Performance of Bacteria-Based Non-encapsulated Self-healing Concrete

G. Vigneswaran, K. Poonguzhali, D. Gowdhaman, A. Sumathi, and A. Rajesh

Abstract This study focuses on the mechanical performance of non-encapsulated self-healing concrete using bacteria by direct application. The influence of *Bacillus subtilus* bacteria on crack healing, compressive strength regains, sorptivity, water absorption, impact strength, and concrete microstructures was examined in this study. M30 grade concrete with a water-cement ratio of 0.45 was used for control specimens. For bacteria incorporated specimens, water content was fully replaced with three different percentage of healing agent. The healing agent comprises of 10, 20, 30% bacterial solution (BS) and 90, 80, 70% nutrient solution (NS) was directly mixed with concrete mixtures with bacillus subtilis bacterial concentration of 10^5 cells/ml and the mixtures were designated as BC 1, BC 2, and BC 3. The concrete specimens were subsequently cured by two methods; wet-dry cycle and full-wet and the results were compared with the control. The cast specimens were immersed in water for 24 h, then held at room temperature for another 24 h in the wet-dry cycle, which was repeated for 28 days. Specimens were immersed in water for 28 days during full-wet curing. However, the curing water was changed every 24 h to ensure that the bacteria had enough oxygen to precipitate calcium carbonate. Results show that the addition of bacteria enhances the mechanical properties compared with control concrete. SEM and XRD results show the micro-structural morphology and the calcium carbonate precipitation.

Keywords Self-healing concrete · Crack healing · Compressive strength · Split tensile strength · Impact strength · *Bacillus subtilus*

G. Vigneswaran · K. Poonguzhali · A. Sumathi (✉) · A. Rajesh
School of Civil Engineering, SASTRA Deemed to be University, Thanjavur, India
e-mail: sumathi@civil.sastra.edu

D. Gowdhaman
School of Chemical and Biotechnology, SASTRA Deemed to be University, Thanjavur 613401, India
e-mail: gowdhaman@biotech.sastra.edu

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1 Introduction

Self-healing concrete or microbial concrete are other names for bacterial concrete. It's a sort of concrete that can create limestone organically to fill in fractures on the surface of any concrete construction. Bacteria that can continuously precipitate calcite can be embedded in concrete to create "Bacterial concrete". A healing agent that works by converting nutrients into limestone by microorganisms implanted in concrete. Concrete wakes up when it is cracked or deconstructed and comes into contact with water and oxygen. With the help of nutrients, the bacteria will multiply and start to produce Calcium carbonate which helps in filling the cracks. The limestone solidifies on the cracked surface. Preparation of bacteria is of three types, i.e., Direct application, encapsulation, and immobilization. CEMI and CEMIII with 60% Ground Granulated Blast Furnace Slag (GGBS) with and without non-encapsulated iron aerobic respiration bacteria, according to [1]. The water absorption velocity was dramatically reduced when a micro agent was added to CEMI and CEMIII concrete samples. When microbial is introduced to concrete in the form of CEMIII, water absorption is reduced by at least 25% [2] represents the research was conducted out with 6 different microbial concentration levels in concrete water mixtures. Concrete specimens were cured for 7, 14, and 28 days. In the samples with a microbial concentration of 10^5 cells/ml of water, a highest of 32% rise in compressive strength, 14% growth in split tensile strength, and 29% enhancement in flexural strength were reported. Kunal et al. [3] identified in their study that after 91 curing days, utilizing 10% bacterial-treated in concrete resulted in 26.6% increases in strength as compared to the control (CC) treatment. Water absorption (64%) and porosity (53%) both decreased significantly, whereas chloride permeability decreased by 22%. According to Luo et al. (2016), adding the Type 1 ingredient to cementitious material reduced compressive strength by roughly 14.7%, 6.8%, and 0.1% after 3,7, and 28 days of curing, respectively. Type 2 admixture causes a compressive strength loss of roughly 1.6 and 2.2% after 3 and 7 days of curing, respectively, and an 8.1% rise after 28 days of curing. After three days, the carbonation depths of control samples for Type 1 and 2 specimens were 6.6 mm, 7.0 mm, and 6.5 mm, respectively. Reference [4] studied the present investigation looked at how Bacillus sp. CT-5, isolated from cement, affected strength as well as durability. Also, in relation to bacterial cells, the compressive strength of cement mortar boosted by 36%. As a consequence of bacterial calcite deposition, treated cubes consumed 6 times less moisture than control cubes. Pachaivannan et al. [5], The strength viz., compressive, split tension, and flexure of 14 days old bacterial concrete was higher than that of CC on the 7th, 14th, and 28th day. Ureolysis of *Bacillus Subtilis* in yeast extract and peptone medium results in $CaCO_3$ precipitation, according to Nguyen et al. [6]. The addition of a microbial adjuvant resulted in a large reduction in permeability of gas of around 70% after 210 days. After 44 days of water immersion, the 400 m fissures in the bacterial concrete were fully closed. De Belie et al. (2015) studied the mechanical properties like compressive and tensile properties of three flexure beams (40 mm × 10 mm × 160 mm) by encapsulating, Bacillus sphaericus in Modified Alginate hydrogel. On addition of 0.5 and

1% of Modified Alginate hydrogel tensile strength was reduced by 15.6 and 30%; compressive strength was reduced by 16.2 and 23.4%. Hence higher dose addition of Modified Alginate hydrogel reduces the mechanical properties. Navneet [7] evaluated the effect of Sporosarcina pasteurii bacteria in silica fume added concrete. Mechanical properties like compressive strength, water absorption and rapid chloride permeability test was conducted. Results indicate that addition of Sporosarcina pasteurii in concrete, Compressive strength was increased by 38.2 MPa for 28 days and 44 MPa for 91 days. Meanwhile, porosity and water absorption capacity are reduced. Nidhi [8] conducted compressive strength and tensile strength for 150 mm × 150 mm × 150 mm cube and 150 mm dia with 300 mm height cylinder specimens. *Bacillus subtilis*, Bacillus megaterium and consortia bacteria were added in concrete specimens. Results concluded that compared to conventional concrete, compressive strength was increased by 14.36, 22.58, and 15.86% and split tensile strength was increased by 25.3, 18.29, and 19.51%. According to Shanmuga Priya et al. [9] by replacing cement with Micronized Biomass Silica (MBS) at 4%, 8%, and 12% and bacteria addition, strength, and durable properties were compared. With 20 ml bacterial solution and cementitious addition of 8% compressive strength was increased by 13.53%, splitting tensile strength increased by 16.38% and flexural strength increased by 13.32%. M. [10] Bacillus paralicheniformis was extracted bacteria from concrete made of Portland pozzolanic cement containing all composites. 28-day Compressive strength of bacteria added Portland pozzolanic cement concrete is 2.8% which is lower than conventional concrete and bacteria added Portland cement type 2 is 1.96% lower than conventional concrete. Water absorption percentage of the two types of cement concrete is 0.07 and 0.19, respectively. Salman Rais et al. [11] used gram-positive aerobic bacteria, Bacillus megaterium in recycled aggregate concrete with supplementary cementitious additions of silica fume and metakaolin. With micro silica and metakaolin additions, 28-day compressive strength ranged from 73 to 93% but for 120 days the compressive strength tends to decrease from 57 to 85%. Ratio of permeability coefficient. For 28 days ranged from 143 to 173% but for 120 days it increased from 163 to 181%. The aim of the current study is to enhance the mechanical performance of bacteria-based non-encapsulated self-healing concrete by direct application adopting two curing stages. The water has been completely replaced by the healing agent. The healing agent constituted 10%, 20%, 30% of *Bacillus subtilus* bacterial solution (BS) and 90%, 80%, 70% of nutrient solution (NS), respectively and specimens were cast and the bacteria concrete results were compared with the CC.

2 Experimental Programme

2.1 Materials and Methods

Ordinary Portland cement (OPC) 53 grade, with a specific gravity (SG) of 3.15 and a fineness modulus of 6.95%, was used confirming IS 12269 [12]. Fine aggregate (FA) river sand was used with a maximum size of 4.75 mm and a SG of 2.65 according to IS 383 [13]. Crushed gravel with a SG of 2.74 was used with sizes 12.5 and 20 mm. ***Bacillus subtilis*** was initially cultured and inoculated in the nutrient medium and Nutrient solution is prepared with Calcium nitrate, urea, and yeast extract concentrations was 5 g/l, 5 g/l, and 3 g/l by cement mass, respectively. Figure 1 shows the bacteria which is prepared in the petri dish. *Bacillus subtilus* properties are shown in Table 1.

Fig. 1 Bacillus subtilus in petri dish

Table 1 Morphological and biochemical characteristics

S. No.	Test	Observation
1	Configuration	Circular lobate
2	Elevation	Flat
3	Pigmented	White
4	Gram reaction	Gram positive
5	Shape of isolate	Rod in chains
6	Margin	Irregular
7	Endospore staining	Central spore

2.2 Preparation of Bacterial Cell Solution

To prepare the media and reagent preparation, 200 ml distilled water is taken in 250 ml conical flask and Luria broth(25g/l) is added to it and autoclave at 121 °C for 20 mins, cool it down for 20mins in Fig. 2. Then 50ml distilled water is taken in 100 ml conical flask, LB Agar(15g/l) is added to it and autoclaved then pour the medium into a sterile petri dish and cool it to solidify as shown in Fig. 3. To prepare the pure culture, pick a single colony from the old culture and do a quadrant streak and incubate the plate at 37 °C for 24 h as shown in Fig. 4. The methodology is that the broth culture is prepared by inoculating the medium conical flask with bacteria and kept in incubator for 24 h and the bacterial solution is prepared in cultured medium in falcon tubes, which are spun in centrifuge machine for about 10 min. The supernatant and pellets were resuspended in saline solution, i.e., NaCl solution

Fig. 2 Broth medium preparation

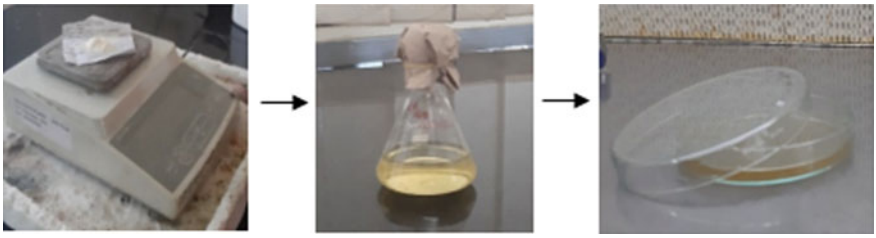

Fig. 3 Reagent preparation

Fig. 4 Pure culture preparation

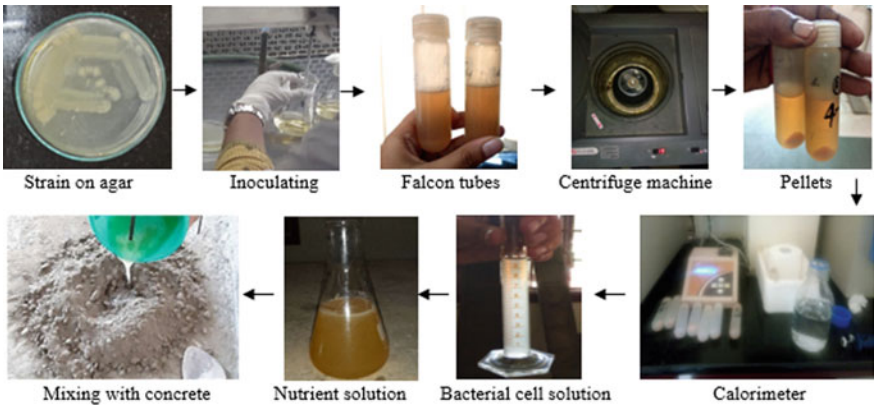

Fig. 5 Preparation of bacterial cell solution

(9g/l). Now, cell concentration is adjusted to 10^7 cells/ml in calorimeter to get the bacterial cell solution as shown in Fig. 5.

2.3 Mix Details and Cast Specimens

Mix design ratio for M30 grade of concrete was obtained as 1:1.70:2.33 as per IS 10262-2019 [14] with fixed water-cement ratio 0.45. In this work, mixes were prepared for different percentage of bacterial solution (BS) of 10%, 20%, and 30% and nutrient solution (NS) of 90%, 80% and 70% respectively. For each mix thespecimens were cast and cured under two different curing methods and the mix proportions details are shown in Table 2. Four different mixes were prepared i.e., Control concrete (CC) which is prepared with ordinary water, Bacterial concrete 10% (BC1), Bacterial concrete 20% (BC2), Bacterial concrete 30% (BC3) is prepared with healing agents by adopting two curing stages i.e., Wet-Dry (WD), Full-Wet (FW). Compressive strength (CS) was determined using 100 mm cube samples, according to IS: 516-1959 [15]. Cylinders of 100 mm diameter and 200 mm height were used for split

Table 2 Mix proportions (kg/m^3)

Mix	Cement	FA	CA	Water (0.45)	
				BS	NS
CC	437.77	811.1	1111.7	196.99	
BC 1	437.77	811.1	1111.7	19.7	177.29
BC 2	437.77	811.1	1111.7	39.4	157.59
BC 3	437.77	811.1	1111.7	59.1	137.89

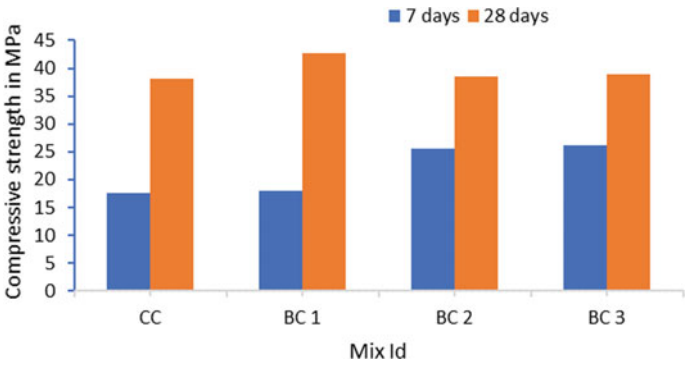

Fig. 6 Compressive strength results for 7 and 28 days

tensile strength (STS). To calculate the impact energy specimens of 150 mm in diameter and 64 mm in height were used in accordance with ACI 544 [16]. Sorptivity test was performed on cylindrical samples measuring 100 mm in diameter and 50 mm in height in accordance with ASTM C1585 [17]. Water absorption was measured using 100 mm cube specimens in accordance with ASTM C642 [18].

3 Results and Discussion

3.1 Compressive Strength (CS)

Figure 6 depicts the CS of cubes after 7 and 28 days. The CS of the BC is higher than the CC under full-wet curing due to the presence of bacteria. The CS of BC is higher than CC after 7 days and 28 days of curing. The increase in CS of BC 1 using *B. Subtilus* for 7 days is 2.43% and for 28 days is 12.12% higher than CC. The percentage increase in CS of BC 2 using *B. Subtilus* for 7 days is 44.55% and for 28 days is 1.23% higher than CC. The percentage increase in CS of BC 3 using *B. Subtilus* for 7 days is 48.52% and for 28 days is 2.52% higher than CC.

3.2 Regained Compressive Strength

Figure 7 shows regained CS of pre-cracked specimen on each mix After the bacterial specimens (BC 1, BC 2, BC 3) were pre-cracked at the age of 3 days under full-wet curing, the bacterial samples are cured under both the curing condition, i.e., Full-Wet and Wet-Dry conditions. After 25 days of curing process, the samples are tested in compression machine until the peak load. The regained CS of BC 1 in FW curing at 25th day was increased by 75.23% and in WD curing at 25th day was

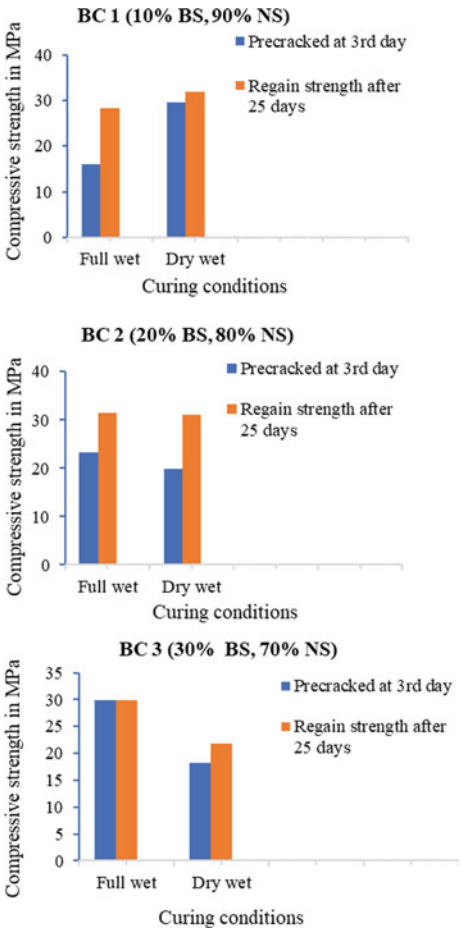

Fig. 7 Regained compressive strength on both curing conditions

increased by 8.31% when especially in comparison to the third day's pre-cracked strength properties. The regained compressive strength of BC 2 in FW curing at 25th day was increased by 35.43% and in WD curing at 25th day increased by 56.81% compared to the 3rd day pre-cracked strength. The CS of BC 3 in FW curing at 25th day was increased by 0.33% and in WD curing at 25th day was increased by 19.58% compared to the 3rd day pre-cracked CS.

3.3 Split Tensile Strength (STS)

The STS is performed after 28 days of full-wet curing and the results are shown in Fig. 8. Due to the addition of bacteria, the tensile strength of (BC) is generally higher than CC. The increase in the tensile strength of BC 1 is 12.13 % higher than

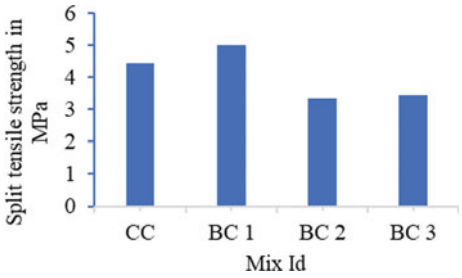

Fig. 8 Split tensile strength for 28 days

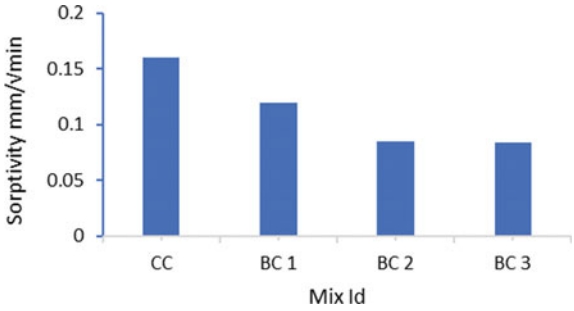

Fig. 9 Sorption coefficient results

the CC. BC samples containing 20 and 30% of bacteria show lesser tensile strength compared to the CC and the BC containing 10% of bacteria.

3.4 Sorptivity

Sorptivity results for CC and BC after 28 days of curing are shown in Fig. 9. The samples without microbes, i.e., CC, clearly demonstrated greater sorptivity than that of the samples with microbes. Sorptivity tends to decrease as microbes are added to the mix, and the setup becomes denser. Because of the enhanced filling potential of the capillaries and gaps, the sorptivity value reduced significantly to much more calcium silicate hydrate powder and excessive curing effectiveness in the mixture [3, 19, 20]. Percentage of sorptivity in BC 1, BC 2, BC 3 was 33.33%, 88.23%, 90.47% compared to the CC.

3.5 Water Absorption

The water absorption percentage of each mix is calculated for full-wet curing method at 28 days. The water absorption percentage gets decreased for the BC compared

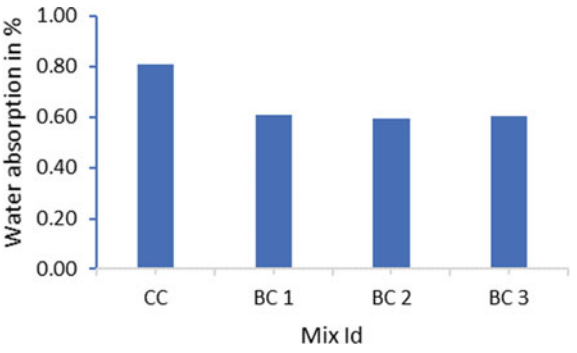

Fig. 10 Water absorption results

to the CC as shown in Fig. 10, presence of bacteria resulting in the filling of tiny capillaries and gaps than CC.

3.6 Impact Strength Test

To identify the impact strength of the concrete according to ACI 544. Impact energy is calculated for the interval of 28 days after full-wet curing. Figure 11 shows the crack pattern which appears on the bacterial and CC specimens. The impact energy was calculated for CC and bacterial specimens (BC 1, BC 2, BC 3) at first crack and final failure, and the results are shown in Fig. 12. Due to the addition of bacteria in the bacterial specimens which has higher number of blows and impact energy compared to the CC at first crack and final failure. The bacterial specimens (BC 3) show higher impact energy compared to Bacterial specimen (BC 2, BC 3). The figure shows the impact energy on each mix and number of blows on each mix. Thus, it concluded that the addition of bacterial spore in the concrete will give maximum strength.

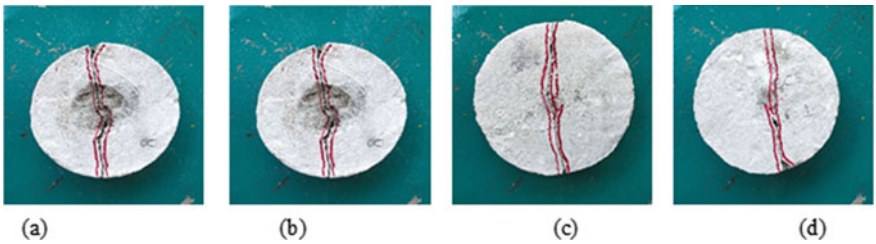

Fig. 11 Crack formation pattern **a** CC, **b** BC 1, **c** BC 2, **d** BC 3

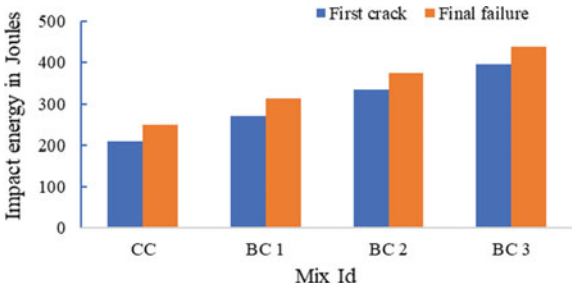

Fig.12 Impact strength test for 28 days

3.7 Visual Observation and Microscopic Observation of Bacterial Concrete

The crack healing efficiency was examined at intervals of 0, 7, 14 days under wet-dry and full-wet conditions for BC 1, BC 2, BC 3. After 28 days of healing period, the samples BC 1, BC 2, BC 3 were gathered in order to visualize the self-healing efficiency shown in Figs. 13, 14, 15, respectively, and microscopic view is shown in Fig. 16.

Table 3 shows the width of the cracks which is before healing and after healing of WD and FW. The crack healing percentage was examined in BC 1 (WD) is about 19% at 7 days, 39% at 14th day, and 84% at 28th day where in BC 2 (WD) is about 21% at 7th day, 44% at 14 days and 94% at 28 days and in BC 3 (WD) was 23% at 7 days, 47% at 14 days and 95% in 28 days, this ensures that cracks are healed fully at 28 days and it was not able to view. Crack healing percentage was examined in BC 1 (FW) is about 19% at 7 days, 39% at 14 days, and 84% at 28 days where in BC 2 (FW) is about 21% at 7 days, 43% at 14 days and 93% at 28 days and in BC 3 (FW) was 22% at 7 days, 46% at 14 days and 94% in 28 days (Table 4).

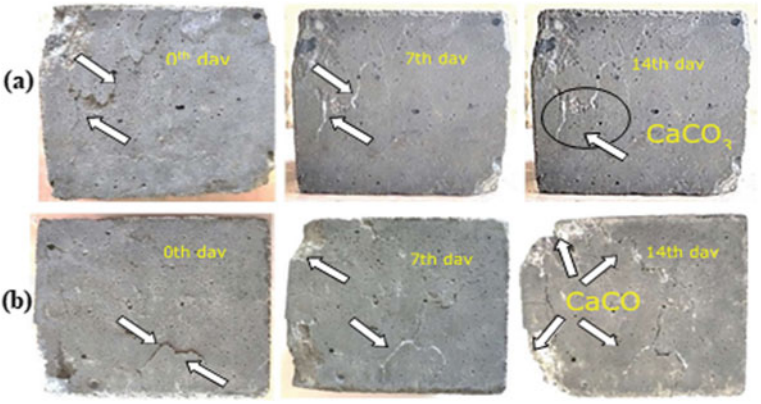

Fig. 13 Visual observations of BC 1 (**a**) cracks under FW and (**b**) cracks under WD

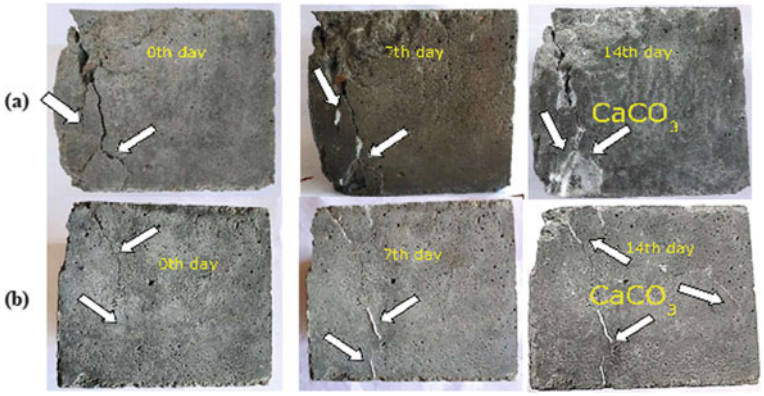

Fig. 14 Visual observations of BC 2 (**a**) cracks under FW and (**b**) cracks under WD

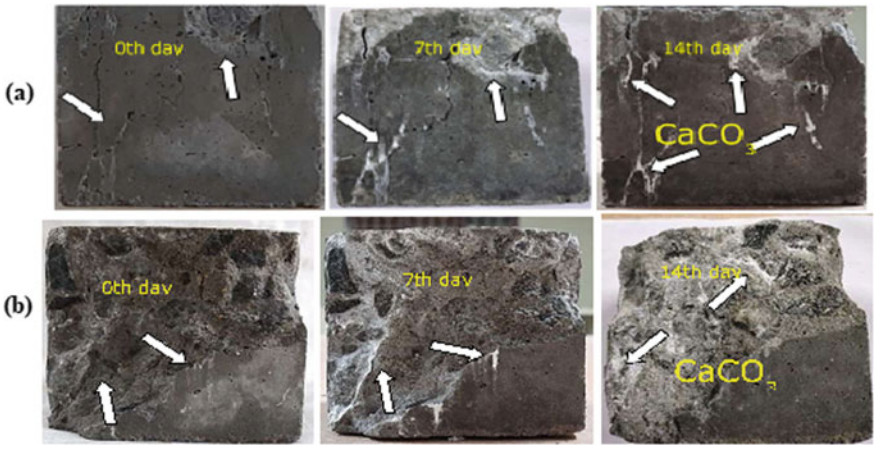

Fig. 15 Visual observations of BC 3 (**a**) cracks under FW and (**b**) cracks under WD

3.8 Scanning Electron Microscope (SEM)

Initially, the fracture was examined microscopically with a compact microscope. SEM analysis has been used to examine the morphology of the crushed test sample after 28 days and define the quality of material precipitated (i.e., calcium carbonate) to even further enhance the structural morphology of its particles [3, 6, 19–23]. To make a comparison of the microstructures of the BC 1, BC 2, BC 3, and CC samples, specimens were tested from the control specimen and the specimens containing bacteria. The SEM images are shown in Fig. 17.

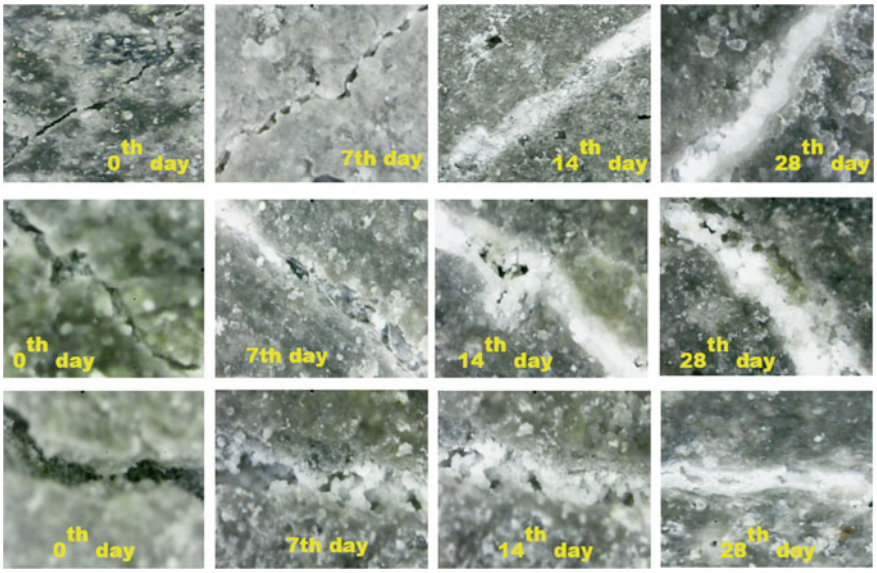

Fig. 16 Microscopic observations of cracks BC 1, BC 2, BC 3

Table 3 Crack width before and after healing of WD

Mix id (WD)	Initial crack width (mm)	Crack healed size (mm)			Crack healing percentage		
		7 Days	14 Days	28 Days	7 Days	14 Days	28 Days
BC 1	0.39	0.315	0.234	0.055	19	39	84
BC 2	0.37	0.295	0.214	0.035	21	44	94
BC 3	0.35	0.275	0.194	0.018	23	47	95

Table 4 Crack width before and after healing of FW

Mix id (FW)	Initial crack width (mm)	Crack healed size (mm)			Crack healing percentage		
		7 Days	14 Days	28 Days	7 Days	14 Days	28 Days
BC 1	0.40	0.325	0.244	0.065	19	39	84
BC 2	0.36	0.285	0.204	0.025	21	43	93
BC 3	0.34	0.265	0.184	0.020	22	46	94

3.9 X-Ray Diffraction (XRD)

XRD findings show a combination of precipitation of nutrients produced by bacteria, which include calcite (Ca), aragonite (AR), and vaterite (Va). Fig. 18 shows the

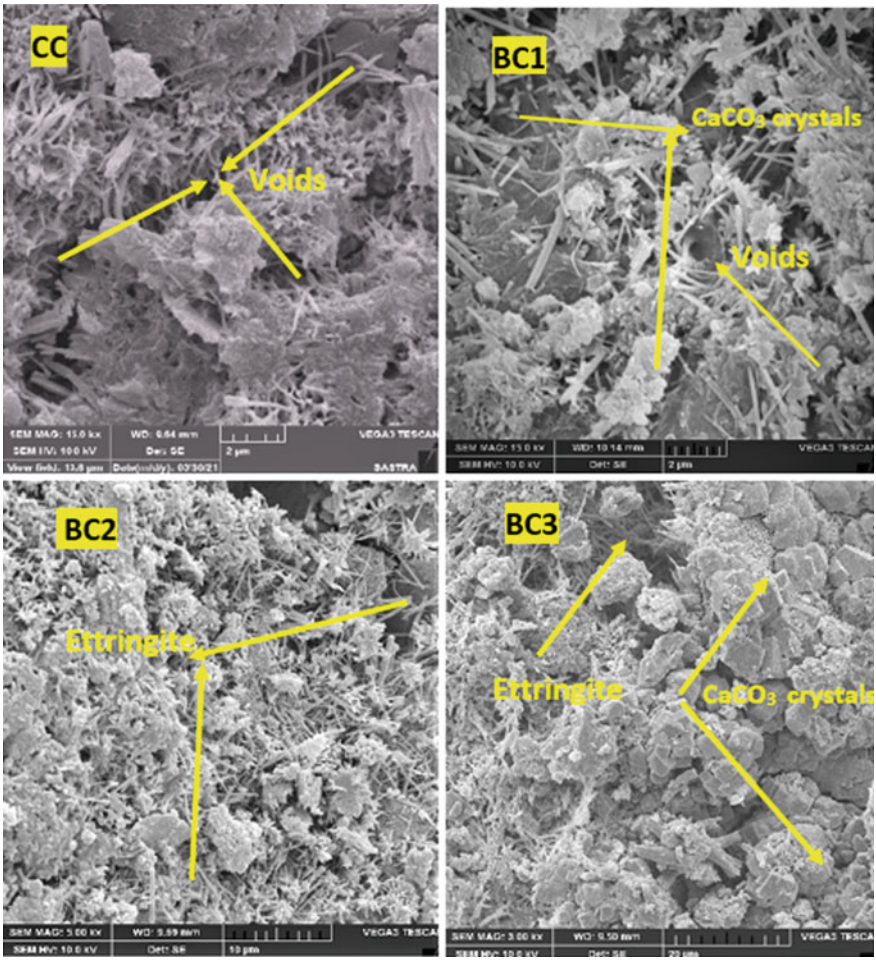

Fig. 17 SEM images

28 days samples of XRD results. In XRD analysis the peak value was obtained for the compound aragonite which is a polymorph of calcium carbonate. This confirms that the precipitated mineral was calcium carbonate-based [16].

4 Conclusions

The current study explores the use of *Bacillus subtilus* and compares the strength, compressive strength regain, crack healing efficiency, and durability of concrete

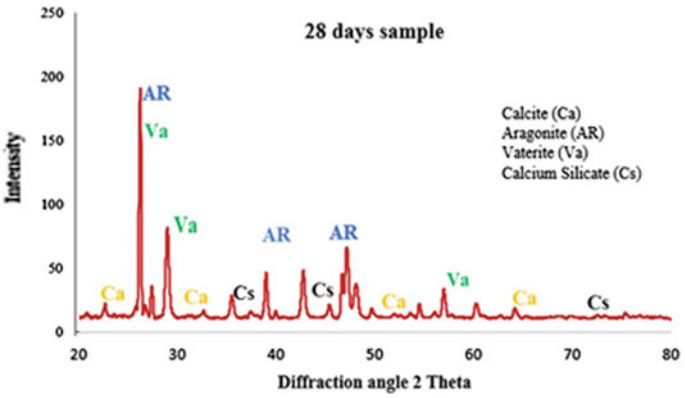

Fig. 18 XRD results

under wet-dry and full-wet curing conditions were studied. The experimental results were analyzed and interpreted, resulting in the following conclusions.

1. The compressive strength and tensile strength of bacterial concrete were much higher than the control concrete. This factor occurs as a result of the development of calcium silicate hydrate inside the pores of concrete, which contributes to increased strength and durability properties.
2. The pre-compressed concrete specimens regained compressive strength at 25 days after being pre-cracked on the third day, which was nearly equal to the characteristic compressive strength. This means that the precipitation of calcium carbonate in fracture areas not only enhances the concrete matrix but also allows it to regain its original strength after failure.
3. Impact energy is calculated for both the normal concrete and bacterial concrete. 30% bacterial concrete and 70% of nutrient solution show higher impact energy compared to other bacterial concrete and normal concrete. As addition of bacteria which increases the impact energy due to $CaCO_3$ precipitation improves the packing of concrete and it delays the propagation of cracks at initial and final stages.
4. At 28 days, exterior repair was observed in the Full-Wet and Wet-Dry pre-cracked samples at approximately 90% and 88%, respectively. Calcium carbonate precipitation in the concrete matrix was caused by bacterial activity that aids in internal curing and it improves the mechanical performance of the concrete.
5. The samples with larger precipitation had reduced water absorption rates, implying that precipitation caused by microbial activity hardens the pores in the concrete matrix, minimizing the volume of water uptake significantly.
6. SEM and XRD analysis confirms the bacteria show different morphological crystals in concrete and the peak value in XRD analysis was obtained for the compound aragonite, which is a polymorph of $CaCO_3$. This demonstrates that the precipitated mineral was composed of calcium carbonate. The influence of

$CaCO_3$ used as a calcium source for this current research may have resulted in a polymorph of calcium carbonate.

Scope of Future Work

The development of bacterial concrete by directly applying, encapsulating, and immobilizing a combination of different types of bacteria to improve the regained strength, crack healing efficiency, and durability properties of concrete with various fibers (steel, basalt, polypropylene, glass) could be the focus of future research.

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