- *Technical Paper* -

ECO-FRIENDLY TECHNIQUE ON NUTRIENT SOURCES AND CAPSULATION FOR BACTERIA-BASED SELF-HEALING CONCRETE

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ABSTRACT

Although concrete is one of the most extensive building material, it cannot prevent cracking caused before hardening or after hardening periods. It is necessary to understand the origin of the cracks and how to repair them efficiently. Hence, self-healing concrete using bacterial mineralization can be a core solution for a combination of self-healing techniques. In this study, we aim to figure out the ability to repair the cracks with 1 mm-width by using *Bacillus subtilis* natto bacteria immobilized in lightweight aggregate. Crack healing was determined through the strength recovery and image analysis method.

Keywords: self-healing concrete, bacteria, calcium carbonate, lightweight aggregate

1. INTRODUCTION

Focusing on incorporating the microorganisms as bacteria within the cement-based matrix [1] to develop self-healing concrete was the first generation of this research topic. Then, as improving approach, suspending endospores were used instead of living cells in activating form to enhance the remaining survival bacteria around 4 months [2]. In recent years, the encapsulation technique has attracted many research groups in all over the world with various materials using. Most of the findings from literature related to microbial induced calcium carbonate (MICP) [2–4] indicate that standard media containing nutrient broth and yeast extract have been used for bacterial growth and precipitation of calcium carbonate ($CaCO_3$). Although these studies show positive results on self-healing ability, the use of this media is expensive and hence reduces the application fields of bacteria-based technology. Therefore, an alternative nutrient with a reasonable cost is necessary to bring self-healing concrete from laboratory-scale specimens to real concrete infrastructures. Preliminary studies [5,6], show that lactose (the main component of milk sugar) can help for the growth of bacteria as well as the urease activity with high efficiency when compared to standard nutrients. One remaining problem, both standard nutrients include nutrient broth and yeast extract, or the alternative lactose can lead to considerable impacts on properties of fresh and hardened concrete. Using lightweight aggregate immobilized bacteria and nutrients can be a solution to these problems. Generally, in six main mechanisms that may cause environmentally induced cracking of the concrete [7], the self-healing effect by bacteria can play an essential role in reinforced and protect the structure. In the case of cracks caused by alkali-silica reactions under high moisture or water ingression, the MICP can occur and seal the damage at the interfaces between cement matrix aggregate. The mineralization of bacteria may also minimize the cracks caused by unsound cement as excessive amounts of unhydrated CaO/MgO. Then, the impact of cracking on durability, especially corrosion, may able to be minimized when the water pathway was closed. Autonomic healing products include $CaCO_3$ and bacterial biomass [6,7], can be formed rapidly after the cracks appear. This mineral layers can act immediately as a barrier to cut off the water access. Then, the autogenous healing phenomenons can have time enough to fill out the volume and close the cracks together. The general mechanism of these processes was summarized in Fig. 1. Moreover, lightweight aggregate (LWA) with high porous volume is an ideal environment for bacteria survival and its activities in the long-term. Also, with the simple treatment technique for immobilization, the marketable LWA can be applied in massive concrete structures with more benefits than encapsulation in hydrogel or polymers.

In this study, we aim to achieve the $CaCO_3$ precipitation by *Bacillus subtilis* natto bacteria in a hash condition with fewer nutrients sources than standard. By using LWA, we also controlled the adverse effects of lactose on the harden and early compressive strength of concrete. Concrete specimens with the artificial crack range up to 1 mm-width were tested the crack closing ability through the water flow experiment. Besides, based on the previous study [8] using course LWA, we compared with the compressive strength development and strength restoration using fine LWA. In addition, “bond strength” between self-healing products and crack flanks is essential and required for upscale

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experiments. We compared the case of healing from inside and outside of $CaCO_3$ to see the characteristic of bacterial mineralization.

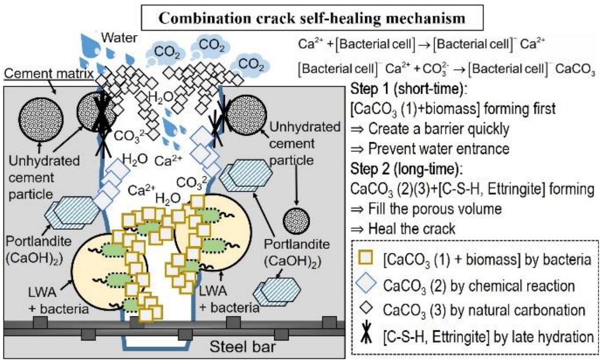


Fig. 1 The mechanism scheme for the combination self-healing effects

2. METHOLODY

2.1 Materials

(1) Bacterial self-healing agent

In this study, the bacteria-based repair solution includes *Bacillus subtilis* natto (Fig. 2), spores stater (10^8 CFU/g), urea (20g/L) and $CaCl_2$ (10g/L). The bacteria-LWA was prepared by following [10]: (i) mix the bacterial solution, (ii) immerse the LWA in solution for 48 h, and (iii) dry the LWA in 40 ºC for 24 h. Note that, the LWA as used as concrete mixture compound has the component proportion: $m_{bacteria}/m_{sugar} = 1.5/1$, $m_{bacteria} = 0.5\ \%m_{cement}$, $m_{urea} = 0.45\ \%m_{cement}$, and $m_{CaCl2} = 0.45\ \%m_{cement}$). The chloride content was kept in a safety limit, according to ACI 318 [11].

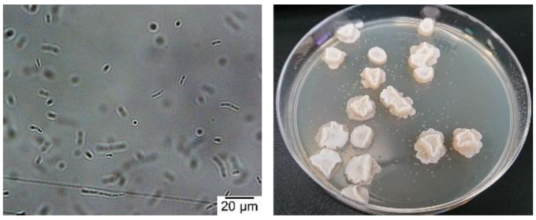


Fig. 2 Microscopic observation and colony development in the petri dish of *Bacillus subtilis* natto

(2) Concrete specimens

Table 1 Properties of LWA and mix proportion

Type of LWA	Coarse	Fine
D_{max} (mm)	20	5
Water absorption (%)	20	10

Amount (kg/m^3 concrete)					
Group	W	C	S	LWA	
				Coarse	Fine
B1	147	370	953	618*	-
B2	153	385	-	714	642*
R1	147	370	953	618	-
R2	153	385	-	714	642

*: LWA with bacteria

As describe in Table 1, concrete specimens with ratio w/c = 0.4 was prepared with two types of commercial LWA immobilized bacteria (B1, B2) to compare with the reference without bacteria (R1, R2) in compressive strength (Φ = 50mm, H = 100mm). For the water flow test, the 14-day concrete specimens were cut into slices (Φ = 50 mm, H = 30 mm) to create the crack under the splitting test.

2.2 Experiments

(1) Compressive strength recovery

For the compressive strength recovery experiment, we test the cracking-healing cycle with 4 cases of cracking-day: 7, 14, 28, and 60-day old of concrete specimens (Φ = 50mm, H = 100mm). Each cycle includes the compression test and 7-day curing in water. Generally, first visible cracks appear and developed at around 50 % of compressive strength. For favourable observation, the load to introduce cracks was defined by 90% of the compressive strength of the "trial-specimen". All of the specimens at the same cracking-age were then compressed under the same load (loading rate = 1.17 kN/s). Repeat these processes with the corresponding value of load for the other case of cracking-day (14, 28, and 60 days). Concrete specimens were measured the ultrasonic pulse velocity before and after compression for each cracking-healing cycle. Repeat this process until the signal from the ultrasonic wave cannot be received.

(2) Water flow test and crack healing ability

For the water flow test, a continuous water flow system (Fig. 3) with a 30 mm water head was applied in 15 minutes. The average crack width was measured by using an optical microscope over curing time. Healing capacity was determine by the ratio of the discharge of water flow after curing time and the water flow before curing. The crack was observed by measured by an optical microscope.

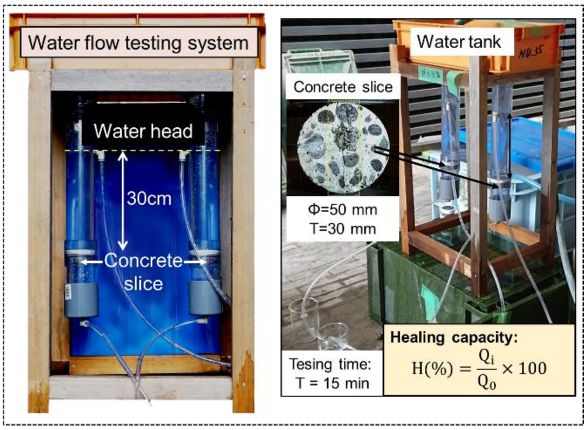


Fig. 3 Water flow testing system set up

3. RESULTS AND DISCUSSION

3.1 Compressive strength recovery

It can be seen that the different period of initial crack creation leads to different compressive strength development (Fig. 4, Fig. 5). Generally, the earlier cracking day (or "younger crack"), the higher strength recovery ability. This behavior can be observed by the change of strength from the 1st to the 4th testing cycle. The increase of compressive strength after 7 days curing in case of 7-day crack and 14-day crack with the increasing rate slow-down over crack old. The 28-day and 60-day crack didn't show this increase; the significant decrease in the 2nd testing cycle instead.

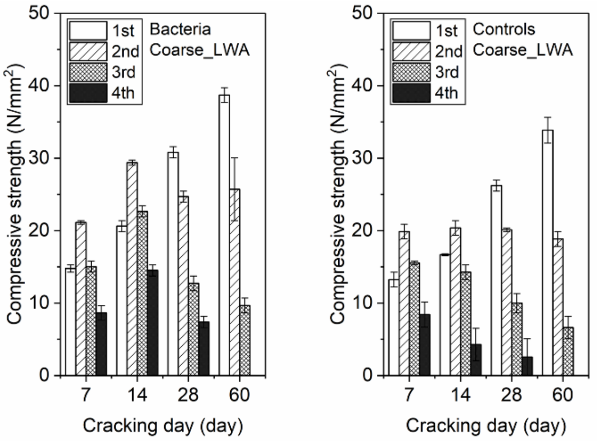


Fig. 4 Recovery strength of concrete specimens at the different initial cracking day in case of using coarse LWA

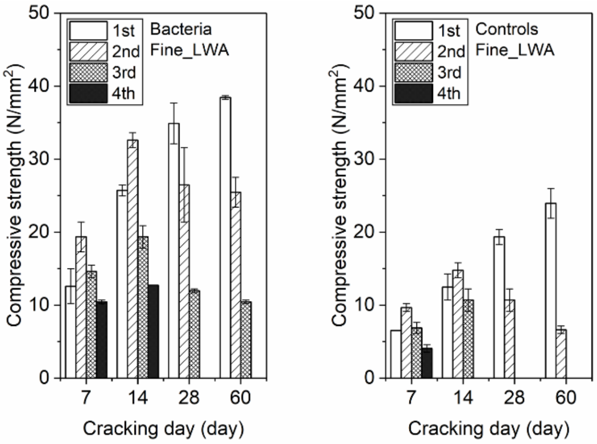


Fig. 5 Recovery strength of concrete specimens at the different initial cracking day in case of using fine LWA

In most cases, before 28-day old, the remaining unhydrated cement particles can react as well as the carbonation and reactions of free lime in the cement matrix, resulted in the densification and slightly increase in compressive strength. In general, 60 μm cracks can be healed autogenously by these processes [12–14]. For a more extended period, the effect of late hydration was almost negligible in strength development. Therefore, the damages caused after 14-day old easily break down the structure and difficult to restore. The higher compressive strength can demonstrate the positive effects of using bacteria in LWA at all cracking days and the number of the cracking-healing cycle that concrete specimens can be taken when compared to the controls without bacteria. Besides, there is no significant difference between the compressive strength recovery between using bacteria in fine LWA and coarse LWA. However, in combination with the discussion above about the ultrasonic pulse velocity, coarse LWA shows a higher ability to enhance the concrete structure and maintain the compressive strength through external damage forces. However, it can be seen that the significantly higher recovery pulse velocity of coarse LWA is not corresponding to the trend of recovery strength. This difference may be explained that the healing product with main $CaCO_3$ as the main component is not enough to create strong bondings, despite it filling out the crack volumes.

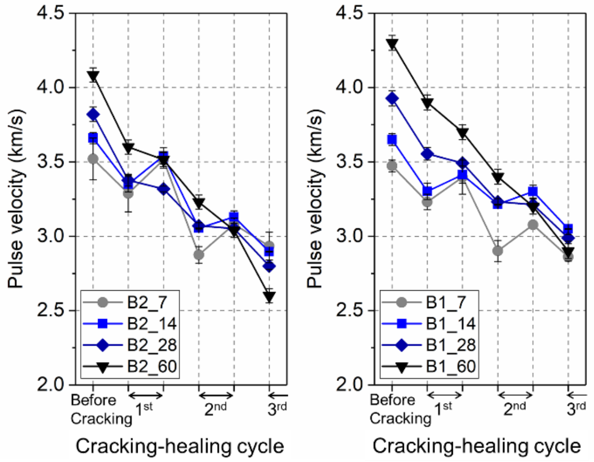


Fig. 6 Ultrasonic pulse velocity through length direction (L = 50 mm) of concrete specimens using two types of LWA with bacteria at the different initial cracking day

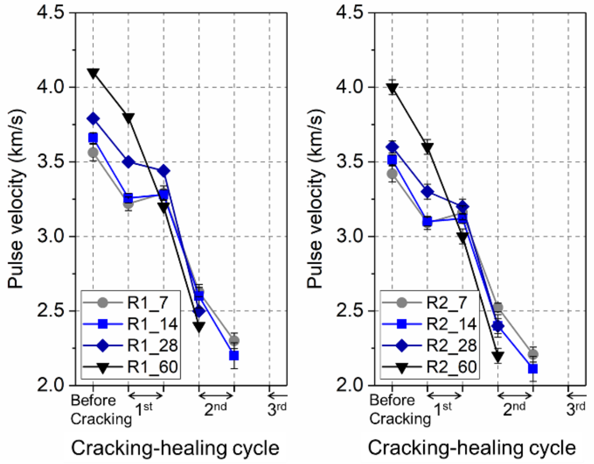
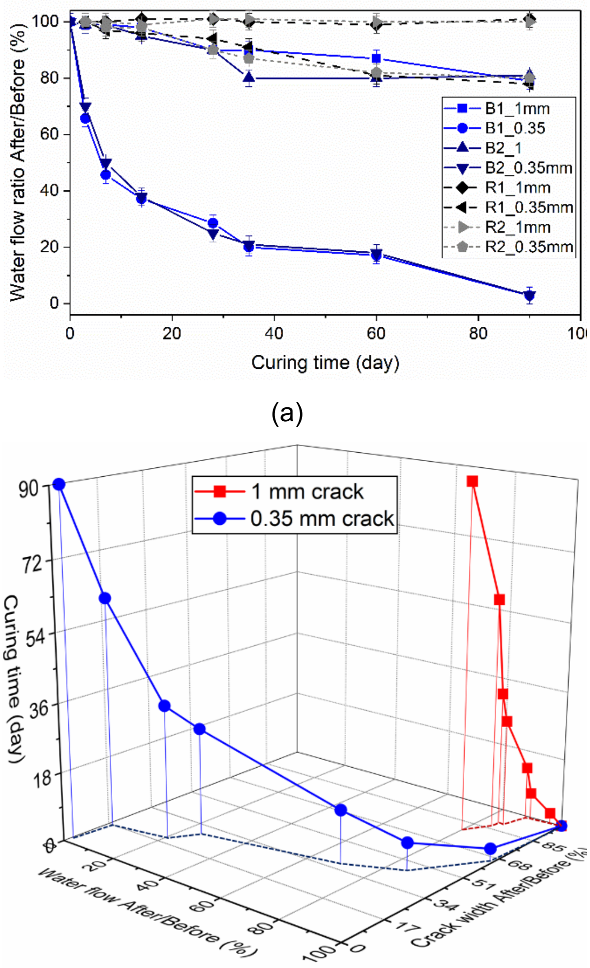


Fig. 7 Ultrasonic pulse velocity through length direction (L = 50 mm) of concrete specimens using two types of LWA without bacteria at the different initial cracking day

The result in Fig. 6 and Fig. 7 show the recovery pulse velocity of two groups of bacteria-LWA compared to the specimens without bacteria. The higher ability of strength restoration by self-healing in case of using coarse LWA can be explained by the remaining amount of healing agent, which depends on the absorption of LWA. We focus on the diameter direction, which perpendicular with the cracks to clarify the crack closure and densification of structure. Through the mixing process, a small number of bacteria and nutrients can react together. These reactions did not occur homogeneously in all positions, resulting in a slight difference in pulse velocity. With the $CaCO_3$ precipitation, the pulse velocity went up to a higher value after every 7 days curing in comparison with the reference specimens. Also, the crack width increased with the increase of damage caused by compression and shear force. Although the pulse velocity decreased and stop at the 3rd cycle, its behavior also shows the enhancement in concrete structure to prevent the broadening of cracks and damages. The recovery pulse velocity can only found in the case of specimens with bacteria in LWA, which caused the $CaCO_3$ precipitation. Note that the coarse LWA made the concrete specimens behave more brittle than using fine LWA.

3.2 Water flow test and crack healing ability



(a)

(b)

Fig. 8 The water flow ratio over curing time (a). The relationship between surface crack width ratio, water flow ratio, and curing time of specimen with bacteria in coarse LWA (b)

Fig. 8 shows the measured water flow over curing time with the change of surface crack width. Generally, the decrease in water flow corresponds with a reduction of crack width after measurements. Despite the difference in healing capacity between 0.35 mm maximum-crack and 1 mm maximum-crack, specimens with bacteria-LWA show positive effects on water preventing. This result also suggests the limit of crack width for self-healing. In the case of the reference specimens without bacteria, the water flow was almost constant. Note that the slope of the graph in the case of 1 mm crack reflects the higher rate of water reduction with the lower rate of surface crack closure. This result may be caused by the structure change inside the crack, which physical observation cannot detect easily. By using the digital microscope, an estimation of the distance was figure out from the healing products inside the crack to the concrete specimen surface (Fig. 9). As a consequence, although the crack volume still not be filled completely, particularly inside of it can be healed, result in minimizing the water pass through. However, this behavior should be studied carefully in more detail. More profound knowledge in this matter can help to upgrade and optimize the engineering self-healing techniques.

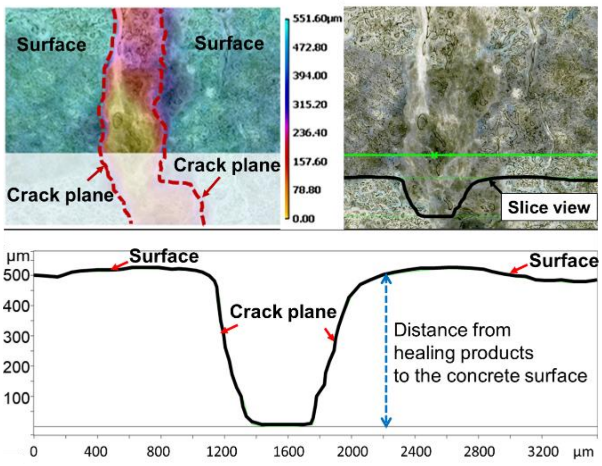


Fig. 9 Crack structure with healing products inside by digital microscopy analysis

After 120 days curing in the water of the cracking day, the crack less than 0.5 mm was almost healed completely. (Fig. 10). In contrast, the crack in the case without bacteria was still as wide as the beginning, despite of the late hydration, natural carbonation, and precipitation of impurities in water. Note that the curing process in water is not continuous. The specimens were taken out for the water flow test and then returned to the water tank. The repeat of this process many times can wash away both the healing products and the other materials inside the cracks, which was not strong bonding enough with the concrete substrate. The remaining mineral crystals can grow up to large-size on the surface of the concrete specimen (Fig. 10-below); it also combined with the biomass to create complex crystalline healing composite products. In the case of cracks in the range 0.5-1 mm, the healing capacity, which was determined by computing estimated the area ratio before/after healing, reached the average limit from 50 % to 20 %. Moreover, the self-healing process depends on various factors, such as the size of specimens, the crack surface, and the curing environment. These matters should be required in further study to achieve a more accurate evaluation in total view.

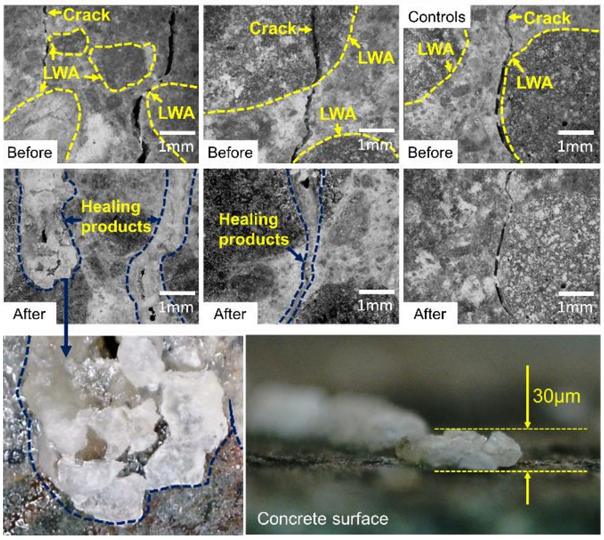


Fig. 10 The closure of cracks in case of crack crossing the bacteria-LWA and across the bacteria-LWA bounder compared with the controls; the large-size crystalline precipitation and the thickness of healing products over the surface of concrete specimen

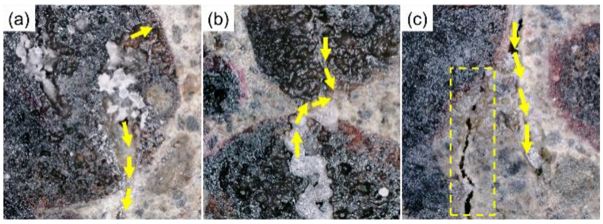


Fig.11 The healing fluid channeled from inside the broken coarse LWA to the outside crack path in the mortar phase (a). The healing fluid occurred in broken coarse LWA particles and connected them across the crack in the mortar phase (b). Littleness healing fluid channeled surrounding the LWA (c)

Fig. 11 demonstrates three particular cases of self-healing in concrete specimens. The first case, also the as ideal mechanism described by literature [2,10–14], when the cracks appear, bacteria in LWA can contact with water and oxygen to react with nutrients to form $CaCO_3$. The transport of bacteria through the crack path can lead to the fluid of mineral products to fill the crack. In the second case, two (or more) broken LWAs can be bridged together by the healing products fluid, result in the prevention of LWA removing from the cement matrix. In the third case, the crack could not break the LWA; instead, it created the split among the cement matrix and the LWA bounder. In this case, the direct contact between bacteria and outside necessary matters was not in a suitable condition. Hence, a little of the bacterial cell may move out with the diffusion as the primary transport mechanism through the LWA cover. As a consequence, this low-rate process cannot help to fill out all of the crack areas. However, these healing products can be a supporting factor to minimize the dissection of LWA, then may help to protect the structure locally. Furthermore, it is required to study the crack closure process with the impacts of surrounding conditions.

3.3 Effect of curing environment on crack healing

Crack healing and closure is a complicated process, depending on various factors both in the concrete mixture and the environmental conditions. Based on the experimental results about the crack width in the range up to 1 mm, we figure out that the healing process by using bacteria can occur via two main stages (Fig. 12). Firstly, the crack width reduction occurred quickly as well as the increase of healing capacity from the first 14 days by the rapid forming of minerals. After that, the healing process turned to the slow-down stage with the developed of precipitated products. As mentioned above, the crack in range 0.5-1 mm cannot be healed with the high capacity as the smaller ones. Therefore, we focused on the period from 60 to 120 days for all-size cracks and found that up to 90 days, the self-healing capacity was almost stable and reached the limit (about 85 %). A nutrient supplement (bacterial solution) was applied, resulting in the increase significantly to over 90 % of healing capacity in just 7 days. These results suggest that in real case applications, the infrastructures in hard conditions (crack formation can repeat easily) should be treated by bacterial solution or necessary chemical compounds after a certain time of using to maintain the high ability of self-healing.

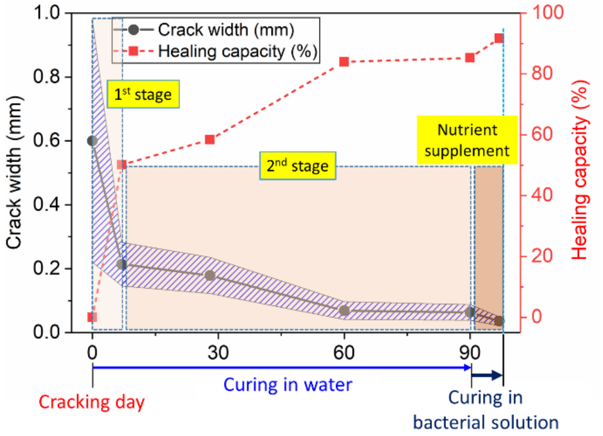


Fig. 12 Crack healing process over curing time and the relationship with the healing capacity

4. CONCLUSIONS

(1) Both fine-type and coarse-type of LWA show the ability to protect the bacteria and maintain the bio-mineralization. However, concrete specimens using coarse LWA have higher ability to restore the damaged structure and enhance the compressive strength due to the biomineralization. By immobilizing bacteria and nutrients in LWA, the negative impact of sugar on the hardening and early-stage compressive strength of concrete was prevent.

(2) *Bacillus subtilis* natto and commercially available with low cost were employed for $CaCO_3$ forming to heal the 0.35 mm maximum-crack width and below with high capacity (over 90%). The crack around 1 mm needs more curing time to get the 10-20% healing capacity.

(3) The self-healing ability shows the high-efficiency time from a 7-day to 14-day period. These results can be figured out by the recovery strength and recovery pulse velocity of concrete specimens. The age of the cracks is an important factor that can affect the self-healing process.

(4) For a long time (over than six months), a nutrient supplement is necessary for the bacteria to renew their growth and activations. The bacterial solution helped to reduce the crack width in a short time for the normal concrete specimens without bacteria in the initial mixture. However, the water flow prevention result is lower than ready-mix bacterial specimens. A combination can lead to high capacity of crack healing and closure.

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REFERENCES

[1] H. Mihashi and T. Nishiwaki, "Development of engineered self-healing and self-repairing concrete-state-of-the-art report," *Journal of Advanced Concrete Technology*, vol. 10, no. 5, pp. 170–184, 2012.

[2] H. M. Jonkers, A. Thijssen, G. Muyzer, O. Copuroglu, and E. Schlangen, "Application of bacteria as self-healing agent for the development of sustainable concrete," *Ecological Engineering*, vol. 36, no. 2, pp. 230–235, Feb. 2010, doi: 10.1016/j.ecoleng.2008.12.036.

[3] C. Joseph, D. Gardner, T. Jefferson, B. Isaacs, and B. Lark, "Self-healing cementitious materials: a review of recent work," *Proceedings of the Institution of Civil Engineers-Construction Materials*, vol. 164, no. 1, pp. 29–41, 2010.

[4] B. Krajewska, "Urease-aided calcium carbonate mineralization for engineering applications: A review," *Journal of advanced research*, vol. 13, pp. 59–67, 2018.

[5] S. Joshi, S. Goyal, and M. S. Reddy, "Influence of nutrient components of media on structural properties of concrete during biocementation," *Construction and Building Materials*, vol. 158, pp. 601–613, 2018.

[6] J. Y. Wang, H. Soens, W. Verstraete, and N. De Belie, "Self-healing concrete by use of microencapsulated bacterial spores," *Cement and concrete research*, vol. 56, pp. 139–152, 2014.

[7] T. C. Hansen, "Causes, Mechanism, and Control of Cracking in Concrete," *ACI, SP-20*, pp. 43–66, 1968.

[8] N. N. T. Huynh, K. Imamoto, and C. Kiyohara, "A Study on Biomineralization using Bacillus Subtilis Natto for Repeatability of Self-Healing Concrete and Strength Improvement," *Journal of Advanced Concrete Technology*, vol. 17, no. 12, pp. 700–714, 2019, doi: 10.3151/jact.17.700.

[9] N. N. T. Huynh, N. M. Phuong, N. P. A. Toan, and N. K. Son, "Bacillus subtilis HU58 Immobilized in micropores of diatomite for using in self-healing concrete," *Procedia engineering*, vol. 171, pp. 598–605, 2017.

[10] H. N. T. Nguyen, K. IMAMOTO, and K. Chizuru, "A novel study of using B. subtilis (natto) for application on self-healing concrete through microbial-induced calcium carbonate precipitation," presented at the Annual Meeting, AIJ, Kanazawa, Japan, 2019.

[11] A. C. I. Committee 318, "Building Code Requirements for Structural Concrete (ACI 318-14): An ACI Standard: Commentary on Building Code Requirements for Structural Concrete (ACI 318R-14): an ACI Report," 2014.

[12] M. Wu, B. Johannesson, and M. Geiker, "A review: Self-healing in cementitious materials and engineered cementitious composite as a self-healing material," *Construction and Building Materials*, vol. 28, no. 1, pp. 571–583, 2012.

[13] K. Van Tittelboom and N. De Belie, "Self-healing in cementitious materials—A review," *Materials*, vol. 6, no. 6, pp. 2182–2217, 2013.

[14] H. W. Reinhardt *et al.*, "Recovery against environmental action," in *Self-Healing Phenomena in Cement-Based Materials*, Springer, 2013, pp. 65–117.

[15] K. Van Tittelboom, N. De Belie, W. De Muynck, and W. Verstraete, "Use of bacteria to repair cracks in concrete," *Cement and Concrete Research*, vol. 40, no. 1, pp. 157–166, 2010.

[16] H. M. Jonkers and E. Schlangen, "Development of a bacteria-based self healing concrete," in *Proc. int. FIB symposium*, 2008, vol. 1, pp. 425–430.

[17] H. M. Jonkers, "Self healing concrete: a biological approach," in *Self healing materials*, Springer, 2007, pp. 195–204.

[18] M. S. Reddy, "Biomineralization of calcium carbonates and their engineered applications: a review," *Frontiers in microbiology*, vol. 4, p. 314, 2013.

[19] K. Vijay, M. Murmu, and S. V. Deo, "Bacteria based self healing concrete–A review," *Construction and Building Materials*, vol. 152, pp. 1008–1014, 2017.