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Research Article

Experimental Investigation on Self-Healing Efficiency of Mortar with *Bacillus subtilis* and *Bacillus cereus*

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With the development of cracks on their surfaces, mortar's service life dramatically shortens. Self-healing concrete by Microbiologically Induced Calcite Precipitation (MICP) is one of the high-tech concretes being used to address these issues. This type of mortar can start biological processes to repair itself and deal with its cracks. The self-healing effectiveness of two different bacteria, in this paper, *Bacillus subtilis* and *Bacillus cereus*, added to the mortar is examined experimentally. In order to conduct this investigation, artificial cracks were made in the mortar. A 3D optical microscope was used to take repeated pictures of the cracked mortar. The mechanical and durability tests conducted on the bacterial mortar were used to gauge the efficacy of self-healing. Mortar samples were left for 7, 14, and 28 days to cure. Compressive strength, flexural strength, water absorption, and sorptivity were measured during various times of the curing process. The test results showed that the mortar with bacteria had an increase in strength and durability compared to the control mix. In the sample of mortar containing bacteria, *Bacillus subtilis* and *Bacillus cereus* a maximum increase of 17.29% and 11.31% in flexural strength, 17.77% and 12.84% in compressive strength were observed and a 34.48% and 26.43% decrease in water absorption in the mortar sample containing bacteria, *Bacillus subtilis* and *Bacillus cereus* at 28 days, respectively. The results of the mortar absorption test showed that the addition of bacteria to the mortar matrix significantly reduced the primary and secondary absorption rates of bacterial mortars B-M-1 and B-M-2. Using a 3D light microscope, the cracks in the bacterial mortar showed that larger amounts of white crystal precipitates were generated that nearly filled the surface of the crack. Overall, *Bacillus subtilis* appeared to be superior to *Bacillus cereus* based on the results of mechanical and mortar durability tests because calcium carbonate precipitates more rapidly.

1. Introduction

The most widely used engineering materials in the construction industry are mortar and concrete because of their durability, strength, and affordability when compared to other building materials [1, 2]. The main drawback of concrete is its low tensile strength, which makes it susceptible to the formation and coalescence of microcracks, which reduces its strength and durability. Tensile strains can be caused by a variety of factors, including tensile loading, plastic shrinkage, and expansive chemical reactions. In addition to weakening concrete, this breaking susceptibility also exposes it to hazardous environmental conditions. These cracks can allow harmful substances to enter, which can cause steel reinforcing to corrode and concrete to deteriorate chemically. Growing cracks as a result of corrosion reduce the strength and stiffness of concrete structures. Both the concrete and the reinforcement in reinforced concrete deteriorate, which results in high maintenance costs. A variety of techniques is used to bridge and stop the spread of cracks, increasing the durability of concrete. However, the majority of solutions, including silicone-based polymers, acrylic resins, and epoxy systems, use costly, often environmentally hazardous ingredients that are incompatible with concrete [3].

Recent studies have identified bio-influenced self-healing concrete as a potential technique for reducing crack propagation [4]. The use of bacteria in the production of concrete is, according to the majority of earlier studies, a significant area of study today. Numerous studies have documented improvements in the mechanical strength and durability of concrete and mortar, as well as the ability of these materials to seal cracks through the biomineralization of various bacterial strains [4–6]. Bioconcrete uses microbial activity to produce mineral compounds that fill cracks in the concrete. By lowering concrete fractures and maintenance costs for reinforced concrete structures, autonomous healing increases structural durability. Because biomineralization is an organic process that benefits the environment while enhancing the compressive strength of broken concrete, it is advised and got huge potential for future concrete works [4–6].

The pH of the concrete, nucleation sites, dissolved inorganic carbon, and the presence of calcium ions throughout the mixture are all important in the production of calcium carbonate, which is directly related to the self-healing process. The efficient self-healing of concrete is influenced by a number of additional elements, including the type of bacteria used, their concentrations, the various curing techniques used, and the material used to incorporate bacteria [7].

In the current study, two bacteria, *Bacillus subtilis* and *Bacillus cereus*, which are noncontagious ureolytic bacteria, are used separately to increase the mechanical strength and durability of cement mortar through a process called microbiologically induced calcite precipitation (MICP).

In comparison to previously reviewed articles, this study used two bacteria, *Bacillus subtilis* and *Bacillus cereus*, to conduct tests on mechanical strength and durability properties, including tests on flexural and compressive strength as well as tests on water absorption, self-healing analysis, and sorptivity. Although the bacterial species is the same as that studied by other researchers, the effectiveness of the bacterial strain depends on the environmental factors and incorporation strategies. Because of this, the authors chose the two bacterial strains to examine their effects on concrete self-healing capacity in Ethiopia's temperate environment.

In the current study, the impact of adding *Bacillus subtilis* and *Bacillus cereus* separately on the cement mortar's compressive strength, flexural strength, water absorption, and sorptivity were assessed. Using a 3D optical microscope, it has been determined how the hardened cement mortar crack recovery works.

2. Materials and Methods

2.1. Materials. Cement, fine aggregate, and water were weighed out to create a batch of mortar mix. The mortar mix contained Dangote Cement Factory's 42.5R-grade Ordinary Portland Cement (OPC) cement. Cement, fine aggregate, and water were used to make the mortar in this study. The cement was 42.5R Ordinary Portland Cement (OPC), made by Dangote Cement Factory, and it met ASTM C150-04 standards. River sand that is clean, well-graded, and readily available in the area was used as the fine aggregate; it has an ASTM C33-compliant specific gravity of 2.75. In order to prepare batches of mortar mix, portable drinking water was also used. According to ASTM Standards, material property tests are conducted on aggregates.

Bacillus subtilis and *Bacillus cereus*, ureolytic-type bacteria that can produce urease during the metabolic process, were provided by the Ethiopian Biodiversity Institute in Addis Ababa for use in this study.

The *Bacillus genus* contains *Bacillus subtilis*, which is the most effective species at converting urea to ammonium carbonate. *Bacillus subtilis* can produce incredibly hardy dormant endospores that are readily found in the top layer of soil in response to nutrient deficiency and other environmental stresses [8]. According to Akindahunsi et al. [9]; the spores produced by *Bacillus subtilis* cells, which are Gram-positive rod-shaped strictly aerobic bacteria, can endure extremely high temperatures for a very long time. An alkaline environment and high mechanical pressure are also protected by this spore formation. According to Khaliq and Ehsan [10]; members of the genus Bacillus can produce spores that can dormant for more than 200 years. Despite being friendly and heavily commercialized by a number of businesses, they have not been used enough in civil engineering applications [11].

Bacillus cereus is a rod-shaped, Gram-positive, facultatively anaerobic, beta-hemolytic, motile, and spore-forming bacterium that is typically found in soil, food, and marine sponges [12]. Its ubiquity in various environments, spore production, and capacity for adaptation to changing circumstances [13].

2.2. Methods

2.2.1. Preparation of Culture Media for Bacteria Growth. *Bacillus subtilis* and *Bacillus cereus* were both grown in a sterile liquid medium that contained peptone powder, yeast extract, beef extract, sodium chloride, sodium bicarbonate, calcium chloride, nutrient broth, and urea. The medium also contained 10 g/L of sodium chloride, 6 g/L of calcium chloride, and 6 g/L of sodium bicarbonate. Adding agar (20 g/L) to the aforementioned elements created the solid medium [14].

The prepared medium was warmed on the hot plate until it began to boil before being used to combine all the ingredients in the larger flask. The media is autoclaved after the ingredients have been boiled and combined in order to sterilize the media. At a temperature of 120°C and a pressure of 15 pascals, the autoclaving procedure was carried out.

Finally, an 18 g/L urea solution that was made in a 250 ml flask was added to the autoclaved urea-free media. The cultured medium was added to six test tubes after being pH-adjusted to 9.5. Each test tube was infused with two bacteria from the gene bank, *Bacillus subtilis* and *Bacillus cereus*, and then incubated at 30°C for 24 hours. Within 24 hours. Using a turbidimeter, the bacteria concentration was measured and adjusted to be 108 cells/ml during the incubation process. One liter of urea-$CaCl_2$ media underwent the same procedure once more. The bacterial solution was measured and adjusted to be 108 cells/ml after incubating for 72 hours at 30°C. It should be noted that the entire culturing procedure was carried out in sterile conditions. Bacterial concrete was prepared by first making the necessary volume of bacterial solution, then adding it to the mixing design with water that was equal to 2% of the cement in volume [15].

2.2.2. Mix Proportion of Mortars. Ordinary Portland Cement (OPC), sand, and water were combined to create the mortar. The mortar was created in accordance with ASTM C348 by mixing cement and sand in a 1 : 2:75 ratio and water to cement in a 0 : 485 ratio. Table 1 displays the compositions of the mortar mixes. In order to prepare samples for bacterial mortar mixing, *Bacillus subtilis* and *Bacillus cereus* were used. A control mortar mix was also made in order to study how adding bacteria affected the mortar mix. In the mortar, 2% by volume of cement and bacteria were combined. For various tests, including the flexural strength test, compressive strength test, water absorption test, and cement mortar sorption test, the mortar was cast in a mold that measured 4 cm by 4 cm by 16 cm. The test specimens were remolded and placed in a wet case after being stored for 24 hours. Before testing, the samples were kept moist.

2.2.3. Test Techniques and Procedures. The objective of this investigation is to examine the mechanical and robustness characteristics of bacterial mortar produced separately using *Bacillus subtilis* and *Bacillus cereus*. 27 mortar samples were cast and tested for flexural strength and compressive strength, and another 18 samples were cast and tested for water absorption and sorptivity of mortars. In order to determine the impact of bacteria on the effectiveness of mortar's crack healing, nine additional samples were cast and examined under a 3D optical microscope. A total of 54 mortar specimens were cast and tested in order to complete the research's objective.

(1) Flexural Strength Test of Mortar. Using cuboid specimens with a geometry of 4 cm 4 cm·16 cm, the compressive and flexural strengths of control and bacterial mortar samples were examined. The test specimens were taken out of the casting framework after 24 hours and kept moist until they were tested. Following seven and twenty-eight days of curing at room temperature, the three-point bending strength was first calculated on each testing date, and then the compressive strength was assessed using a loading frame. The broken half prisms that were put through a flexure test were kept damp until they went through a compression test.

Table 1: Mix composition of mortar samples.

S. no	Ingredients	Units (kg/m^3)
1	FA	2016.0
2	OPC	840.0
3	Water	129.0

As per ASTM C348, the flexural strength of mortars with 4 cm × 4 cm × 16 cm can be calculated by

$$S_f = 0.0028P, \tag{1}$$

where S_f = flexural strength, MPa. P = total maximum load, N.

(2) Compressive Strength Test of Mortar. Following the flexural strength test, the remaining prism halves were used once more to conduct the compressive strength test in accordance with ASTM C349-02 specifications. Utilizing compression test equipment, the test was carried out on the faces' smooth side. Up until failure in fracture, the load increment is applied smoothly throughout the entire load application.

As per ASTM C349, the test specimens' portions of prisms made and broken in flexure in accordance with ASTM C348 can be used to calculate the compressive strength of mortars.

$$S_f = 0.00062P, \tag{2}$$

where S_c = compressive strength, MPa, P = total maximum load, N.

(3) Water Absorption Test of Mortar. The amount of water absorbed by the mortar while the mortar prisms are completely submerged in the water was measured using the water absorption test in accordance with ASTM C642-97. The mortar specimens are oven dried for 24 hours at 1100 C, then allowed to cool and weighted. The weight was noted as the dry weight (W_1). The specimen was then left in water for the following 72 hours at room temperature. The specimen's wet weight (W_2) was then recorded as being this weight.

$$\text{Water absorption (\%)} = \left(\frac{W_2 - W_1}{W_1}\right) * 100, \tag{3}$$

where W_1 = oven dry weight of mortar specimen, kg, W_2 = wet weight of mortar specimen, kg.

(4) Sorptivity Test of Mortar. In accordance with ASTM C1585-04, this test method is used to ascertain the rate of water absorption (sorptivity) by hydraulic cement mortar by calculating the increase in mass of a specimen as a function of time after water absorption when only one surface of the specimen is exposed to water. During initial contact with water, capillary suction dominates water ingress through the exposed surface of the specimen, which is submerged in water.

Test samples from the three mortar categories (C-M-0, B-M-1, and B-M-2) were dried in room temperature for 24 hours after 28 days of curing. To ensure one-way flow through the specimen, the sides were sealed with an epoxy coating. Each specimen only had one surface in contact with the 3–5 mm-deep water. The 40 mm × 160 mm side of each test prism was submerged in water at a level height of 3 to 5 mm above the base of the test mortar prisms. Each sample's water absorption was measured at predetermined intervals of 1, 5, 10, 15, 20, and 30 minutes, 1, 2, 3, 4, and 5 hours, and 1, 2, 3, 4, 5, 6, and 7 days. Specimens were immediately submerged again after each measurement. At the conclusion of each interval, the mortar samples were removed from the water, and the new weight was calculated after a fresh towel had been used to dry the submerged surface. By weighing the mortar samples and re-immersing them, this procedure was repeated for up to 7 days.

$$S = \frac{\mathbf{I}}{\sqrt{\mathbf{t_e}}}, \quad (4)$$

where S = sorptivity coefficient in mm/min$^{0.5}$, t_e = elapsed time in min.

In this case, the following formula can be used to determine the total water absorption per unit area of the inflow surface.

$$\mathbf{I} = \frac{\Delta \mathbf{w}}{\mathbf{A_s} * \boldsymbol{\rho}}, \quad (5)$$

where ΔW = change in weight of cube after the elapse time = $W_2 - W_1$. W_1 = dry weight of mortar prism in grams; W_2 = weight of mortar prism after t time capillary suction of water in grams, A_s = surface area of the specimen through which water penetrated; and ρ = density of water

3. Results and Discussion

This section reports the results of tests for flexural strength, compressive strength, water absorption, sorptivity, and three-dimensional optical microscopic imaging.

3.1. Flexural Strength Test of Mortar. At 7, 14, and 28 days after curing, the bending strengths of control and bacterial mortars were evaluated. As shown in Figure 1 below, the flexural strength test results showed that the bacterial mortar was stronger than the control mortar. When the flexural strength of mortar containing *Bacillus subtilis* bacteria, B-M-1, is compared to control mortar after 7 days, 14 days, and 28 days, it increases by 9.68%, 13.82%, and 17.29%, respectively. Similar increases of 7.17%, 9.42%, and 11.31% were noted in mortar containing *Bacillus cereus* bacteria, B-M-2, for periods of 7 days, 14 days, and 28 days, respectively. When two bacterial mortars were compared to one another at 7, 14, and 28 days, the *Bacillus subtilis* (B-M-1) mortar increased by up to 2.34%, 4.01%, and 5.37%. As seen in earlier studies, microbially induced calcite precipitation fills the pores in mortar, increasing the flexural strength [1, 3, 4, 7, 16, 17]. Bacterial mortar has generally been shown to have a higher flexural strength.

3.2. Compressive Strength Test of Mortar. At 7, 14, and 28 days after curing, the compressive strengths of the bacterial and control mortars were evaluated in this study. In comparison to the control mortars over time, the compressive strength test results for the bacterial mortar showed an increase in strength, as shown in Figure 2. A maximum compressive strength of 8.60 MPa, 23.13 MPa, and 27.67 MPa were observed at 7, 14, and 28 days, respectively, in samples of bacterial mortar containing *Bacillus subtilis*, B-M-1, according to the results. The compressive strength of the B-M-1 increased by 7.22%, 9.92%, and 17.77% at 7, 14, and 28 days, respectively, when compared to control mortar specimens. In addition, *Bacillus cereus*, B-M-2-containing bacterial mortars revealed maximum compressive strengths of 8.43 MPa, 22.38 MPa, and 26.52 MPa at 7, 14, and 28 days, respectively.

Comparing the compressive strength of the B-M-2 to control mortar specimens at 7, 14, and 28 days, respectively, revealed increases of 51%, 69%, and 121%. Similar outcomes are seen in as the addition of bacteria increases the compressive strength of the mortar. The compressive strength of two bacterial mortars was compared, and at 7, 14, and 28 days, the mortars with *Bacillus subtilis* (B-M-1) increased by up to 1.96%, 3.32%, and 4.36%, respectively, compared to the bacterial mortar with *Bacillus cereus* (B-M-2). The increase in compressive strength is the result of ureolytic bacteria's hydrolysis of urea, which results in the production of carbonate ions [1–3, 7, 16–22].

3.3. Water Absorption Test of Mortar. Both the control mortar and the bacterial mortar mixes underwent a water absorption test after 28 days of curing. According to the findings, when bacteria such as *Bacillus subtilis* (B-M-1) and *Bacillus cereus* (B-M-2) are added to mortar, the amount of water that is absorbed is less than when conventional mortar is used. Water absorption decreases from 11.85% in control mortar samples to 8.62 and 9.37% in B-M-1 and B-M-2 bacterial mortar mix samples after 28 days. Figure 3 depicts the water absorption test results.

At 28 days, the water absorption of the bacterial mortars was decreased by 37.48% and 26.43% for B-M-1 and B-M-2, respectively. The bacterial mortar with *Bacillus subtilis* (B-M-1) decreased by up to 8.73% compared to the bacterial mortar with *Bacillus cereus* (B-M-2) at 28 days when water absorption of the two bacterial mortars was compared to one another. According to test results, adding bacteria to the mortar matrix significantly reduced the amount of water that the control mortar absorbed. Because calcite sediments accumulate in mortar pores as a result of the presence of bacteria, the mortar absorbs less water. Similar outcomes are attained in [5, 16, 18–21, 23–25], as the presence of bacteria increases the ability of mortars to absorb water.

3.4. Sorptivity Test of Mortar. After 28 days of curing, the sorptivity test for both the bacterial and control mortar mixtures was conducted. In comparison to conventional mortars, the results show that both *Bacillus subtilis* (B-M-1) and *Bacillus cereus* (B-M-2) bacteria-infused mortars have

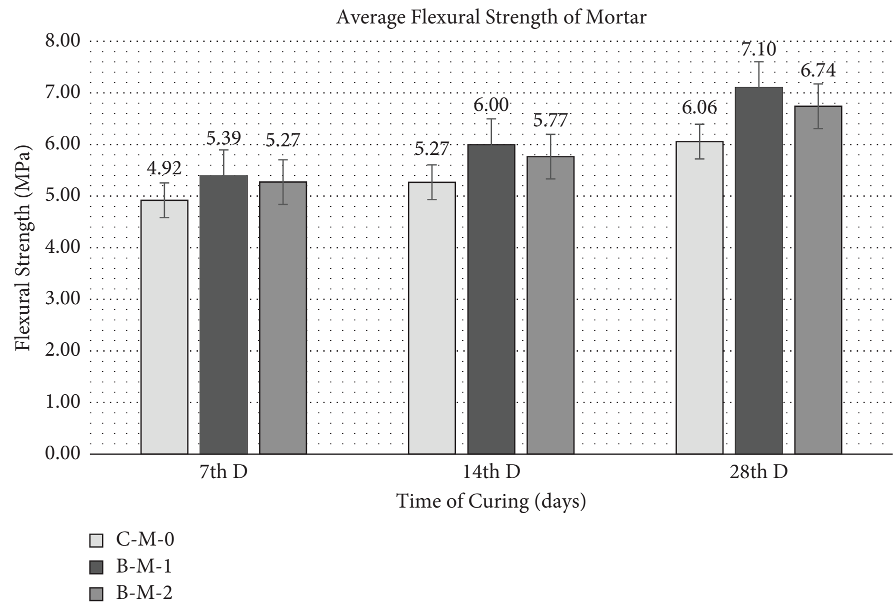


Figure 1: Flexural strength of mortar specimens at 7, 14, and 28 days.

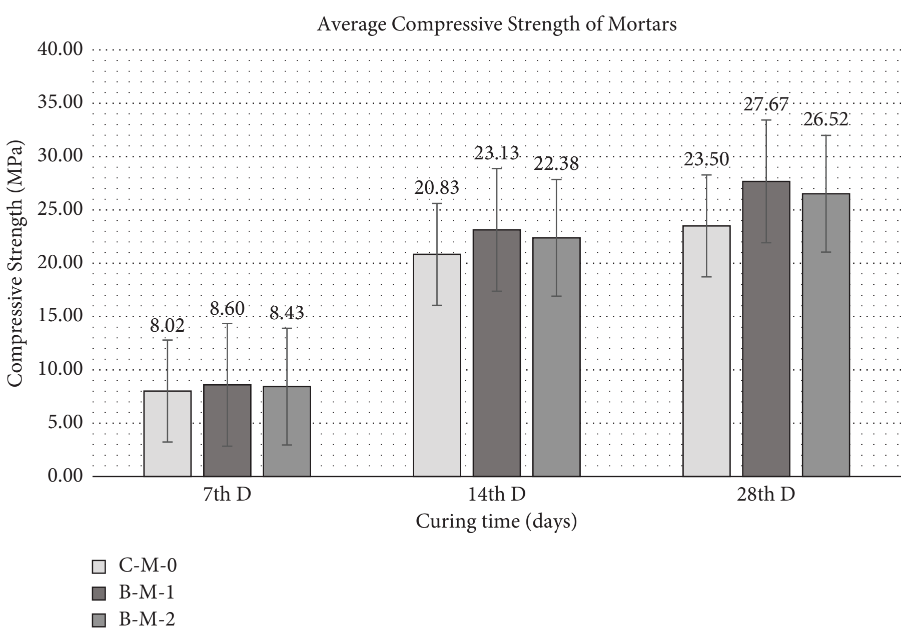


Figure 2: Compressive strength of mortar specimens at 7, 14, and 28 days.

lower initial and secondary absorption rates. Figure 4 shows the analysis outcomes of the bacterial and control mortar sorptivity tests.

tAt 28 days, the initial rate of bacterial mortar absorption for B-M-1 and B-M-2 was reduced by 82.14% and 50%, respectively. The secondary rate of absorption for mortars inoculated with the bacteria B-M-1 and B-M-2, respectively, also decreased by 75% and 40%. Mortars containing *Bacillus subtilis* had lower initial and secondary rates of absorption than mortars containing *Bacillus cereus* in terms of the two bacterial species, by 21.43% and 25%, respectively. According to test results, adding bacteria to the mortar's matrix significantly decreased the primary and secondary rates of absorption of bacterial mortars B-M-1 and B-M-2. Due to the deposit of calcite sediments in mortar pores, the incorporation of bacteria in mortar decreases its rate of water absorption. Similar results are obtained in [5, 6, 19, 20, 26–36], as the inclusion of bacteria increases the rate at which mortars absorb water.

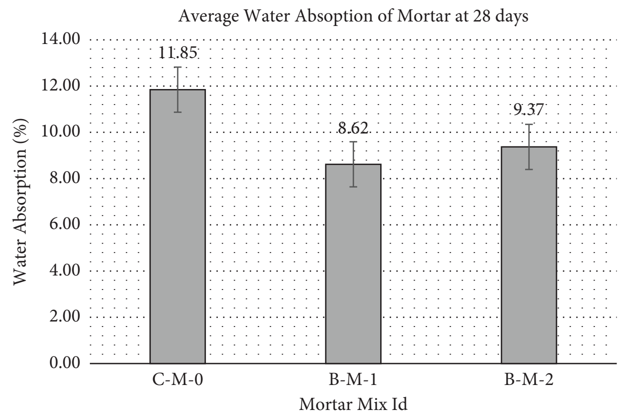


Figure 3: Water absorption test result of mortar specimens at 28 days.

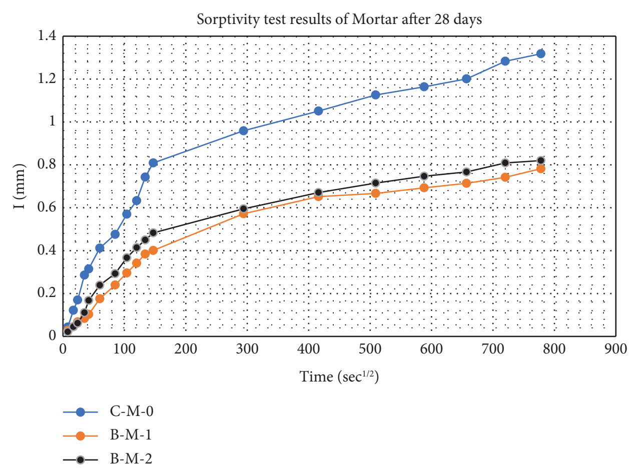
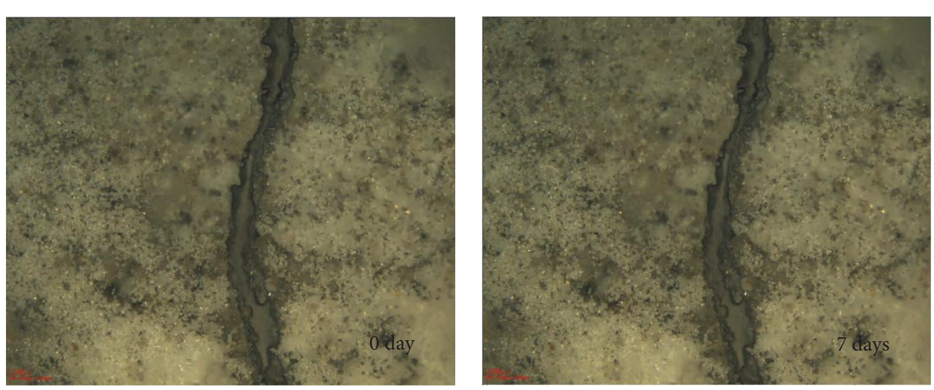


Figure 4: Sorptivity (rate of absorption) test result of mortar specimens at 28 days.



(a)

(b)

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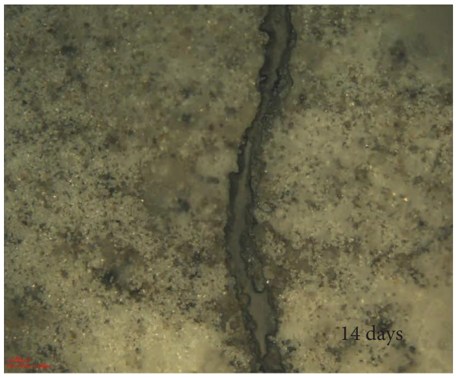

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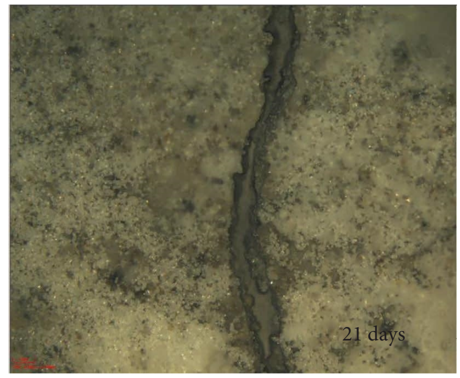

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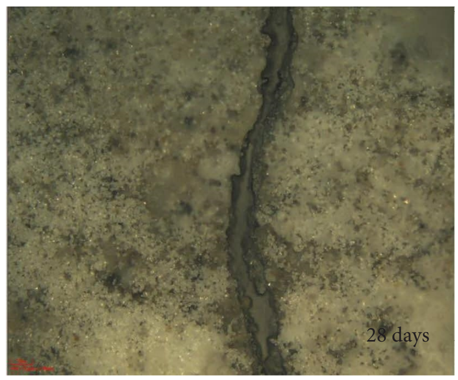
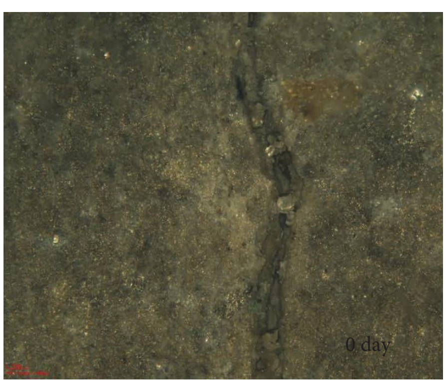


(e)

FIGURE 5: Images from 3D optical microscope for evolution of crack healing of control mortar (C-M-0) during (a) 0 day, (b) 7 days, (c) 14 days, (d) 21 days, and (e) 28 days.



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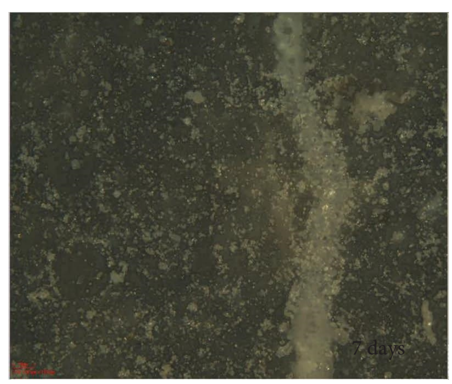

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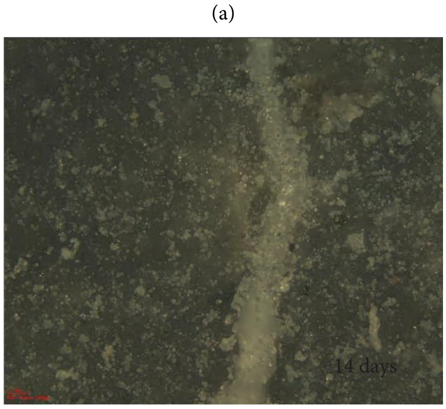

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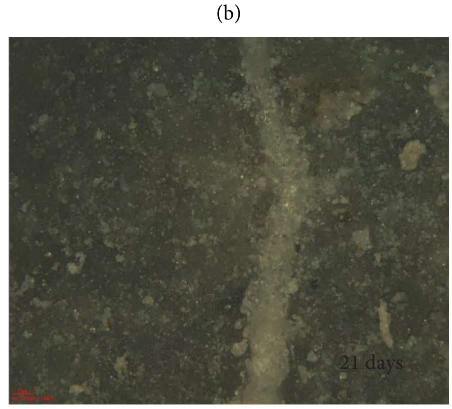

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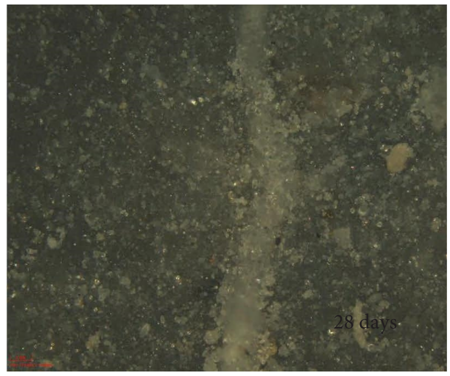
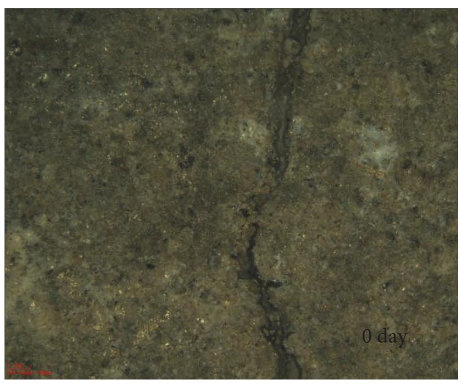
FIGURE 6: Continued.



(e)

Figure 6: Images from 3D optical microscope for evolution of crack healing of mortar with *B subtilis* (B-M-1) during (a) 0 day, (b) 7 days, (c) 14 days, (d) 21 days, and (e) 28 days.



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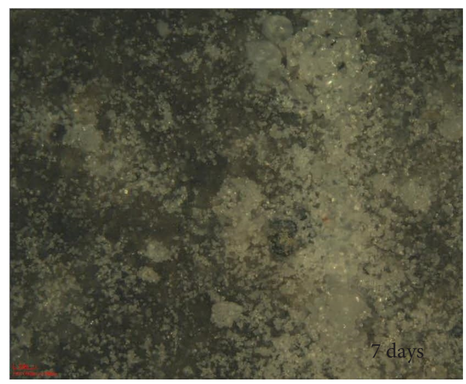

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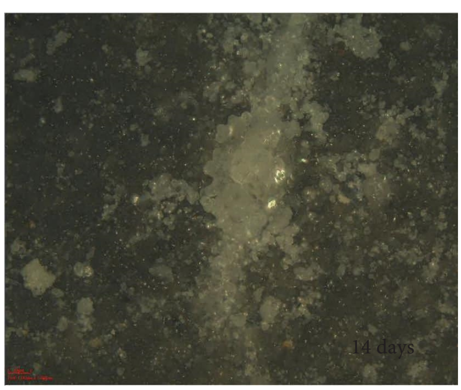

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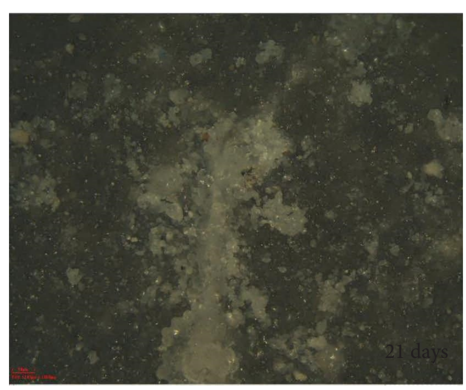

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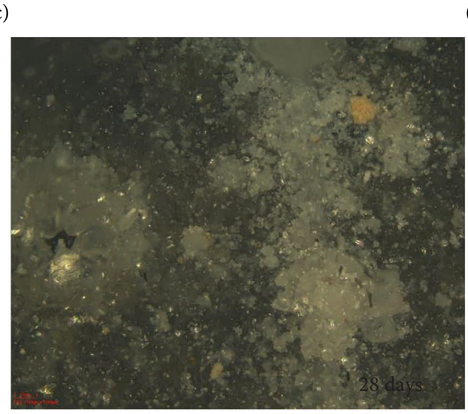

(e)

Figure 7: Images from 3D optical microscope for evolution of crack healing of mortar with *B cereus* (B-M-2) during (a) 0 day, (b) 7 days, (c) 14 days, (d) 21 days, and (e) 28 days.

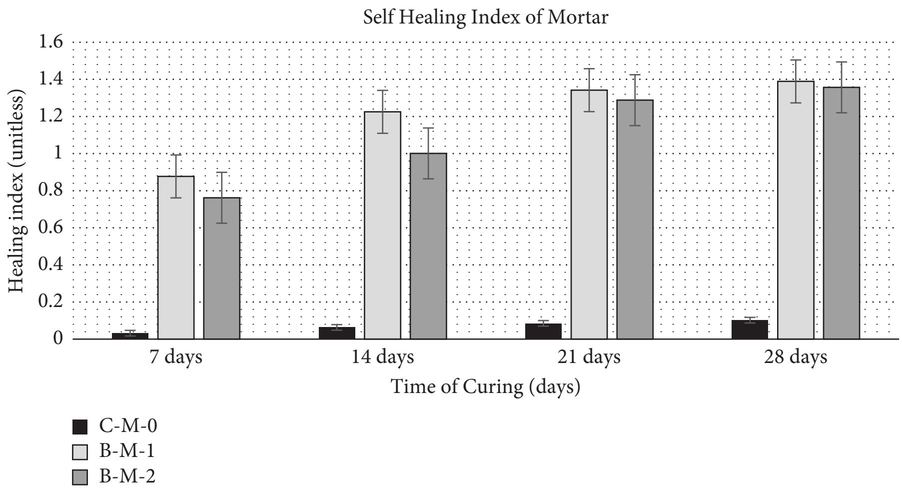

Figure 8: Healing index result of mortar specimens from 3D optical microscope.

3.5. Self-Healing Analysis of Mortars at Microlevel. Using a compression testing machine on nine samples of control and bacterial mortar with dimensions of 2.5 cm × 4 cm × 4 cm, visible cracks were introduced in this study after curing for seven days. The precracked specimens were observed by a 3D optical microscope at AASTU at 0 day, 7 day, 14 day, 21 day, and 28 day after cracking to assess the effectiveness of the self-healing process. Significant self-healing was evident in these cracks, and larger amounts of white crystal precipitates were produced, nearly filling the crack surface.

The surface of the samples had the healing substance, white crystals, inlaid with self-healing bacteria, as shown below in Figures 5–7. Few researchers have observed and discussed the formation of these therapeutic compounds [29, 37–41] as a result of bacterial transformation of calcium chloride into calcium carbonate.

According to the findings shown in Figure 8 in the following, the specimens C-M-0, B-M-1, and B-M-2's surface crack healing efficiency indexes after immersion in water for up to 28 days were checked at a seven-day interval. According to the results, after 28 days of curing, B-M-1 had the highest normalized healing index (1.389), followed by B-M-2 (1.357). Calcium carbonate formation was the primarily cause of this phenomena.

4. Conclusions

This study investigated how the behavior of the bacteria *Bacillus subtilis* and *Bacillus cereus* affected the mortar's capacity for self-healing. In the current study, the mechanical characteristics and durability traits of mortar were examined and quantified. At various points during the curing process, tests were done to measure properties such as compressive strength, flexural strength, water absorption, and sorptivity. In addition to the tests mentioned above, cracked mortar surfaces were regularly checked with a 3D optical microscope. The primary research findings of the current research work are then presented.

(1) The flexural strength of mortars was increased by individually applying bacterial agents. For 7 days, 14 days, and 28 days, respectively, the flexural strength of the *Bacillus subtilis* specimens increased by 9.68%, 13.82%, and 17.29%. Similar to this, *Bacillus cereus* exhibits a percentage increase of 7.17%, 9.42%, and 11.31% for 7 days, 14 days, and 28 days, respectively, in comparison to the control mortar.

(2) Mortar's compressive strength is improved at 7, 14, and 28 days by the addition of bacteria. The maximum 28-day strengths of the bacterial mortars B-M-1 and B-M-2 were observed to be 27.67 MPa (about 17.77%) and 22.38 MPa (about 12.84%) higher than those of the control mortars, respectively.

(3) Water absorption was lower in the mortar treated with the bacterial solution than it was in the control mortar. At 28 days, for B-M-1 and B-M-2, respectively, the bacteria addition reduced water absorption by 37.48% and 26.43% when compared to the control mortar.

(4) The results of the mortar's sorptivity tests showed that adding each bacterium separately to the mortar matrix significantly decreased the initial and secondary rates of bacterial mortar absorption.

(5) White crystal precipitates were being produced in greater quantities in bacterial mortar cracks, and the surface of the crack was almost completely healed. At 28 days after curing, B-M-1 had the highest normalized healing index (1.389) compared to the control mortars, while B-M-2 had the lowest (1.357).

(6) *Bacillus subtilis* seems to be superior to *Bacillus cereus* in mortar mechanical strength and durability results.

Data Availability

All the data used in this study are included in the manuscript.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

All authors have contributed equally to this work.

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