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Highlights:

- Use of Bacillus cibi was successful in producing a self-healing concrete.
- Encapsulation of *Bacillus cibi* with lightweight aggregates had the largest crack healing after 35 days.
- Addition of *Bacillus cibi* improved the compressive strength of the concrete.
- Using *Bacillus cibi* with no encapsulation showed the highest water permeability recovery.

Abstract. One of the main challenges related to concrete is the formation of cracks, which can greatly diminish its strength and decrease its service life. Repair costs to mitigate these cracks can be high. This study investigated the use of *Bacillus cibi* to produce a self-healing bio-concrete capable of repairing cracks by itself through microbial activity. Bacteria were introduced into concrete by direct incorporation and encapsulating it in lightweight aggregates (LWA) and diatomaceous earth (DE). Samples of concrete cylinders were tested to quantify crack healing, compressive strength, and water permeability. The results showed that bio-concrete prepared with the LWA encapsulation method was able heal the largest crack width at 0.541 mm after 35 days of wet-dry cycle. Bio-concrete produced from bacteria with no encapsulation exhibited significant enhancement in 28-day compressive strength (57.28 MPa) compared to normal concrete (54.78 MPa) and produced the highest hydraulic conductivity recovery at 85.04% after 35 days of healing. The XRD analysis showed that the bacteria did not cause any major changes to the concrete.

Keywords: Bacillus cibi; bio-concrete; cracks; encapsulation; self-healing concrete.

1 Introduction

Concrete is the most widely used manufactured construction material in the world [1-5]. Despite its popularity, concrete has one major flaw: it has low tensile strength, which makes formation of cracks inevitable due to the action of different loads. Cracks can greatly diminish concrete strength and decrease its service life; thus, different methods are being employed to control crack propagation in

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concrete. However, most of the methods used are chemical-based, such as the use of epoxy, acrylic resins, and silicone-based polymers. These materials are generally not compatible with concrete, expensive, and mostly hazardous to the environment [1]. In addition, Khushnood, *et al.* [3] indicate that most of these methods are applied on the surface and are applicable to macrocracks only, and Vijay, *et al.* [6] note that conventional methods do not commonly address cracks with size less than 0.8 mm. Furthermore, repair of cracks is a costly operation. Therefore, there is a need to find a sustainable way of healing cracks that eliminates the need of manual intervention and involves less costs [7].

Self-healing concrete is an emerging alternative to concrete crack healing. In this technology, concrete is capable of healing cracks on its own without external intervention. Vijay, *et al.* [6] mention that self-healing approaches to concrete have shown promising results in remediating early formation cracks. As a result, self-healing concrete is preferred over conventional techniques. Khaliq and Ehsan [1] state that self-healing concrete increases structural durability through reduction of concrete cracks, thereby reducing maintenance for reinforced concrete structures.

A self-healing material is described as one that is capable of repairing itself back to its original state. Generally, there are two types of self-healing in concrete: autogenous and autonomous self-healing. Autogenous self-healing involves the hydration of cementitious products within the matrix. In this process, no additional foreign material is added to the concrete mixture. Autogenic selfhealing is the natural ability of normal concrete to repair itself when moisture interacts with non-hydrated cement clinker in the cracks. Old concrete structures have been observed to have autogenic self-healing of cracks [6]. This is evident in these structures, which have remained standing despite having limited maintenance throughout their lifespan. However, there are several weaknesses to autogenous self-healing. One of these is the limitation of the crack width that can be healed: it is generally effective when the crack width is only between 0.01 to 0.10 mm and sufficient exposure to humidity is available to the concrete, as shown in the study conducted by Rauf, et al. [5]. Autonomous self-healing, on the other hand, is based on the embedment of unconventional engineered additions to the concrete mixture. These additions can be bacteria, admixtures, or polymers to facilitate crack healing [3,5]. This type of self-healing remains effective even with crack widths greater than 0.5 mm.

Some direct benefits of concrete self-healing include the reduction of the rate of deterioration, extension of service life, and reduction of repair frequency and cost over the life cycle of concrete infrastructure. Li and Herbert [8] emphasize that the use of self-healing concrete also leads to enhanced environmental sustainability in concrete since fewer repairs indicate a lower rate of material

resource usage and reduction in energy consumption and pollutant emission in material production and transport.

Among the various autonomous self-healing techniques, microbial self-healing of concrete, or bio-concrete, is generally considered as advantageous and has been popular in recent concrete research (Zhang, *et al.* [2]). Bio-concrete is a type of self-healing concrete that uses microbes or bacteria that precipitates calcium carbonate in cracks. Bacteria in concrete have been known not only to repair cracks but also improve the mechanical performance such as the compressive and the tensile strength of the concrete. In addition, Rauf, *et al.* [5] state that it contributes to CO_2 reduction by consumption of bacteria. Recent studies by Wu *et al.* [4], Rauf, *et al.* [5], and Vijay, *et al.* [6] have shown the contribution of microbial mineral precipitation, as a result of metabolic activities of favorable microorganisms, in improving the overall behavior of the concrete. Bio-concrete involves healing of cracks by production of mineral compounds through microbial activity in the concrete. This is preferred over other self-healing methods because it is a natural process that can be considered environmentally friendly [1].

Studies on self-healing are a relatively new field in concrete technology. At present, the understanding of microbial self-healing in concrete is still in the laboratory stage. Further studies are needed to improve the efficiency of self-healing and accelerate the healing process.

This proposed research fills in existing gaps and limitations of published studies, particularly in determining the effect of the self-healing material on the durability of the concrete. This study investigated the viability of using a novel bacterium, *Bacillus cibi*.

2 Materials and Methods

2.1 Bacteria Preparation

The bacteria used in this study, *Bacillus cibi*, were obtained from the Department of Medical Microbiology of the University of the Philippines (UP), Manila. They were placed in a nutrient slant and brought to UP's Natural Sciences Research Institute, where they were processed in a nutrient broth. The bacteria were characterized to verify their identity using BIOLOG ID. The procedures indicated by Zimbro [9] were followed for the processing of the nutrient broth. The nutrient slant was subcultured and introduced into a solution of beef extract and peptone after 24 h with a concentration of 3.0 g/L and 5.0 g/L respectively. It was then incubated for 24 h at 35 °C in an aerobic environment. The bacterial concentration was measured to be $1.0x10^9$ cells/mL.

2.2 Encapsulation and Test Cases

Test cases were done to compare four different encapsulation techniques: 1) normal concrete (NC) for control, 2) no encapsulation of bacteria (BNO), 3) encapsulation of bacteria using diatomaceous earth (DE), and 4) encapsulation of bacteria using lightweight aggregates (LWA). These four test cases had the same mix proportion and amount of nutrient broth. The number of bacteria and encapsulation per cylinder were based on the literature. Neeladharan [10] recommends 10 mL of nutrient broth per concrete cylinder, while Wang, *et al.* [11] concluded that the optimal concentration of DE for immobilization is 60% w/v (g of DE per mL of solution). The amount of 50g LWA per 10 mL of solution was used based on empirical testing. The mix proportion for 10 pcs of 4" x 8" cylinders used per test case is shown in Table 1.

 Table 1
 Test cases, encapsulation, and mix proportion used for bio-concrete.

Test Case	Coarse Aggregates (kg)	Fine Aggregates (kg)	Water (kg)	Cement (kg)	Bacterial Solution (mL)	Encapsulation (g)
NC	18.238	15.903	5.965	13.292	-	-
BNO	18.238	15.903	5.965	13.292	100	-
DE	18.238	15.903	5.965	13.292	100	60
LWA	18.238	15.903	5.965	13.292	100	500

2.3 Crack Propagation and Self-Healing

The samples were loaded in a splitting tensile test until microcracks were seen on the surface. The samples were initially healed for 14 days using a wet-dry cycle, where the samples were immersed in water for 2 h and air dried for 22 h. In order to accelerate the healing, they were put in a wet curing environment after 14 days. The healing period ended after 35 days. The cracks were observed using a digital microscope to obtain more accurate measurements of the cracks. Parts of the cracks were marked to have a basis for comparison in the following days; marks were put on the largest width of the cracks. The cracks were observed every 7 days of healing.

2.4 Water Permeability

The water permeability of the cylinders was tested in accordance with ASTM D5084 [12]. The falling head permeameter used is shown in Figure 1. The water permeability was tested before cracking, after cracking, and every 7 days of healing.

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Figure 1 Falling head permeameter.

The hydraulic conductivity of the cylinder is given by:

$$k = \frac{a * L}{A * \Delta t} \ln\left(\frac{h_1}{h_2}\right) \tag{1}$$

where k is the hydraulic conductivity (m/s), a is the cross-sectional area of the reservoir containing the influent liquid (m²), L is the length of the specimen (m), A is the cross-sectional area of specimen (m²), Δt is the interval of time over which the flow occurs from h₁ to h₂ (s), and h₁ and h₂ are the head losses across the permeameter at t₁ and t₂, respectively (m).

To determine the recovery of water permeability, the following formula is used:

$$\% Recovery = \frac{AC}{AC - BC} * 100\%$$
⁽²⁾

where AH is the hydraulic conductivity of the sample after healing (m/s), AC is the hydraulic conductivity of the sample immediately after cracking (m/s), and BC is the hydraulic conductivity of the sample before cracking (m/s).

2.5 Compressive Strength

After curing for 28 days, the cylinders were tested for compressive strength in accordance with ASTM C39 [13]. Three samples were tested per test case. After 35 days of healing, the samples were tested again for compressive strength to determine their recovery.

3 Results and Discussion

3.1 Cell Count

After soaking the bacteria in DE and LWA, a cell count was done in order to determine if the encapsulation would be able to provide adequate protection for the bacteria. The cell counts on the nutrient broth, DE, and LWA were 1.00×10^9 cells/mL, 1.60×10^8 cells/mL, and 2.80×10^6 cells/mL, respectively.

DE showed a high cell count, making it a suitable encapsulation agent. In comparison, LWA showed a lower cell count because the cells were only counted on the surface; the full cell count should be larger if the cells inside the voids were also counted.

3.2 Compressive Strength Analysis

The concrete's strength was determined after curing the samples for 28 days, as well as after 35 days of healing to determine the % Recovery. The average compressive strengths are shown in Table 2.

Test Case	28-Day Strength (MPa)	After Healing Strength (MPa)	% Recovery	
NC	54.775	27.708		
BNO	57.281	32.234	56.27 %	
DE	54.940	35.121	63.93 %	
LWA	51.209	33.751	65.91 %	

Table 2Average compressive strength.

Using ANOVA one-way analysis with 95% confidence showed that the average compressive strengths had a statistically significant difference. Using bacteria in concrete (BNO) showed a 4.58% increase in the 28-day compressive strength compared to NC. This is due to the additional calcium carbonate produced by the bacteria when it was in contact with water during the mixing process. Meanwhile, the average compressive strengths recorded for DE and LWA were close to the results of NC, since the encapsulation served as a protective barrier for bacteria, which means there was no bacteria-to-water contact during the mixing process and therefore no additional calcium carbonate was produced.

LWA had the highest compressive strength recovery of 65.91%, while DE came second with a high strength recovery of 63.93%. The recovery of normal concrete can be attributed to the hydration of cement particles due to the incomplete hydration process in the early stage and swelling of the cement matrix. These results confirm the study by Muhammad, *et al.* [14], where it was established that the compressive strength can be regained up to 60% after self-healing.

3.3 Water Permeability Analysis

The concrete cylinders were subjected to a falling head test to determine their hydraulic conductivity, which is a measure of water permeability. The results are shown in Figure 2.

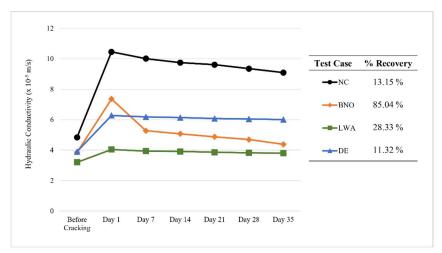


Figure 2 Water permeability recovery.

The results are similar to the study of Chen, et al. [15], where the hydraulic conductivity was also in the range of 10⁻⁵ m/s. BNO showed a high water permeability recovery of 85.04%. The small recovery of LWA and DE is attributed to the small difference in hydraulic conductivity before and after cracking, which made the permeability harder to recover. Since the water permeability is directly proportional to the amount and size of the cracks, not to mention that those cracks need to let bacteria or their encapsulation pass through for healing to occur, a small difference in hydraulic conductivity entails that there are only few cracks present on the top of the sample. Hence, there is a smaller chance for water to pass through the diatomaceous earth powder or lightweight aggregate. There was also a sample in DE that had large cracks such that when water passed through it, it made the cracks larger. This is why BNO had a significantly higher recovery than the other bio-concrete. Bacteria are present throughout the whole cylinder and therefore it is very likely that bacteria are present in the cracks. Another reason is that the BNO samples had a larger difference of hydraulic conductivity before and after cracking, which means that numerous cracks were developed. This observation also explains why NC showed little recovery. Even though the difference in hydraulic conductivity before and after cracking was large, it had recovered only a small percentage of its hydraulic conductivity after 35 days.

3.4 Crack Self-Healing Analysis

In all samples of NC, no crack healing was observed on the sides of the concrete. Some samples showed some healing on the bottom of the samples, but this was just some particulate matter that stuck to the bottom of the samples when it was put in wet curing, which was easily dislodged when water was sprayed on it. There was no actual healing visible on the samples. Longer healing and more exposure to water is needed for crack healing to be observed on the samples.

The trend of healing was similar for all cases where healing was observed to have started at day 21. The unpredictability of the self-healing is apparent since BNO and LWA had a slow improvement in crack healing, while DE had cracks suddenly filled at day 28. The location of the cracks healed was also random, which is why in LWA, unmarked cracks were observed to start healing at day 14.

The cracks filled in these test cases measured around 0.500, with LWA having the largest crack width filled at 0.541 mm, while the largest crack widths healed by BNO and DE were 0.500mm and 0.527mm, respectively. These crack widths having healed confirms the studies made by previous researchers that were also able to obtain crack healing at around 0.500 mm, as can be seen in Figure 3.

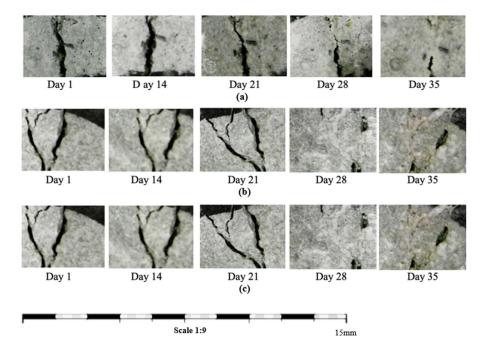


Figure 3 Crack images for (a) BNO, (b) DE, and (c) LWA.

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Precipitation was also observable on the concrete surface of the BNO sample, as shown in Figure 4, where the precipitate had a yellowish color, with excess precipitate coming out of the cracks.

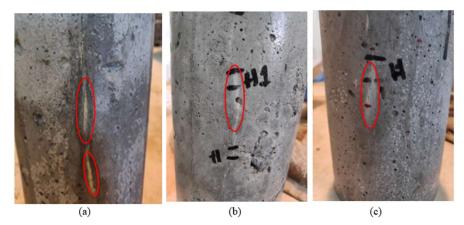


Figure 4 Bacteria precipitation in cracks for (a) BNO, (b) DE, and (c) LWA.

3.5 X-Ray Diffraction (XRD) Analysis

The highest peak in the XRD analysis for all test cases was obtained at a 2 theta (2θ) value of around 28°, i.e., very close to 29.455°, the established value of 2 θ for pure calcite according to the results of the study conducted by Harrington [16]. The slight difference in 2 θ value could be due to impurities in the samples since they were exposed in an open environment. This shows that the samples were calcium carbonate in matter and that the bacteria did not change the composition of the concrete.

The chemical composition of the concrete for the different test cases is shown in Table 3. The overall composition of the concrete may not have changed but there were small changes in its chemical properties, such as the major components of cement, which are alite and belite. The increase in these components when using encapsulations shows that using lightweight aggregates and diatomaceous earth had higher early strengths but low hydration rates. The increase in limestone content of the bio-concrete with or without encapsulation meant that it may have had high early age strength, the rate and capacity of bleeding decreased considerably, and it was less susceptible to a lack of curing [17]. The change in the amount of cementitious compounds such as pozzolan and fly ash also has an effect on the properties of fresh concrete, such as an increase of workability, lower heat of hydration and retard setting time when fly ash increases. However, it may have low early age strength and reduced resistance to deicer salt scaling and carbonation of concrete [18] compared to LWA.

Components	NC	BNO	LWA	DE
C3S-Alite	1.00	0.00	1.18	2.58
C2S-Belite	0.00	0.00	1.90	1.00
C4AF- Ferrite	3.32	4.09	3.38	3.29
C3A- Aluminate cubic	0.00	0.00	0.00	0.00
C3A-Aluminate ortho	0.00	0.00	0.00	0.00
Free Lime	0.00	0.00	0.00	0.00
Calcium hydroxide-Portlandite	12.21	12.74	11.86	12.70
Magnesium Oxide- Periclase	0.34	0.42	1.15	0.58
Potassium sulfate, beta-Arcanite	5.70	5.99	4.49	6.42
Calcium sulfate dihydrate-Gypsum	0.54	0.46	0.38	0.60
Calcium sulfate hemihydrate-Bassanite	2.99	2.09	1.69	2.11
Calcium sulfate-Anhydrite	0.00	0.00	0.00	0.00
Limestone	7.35	8.65	11.06	8.28
Silicon Oxide-Quartz	4.12	4.71	3.40	3.76
Pozzolan	58.71	60.85	53.98	58.63
Fly Ash	3.74	0.00	5.54	0.00
Standard	0.00	0.00	0.00	0.00

 Table 3
 Chemical composition of the different test cases in percent (%).

4 Conclusion

This is the first study to utilize *Bacillus cibi* for self-healing of cracks in concrete. Based on the results achieved during this study, the use of *Bacillus cibi* was successful in producing a self-healing concrete. Additionally, using lightweight aggregates as an encapsulation technique showed the highest compressive strength recovery at 65.91% and the largest healed crack width at 0.541 mm. Using bacteria with no encapsulation showed the highest water permeability recovery at 85.04% and that adding bacteria to the concrete improved the 28-day compressive strength of the concrete and did not lead to any major changes in the composition of the concrete.

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