier technology is used to improve the survival ability of microorganisms and the long-term self-healing effect of cracks. In addition, the mineralization process of microorganisms has been studied in crack zone, and the microbial self-healing technology has been applied to specific projects. The conclusions are as follows.

(1) For the small cracks on surface and the large cracks in concrete, the surface coating and the filling techniques were developed to achieve good repair effectsrespectively. In addition, aiming at sovling the problem of emision of ammonia in *Bacillus pasteurii* mineralization process, a system based on bacteria fixing carbon dioxide from atmosphere had been developed.

(2) The self-healing of cracks with the width of less than 0.3 mm could be repaired by addition of *Bacillus alkalophilus* directly into concrete, and the self-healing of cracks with the width of less than 0.5 mm could be repaired by *Bacillus mucilaginosus*. However, these two kinds of microbial self-healing systems had a good self-healing effect on the early age cracks, but the self-healing effect on the later age cracks was very limited.

(3) Comparing the repair systems of *Bacillus alkalophilus* and *Bacillus mucilaginosus*, it was found that the controlling step for self-healing rate in *Bacillus alkalophilus* repair system was the enzymatic reaction of the bacteria on the substrate. However, in *Bacillus mucilaginosus* repair system, the control step was the diffusion of CO_2 from air into the crack.

(4) Among several carriers and immobilization methods, fiber immobilization could fix calcium ions and deposit calcium carbonate, but it did not meet requirments of high protection and low-cost. The uniformly carried spores could only reach the healing depth to 5 mm. However, the core-shell structural immobilization was designed to be flexible, and the healing depth was improved to about 20 mm.

(5) In the process of self-healing of crack, there are a series of steps, such as the germination of spores, the growth, the reproduction of cells and the production of enzyme.The enzyme activity was affected by many factors such as temperature, pH, and calcium ion concentration. In addition, bacterial cells could be used as the nucleation sites to regulate nucleation during the mineralization and deposition process, while the organic matter generated by bacteria in the metabolic process could delay nucleation and also regulate the morphologies of crystals.

(6) Compared with the chemical calcium carbonate, biological calcium carbonate had a smaller grain size. Howecer, in the presence of organic matter, biological calcium carbonate tended to agglomerate and formed larger particles. Meanwhile, the adhesion and cementing force of biological calcium carbonate were greater than that of chemical calcium carbonate. In addition, it was easier to detect $CaCO_3$ after Eu^{3+} doping labeling.

(7) The experiences of engineering application showed that water conservancy engineering, underground structure, ocean engineering and so on were the most suitable applications for microbial self-healing concrete, which would have a broader application market in the future.

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