



Investigation in manufacturing selfhealing cementitious materials using microbial technology

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Abstract: Small cracks are inherent in cementitious-based structures. This causes deterioration of buildings, reducing the bearing capacity of structures due to the invasion of harmful substances as well as corrosion of reinforcement in cementitious material. Therefore, the cementitious material needs to be restored and these voids filled. Selfhealing cementitious material has been strongly developed for long time in the world. However, this technology has not been popular in Vietnam. Therefore, the aim of this paper is to find the appropriate mix-design for creating self-healing cementitious material in Vietnam conditions. Experimental results show that the mixture including bacillus subtilis and calcium lactate can be used for creating the self-healing cementitious materials. Mixing directly the mixture in the cement is selected to manufacture the self-healing cementitious materials. It seems that the width of crack affects more on healing ability than the length of crack.

Keywords: Self-healing mortar, cementitious material, Bacillus subtilis, calcium lactate.

1. Introduction

Reinforced cementitious materials is a type of composite material combined by cementitious materials and steel, in which cementitious material and steel participate in bearing forces. In Vietnam, there are many reinforced cementitious materials works built with structural problems such as cracks appearing during construction and use. There are many causes leading to the cracking of reinforced cementitious material such as: cracking due to structural deformation, plastic stability, shrinkage, reinforcement corrosion, reinforcement expansion, etc. Cracks affecting the safety of the structure need to be treated or reinforced to ensure safety for construction works.

There are many methods to strengthen

cracked structures in reinforced cementitious material, but they are expensive and time consuming. One of those measures is the method of using microorganisms to cure cementitious material. Cementitious material is a highly alkaline material with (pH>12) so the microorganisms used need to be tolerant of this environment [1]. Microorganisms create calcium carbonate precipitates that bind other materials such as gravel and sand in cementitious material thereby helping to fill cracks [2]. The participation of microorganisms in the calcium precipitation process increases the strength and durability of cementitious material [3].

The main mechanism for precipitation of $CaCO_3$ is the breakdown of urea by microbial urea

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Received: 19/05/2022 Accepted: 20/06/2022 Published: 26/06/2022 enzymes. Microorganisms produce urea fertilizer, which catalyzes urea to carbonate and ammonium and which increases the pН carbonate concentration in the environment surrounding the bacteria. These substances are hydrolyzed to ammonia NH_4^+ and carbonic acid CO_3^{2-} leading to the formation of calcium carbonate when combined with a medium rich in Ca2+ ions of calcium carbonate in cementitious material. The process of hydrolysis of urea $CO(NH_2)_2$ to carbonic acid CO_3^{2-} and ammonium NH_4^+ is as follows [5]

$$CO(NH_2)_2 + H_2O \xrightarrow{enzym} NH_2COOH + NH_3$$
(1)

 $NH_2COOH + H_2O \to NH_3 + H_2CO_3$ (2)

$$H_2CO_3 \leftrightarrow HCO_3^- + H^+ \tag{3}$$

 $2NH_3 + 2H_20 \leftrightarrow 2NH_4^+ + 20H^-$ (4)

 $\begin{array}{r} HCO_{3}^{-} + \ H^{+} + 2NH_{4}^{+} + 2OH^{-} \\ \leftrightarrow CO_{3}^{2-} + 2NH_{4}^{+} + \ 2 \ H_{2}O \end{array}$ (5)

The cell wall of microorganisms (cell) $-Cell + Ca^{2+} \leftrightarrow Cell - Ca^{2+}$ is negatively charged, so bacteria absorb Ca²⁺ cations from the cementitious material environment to form Ca²⁺ ion membranes. The Ca²⁺ ions react with the carbonate anion CO₃²⁻ to precipitate calcium carbonate at the cell surface of the microorganisms [1] (Figure 2). This process can be described through chemical equations (6) and (7)

$$-Cell + Ca^{2+} \rightarrow Cell - Ca^{2+}$$

$$-Cell + Ca^{2+} \rightarrow Cell - Ca^{2+}$$
 (6)

$$Cell - Ca^{2+} + CO_3^{2-} \rightarrow Cell - CaCO_3 \tag{7}$$

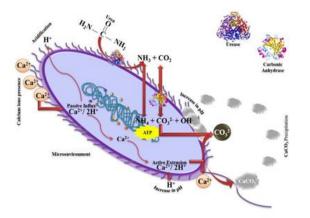


Fig 1. Diagram of calcium carbonate precipitation produced by microorganisms [4]

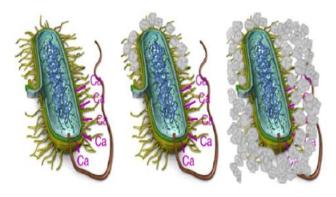


Fig 2. Carbonization of microbial cell walls [6]

А series of studies on self-healing cementitious material in general and self-healing cementitious material using microorganisms in particular have been scrutinized worldwide. For example, the study of Tanvir Qureshi and Abir Al-Tabbaa [7] has shown in quite detail the impact of microorganisms on the self-healing process of cement materials. In particular, mainly focus on self-healing cementitious material in traditional ways such as using fibers, polymers, minerals and additional additives, as well as some new nontraditional ways such as using capsules. with polymeric agents, minerals and microorganisms. Many other studies have also focused more on the use of microorganisms for self-healing cementitious material materials. That is the study of Kunamineni Vijay et al. [8], H.M. Jonkers & E. Schlangen [9], these studies have elaborated on the microbial self-healing process with hydration and calcite precipitation reaction to fill cracks in the structure. cementitious material. Accordingly, the study of Mohamed Alazhari et al. [10] also clarified the suitability of the microbial capsule system and determined their necessary ratios necessary to ensure the self-conforming process. More fully assessed, research by J.Y. Wang et al. [11]. By focusing on research, examining the self-healing process of cementitious material materials when using micro-capsules containing microorganisms, the author has demonstrated the effectiveness of the method of using microorganisms to bring the self-healing efficiency for cementitious material samples is much higher than that of other methods.

With the method of using microorganisms for self-healing cementitious material materials, the

problem is that the specific bacteria selected must be able to withstand the high alkalinity of the cement and the compressive pressure inside the structure. Along with that, it is also necessary to have a source of nutrients available to provide for the activity of microorganisms. A series of tests have been carried out towards the application of self-healing cementitious material into practical use. Experiments by [6] Wiktor and Jonkers (2011) have shown that cracks up to 0.46 mm can be completely filled with self-healing cementitious material using microorganisms, however effective of this method can be limited if bacterial spores lose their ability to survive in overly dry environments. In addition, Wiktor and Jonkers [12] conducted experiments also with Bacillus pseudofirmus and Bacillus cohnii spores that demonstrated self-healing ability based on visual observation of results from calcium carbonate precipitation reactions on Cracked surfaces after 100 days of immersion in water. Huang and Ye (2011) [12] added 5mm capsules containing sodium silicate to the cement mix, the cementitious material structure then showed a recovery of mechanical properties including flexural strength and strength after being damaged by the threepoint bending test. Thereby, microbial self-healing cementitious material has demonstrated that their recovery is accompanied by recovery in both mechanical and transmission properties, however, it may not be achieved. 100% efficiency as well as the need to minimize the repetition of failures and recovery processes [13] [14] [15]. Currently, the two main methods used to introduce microorganisms into cementitious material are direct mixing of microorganisms and packaging of microorganisms into pellets and mixing into cementitious material. These two methods have their own advantages and disadvantages, the direct mixing method has the advantage of being simple, non-polluting, but it is necessary to have measures to ensure the survival of bacteria. In contrast, mixing pellets requires high requirements in terms of packaging technology as well as complexity in the mixing process to ensure no

breakage as well as evenly distribute the pellets in the cementitious material [16]. However, the method of mixing tablets in the form of tablets ensures the survival of bacteria, so it can be applied to many types of components under the effect of many types of loads. There are many types of bacteria studied and applied in the world such as Bacillus sp. CT-5, Bacillus megaterium, Bacillus subtilis, Bacillus aerius, Sporosarcina pasteurii, AKKR5 and Shewanella Species.

A series of studies in Vietnam have analyzed and evaluated to bring self-healing concrete into application for construction projects across the country. An article on the Center for Information and Statistics of Science and Technology of Ho Chi Minh City [17] mentioned an overview of selfhealing concrete materials as well as the current status of research and application of this material on world. Going a little deeper, a research essay has been done by students of Ho Chi Minh City University of Technology [18] on the topic "Application of biology in construction day" has initially entered the work. The study of self-healing concrete under the influence of microorganisms and an overview of the development direction of this material in Vietnam. In addition, the study by Le Quynh Loan and his colleagues published in the Scientific Journal of Ho Chi Minh City University of Education [19] clarified and described the calcite precipitation reaction with the influence of bacteria from cementitious samples. In more detail, Nguyen Ngoc Tri Huynh and Nguyen Khanh Son, from Ho Chi Minh City University of Technology, have conducted quite complete studies and assessments on self-healing concrete applying the bioactive mechanism of concrete bacteria Bacillus subtilis [20]. This study demonstrated the effect of bacteria in the self-healing process, as well as the improvement of the mechanical and mechanical properties of this self-healing concrete material.

Overall, the domestic investigations have not clarified the mixing method and mixing materials into concrete to make self-healing concrete. Through detailed research overview, this study proposes to use direct mixing technology with the advantages of easy implementation. In addition, the main aim of this study is to propose mixing materials including type of bacterial and type of nutrient to successfully manufacture self-healing concrete in Vietnamese conditions with domestic material.

2. Experimental methodology

2.1. Selection of materials, microorganisms and fabrication nutrients

The aim of this paper is to initially evaluate the success of self-healing cementitious materials in Vietnam condition with domestic materials. Therefore, the cement mortar mixture was used. The mix design of mortar includes sand 581 kg/m³, cement 450 kg/m³ and water 180 kg/m³. The cement used in the research is Xuan Thanh PCB 40 cement. This is a mixed Portland cement that meets the requirements of technical standards according to TCVN 6260:2009. Sand meets the Standard requirements TCVN 7570:2006.

 Table 1. Grain composition of sand

	1	
Size of sieve	Amount of residue	
	accumulated on sieve (%	
	mass)	
2,5 mm	From 0 to 20	
1,25 mm	From 15 to 45	
630 mm	From 35 to 70	
315 mm	From 65 to 90	
140 mm	From 90 to 100	
Amount through		
sieve 140 mm,	10	
not more than		

The selected microorganisms are in accordance with the standard conditions of Vietnam. In this study, the author used the most widely used and popular family of bacillus microorganisms to make self-healing cementitious material by microorganisms [21]. Through the study, the source of bacillus subtilis in water can be divided into 3 types as illustrated in Figure 3 [22].

Bacillus subtilis microbial products in water exist in 3 main forms: (i) pure form in seed tubes (Figure 3a), (ii) powdered preparations for food technology and medicine (Figure 3b), (iii) powder form for agricultural use as animal feed or as a decomposing agent in aquaculture ponds (Figure 3c). In this study, the authors used 2 preparations shown in Figure 3b and Figure 3c for experimentation because these are two products with low cost and relatively large number of spores



Fig 3. Sources of bacillus subtilis in water

2.2. Nutrient source for microorganisms in cementitious material.

Microorganisms in order to survive and grow and create calcite minerals in Cementitious material need a suitable nutrient environment. There are 2 nutrient mediums: urea-containing (type 1) and urea-free (type 2). For type 1 nurturing environments, several studies from around the world are summarized in the following tables

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Table 2.	Composition of nutrient medium type 1
for bacillus subtilis	

Component	Quantification (g)
Microbiology Bacillus subtilis	-
Urea	7.0
Nutrient Broth (Nutrition soup)	2.1
(NH ₄) ₂ CO ₃	7.0
CaCl ₂ .2H ₂ O	5.0

Component	Quantification (g)	Rate (%)
Microbiology Bacillus subtilis	2.25	22.61
Urea	2	20.10
Nutrient Broth	0.5	5.03
NH₄CI	0.2	2.01
CaCl _{2.2} H ₂ O	1	10.05
NaHCO ₃	4	40.20

Table 3. Composition of nutrient medium type 2for bacillus subtilis

For type 2 nutrient medium, several mixtures are used: nutrient broth (beef broth + peptone + NaCl) [27], Mueller Hinton (MH) or Trypton Soy Broth broth. [28], yeast extract medium [29], or type 3 contains only calcium lactate $C_6H_{10}CaO_6$ [22], [30]. Among these materials, calcium lactate $C_6H_{10}CaO_6$ has the cheapest price and is easy to find in Vietnam market.

2.3. Mixing self-healing mix design

The nutrient medium type 1, the research group used to create the precipitation of CaCO3 The substances used to create the type 1 nutrient mixture (Figure 4). The composition is used as shown in (Table 3) with 2 types of bacillus subtilis



Fig 4. Substances used in the preparation of mixtures of nutrient media type 1

Proceed to mix the above substances and two types of bacillus subtilis preparations (Figure 5). The two types of microbial preparations bacillus subtilis correspond to two densities of 2.05×10^{10} cfu/g and 2.57×10^{9} cfu/g, respectively. These densities were diluted in 1000 ml of water also with a mix of one nutrient medium



Fig 5. Experiment on mixing and precipitation of calcium carbonate of two microbial preparations bacillus subtilis in a mixture of nutrient medium type 1

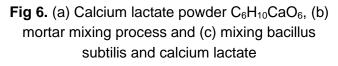
Experimental results of mixed solution with initial bacillus subtilis bile 2.05×1010 cfu/g did not appear calcium carbonate precipitate while solution with initial density of bacillus subtilis 2.57×10⁹cfu/g appeared precipitate of calcium white carbonate suspension transparently on the surface of the solution as well as inside the solution. It can be seen that even under normal conditions, bacillus subtilis can also produce CaCO3 precipitates. Nutrient group type 3 is calcium lactate C₆H₁₀CaO₆ mixed with two types of bacillus subtilis concentration directly into the cementitious material mixture to cure cementitious material. This study proposes to use direct mixing in mortar mixture with the advantages of easy implementation.

2.3. Testing of the healing ability of cementitious material

The evaluation of the success of the mixture of bacillus subtilis and calcium lactate $C_6H_{10}CaO_6$ in the manufacture of self-healing cementitious material will be initially investigated with a mortar mixture of sand 581 kg/m³, cement 450 kg/m³ and water 180 kg/m³. The ratio of microbiological/nutritional mixing is 1:1, with the

microbial content used at 4 kg/m³. Two microbial densities were used Calcium lactate powder $C_6H_{10}CaO_6$, microorganisms are mixed directly with cement, sand and water (Figure 6).





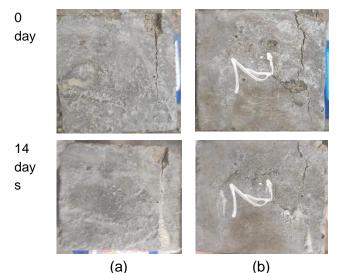
The experimental samples were molded with 2 types of cylinders of size 10×30 (cm×cm) and cube samples 5×5×5 (cm×cm×cm). The samples were de-moulded after 24h and cured in a saturated water atmosphere at room temperature. After 7 days, the samples were loaded until cracks appeared. After cracking, samples were immersed in water under normal environmental conditions and observed after 7 days and 14 days of immersion in water saturated environment. The results of the experiment with 2 types of nutrient media are as follows:



Fig 7. Healing ability of mortar mixture containing bacillus subtilis 2.57×10⁹ cfu/g

Experimental results (Figure 7) showed that the crack healing ability of cement mortar samples

using bacillus subtilis density was 2.57×10^9 cfu/g, the precipitation of CaCO₃ occurred strongly after 14 days of age, the cracks were is filled, small cracks are gone. Observing visually, the calcite content is much precipitated after 14 days in comparing with 7 days.



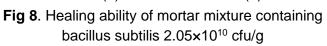


Figure 8 shows the test results of a mortar mixture containing bacillus subtilis with a density of 2.05×10^{10} cfu/g, showing that after 14 days the cracks appeared a lot of CaCO₃, however the healing of these cracks take place unevenly on the location of the cracks. Moreover, it seems that the width of crack affects more on healing ability than the length of crack. In fact, with different length of cracks in two case mixture of bacillus subtilis density, the cracks can be healed but large and deep crack cannot be totally filled (Figure 8a).

The above image results show that bacillus subtilis with a density of 2.05×10^{10} cfu/g is capable of forming calcium carbonate precipitates stronger than bacillus subtilis a density of 2.57×10^9 cfu/g. The same microbial content used, but the precipitation occurred by bacillus subtilis with a density of 2.05×10^{10} cfu/g not only filled the cracks but also appeared precipitated outside of the mortar sample. The size of samples helps to evaluate the length effect of crack on the healing process of mortar. However, the shape of samples doesn't have the effect on the healing process of mortar.

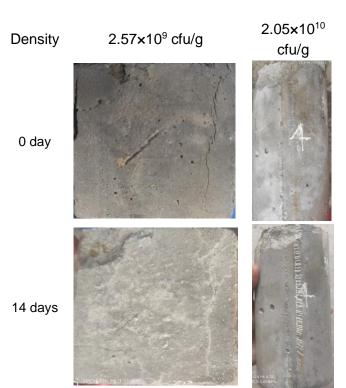


Fig 9. Comparison results of the ability to fill cracks by two concentrations of bacillus subtilis

3. Conclusion

The use of bacterial for healing cracks in cementitious material is highly feasible. The domestic mixture including bacillus subtilis and calcium lactate can be easily found in Vietnam and are used to manufacture successfully the selfhealing cementitious materials. Mixing directly this mixture in the cement is selected to manufacture the self-healing cementitious materials. It seems that the width of crack affects more on healing ability than the length of crack.

More investigations need to be performed to evaluate size effect of crack, age of samples on the healing ability of cementitious materials.

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