Developing the solution of microbially induced CaCO₃ precipitation coating for cement concrete

Nguyen Ngoc Tri Huynh, Nguyen Quynh Nhu, Nguyen Khanh Son*
Faculty of Materials Technology, Ho Chi Minh City University of Technology - VNU-HCM
Building C4, 268 Ly Thuong Kiet street, District 10, Ho Chi Minh city, Vietnam

*Corresponding author: ksnguyen@hcmut.edu.vn

Abstract. The term of microbially induced calcium carbonate precipitation or biomineralization usually link to phenomenon of self-healing in cement-based material due to metabolism bacteria. In this paper, we investigated on the water sealing performance of MICP coating for concrete surface treatment. Prepared bacterial solution (Bacillus subtilis HU58 and cement paste) was painted on cement substrate. After curing several days under high humidity condition, thin layer 0.1-0.5 mm of precipitated product could be recognized by naked eyes. Material analysis of precipitated product revealed the presence of both calcite and aragonite mineral. In addition, result of water sealing test on coated mortar specimens helped us to clarify the positive effect of the MICP coating. From that, sustainable solution of bacteria-based coating for surface treatment of concrete structure can be opened for discussion.

1. Introduction

Concrete material based on Portland cement is the most commonly used building material in the world. However, different cracks generated at the both early and later age of set cement paste perform specific effect on the durability of concrete structure. Consequently, maintenance of concrete structures is recurrent and costly, but it is inevitable to ensure the designed performance of concrete. Recently, due to its originality and its sustainability, self-healing concrete has gained much attention from researchers around the world. The self-healing mechanism of bacterial concrete is autogenous by healing agent of microbiologically-induced calcium carbonate precipitation. In almost case, the object of autogenous self-healing is micro cracks that are generated in the hardened cement matrix and also to prevent open cracks that are developed from the initial one due to internal stress or external loading [1,2]. Precipitated calcium carbonate from biomineralization process permit to block gradually the crack width until the decay step of reaction.

The circumstance of self-healing or self-sealing is actually inspired from the natural bone regeneration [3]. Three controlling factors of biomineralization process include locally concentrated microorganism, humidity due to water penetration through micro-cracks and locally presented nutrient component. According to Al-Thawadi, there are four main steps in this process [3]: (i) hydration of urea; (ii) increasing of micro-environment; (iii) absorption of Ca²⁺ into bacterial spore wall; (iv) formation and development of crystallized calcium carbonate. As reported in the literature study, healing ability may have occur in crack widths of 0.1 to 1 mm. Time of efficacy was between 3 and 14 weeks depending on the use of different bacteria species [4–6]. In our previous study [10,11], we showed the evidence of
healing agent inside an artificial crack 1.5 mm in width of bacteria modified mortar. *Bacillus subtilis* HU58 and microporous diatomite as supporting materials were used to prepare pellets of diatomite immobilized bacteria. The results of water permeability test confirmed the positive effect of remediating cracks on the degree of impermeability of mortar specimens.

In general, concrete surface treatment help us preventing the direct contact between hardened cement and aggressive environment. As a consequence, gradually penetration of corrosive agent can be limited. Last could have an impact on the durable behaviour of concrete structure and reinforcement. It is a fact that surface treatment by using an organic agent is now the most popular solution. However, there are still concerns about the limited life span and about its consistency with the cement substrate [10,11]. For the inorganic substance, recent publication reported the use of silicate-based solution for concrete hardener and/or stain. Considering its consistency there is a guarantee due to the formation of chemical bond with hydrated cement. Based on the same mechanism, in this paper we have developed a coating solution with microbially induced CaCO₃ precipitation. We name the expected coating due to biomineralization as bacteria-based coating. Material analysis techniques such as XRD, SEM were adopted to characterize the generated carbonate mineral. The effect of curing condition and coating thickness will be determined by observing mortar specimens after surface treatment. Specific discussion on the performance of water sealing of cement mortar was also reported via a water absorption test.

### 2. Materials and experiments

We prepared bacterial solution from urea, CaCl₂, *Bacillus subtilis* HU58 (10⁹ cfu/g). Portland cement and tap water. As reported in the previous study, the mixing proportion of dry mixture of bacteria and nutrient is given in table 1. To apply on mortar surface, we prepared liquid solution of bacteria and Portland cement paste. In order to improve the coating process and determine the influence of the calcium source on the thickness of the carbonate layer, three mixed solutions were distinguished by solid/water 1.1. After mixing, prepared bacteria-based solution was stored in laboratory for 30 minutes before applying on cement mortar.

**Table 1. Three mixtures of bacteria-based solution**

| wt. % | Solid | Urea | Calcium chloride | Portland cement | Tap water | Solid/Water ratio |
|-------|-------|------|------------------|------------------|-----------|------------------|
|       | *Bacillus subtilis* |      |                  |                  |           |                  |
| B1    | 8.0   | 8.0  | 4.0              | 32.0             | 48.0      | 1.1              |

For casting mortar specimen, we used Portland cement PC40 – Vicem Ha Tien 1, standard sand, and tap water. Those materials went through series of quality control tests conforming to the requirement of national standard. After 28 days of curing period for strength development, those specimens could be served as mortar substrate for the next step of surface treatment. We used two categories of mortar specimens, including cube specimen 40x40x40 mm and round slice 90x20 mm specimen. A simple painting method has been adopted to apply bacteria-based solution on mortar. After painting, mortar specimens were cured in a seawater tank for biomineralization.
An X-ray diffractometer (D8-Advanced-Bruker) was employed to identify the mineral composition of the precipitated product. Optical microscope could be used to recognize the coating thickness and adhesion between precipitated CaCO$_3$ and cement substrate. Combined SEM/EDX analysis was used to identify the phase elements of precipitation product. To test the performance of water sealing, we conducted test of water absorption as shown in figure 6. The principle of test is quite simple where the percentage of weight gain due to water absorption through the bottom surface of mortar cube was measured. The bacteria-based coating layer was covered such bottom surface. Meanwhile, the top and surrounding surfaces of mortar cube were covered with epoxy resin.

3. Results and discussion

3.1. Result of CaCO$_3$ precipitation on the cement substrate
In the first step, we observed the formation of precipitated CaCO$_3$ by bacteria and nutrient on the mortar specimens $\phi$90x20 mm. In this experiment, first we wrapped a rubber ring around the round slice specimens of mortar. Then, we let flow the prepared solution of bacteria nutrient and water (as given in table 1 without cement paste) on the top surface of mortar specimens. After that, we kept those bacterial mortar in a chamber of humidity 7 days for curing step. Figure 3 shows the photos taken from the upper surface of controlled mortar and bacterial mortar. The colour of bacteria-based coating sample is distinguishable with the reference one. A layer of white colour mineral covered top surface of the coated specimen after 7 curing days. Also, we could highlight out some pores position on the cross-section of controlled mortar. That was not the case of mortar specimen with bacteria-based solution. Regarding to the SEM cliché, we recognized the filling effect of precipitated product into pores position. Additionally, result of combined EDX/SEM analysis revealed the presence of different elements Ca, C, and O. It seems that they exist linkage between coating material and the precipitated calcium carbonate by bacteria metabolism. Those generated material helped us fixing deterioration of hydrated cement i.e. cracks, pores...

Figure 2. Cross-sectional image of round slice mortar with and without bacteria-based coating layer.

3.2. Result of characterization of bacteria-based coating layer
As we mentioned in the above paragraph, the self-sealing of failures on the hardened mortar with the presence of bacteria-based solution. For the purpose of surface treatment of cement concrete by bacteria, we must focus on remediating micro-cracks mainly due to shrinkage during hydration reaction of cement. To do that, in the following steps we need to characterize bacteria-based coating layer with regards to different controlling factors such as time of specimen curing, bacteria concentration and method of painting. Also, we need to highlight that the context of research consisted of improving the global durability of cement concrete in marine environment through surface treatment. That means we tested ability of bacteria-based coating while exposing to natural seawater in a curing tank of laboratory.

3.2.1. Optical microscope image
As shown in figure 3, before treatment, micro-cracks generated on the surface of mortar sample. After 3 days of curing period, it seems that a homogeneously layer of bacteria-based coating appeared and
covered the surface. Zooming area shows the morphology of this formed layer similar to morphology of calcium carbonate.

**Figure 3.** Illustration photo by optical microscope (x400) of bacteria-based coating layer on the substrate of mortar cube.

### 3.2.2. Coating thickness

Considering the effect of curing time on the bacteria-based coating layer, we observed under optical microscope the generating product of precipitation by bacteria metabolism after 1-3 and 14 days of curing period. Mortar cube was painted with the bacteria-based solution. As shown in figure 4, we highlight the evolution of coating thickness in function with time of curing. After 1 day, it seems that a very thin layer #0.1 mm of precipitated product covered on the mortar substrate. After 3 days, we observed #0.5 mm of coating thickness that was appeared on the mortar substrate. And particularly, two weeks continuously cured in high humidity condition led to generate #2.5 mm coating layer of calcium carbonate mineral. The evidence of its compactness and its integrity could be easily figured out while observing white crystallized calcium carbonate. More the bacteria-based coating layers are stable, more they prohibit the penetration of aggressive agent from the surrounding environment.

**Figure 4.** Illustration photo by optical microscope (x100) from side view of bacteria-based coating layer after curing 1, 3 and 14 days in seawater

### 3.2.3. Compositional analysis

We adopted XRD technique for analysing phase composition of precipitated product. Result of XRD pattern was shown in figure 5. They appeared major peaks $2\theta = 29.5; 47.5$ and 48.5 of crystallized calcite product (ICDD # 05-0586). Additionally, others peaks of aragonite were also found on the pattern, $2\theta = 26.2, 27.2, 33.1$. The most logical explanation for the obtained result is based on the fact that dissolving Mg$^{2+}$ in sea water facilitated the aragonite precipitation instead of common calcite product. In their turn, the presence of both calcite and highly crystallized aragonite allow it enhancing the compactness of the generated coating layer.
3.3. Performance of water sealing of cement mortar covered with bacteria-based coating

We reported measurement of water absorption in figure 6. Test was conducted on mortar specimens after 3 and 14 days of curing time. As might be expected, measured water absorption increased in function with time due to pressure gradient force. Bacteria-based coating contributed preventing water ingress into mortar specimen. And that could help us to explain the difference between the result of controlled and bacteria-based coating mortar. Between 3 and 14 days mortar specimens, they existed a significant difference of the measured value of water absorption. That might be explained by the increasing coating layer thickness in the case of 14 days cured mortar. Also, after 10-20 hours of testing time, curves result on the right-hand diagram seemed to be more stable.

4. Conclusion

For concluding, we shown in this study the development of a solution of microbially induced CaCO₃ precipitation coating for cement concrete. The purpose of research is given in the context of ability of
bacteria-based coating while exposing to natural seawater in a curing tank of laboratory. The bacteria-based solution was prepared with suitable culture media and mixing proportion. For the result of test on the CaCO₃ precipitation by bacteria metabolism, a thin layer of calcium carbonate product was formed on the mortar substrate after 7 days of curing. For the result of characterization of bacteria-based coating layer, we figured out that after 3 and 14 days of curing period around 0.5 and 2.5 mm of coating thickness appeared on the cement substrate. For the result of test on the performance of water sealing of cement mortar with bacteria-based coating, we highlighted the positive effects of such generated coating on the measured water absorption. In the future study, we should scale up this laboratory proven result for concrete surface treatment. That might contribute effectively to enhance durability of concrete material structure under severe condition.

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