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In situ Restoration of the Surface Defects on Cement-based Materials by Bacteria Mineralization with Spraying Method

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Abstract: Defects of cement-based materials can be restored by microbial carbonate precipitation, but in order to accelerate the completion of the mineralization process, previous studies all adopt the approach of immersion in bacterial liquid, which can not be applied for *in situ* repair. We investigated micro-environment, basophil-domestication of microorganism and effective absorption of micro-organisms by cement-based materials, and adopted spray technology to conduct *in situ* repairs on the defects on the surface of cement-based materials and enhance the repair process operability. Through microbial carbonate precipitation in the defects by spraying bacteria liquid, 100 μm thickness of calcium carbonate film can be deposited on sample surface and in defects holes' microenvironment within 3 to 5 days. The capillary water absorption coefficient of specimen surface is 77% lower than the value before repair. The repairing effect is remarkable which makes it possible to conduct on-site repairs.

Key words: calcite; bacteria; mineralization; *in situ* restoration; spray

1 Introduction

Calcite is the most extensively-distributed natural mineral material on the earth, and it is the main component of limestone and marble which accounts for about 4% of the earth's crust mass. It is a major mineral in natural diagenetic formation. In recent years, researchers find with surprise that a lot of micro-organisms in nature can also complete mineralization formation of calcite in external cells. The mineralization process is known as MCP (microbial carbonate precipitation)[1-3].

The utilization of MCP technology in the protection and repairs on stones, cement-based materials, historic on buildings by bio-mineral layer of calcium carbonate is the most intensive application direction in recent years. Since these building materials are exposed to natural environment, due to mutual effect of loads and environmental factors, the surface is prone to have loosen and spalling defects. And there are even micro-cracks inside the materials. If these defects can not be repaired in time, the external water and aggressive media will gradually infiltrate from the surface defects which will ultimately lead to accelerated deterioration of material properties[4,5]. Calcite is one of the most stable minerals in nature, and it has superior repairing and protective performances for these natural stones or artificial stones.

In 1973, Spanish professor Boquet first found that the isolated bacteria from soil could achieve mineralization of calcium carbonate deposition in the laboratory[6]. In 1974, professor Adolphe also isolated microorganisms from the limestone which had the same characteristics. Some researchers began to conduct in-depth studies on the mineralization mechanism of this microorganism continuously for a long time[7]. Until 1990, professor Adolphe first proposed that it can be applied as a surface protection component and applied for a patent[8]. In 1999, all national research organizations which were represented by French Calcite Bioconcept Company began to develop

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microbial remediation technology, but it still focused on the surface repairs of limestone and other stone materials[9]. In 2008, De Muynck began to apply this technology to the surface repair of the cement-based materials[10,11].

The researchers immersed samples in bacteria culture medium for a long-term, and the bacteria would continue to reproduce decomposition of the substrate in the process and generate CO_3^{2-} and NH^{4+}. While the environmental pH value increased, the bacteria cell interface had negatively-charged SM (water soluble organic matter) which had been chelating Ca^{2+} and induced local crystal anions (CO_3^{2-}) . The concentration was further increased to attract more Ca^{2+}, until the crystal precursor concentration increased to a concentration which was conductive for nucleation. It would slowly produce mineralization of $CaCO_3$ deposition which is attached to the surface of specimen as described in Eq.(1) to Eq.(3). This technology could effectively prevent the external water and aggressive media from infiltrating the defects on cement-based materials and then achieve the repair protective effects.

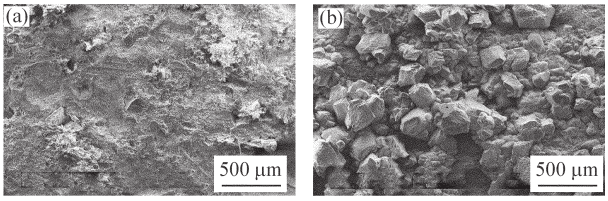
$$CO(NH_2)_2+2H_2O \rightarrow CO_3^{2-}+2NH_4^{+} \quad (1)$$

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

$$Cell-Ca^{2+}+HCO_3^{-}+OH^{-} \rightarrow Cell-CaCO_3+2H_2O \quad (3)$$

It should be noted that the microbial mineralization in nature is slowly accompanied by its metabolism. It often takes years or even centuries to complete. In order to artificially accelerate the completion of the mineralization process, the researchers all adopted nutrient solution culture methods. Through immersing the specimen in bacterial liquid, it can provide adequate conditions for growth and mineralization, shorten cycle of mineralization, form a dense layer of calcite mineralization successfully on the surface of cement-based materials within 5-28 days to achieve the purpose of protection, as shown in Fig.1. However, the adoption of immersion technology can not meet the on-site requirements of surface defects repair which makes this technology not be applicable for *in situ* repair[12-14], and it has been remained at the laboratory status.

In order to break the bottleneck of this technology and discard immersing techniques, we should address the following two questions: The alkalophilic ability of microorganisms, since cement-based materials have high alkaline properties, in which microorganisms can not grow and complete mineralization, so we must rely on immersing process to reduce environmental pH; The adhesion growth capacity of microorganisms. Since microorganisms can not grow on the surface of cement base, their growth must rely on immersing in culture medium. Through the action of gravity they remain in the upper surface of the specimen, therefore we can not complete the immersion repairs on the side of specimen.



(a) Untreated specimens surface (b) Treated specimens surface

Fig.1 Scanning electronic micrograph images of bacterially deposited calcite layer on the surface of mortar specimens with immersing method[10]

This paper went through rapid supply of micro-environment, basophil-domestication of microorganism and effective absorption of micro-organisms by cement-based materials and tried to adopt spraying *in situ* repair on the surface defects of cement-based materials to enhance the process operability.

2 Experimental

2.1 Cement paste specimens

Cubes with side length of 30 mm were prepared with ordinary Portland cement (PO32.5). Prisms were made with water-to-cement ratios (*w/c*) of 0.45, and were cured for 7 days at room temperature (20 ℃) prior to surface treatment.

2.2 Microorganisms and growth conditions

According to our previous work[15], a kind of bacteria with a high urease activity and harmless to human being was adopted. It could form calcium carbonate crystals in liquid culture, which was consisted of 5 g/L peptone, 3 g/L meat extract and 20 g/L urea. Bacteria B were incubated at 30 ℃ by shaking culture at 170 rpm for 24 h.

2.3 Bacteria B basophilic domestication

Appropriate pH environment for Bacteria B growth and reproduction was from 7 to 9, then the environmental pH continues to rise and growth of Bacteria B was increasingly inhibited. In 48 h there was a sharp decline in the maximum cell concentration, and the growth and reproduction capability rapidly decrease. and the In this paper, it gradually adopted domestication to improve the basophilic adaptability of Bacteria B under the conditions of high-alkaline. The

method was as follows: Add 1.5 g/L of agar preparation of solid medium in liquid culture, adjust pH to 10.0, inoculate and keep static culture plate at 30 ℃ and 170 r/min for 72 h. Take Bacteria B colonies which remain growth in the high alkaline environment to multi-tag test tube and shake culture. After 24 h take it out and measure the bacterial concentration X_{24h}. Screen the colonies which grow well as mother bodies and once again inoculate to pH 11.0 solid medium. Put them in static culture, shake culture and conduct second screening as the approaches which have been mentioned above, it would show that the excellent colony alkalophilic bacteria were repeatedly locating at pH12.0 and pH13.0. They all had gradual acclimation of alkalophilic under different environment.

2.4 Repairing methods of spraying bacteria liquid

The methods were: Infiltrate the cultured bacteria spray into the surface of cement-based materials; make mineralization deposition of calcium carbonate under the microenvironment of specimen holes to achieve the goals of repair and protection. Since it lacks of adequate nutrition source for the growth and reproduction of strains and the rehabilitation of the environment protection, spraying technology needs to spray strains and urea/Ca^{2+} repairing liquid on the specimen surface several times to ensure the continuous microbial mining acting on the specimen surface.

2.4.1 Repair by spraying alkalophilic domesticated bacteria

Compare with immersion repairing technology, spraying repair needs the target alkalophilic bacteria to have strong capacity of high alkalophilic capacities. This can ensure the continuation of mineralization by bacteria among micro-gaps of alkalophilic cement-based materials. This article compared the effects of Bacteria B which go through original strains and alkalophilic domesticated strains spraying on cement-based materials, which made 1 M urea/Ca^{2+} solution into repair fluid to spray on the surface of cement samples. After every 6 hours, spray one time to the specimen surface, continue for 2 d, and then put it aside for 5 d, observe the coating situation on the surface of the specimen.

2.4.2 The concentration of bacteria liquid

The concentration of the freshly-cultured broth was obtained by the measurement of OD values and cell centrifugation. Different concentrations of bacilli repairing fluid with 0.4×10^8, 4×10^8, 40×10^8, 400×10^8 cell/mL were prepared, in which the concentration of urea/Ca^{2+} was 1 M. The spraying process was adopted, which is described in 2.4.1, to conduct defect repair and protection on the cement surface. The repairing effects can be manifested by the capillary water absorption coefficient k after drawing and calculation.

2.4.3 Urea/ Ca^{2+} concentration

Different concentrations of urea/ Ca^{2+} (0.50, 0.75, 1.00, 1.25, 1.50, 1.75, and 2.00 M) were selected to make repairing solution, in which the bacterium cell concentration was uniformly 40×10^8cell/mL. The spraying technology in 2.4.1 was adopted to conduct repair and protection on cement surface defects. The repair effects and water absorption coefficient after capillary is represented by k.

2.4.4 Spraying sequence

The spraying sequence was: First adopt bacteria liquid spraying and then adopt urea/ Ca^{2+} spraying, and the spraying of urea/ Ca^{2+} and then bacterium spraying these two processes, bacterium cell concentration are still 40×10^8 cell/mL, while urea/ Ca^{2+} concentration chooses 0.50, 0.75, 1.00, 1.25, 1.50, 1.75, and 2.00 M multiple levels to determine the capillary water absorption coefficient of samples which takes K as a coefficient for that.

2.4.5 Premixed spray of Ca^{2+}

10, 20 and 40 mM of urea/Ca^{2+} was adopted to mix with 40×10^8 cell/mL bacteria liquid. After spraying 1 M of urea/Ca^{2+} repair fluid was sprayed to determine capillary water absorption coefficient k to represent the repairing effect.

2.4.6 Spraying times and spraying time interval

The two processes mentioned above were adopted to make repeated sprayings every 12 h. After 12 h of each spray and before the next spraying samples should be taken to measure the capillary water absorption coefficient *k* to represent the repair effects. An investigation on the influence of spraying frequency and coating interval on the mineralization effects of repairs was conducted.

2.5 The evaluation of repairing effects

In this paper, film thickness, SEM, XRD and capillary water absorption coefficient *k* before and after repair were measured to make evaluation on the effects of bacterium spray on the surface defects of cement-based materials.

2.5.1 Thickness of the bio-film

The specimens with bio-deposition on mortar surface were observed under optical microscope to determine the thickness of the bio-film.

2.5.2 Scanning electron microscopy (SEM) analysis

The morphology and mineral composition of the deposited $CaCO_3$ crystals were investigated with

scanning electron microscopy. SEM micrographs were obtained using a HITACHI X-650 apparatus. Samples were coated with gold prior to examination.

2.5.3 XRD analyses

Individual samples with bio-precipitated $CaCO_3$ layer were scanned by X-ray diffraction (XD-3A, SHIMADZU, AMERICA), which was used to analyze the crystal structure of the precipitation.

2.5.4 Coefficient of capillary suction with bacterially deposited layer on cement paste surface

In this paper, the surface capillary water absorption rate J and the capillary water absorption coefficient k were determined to characterize the protective effect of calcium carbonate film on the cement surface. The cement specimens were put in the 70 ℃ drying oven until the mass loss rate of quality was less than 0.1% within 24 h. They were removed and sealed with wax on the four sides after weighing to ensure one-dimensional water absorption by the cement during the process of capillary water absorption. A holder was put in the flat-bottomed container, taking cement specimen coats face down, adding water slowly into the container until the liquid level was above the bottom of test block (10 ± 1) mm. To remove it at different time intervals, quickly dry the water on the specimen surface, weigh and record their quality changes. The water absorption of specimen can be characterized in Eq. 4, so the capillary water absorption coefficient of spacemen can be obtained by linear calculation slope.

$$\frac{Q}{A} = k\sqrt{t} \qquad (4)^{[10]}$$

where Q is the water absorption of specimen, g; A is the surface area of water absorption, m^2; t is the time of water absorption, h; k is the coefficient of capillary suction of specimen, $g/(m^2 \cdot h^{1/2})$

3 Results and discussion

3.1 Basophilic domestication of Bacteria B

The colonies of Bacteria B which still had good growth characteristics in pH = 10.0 solid medium were put into the pH = 10.0 nurturing liquid medium for further growth. Its cell concentration was determined after 24 h, as shown in Fig.3(a). It can be seen from the figure that different colonies show varying ability to adapt to the high alkaline environment. The concentration of major colonies culture is below 3.62×10^8 cell/mL after 24 h, while the sample colonies 4 manifests excellent alkalophilic capacity. After 24 h culture inoculation, the concentration reaches to 5.12×10^8 cell/mL, it increases by nearly 40% compared with the average increase level. Sample 4 colonies, were stored under the pH 11 environment to make basophilic domestication, the experimental results are shown in Fig.2(b).

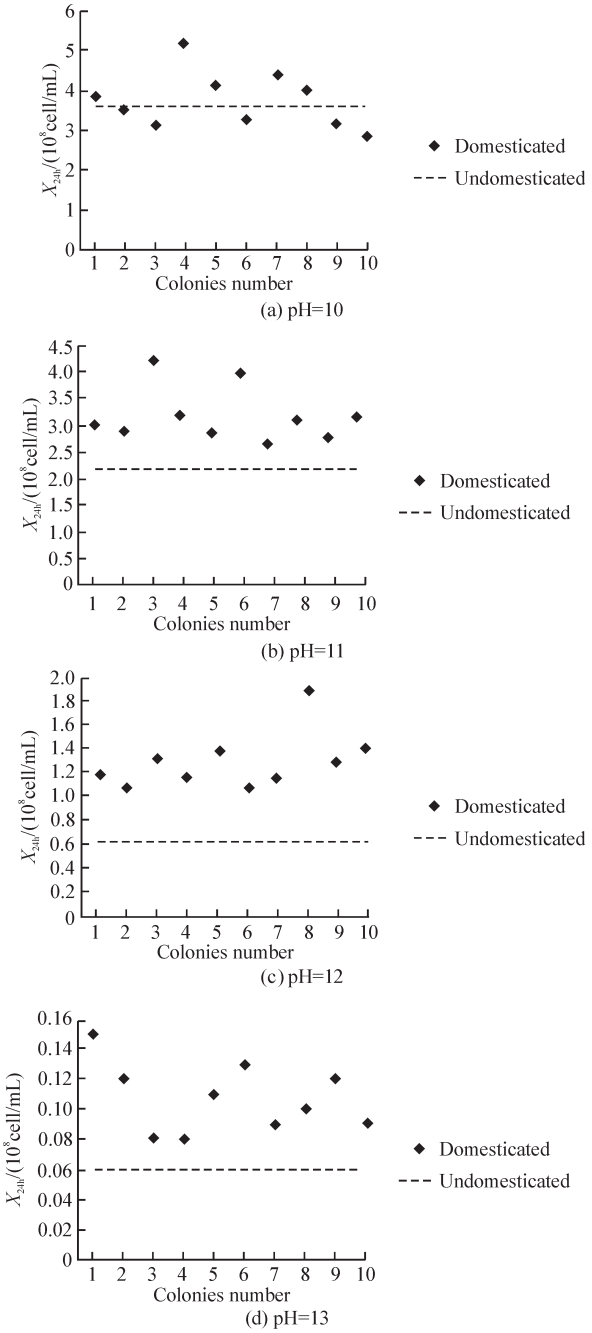


Fig.2 Domesticated bacteria B culture under different pH

As it can be seen from Fig.2(b), after strains inoculation in the first round of basophilic domestication, under the environment of pH = 11.0, relative to domesticated colonies, it has a good overall performance of alkalophilic capacity. The highest increases are nearly 94%, but there are still some

differences between individuals. Store the 3 best samples of alkalophilic capacity (24 h strains culture concentration is 4.18×10^8 cell/mL). At the same time since the pH 12 environment needs to conduct repeated basophilic plate of acclimation, the experimental results are shown in Fig.2(c).

As it can be seen from Fig.2(c), the strain's basophilc capability in the pH = 12.0 environment will be further enhanced, in particular, 24 h broth culture samples 8 has an concentration of 1.89×10^8 cell/mL. It increases by about 200% before carrying out domestication. Saving sample 8 as mother body and conducting flat scheme basophilic repeated domestication under pH 13, the experimental results are shown in Fig.3(d).

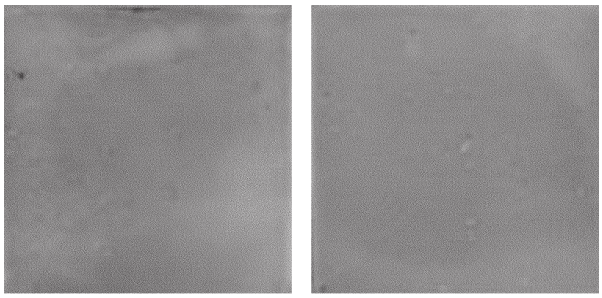
Table 1 X_m of undomesticated and domesticated bacteria B in different pH environment (10^8 cell/mL)

pH	10.0	11.0	12.0	13.0
Undomesticated bacteria B	3.62	2.16	0.62	0.06
Domesticated bacteria B (with 2% inoculation volume)	5.12	4.18	1.89	0.15
Domesticated bacteria B (with 10% inoculation volume)	9.42	8.52	4.87	1.18

In pH13 environment, alkalophilic capacity of strains is further enhanced before domestication. But the absolute growth of strains has significant declines, and the effect of alkalophilic acclimation is not obvious. Table 1 compares the strains' growth situation before and after acclimation in high alkaline environments. After gradual basophilic domestication, Bacteria B significantly improves the alkali capacity. Under pH10, 11, 12 environment, its growth has a significant increase compared with the situation before domestication. Especially in the environment of pH12, the maximum cell concentration increases by about 200%, while at pH13 environment, there are signs of strain growth and reproduction. This is mainly because a small number of Bacteria B cells mutate under environmental stress, resulting in mutations which are closely related with the alkali resistance. In high alkaline environment, the cells activate a different set of regulatory mechanisms, such as K -Na pump gradient reverse transport mechanism and Na gradient to induce cell to adapt to a high alkaline environment.

3.2 Restoration of defects on cement- based materials surface by alkalophilic domesticated strains with spraying methods

Spray repairing methods were described in 2.4.1, which make continuous spray for 2 d and then put it aside to 5 d. We find there is a thin layer on the surface of strains which go through alkalophilic domestication. While there are no significant changes in original strain spray, as shown in Fig.3.



(a) Untreated specimen (b) Treated specimen

Fig.3 The specimen with bacterially deposited layer on its surface

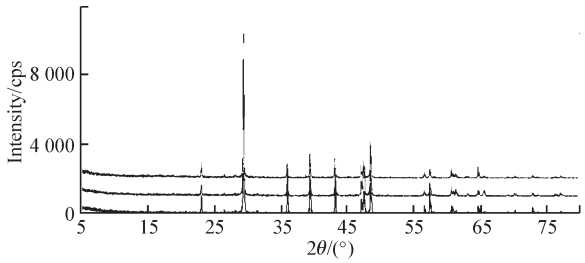


Fig.4 X-ray diffraction (XRD) patterns of the deposited layer on the specimen surface

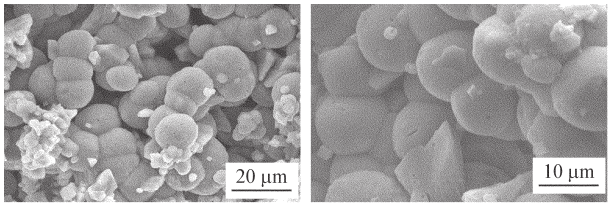


Fig.5 Scanning electronic micrographs of calcite layers with spraying method

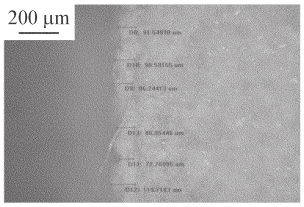


Fig.6 Thickness of the deposited layer on the specimen surface

The successful cement coating, after XRD qualitative analysis, is confirmed to be calcium carbonate, and it is a stable calcite crystal as shown in Fig.4. After spraying the specimen and observing under scanning electron microscope, the morphology shape and accumulation situation of $CaCO_3$ particles deposited on the surface of cement dense case are shown in Fig.5. As spraying has a high bacterial concentration, in micro-voids environment, water-

dissolved matters have very high concentrations of organic carbon. The growth process of calcium carbonate crystal is obviously regulated by organic molecules of protein which are easy to produce multi-level growth and mutual aggregation to form more regular spherical balls with diameters within the range of 5-10 μm. They are closely linked with each other. The measured film thickness under optical microscope is shown in Fig.6. Spraying method mainly forms micro-liquid surface on the top surface through tension. The strains which form in this environment will be difficult to achieve growth and reproduction and reach the peak again. So the provisions for high concentration of strains in early supply can make enzymatic sustaining in a certain time range. Then there are depositions of calcium carbonate particles. As restricted by deposition environments, the calcium carbonate films are relatively thin which are about 70 - 100 μm.

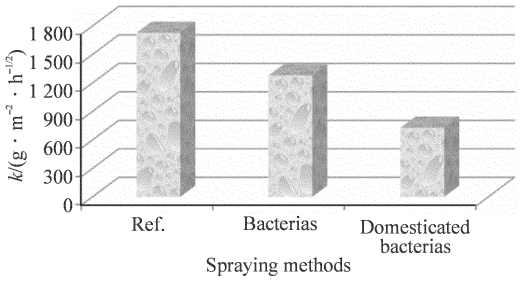


Fig.7 The coefficient of capillary suction with bacterially deposited layer on cement paste surface

In order to further characterize the repairing effect of bacterial liquid spraying, as is described in 2.5.4 in this article, continuous measurements were made on the water absorption volumes with cement-based materials before and after spraying, by calculating and drawing capillary water absorption coefficient k and the results are shown in Fig.7. The application of alkalophilic bacterium domestication by spraying on cement specimens after the repair of capillary water absorption coefficient k is decreased from 1 728 to 731 g/(m^2•$h^{1/2}$) and the reducing rate reaches over 58%. But non-domesticated strains have a decreasing rate of 26% alkalophilic reducing rate after spraying which originates from its enzyme which is restricted in the high alkaline environment of cement-based activity.

It shall also be pointed out that the materials of bacterium liquid spray are almost the same as the materials which are used in immersion approach. The difference is its high concentration of bacilli culture, many times of continuous spray on the surface of the specimen to form calcium carbonate micro-environment. Since there are inadequate supplies of nutrient source, urea and Ca^{2+} and strains are extremely prone to loss on the surface of cement specimen, strains have slow metabolism and deposition. The environmental conditions for deposition are inadequate; there are a relatively small amount of calcium carbonate deposition and thin film. Considering the perspective of capillary water reducing coefficient, the effects of protective film are not as good as immersion approach.

3.3 The influence of component ratio of bacterial liquid on the restoration effects

3.3.1 Concentration of bacterial liquid by spraying

The spray repairing process was adopted, due to its lack of rich culturing environment of bacterial liquid, in addition to the alkalophilic capacity of strains, the cell concentration for spray of bacterial liquid can also directly affect the repairing results. As is described in 2.4.2, the paper makes use of different spray concentration of bacterial liquid to prepare fluids and the concentration of urea/Ca^{2+} is 1 M. The capillary water absorption coefficient k after and before spraying can characterize the repairing results, which are shown in Fig.8.

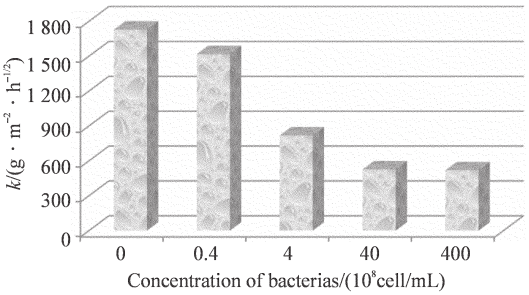


Fig.8 The effect of bacteria B concentration on the coefficient of capillary suction

It could be seen from the figure that along with increasing concentration of spraying bacterial liquid cells, the capillary water absorption coefficient of cement-coated specimen shows a downward trend. The higher the spraying of bacterial liquid concentration, there will be more and more microorganism which gather together and play the role of enzymes on the cement surface, the mineralization repairing effect will be more prominent. When spraying bacterial concentration increases to 400×10^8 cell/mL, the repairing effect will get little improvement, taking the economic cost of repairing liquid into account. It is better to spray the bacterium cell with concentration of 400×10^8 cell/mL.

3.3.2 Concentration of urea/ Ca^{2+} by spraying

The other main component of spray repairing solution is to provide the source of the urea nitrogen cycle and metal ions Ca^{2+}. Spray concentration of urea/ Ca^{2+} has obvious regulating role in mineralization repairing effect. The bacterial cell concentration will be optimized for 40×10^8 cell/mL, adjusting urea/ Ca^{2+} concentration in spray of repairing fluids. Before and after spraying the capillary water absorption coefficient of the specimen *k* manifests the repairing effects of film coating, which is shown in Fig.9.

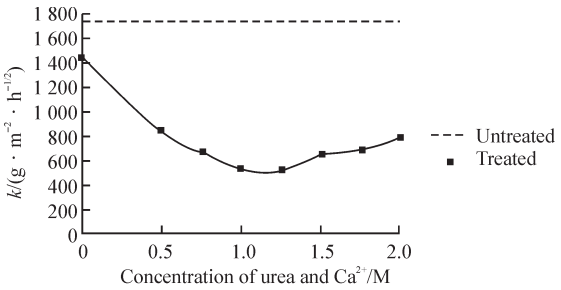


Fig.9 The effect of urea/ Ca^{2+} concentration on the coefficient of capillary suction

It could be seen from the figure that when bacterial liquid spraying was adopted alone without urea/ Ca^{2+}, the capillary water absorption coefficient of the specimen also showed a slight declines, mainly due to the spraying of high concentration of bacilli coating, making the cells of strains continue to penetrate into the surface of cement-based materials. They act as thin fiber body and make the surface structure relatively more compact. However, along with the urea/ Ca^{2+}'s involvement in different concentrations, mineralization role of bacterial cells began to appear and form more stable and dense calcite in micro-voids, so that the capillary water absorption coefficient of specimen has been continuously reduced.

But when the concentration of urea/ Ca^{2+} is further increased to more than 1.5 M, due to Ca^{2+} absorption role in the cell body wall, the mineralization of intracellular enzyme begins to be inhibited by varying degrees. The repairing effects are not as effective as the former one. Therefore, in the context of this paper, selecting the urea/ Ca^{2+} solution spray within concentration range of 1.00 - 1.25 M can obtain a better repairing results. The capillary water absorption coefficient of specimen after coating is reduced to 513 $g/(m^2\bullet h^{1/2})$ and the reducing rate can reach up to 70%.

3.4 The influence of bacterial liquid spraying process on the restoration effects

Based on the optimized ratio of sprayed liquid for repairing, the improvements on spraying process are also conducive to the sustainable and stable microbial conducive mineralization. Thereby it can improve the actual repairing effects.

3.4.1 Spraying sequence

The spraying sequences for repairing liquid surface have various mineralization repairing effects on cement specimens. In order to optimize the repairing process and enhance the effects of mineralized repair, two processes were compared, one with bacterial liquid spray first followed by urea/Ca^{2+} spray, and the other with urea/Ca^{2+} spray first followed by bacterial liguid spray. The spraying repair effects are shown in Fig.10.

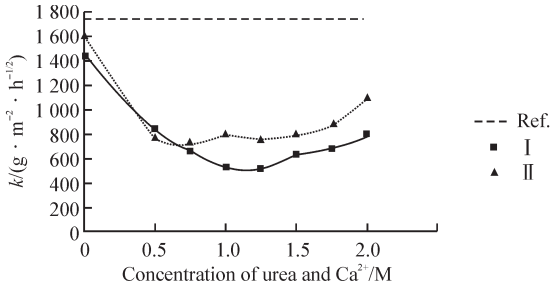


Fig.10 The effect of spraying order of repair liquid on the coefficient of capillary suction (Ⅰ-1st bacteria, 2nd urea/Ca^{2+}; Ⅱ-1st urea/Ca^{2+}, 2nd bacteria)

As it can be seen from the figure, bacterial liquid is superior to urea/ Ca^{2+} first spraying on the specimen surface which is more conducive to enhance mineralization repairing effect. Bacterial cells of Bacteria B 1 - 3 μm are in long pole shapes, bacterial liquid's advance spray can make cells gather around the defects holes of cement-based materials. In certain ranges it can form the enzyme within a certain range mineralized micro-environment. If urea/ Ca^{2+} repairing solution is sprayed before the bacterial liquid, it will be difficult to make strains' penetration. It is difficult to penetrate the original depth and concentration, thus affecting the effects of mineralization repairing.

3.4.2 Premixed-spray of Ca^{2+} adsorption

In the earlier studies, it proves that since Bacteria B's cell membrane interface has water-soluble organic matter, so it has negative charge. In order to increase the retention volume of bacterial cell on the surface of cement-based materials, there should be a pre-spray of small amount of Ca^{2+} which is more conducive to let bacterial adsorption defects appear in defects environment, thereby reducing the strains loss and enhancing the effect of mineralization repairs. This paper makes use of 10, 20 and 40 mM of urea/Ca^{2+} to mix with 40×10^8 cell/mL bacterial liquid, then sprays again with 1 M of urea/Ca^{2+} repairing fluid. The measurements of the capillary water absorption

coefficient k were made to characterize the fixed effects, which are shown in Fig.11. As it can be seen from the figure, with the application of 20 mM of urea/Ca^{2+} to have premixed spray with bacterial liquid, after cement specimens are sprayed, capillary water absorption coefficient k is decreased most significantly which is about 429 g/(m^2•$h^{1/2}$). The reduced rate is of 75% and the restoration effect is more significant.

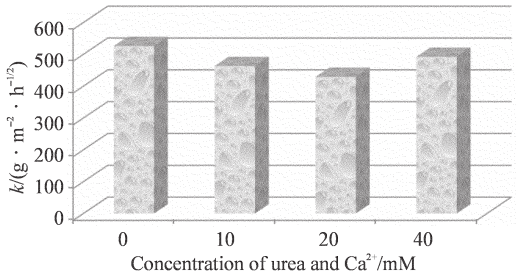


Fig.11 The effect of urea/ Ca^{2+} concentration of pre-spraying on the coefficient of capillary suction

3.4.3 Spraying times and spraying time intervals

During actual spraying process, the less spraying time may be unable to guarantee the mineralization deposit on the defects with sufficient quantities of calcium in cement-based materials. Since there are too complicated spraying times resulting in the complicated repairing process and increasing rehabilitation costs, this paper adopts to first spray bacterial liquid and then spray urea/Ca^{2+} solution in the concentration of 20 and 1 mM for the adsorption of bacterial liquid. Then the effects of spraying frequency and spraying time intervals on mineralization repair efficiency were investigated. The results are shown in Fig.12.

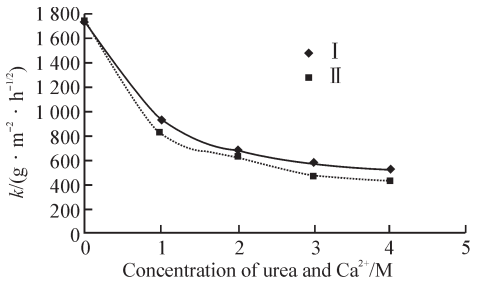


Fig.12 The effect of spraying times of repair liquid on the coefficient of capillary suction (Ⅰ- bacteria; Ⅱ- bacteria with 20 mM urea/ Ca^{2+})

It can be seen from Fig.12 that during the 1st spraying, no matter strains or repairing liquid are prone to penetrate into the surface of cement-based materials. The repairing effects are the most obvious one. The capillary water absorption coefficient of the specimen is about 50%, along with the mineralization deposition of calcium carbonate in the sample pores; it will be quite difficult to conduct several follow-up spraying liquid penetrations. After spraying 3 to 4 times, the capillary water absorption coefficient of the specimen will maintain a constant level and there are no significant improvements.

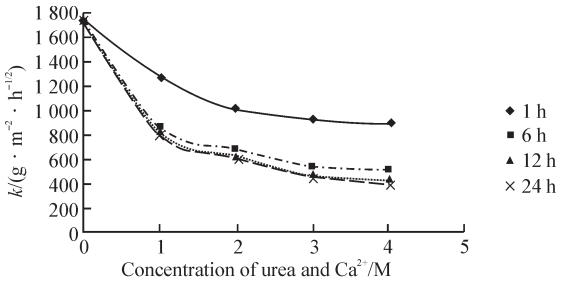


Fig.13 The effect of spraying time interval on the coefficient of capillary suction

With the same amount of spraying and the same number of spraying times, different spray intervals have various influences on mineralization repairing effects of the final specimens. In this paper, 1, 6, 12 and 24 h were taken as time interval to spray repairing fluid. Before the spraying of the second specimen, the capillary water absorption coefficient k was determined to represent the repairing effectiveness and the experimental results are shown in Fig.13. The time interval before and after spraying is too short and the enzymatic mineralization in last round of strain has not ended yet. There are water contents among porosity defects at the same time and the penetration of second round of repair solution is even more difficult. So the expected repairing effect can not be achieved. Extended spray intervals can assure a nearly saturated state of bacilli on the specimen surface or there is sedimentary mineralization in the defects holes. From Fig.13 it can be seen that two spray intervals of 6 h can guarantee the restoration and repairing effects. 12-24 h is better for film specimen repairing, the capillary water absorption coefficient k is reduced from 1 728.0 to 399.7 g/(m^2•$h^{1/2}$).

4 Conclusions

The method of mineralization of calcium carbonate deposition process of bacterial immersion for repairing the surface defects of cement-based materials is only applicable for protective coating on the immersing specimen and can not be applied in the defects repairing of construction materials. The application prospects of MCP technology in practical engineering must depend on the breakthrough of repairing process.

a) This paper was based on the alkalophilic domestication of microorganisms which can make the cement-based materials remain growth characteristics under high-pH environment, particularly in pH12 environment, and within 24 h the maximum cell concentration can reach to 4.87×10^8 cell/mL which improves by about 7 times. That ensured the material validities after spraying the internal cement-based materials.

b) Because of the secretion of water soluble organic matter at Bacteria B cell membrane interface, it had negative charge. In order to increase the retention volumes of bacterial cell on the surface of cement-based materials, pre-spray of small amounts of Ca^{2+} (20 mM) was made, which will be more conducive to bacterial cells in various defects environment, thereby reducing the strain losses and enhancing the effect of mineralization repairs.

c) A kind of technology which adopts bacterial liquid spray to make *in situ* mineralization of repairing defects on the surface of cement-based materials was developed 20 mM Urea/Ca^{2+} was sprayed on the specimen surface to be repaired, after 0.5 - 1 h spray the high concentration of 40×10^8 cell/mL Bacteria B was obtained through the centrifuge technology, then 1.0 - 1.25 M Urea/Ca^{2+} + repairing solution was sprayed until the specimen surface was in a saturated state. By spraying once every interval 12 - 24 h time, after spraying 3 to 4 times, there would be 100 μm thickness of sedimentary layers of calcium carbonate on the defects and surface of the specimen and sediment in the hole micro-environment. The capillary water absorption coefficient of the specimen surface was reduced from 1 728.0 g/(m^2•$h^{1/2}$) to 399.7 g/(m^2•$h^{1/2}$), the decrease rate was 77%. It has achieved significant repairing effect, and more operability compared with immersing process which makes the MCP possible for overcoming the technology shortcomings of existing on-site constructions.

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