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MECHANICAL AND PHYSICAL BEHAVIOR OF SELF-HEALING CONCRETE USING *BACILLUS MEGATERIUM* BACTERIA

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ABSTRACT: The *Bacillus megaterium* bacteria synergy on concrete mechanical and physical properties to enhance durability and strength through self-healing is studied. Two *Bacillus megaterium* concentration variations, variations of 4% and 8% to water volume, were added to fresh concrete based on the substitution method. NC0 stands for 0% bacteria and functions as a control specimen, while SHC4 and SHC8 represented 4% and 8% bacteria content, respectively. The primary focus was to analyze the compressive strength, density, permeable voids, and water absorption behavior at ages 28 and 56 days. Results indicated that the bacteria significantly improved the mechanical properties of hardened concrete. SHC4 and SHC8 exhibited a compressive strength increase of 8% and 14% at 28 days and 15% and 19% at 56 days compared to NC0. This strength increase resulted from permeable voids and water absorption reduction, as well as an improved aggregate-to-mortar ITZ bond due to the formation of bacteria-produced $CaCO_3$, which filled the voids. Reduction in permeable voids and water absorption were 7% to 17%, while density improvement was up to 10% at 28 days. A higher bacteria content consequently produced a better void-filling mechanism. The SHC8 with 8% *Bacillus megaterium* was proven more effective than SHC4. The 56-day specimens revealed that a significant concrete performance enhancement resulted from the development of $CaCO_3$ deposits over time. It is interesting for further studies to determine the bacteria effectiveness convergence as a function of hardening time. This research highlights the potential of biological approach methods for developing sustainable and resilient construction materials.

Keywords: Concrete, Bacillus Megaterium, Mechanical and physical behavior, Permeable void, $CaCO_3$

1. INTRODUCTION

Concrete is among the most extensively utilized construction materials, renowned for its ease of application and high compressive strength [1,2]. Nowadays, sustainability and environmental awareness have become major priorities, and the green concrete concept includes all innovations to minimize the use of natural resources. Cement conservation as a major CO_2 contributor was approached by reducing cement use and improving concrete integrity. Attempts to substitute cement with natural products such as rice husk [3-5] were conducted with good results, while bamboo leaf ash was proven to improve durability and concrete strength [6-8].

One of the prominent weaknesses of concrete is its low tensile strength, resulting in microcracking even at very low strain levels [9]. These cracks can compromise the structural integrity and durability. An approach to rejuvenate and restore the performance of concrete was sought, such as incorporating bacteria into concrete mixtures to create self-healing concrete [10].

Self-healing concrete (SHC) is an advanced technology that enables concrete to autonomously repair its cracks through the incorporation of bacterial agents [10-12]. The self-healing process occurs with the help of water and organic materials or nutrients that produce nutrients for bacterial activity [13]. This research employs *Bacillus megaterium*, a bacterium capable of thriving in high-pressure and alkaline conditions [14].

Previous research has demonstrated that the urease enzyme in *Bacillus megaterium* can convert urea into ammonium carbonate as precipitation of calcium carbonate ($CaCO_3$), which effectively seals concrete cracks and binds its materials [15]. Previous studies found that the calcium carbonate deposits enhance the material's durability [16-18].

A previous study employed *Bacillus megaterium* bacteria to create self-healing concrete, incorporating the bacteria directly into the concrete. This approach resulted in a 24% increase in compressive strength, indicating that *Bacillus megaterium* can be directly used in concrete to enhance its compressive strength. Additionally, the study highlighted the bacterium's ability to improve the concrete's durability [19].

Another investigation was conducted to examine the effect of Bacillus subtilis and *Bacillus megaterium* on the compressive strength of concrete. The findings revealed that self-healing concrete treated with *Bacillus subtilis* and *Bacillus megaterium*, as well as untreated concrete, demonstrated compressive strengths of 52 MPa, 57 MPa, and 44 MPa, respectively. Consequently, it was concluded that the incorporation of these bacteria enhances the compressive strength of concrete [20].

The impact of incorporating *Bacillus megaterium* into the concrete was investigated in another study. Tests showed that the maximum crack that could be covered was 0.4 mm in concrete, aged 28 days, with an increase in compressive strength of 17.28%. It was concluded that *Bacillus megaterium* can be directly applied to the concrete mixtures as an agent to improve durability. This study underscored the potential of bacteria to enhance structural integrity of the concrete [14,21].

Additional studies have also proven bacteria's ability to increase concrete strength. Test results proves that the use of 1%, 3%, and 5% Bacillus bacteria as a partial substitution for mixing water in the concrete mixtures can increase the strength of concrete up to 6.45%, 11.71%, and 13.04%, respectively, compared to the normal concrete without bacteria [22].

Previous studies suggest that incorporating *Bacillus megaterium* bacteria into concrete mixtures can influence the material's properties [14,21]. This research seeks to evaluate and compare the mechanical and physical characteristics of the modified concrete. The investigation focused on the concrete's compressive strength at 28 and 56 days and its physical properties: permeable voids, absorption, and density of the hardened concrete. The bacteria were introduced to the concrete mixture with a concentration of 0%, 4%, and 8% as substitutes for water. For the compressive strength assessment, cylindrical specimens 100 by 200 mm were prepared, while fragments of the hardened concrete cylinders were used for permeable voids, absorption, and density determination. The resulting data were analyzed to conclude the effects of the bacteria on the concrete.

2. RESEARCH SIGNIFICANCE

The utilization of *Bacillus megaterium* bacteria aims to enhance concrete's mechanical and physical characteristics. In earlier studies, 5% of *Bacillus megaterium* bacteria were incorporated directly into the concrete mix, partially replacing the mixing water. However, most of these studies focused solely on the mechanical properties and did not concurrently investigate their impact on physical properties. In this research, addition up to 8% of these bacteria demonstrated the potential to enhance the physical and mechanical behavior of concrete. The findings are expected to contribute valuable insights into advancing sustainable concrete concepts, offering sufficient mechanical and physical capabilities through a biological approach for the engineering community.

3. EXPERIMENTAL METHODS

3.1 Variation of Concrete Specimen

The concrete specimens incorporate varying concentrations of bacteria of 0%, 4% and 8%. Table 1 details the different test specimens utilized in this research. These variations are crucial for evaluating the concrete's response to the bacteria in terms of strength and durability. The different bacterial concentrations help assess how the presence of bacteria influences the concrete's overall properties.

Table 1. Variation of concrete specimen

	Bacteria Content	Specimen Code	Testing Properties
Normal concrete (control)	0%	NC0	Mechanical
			Physical
Self-healing concrete (SHC)	4%	SHC4	Mechanical
			Physical
	8%	SHC8	Mechanical
			Physical

By systematically analyzing these concentrations, the study aimed to provide valuable insights into *Bacillus megaterium*'s potential for enhancing concrete performance.

3.2 Compressive Strength Test

The compressive strength test was carried out by using 100 × 200 mm concrete cylinder specimens after a curing period of 28 and 56 days, respectively. Furthermore, the test was conducted in accordance with ASTM C 39 and calculated using Eq. (1).

$$f_c' = \frac{P}{A} \tag{1}$$

f_c' in MPa = compressive strength
P in N = ultimate load
A in mm^2 = specimen area

3.3 Permeable Void and Absorption Tests

Concrete with low permeable voids can resist the penetration of water vapor into the hardened concrete matrix, thereby increasing its strength and durability. The standard used to determine the permeable void and absorption value of the hardened concrete is the ASTM C 642-06. The Permeable void and absorption test were carried out based on the ASTM C 642-06 and calculated using Eq. (2) and Eq. (3).

$$Abs\ (\%) = \frac{C - A}{A} \times 100 \tag{2}$$

$$PV\,(\%) = \frac{g_2 - g_1}{g_2} \times 100 \quad (3)$$

$$g_1 = \frac{A}{C - D} \times \rho \quad (4)$$

$$g_2 = \frac{A}{A - D} \times \rho \quad (5)$$

Abs in % = absorption after immersion and boiling
PV in % = permeable void (%)
A in gr = sample weight in oven dry condition
C in gr = sample weight in dry surface condition after soaking and boiling
D in gr = sample weight in water after boiling
ρ in Mg/m^3 = density of water = 1 Mg/m^3

3.4 Density Test

The density test was conducted to verify the mechanical and physical properties of hardened concrete. Fragments from the hardened concrete samples were taken and investigated based on the Archimedes principles through mercury submersion (Fig. 1). The density was calculated using Eq. (6). The mercury specific gravity was taken as 13.60 gr/cm^3

$$Density\ (\text{kg/m}^3) = \frac{mass}{volume} = \frac{A}{(B \div C)} \times 1000 \quad (6)$$

A in gr = concrete specimen mass
B in gr = mass of spilled mercury
C in gr/cm^3 = mercury specific gravity

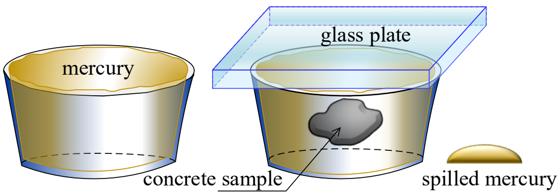

Fig. 1 Mercury submersion for concrete volume determination

4. RESULTS AND DISCUSSION

4.1 Compressive Strength

Compressive strength tests were conducted on concrete samples after the curing period and at the concrete ages of 28 and 56 days. The 28-day compressive strengths were 33.98 MPa for NC0 (control), 36.73 MPa for SHC4, and 38.57 MPa for SHC8, showing increases of 8.09% for SHC4 and 13.50% for SHC8. At 56 days, NC0, SHC4, and SHC8 reached 34.65 MPa, 39.79 MPa, and 41.29 MPa, reflecting increases of 14.84% and 19.17% for SHC4 and SHC8, respectively. Compressive strength test results are shown in Fig. 2.

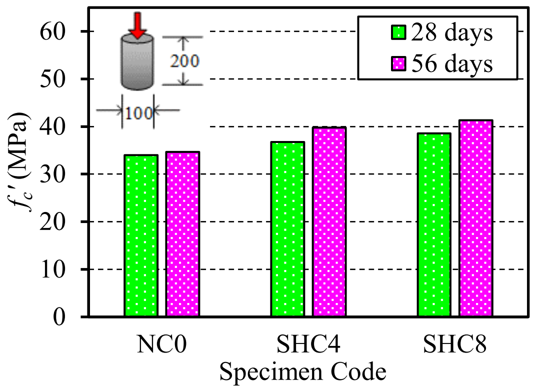

Fig. 2 Compressive strength test result of specimens

The enhancement in compressive strength is ascribed to the bio-mineralization process of calcite ($CaCO_3$) on the cell surfaces and within the pores of the concrete matrix. The deposition of $CaCO_3$, which effectively fills voids within the concrete, further contributes to the increased compressive strength [23,24].

Bacillus megaterium bacteria act as a nucleation site for bacterial cells that have negatively charged groups, attracting divalent ions such as Ca^{2+}, and Mg^{2+} [25]. The urease enzyme then catalyzes urea into ammonia (NH_3) and carbonate (CO_3^{2-}), which reacts with the attracted divalent Ca^{2+} ion, forming calcium carbonate ($CaCO_3$) precipitation [18]. The calcium carbonate acts as a seal and filler in cavities [25]. Calcium carbonate produced by bacterial agents, helps the concrete form an excellent packing and compaction with other concrete constituent materials so that concrete can produce much greater strength compared to the control specimens [25].

Previous research demonstrated that incorporating *Bacillus megaterium* in concrete can enhance compressive strength up to 11.30% [23]. Similarly, [26] found that *Bacillus megaterium* increases concrete compressive strength by up to 14.80%. Recent studies also confirmed that using *Bacillus megaterium* bacteria results in a 17.28% increase in compressive strength compared to control concrete [14]. Additionally, the use of *Bacillus megaterium* has been shown to yield a compressive strength of 46.68 MPa, representing a 22.50% improvement over control samples [25]. Previous research consistently indicates that *Bacillus megaterium* bacteria can boost concrete compressive strength by 6% to 25%, aligning with the findings of this study, which observed increases ranging from 8.09% to 19.17%.

4.2 Permeable Void and Absorption

The incorporation of *Bacillus megaterium* in concrete also influences its absorption rate and permeable voids number. Permeable void testing on

28-day-old concrete samples revealed that NC0, SHC4, and SHC8 had permeable void percentage of 3.55%, 3.29% and 3.15%, respectively. These results indicate a reduction in permeable voids for SHC4 and SHC8 compared to NC0, with decreases of 7.39% and 11.41%, respectively. A similar trend was observed in 56-day concrete samples, where NC0, SHC4, and SHC8 exhibited permeable void percentages of 3.50%, 3.04% and 2.91%, respectively. The permeable void values for SHC4 and SHC8 at 56-days showed a further reduction compared to NC0 of 13.30% and 16.75%, respectively.

The reduction in permeable voids in concrete leads to a decrease in its water absorption. Absorption tests were conducted on concrete samples at 28 and 56-days. At 28-days, the absorption percentages for NC0, SHC4, and SHC8 concrete were 1.47%, 1.36% and 1.30%, respectively. This represents a decrease in absorption of 7.50% for SHC4 and 11.56% for SHC8 compared to NC0. At 56-days, the absorption for NC0, SHC4, and SHC8 concrete were 1.45%, 1.25% and 1.20%, respectively. These results indicate a further reduction in absorption of 13.46% for SHC4 and 16.95% for SHC8 compared to NC0. The results of the permeable void and absorption tests are displayed in Fig. 3 and Fig. 4.

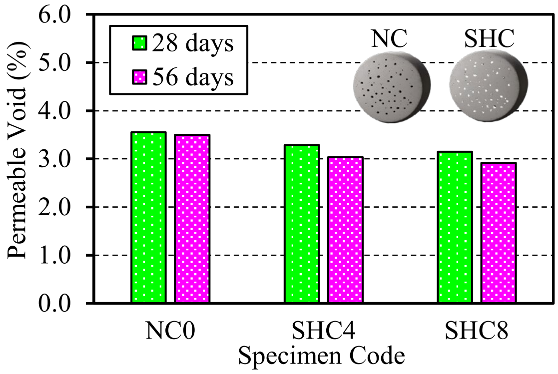

Fig. 3 Permeable void test result

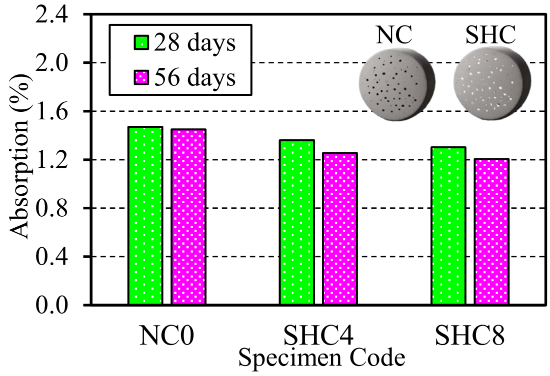

Fig. 4 Absorption test result of specimens

Recent investigation discovered that concrete mixed with *Bacillus megaterium* produces fine networks within the pores of the concrete [23]. This network, a calcified filament formed through bacterial metabolism, results in $CaCO_3$ biomineral deposits that act as fillers in concrete cracks, reducing water absorption and porosity. Another research demonstrated that using Bacillus bacteria can lower the water absorption value of concrete by 14.70% to 23.50% compared to control concrete [27]. Similarly, [28] observed a 9.72% decrease in water absorption in concrete mixed with *Bacillus subtilis* bacteria as a partial water substitute. Understanding these mechanisms is crucial for optimizing the physical performance of the concrete in various environmental conditions.

Other studies confirm that bacteria as self-healing agents in concrete can produce $CaCO_3$ compounds, filling cavities and thus reducing permeable voids and water absorption [29-31]. The results of permeable void tests show a linear relationship with water absorption test values, as permeable voids values decrease, so does the water absorption values [29] The correlation between both permeable voids and absorption value are depicted in Fig. 5 and Fig. 6.

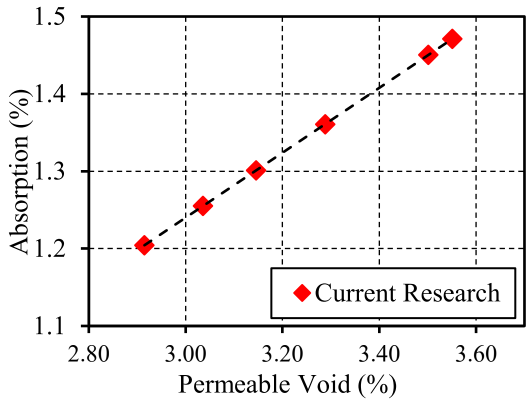

Fig. 5 Correlation of permeable voids and absorption

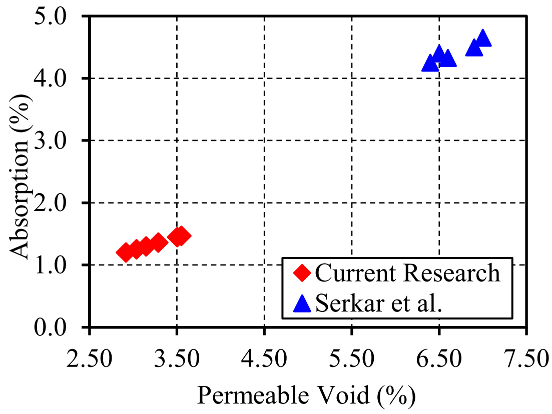

Fig. 6 Permeable void and absorption responses

4.3 Correlation of Compressive Strength, Permeable Void, and Absorption

The test results reveal a correlation between compressive strength and the obtained absorption and permeable void values. It is observed that higher compressive strength correlates with lower permeable void and water absorption. This relationship is supported by several previous studies indicating that an increased in compressive strength leads to a reduction on absorption and permeable void values [29,31,32]. Understanding this interrelationship is essential for optimizing concrete mixtures to enhance both structural integrity and durability for various applications. Data from conducted tests presented in Fig. 7 and Fig. 8 demonstrate a strong correlation between compressive strength (f_c') and permeable voids (*PV*), as well as absorption (*Abs*) value.

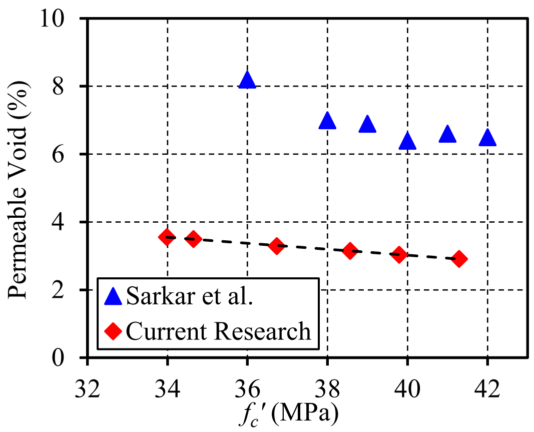

Fig. 7 Correlation between compressive strength and permeable voids

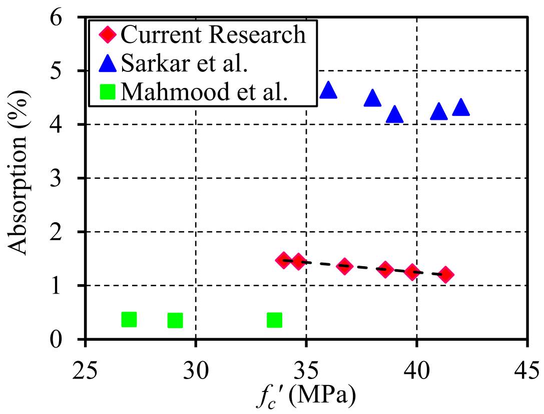

Fig. 8 Correlation of compressive strength and absorption

4.4 Density

Research findings from [23] and [29] suggest that incorporating *Bacillus Megaterium* bacteria as a healing agent in concrete increases its density through the production of $CaCO_3$ deposits. This was confirmed through density testing conducted on hardened concrete samples. Density or specific gravity testing on concrete samples NC0, SHC4, and SHC8 resulting on density values of 2203.14 kg/m³, 2348.15 kg/m³ and 2417.39 kg/m³, respectively. SHC4 concrete exhibited a 6.58% increase compared to NC0, while SHC8 concrete showed a density increase of 9.75% compared to the NC0 control sample.

According to [31], Bacillus bacteria can increase density by 6.25%. The density value obtained correlates directly with the results of the compressive strength tests conducted as in Fig 9. Higher concrete density corresponds to higher compressive strength values, consistent with findings from prior research [23,29,31].

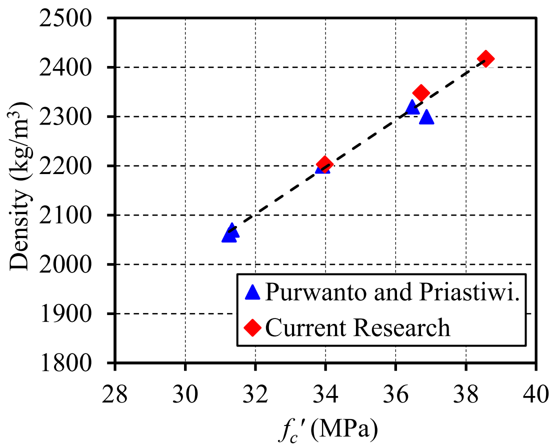

Fig. 9 Correlation between compressive strength and density

The calcium carbonate ($CaCO_3$) precipitation produced by *Bacillus megaterium* fills the voids within the concrete matrix. This process improves the overall density and durability of the concrete. As the number of bacteria in concrete increases, so does the precipitation of $CaCO_3$ [33].

4.5 Failure Mode

The enhancement of compressive strength is also influenced by improvements of the Interfacial Transition Zone (ITZ). The deposition of calcite ($CaCO_3$) produced by *Bacillus megaterium* in the concrete matrix results in enhancements in the ITZ area between the aggregate and the cementitious matrix [23]. Repairing and compacting the ITZ area between the aggregate and binder in concrete has been shown to improve the mechanical properties of the concrete [23,34]. These findings were validated by visual observations of the failure areas in the concrete samples.

Concrete mixed with 4% (SHC4) and 8% (SHC8) bacterial substitution exhibited a higher number of aggregate failures due to fracture if compared to NC0. NC0 mode of failure was predominantly characterized by ITZ debonding. This disparity is attributed to the $CaCO_3$ crystals produced by *Bacillus megaterium*, which enhance the mechanical bond in the ITZ area, thereby improving the mechanical and physical properties of the concrete. These improvements not only increase the compressive strength but also reduce permeability and water absorption, further enhancing the durability and longevity of the concrete. Visual observation of the differences in the fracture plane of specimens is presented in Fig. 10. It can be clearly seen that the number of fractured aggregates increased substantially as a function of bacteria content. Fig. 11 underlined the differences in failure mode; for the debonding case, a clear, unbroken surface is seen on the aggregate plane, while the opposing face shows the mortar (Fig. 11a). A fractured aggregate surface is seen for the aggregate fracture failure mode, and an exact image is displayed on the opposite plane (Fig. 11b).

The failure pattern due to interface debonding occurs when the ITZ between the aggregate and the binder matrix detaches, characterized by cracks passing through the aggregate without splitting the aggregate [35]. Additionally, [35] Also described a failure pattern due to aggregate fracture, which is marked by cracks passing through and splitting the aggregate, indicative of a cohesive failure within the aggregate material itself.

Calcium carbonate ($CaCO_3$) crystals produced by bacteria within the concrete pores form strong bonds with the aggregate surfaces, creating robust connections within the cavities. These layers subsequently fill and cover the voids in the concrete [32]. It is also noted that the adhesion strength between $CaCO_3$ crystals significantly enhances the interfacial cohesion of the constituent materials, ultimately improving the specimen's mechanical capabilities [32].

The $CaCO_3$ crystals produced by *Bacillus megaterium* provide additional bonding in the ITZ area, thereby enhancing the overall mechanical properties of the concrete. Moreover, this improved bonding in the ITZ area not only increases the concrete's compressive strength but also contributes to its durability and longevity. The formation of $CaCO_3$ crystals also helps to seal micro-cracks in the concrete, acting as a self-healing mechanism. The presence of $CaCO_3$ crystals reduces the permeability and water absorption of the concrete, making it more resistant to environmental degradation.

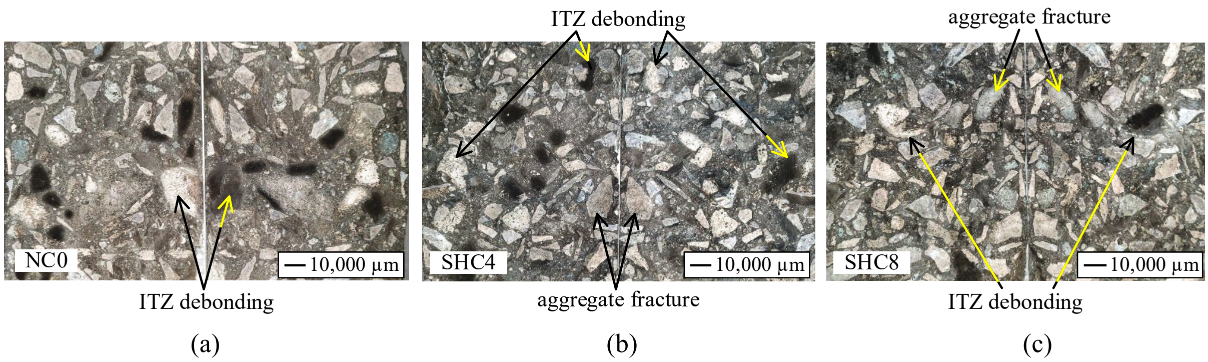

Fig. 10 Visual failure analyses: (a) NC0; (b) SHC4; (c) SHC8

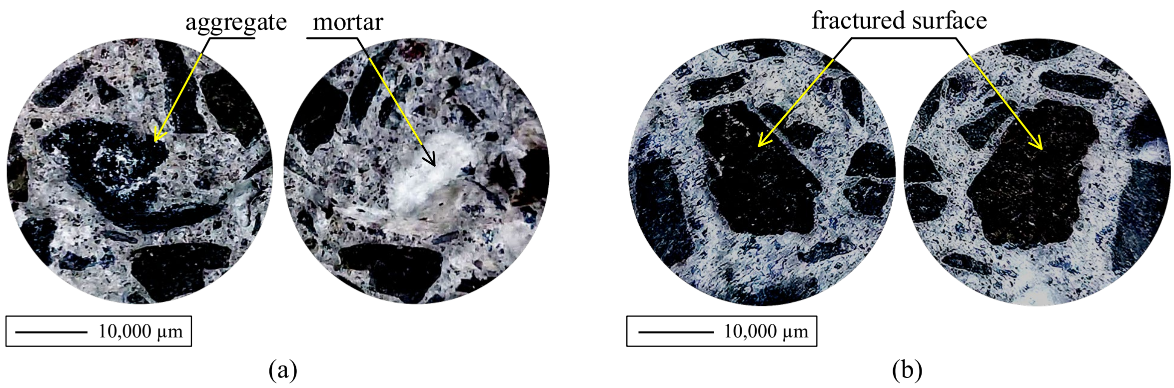

Fig. 11 Specimen failure mode: (a) ITZ debonding; (b) Aggregate fracture

4.6 Precipitation of $CaCO_3$

The study on the hardened concrete surface proved the formation of $CaCO_3$ (Fig. 12). Fig. 12a demonstrates that no CaCO3 precipitation as a result of bacteria activation was detected on specimen NC0. The surface was characterized by the greyish surface typical for cement-based products. The SHC4 specimen had clear white spots on the hardened concrete surface, which were detected as $CaCO_3$ deposits (Fig 12b). The number of white spots became more pronounced in vivid form and increased in number for SHC8, as seen in Fig. 12c. In line with these findings, the formation of $CaCO_3$ was proven as a fact and positively influenced the performance of concrete.

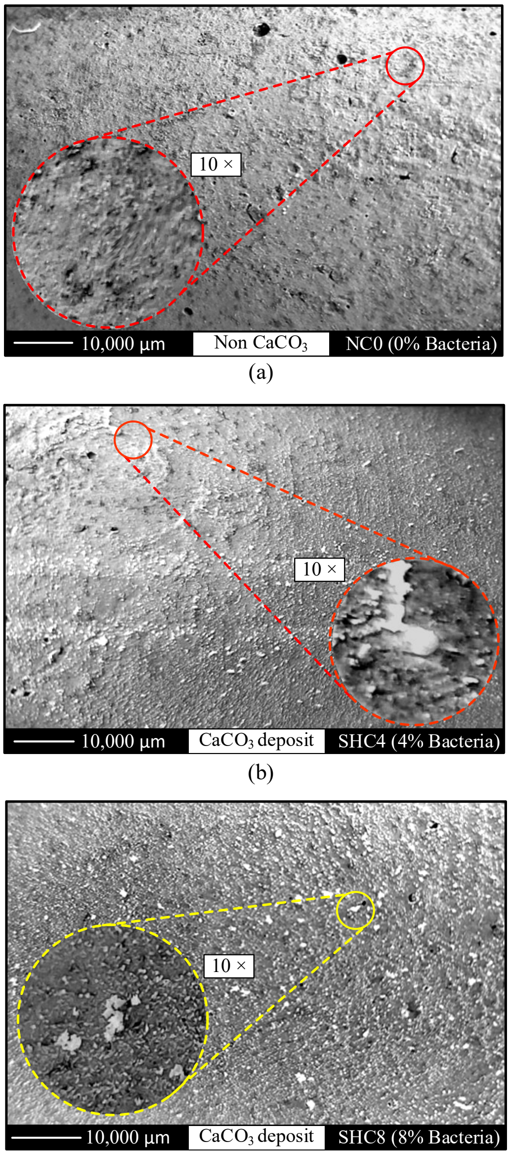

Fig. 12 $CaCO_3$ deposits surface details: (a) NC0; (b) SHC4; (c) SHC8

5. CONCLUSIONS

Based on the comprehensive results, several key conclusions emerge:

1. The presence of *Bacillus megaterium* increases the concrete compression strength at 28 and 56-days. The increase at 28-days is 8% for SHC4 (4% bacteria substitute to water), and 14% for SHC8 with an 8% bacteria content. For a 56-day concrete SHC4 produced a 15% strength enhancement (7% increase rate to the 28-days sample), and 19% for SHC8 (6% increase rate to the 28-days specimen). Although the bacteria remain active for 56 days, it is demonstrated that the additional increase of 4% bacteria to SHC4 is less effective than adding the same amount to NCO for both 28 and 56 days. Using a larger bacteria content increased the $CaCO_3$ formation, and an optimum could be reached since the livelihood of these bacteria is time-sensitive.
2. Incorporation *Bacillus megaterium* bacteria to concrete reduces permeable voids and water absorption of concrete. At concrete age 28-days the permeable voids were reduced with 7% and 11% for SHC4 and SHC8; the reduction was 13% (SHC4) and 17% (SHC8) at 56-days. The improvement rates were consistent with the numbers observed from the compression strength behavior. The *Bacillus megaterium* continued to produce $CaCO_3$ with a constant rate, but again, the effectiveness of adding the additional 4% to SHC4 was only half of adding the bacteria to NCO.
3. Underlining the findings of the compression strength improvement and permeable void reduction, the absorption lessening was measured 8% (SHC4) and 12% (SHC8) at 28-days, and 13% and 17% for SHC4 and SHC8 at age 56-days respectively. The improvement rate for both bacteria content overtime was stable. Long-term bacteria activity made this method extremely useful for wet-environment concrete structure in terms of corrosion protection and crack control.
4. The working mechanism of *Bacillus megaterium* was underlined by the evaluation of concrete's failure mode and surface inspection. The specimen NCO was predominantly marked by ITZ debonding failure, the addition of bacteria altered the failure pattern to a combination of ITZ debonding and aggregate fracture. This was due to the increase in bond strength between the mortar and aggregates. The higher the bacteria content, the more pronounced the number of fractured aggregates ratio to ITZ debonding. The visual formation of $CaCO_3$ was seen on the surface of harden concrete as white spots filling the voids. The NCO did not display any of these white marks, while an increase in white $CaCO_3$ deposit's number and distinguishing boundaries was presence, as the bacteria content became greater.

5. The enhancement of concrete performance is a function of bacteria content and time. However, the increase in concrete strength is not linearly dependent on the bacteria percentage. Further research is required to determine the optimum bacteria percentage that could effectively improve the strength and reduce permeable voids and absorption rate. The number is predicted to lay between 4% and 8%. The study also needs to explore the function of time to the productiveness of $CaCO_3$ since the study suggested that the bacteria continued to evolve over time up to 56 days, but limitations could apply to the lifespan of *Bacillus megaterium.*

6. ACKNOWLEDGMENTS

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