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Enhancing Structural Resilience: Microbial-Based Self-Healing in High-Strength Concrete

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Abstract

Concrete's weak tensile strength renders it susceptible to cracking under prolonged loads, leading to reduced load-bearing capacity and reinforcing bar corrosion. This study investigates the effectiveness of microbial-based self-healing in high-strength concrete, focusing on two bacterial strains: *Sporosarcina koreensis* and *Bacillu*s. Results demonstrate significant enhancements in micro- and macro-physical properties of high-strength bacterial concrete with *Bacillus flexus* and *S. koreensis*, surpassing the control. *Bacillus flexus*-infused concrete exhibits a remarkable 21.8% increase in compressive strength at 7 days and 11.7% at 56 days. Similarly, *S. koreensis*-treated concrete shows 12.2% and 7.4% increases at 7 and 56 days, respectively. Enhanced crack healing occurs due to calcite precipitation, confirmed by X-ray diffraction and scanning electron microscopy. Both bacterial strains achieve crack closure within 42 days, with widths of 259.7 µm and 288.7 µm, respectively. Moreover, bacterial concrete from these strains excels in durability against water, acid, and salt exposure, surpassing control concrete. These findings emphasize microbial-based self-healing's potential in high-strength concrete, providing a practical strategy to enhance structural resilience and extend concrete infrastructure lifespan.

Keywords *Bacillus flexus*, Compressive strength, Concrete durability, High-strength concrete, Microbial self-healing, *Sporosarcina koreensis*

1 Introduction

High-strength concrete (HSC) has gained widespread recognition for its enhanced mechanical properties, making it suitable for demanding structural applications (Krishnapriya et al., 2015). However, like conventional concrete, HSC is susceptible to cracking under various stress conditions, which can compromise its durability and load-bearing capacity (Iheanyichukwu et al., 2018). The resulting cracks not only diminish load-bearing capacity but also trigger environmental and economic issues, including carbon emissions during repair (Rosewitz et al., 2021). Additional weakness of concrete structure is the presence of voids and fissures in its body matrix. The circumstance of such voids in the matrix of concrete plays substantial role in determining its

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mechanical properties and durability (Feng et al., 2021; Khaliq & Ehsan, 2016).

The concept of self-healing concrete, facilitated by microbial activity, has emerged as a promising avenue to address these challenges, aiming to autonomously fill and repair such voids and cracks via bio-mineralization. In this process, bacteria induce the conversion of calcium particles within the cement composite into calcium carbonate, calcite. Precipitation and deposition of calcite due to microbial activity of bacteria seals such voids and fissures in the concrete body matrix. This in turn advances the mechanical properties and durability of concrete as well as healing rate of crack formed due variously initiated stress (Rohini & Padmapriya, 2021; Singh & Gupta, 2020). These bacteria must exhibit robust urease activity, withstand high pH levels, and endure mechanical stress within the concrete matrix (Rohini & Padmapriya, 2021; Rosewitz et al., 2021).

Moreover, bacterial concrete offers the potential for sustainable construction, serving the dual purpose of mitigating the environmental impact of concrete while enhancing structural resilience. The incorporation of waste materials into high-strength concrete, complemented by microbial-based self-healing capabilities, presents an avenue for environmentally responsible construction (Jahami & Issa, 2023). This approach can have a positive impact on indoor air quality and the well-being of building occupants, as it leverages sustainable, cost-effective, biodegradable, and low-carbon technologies (Jahami et al., 2022; Thangaraj Sathanandam, 2017).

Multiple research studies have demonstrated the enhancement of normal-strength concrete's mechanical properties when bacterial strains are incorporated into the mix. Bio-concrete with a 30 MPa grade, containing *B. flexus* at a concentration of 105 cells/ml, exhibited significant improvements compared to standard concrete. After 28 days of curing, it displayed an increase of more than 40% in compressive strength, over 30% in flexural strength, and more than 10% in split tensile strength (Andalib et al., 2016). Another researcher investigated the impact on concrete's mechanical strength by introducing the rod-shaped ureolytic bacterium Bacillus Subtilis at a concentration of 108 cells/ml. The study revealed that bio-concrete mixes with a 40 MPa grade achieved a 22% increase in compressive strength, a 16% increase in split tensile strength, and an 11% increase in flexural strength after 28 days of curing (Durga et al., 2020). Study was also conducted on the compressive strength and self-healing capacity of concrete with the addition of two different Bacillus species and *D. salina* algae (Osman et al., 2021). Early age examinations demonstrated a higher percentage increase in microbial concrete's mechanical properties, averaging 35%, compared to later age examinations, which showed an average increase of only 8%. This underscores the time-dependent effectiveness of directly incorporating bacteria into concrete.

Furthermore, the integration of microbes with concrete enhances the filling of voids in the concrete microstructure. Scanning Electron Microscopy (SEM) findings indicated that the rod-shaped bacterium Bacillus Subtilis HU58 performed calcite precipitation and effectively restored cracks with a width of approximately 1.8 mm after 28 days of curing (Nguyen Ngoc Tri Huynh, 2017). Bacterial species Bacillus sp. CT-5 successfully repaired concrete cracks with a depth of 27.2 mm (Varenyam Achal, 2013). The effectiveness of healing is dependent on bacterial concentration, with crack widths of 0.6 mm, 0.9 mm, and 1.2 mm being repaired with concentrations of 103 cells/ml, 105 cells/ml, and 107 cells/ml of Bacillus Subtilis (MTCC 441), respectively (Mondal & Ghosh, 2018). X-ray diffraction (XRD) analysis was employed to confirm the precipitation of calcite in the filling deposition (Chen et al., 2019; Feng et al., 2021; Khaliq & Ehsan, 2016).

While microbial-mediated self-healing has been extensively studied in conventional concrete (Algaifi et al., 2021; Andalib et al., 2016; Chen et al., 2019; Feng et al., 2021; Khaliq & Ehsan, 2016; Luo et al., 2015; Mondal & Ghosh, 2018; Rao et al., 2015), its applicability and effectiveness in high-strength concrete (HSC) require further investigation. High-strength concrete has a denser microstructure and often contains supplementary cementitious materials, affecting the availability of nutrients and the growth of bacteria (Jonkers et al., 2010). Therefore, understanding how microbial activity functions within the specific context of HSC is essential.

Bacillus species, renowned for their robust urease activity, have demonstrated their ability to facilitate calcium carbonate precipitation in normal-strength concrete. In a similar vein, Sporosarcina strains have exhibited promising outcomes in conventional concrete. Nevertheless, a comprehensive grasp of the compatibility and effectiveness of these strains in the realm of high-strength concrete remains an ongoing endeavor (Khan et al., 2023). Moreover, the long-term durability and sustainability of these improvements require further assessment (Bhutange & Latkar, 2020).

This study thus investigates the influence of microbial activity on self-healing properties in high-strength concrete. Specifically, it studies the calcium carbonate precipitation abilities of two bacterial strains, *S. koreensis* and *B. flexus*. These strains are introduced into high-strength bacterial concrete, and their effects on micro- and macro-physical properties are systematically analyzed and compared against control specimens.

Notably, this research addresses a gap present in the current literature, by scrutinizing the self-healing potential intrinsic to high-strength concrete. This distinctive contribution advances the comprehension of microbial-based self-healing, accentuating its adaptability across a wider spectrum of concrete formulations, thus offering novel insights into enhancing concrete's durability and sustainability.

2 Methodology

2.1 Materials

The concrete production experiment utilized a combination of ordinary Portland cement, coarse aggregate, fine aggregate, tap water, and a chemical admixture. The physical attributes of the cement are detailed in Table 1. Fine aggregate was composed of natural sand, with a maximum size of 4.75 mm, while crushed stone with a maximum nominal size of 12.5 mm served as the graded coarse aggregate. To assess the aggregates' physical characteristics, ASTM standards were followed, and the summarized results are presented in Table 2. Furthermore, the sieve analysis, aligned with the ASTM C 33 standard grading requirement (ASTM33, 2016), yielded the results depicted in Fig. 1. The particle size distribution of the aggregates conformed to the specified upper and lower limits, thus confirming their suitability for concrete components.

The chemical admixture employed was Muraplast SP1 High Range Water Reducing Superplasticizer, conforming to ASTM C494 standards. In line with previous studies (ACI, 2008; Schaefer, 1995), a dosage of 16 oz./cwt of HRWR was adopted for this research. The inclusion of tap water was essential to achieve the intended consistency and promote the hydration process of the concrete mix.

2.2 Isolation, Identification, and Culturing of Bacteria

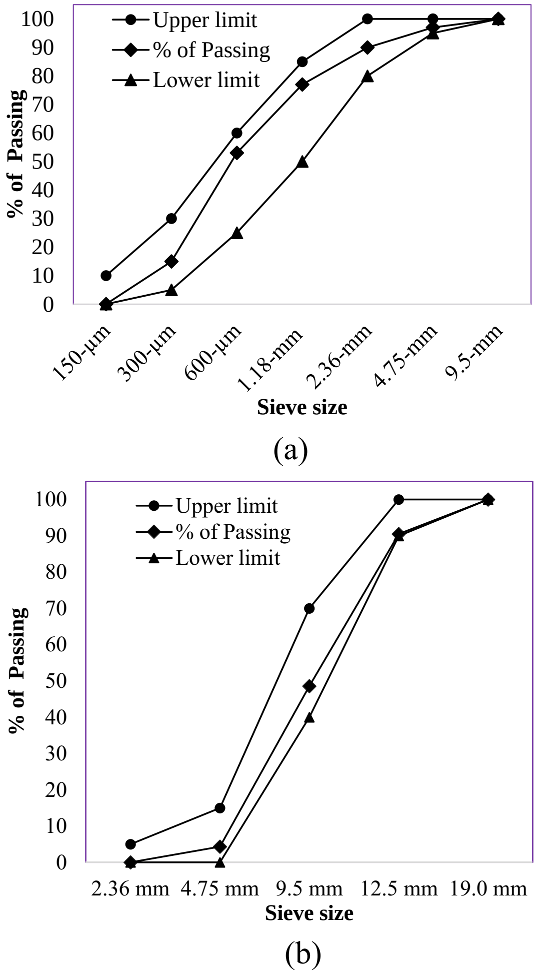
The bacterial strains, *B. flexus* and *S. koreensis*, were isolated from cementitious soil originating in the Central Rift Valley of Ethiopia, commonly utilized in cement industries. The isolation, identification, and culturing procedures were conducted at the gene bank of the Ethiopian Institute of Biodiversity. Preservation of the bacteria occurred within a nutrient agar medium formulated with 6 g peptone powder, 6 g yeast extract, 6 g beef extract, 6 g sodium bicarbonate, 10 g sodium chloride, 2 g calcium chloride, 6.5 g nutrient broth, and 18 g urea per 1000 ml of water, consistent for both bacterial strains. Calcium chloride encompassed as a source of calcium, whereas urea used for the facilitation of urease activity.

Table 1 Summary of physical properties of cement

Properties	Test result	Standards
Fineness (%)	94.6	(ASTM C184-94e1)
Specific gravity (g/cc)	3.15	(ASTM C188)
Normal consistency (%)	28.00	(ASTM C187)
Initial setting time (min)	66	(ASTM C191)
final setting time (min)	310	(ASTM C191)

Table 2 Summary of physical properties of aggregates

Properties	Fine aggregate	Coarse aggregate
Bulk unit weight (Kg/m^3)	1550.0	1619.5
Specific gravity (g/cc)	2.60	2.89
Absorption capacity (%)	1.37	0.88
Moisture content (%)	1.895	0.863
Fineness modulus	2.68	2.57



(a)

(b)

Fig. 1 Gradation of **a** fine aggregate **b** coarse aggregate

Cultivation transpired at 38 °C with a pH value of 9.5 within the laboratory environment.

Both *B.s flexus* and *S. koreensis* are characterized as gram-positive, alkaliphilic, aerobic bacteria with the capacity to generate urea. These attributes enable their endurance in harsh, highly alkaline environments for extended periods. The urease activity exhibited by both strains facilitates the hydrolysis of urea $[CO(NH_2)_2]$ into ammonia (NH_3) and carbamate (NH_2COOH), a pivotal process (Kwon et al., 2007; Rao et al., 2015). This enzymatic action aids in Microbial-Induced Calcium Carbonate Precipitation (MICP), a metabolic-driven process through which microorganisms extracellularly form calcium carbonate (Ryparová et al., 2021). The rate of calcium carbonate precipitation hinges on factors such as the calcium content available within the concrete matrix and external conditions. These include the pH level of the concrete composition, the presence of liquefied carbon, and the existence of nucleation sites usually cell walls of bacteria where bacterial metabolic processes foster the formation of calcite (Iheanyichukwu et al., 2018; Osman et al., 2021; Rauf et al., 2020; Ryparová et al., 2021).

2.3 Concrete Mix Design and Sample Preparation

The ACI 211.4R-08 guideline was employed as a universally applicable methodology for selecting mixture proportions in high-strength concrete and subsequently optimizing these proportions through trial batches (ACI, 2008). The concrete mix design for grade C-60 Mpa, with a water-to-cement ratio of 0.3, was conducted according to the ACI 211.4R-08 guideline (ACI, 2008). The mix proportions of ingredients for high-strength concrete are detailed in Table 3. Standard cube specimens measuring 100 mm × 100 mm × 100 mm were utilized for casting specimens for various tests, including compressive strength, acid resistance, ultrasonic pulse velocity, water absorption, and salt resistance. For the flexural strength test, a specimen measuring 500 mm × 100 mm × 100 mm was employed. Three types of mixtures were prepared: a control mix devoid of bacteria, and two bacterial concrete mixes containing *B. flexus* and *S. koreensis*. Three samples were prepared and tested for each specific test, and the average test result was reported (ASTM, 2020).

Table 3 Mixture proportions of concrete grade C-60 Mpa

No	Content	Batch weights (Kg/ m^3)	Ratio with cement
1	Cement	644.3	1
2	Fine aggregate	504.5	0.78
3	Coarse aggregate	1101.3	1.71
4	Water	200.4	0.3

Various methods exist for incorporating bacterial strains into concrete mixes, with two common approaches being direct addition of cultured bacterial strains and the use of encapsulated bacterial strains (Gupta Souradeep, 2017). In this research, the direct mixing method for supplying bacterial solutions was chosen due to its simplicity and compatibility with the characteristics of *B. flexus* and *S. koreensis*, which can endure harsh environmental conditions, including high pH values up to 9 (Kwon et al., 2007; Rao et al., 2015). Furthermore, the direct application of bacteria has been shown to enhance the mechanical strength of concrete (Andalib et al., 2016; Kunamineni Vijay, 2017).

The optimal concentration of bacteria for enhancing concrete resilience typically falls within the range of 105–107 cells/ml of mixing water, while more effective crack healing occurs at higher bacterial cell concentrations of 108–109 cells/ml of mixing water (Mondal & Ghosh, 2018; Varenyam Achal, 2013). In the study of concrete with a 50 MPa cylindrical compressive strength, manufactured with five different bacterial concentrations (ranging from 10×10^5 to 50×10^5 cells/ml of mixing water) of Bacillus Megaterium, a bacterial concentration of 30×10^5 cells/ml was found to be the optimal choice for enhancing compressive and flexural strength, as well as promoting microbial activity (Andalib et al., 2016). The primary objective of bacterial concrete is crack healing without negatively affecting compressive strength, especially for high-strength concrete (HSC). Therefore, based on the above findings, a concentration of 30×10^5 cells/ml of mixing water was incorporated for bacterial concretes. Turbidimetry was employed to establish a bacterial growth curve to determine the optimum concentration.

Following particular batching of ingredients, a mixing machine was utilized for producing fresh concrete to be cast in prepared molds. The mixing method adhered to ASTM C94/C94M-23 standards. The slump test results of freshly mixed concrete were 115 mm for control, 134mm for *B. flexus*, and 120 mm for *S. koreensis*, all in accordance with ACI 211.4R-08 guidelines. The assessment of macro-physical properties in self-healing high-strength concrete encompassed parameters such as compressive strength, flexural strength, acid resistance, ultrasonic pulse velocity, water absorption, and salt resistance. The microscopic physical properties were investigated through analysis conducted via a 3-D optical surface morphology microscope, scanning electron microscopy (SEM), and X-ray diffraction techniques.

3 Results and Discussions

3.1 Compressive Strength Test Result

Compressive strength assessments of the concrete adhered to BS EN 12390-3 (Standard, 2009), conducted

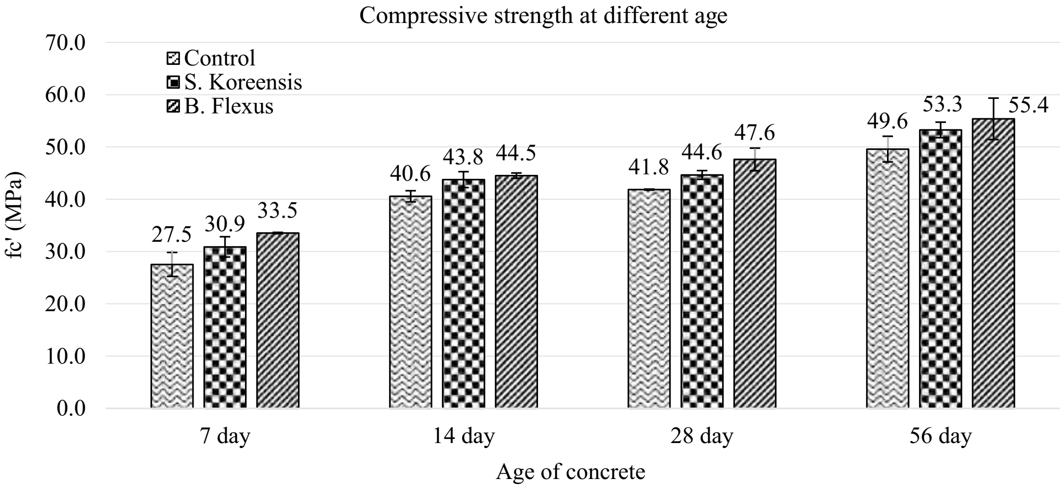


Fig. 2 Compressive strength of concrete at different age

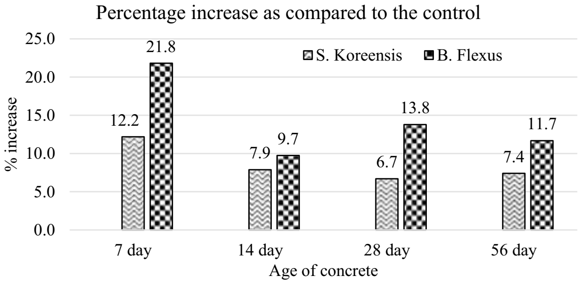


Fig. 3 Percentage increase in compressive strength as compared to the control

at 7, 14, 28, and 56 days, are illustrated in Fig. 2. The comparative compressive strength performance gains of the bacterial concrete, relative to control concrete, is also depicted in Fig. 3.

At the 7-day curing stage, bacterial concrete featuring *B. flexus* exhibited a substantial increase of 21.8%, while bacterial concrete incorporating S. koreensis demonstrated a notable 12.2% surge compared to control specimens. At 14, 28, and 56 days of curing stages, the bacterial concrete incorporating *B. flexus* showed improvements of 9.8%, 13.8%, and 11.7%, respectively, relative to the control. Similarly, at the corresponding curing stages, the bacterial concrete with S. koreensis exhibited increases of 7.9%, 6.7%, and 7.4%, respectively, in comparison to the control. Consequently, bacterial concrete with *B. flexus* displayed superior efficacy in augmenting the strength of high-strength concrete. However, it is worth noting that concrete containing *S. koreensis* also demonstrated notable enhancement when contrasted with the control. These findings are in alignment with previous studies focusing on the enhancement of mechanical strength in normal-strength concrete (Andalib et al., 2016; Durga et al., 2020; Rao et al., 2015). Furthermore, in the context of comparing concrete compositions that incorporated waste glass sand (WGS) as a partial replacement for fine aggregates (FA), it is noteworthy that concrete containing a 50% substitution of WGS for FA exhibited a 27% increase in compressive strength relative to conventional concrete (Jahami et al., 2022). This integration of microbial self-healing with sustainable waste materials for the development of environmentally friendly, high-strength concrete presents a promising avenue for future research exploration.

3.2 Flexural Strength Test Result

The flexural strength assessments of both bacterial concrete and control specimens were conducted at 14 and 28 days, following ASTM C78 standards (ASTM International, 2018). The results of this evaluation are presented in Fig. 4, with the comparative increase in flexural strength of bacterial concrete in contrast to standard concrete illustrated in Fig. 5.

Fig. 5 reveals that the inclusion of bacteria in the concrete mixture led to advancements in flexural strength. Specifically, concrete incorporating *B. flexus* exhibited an escalation in flexural strength by 6.0% and 9.0% at 14 and 28 days, respectively, in comparison to the control specimens. Likewise, concrete infused with *S. koreensis* bacteria displayed enhancements in flexural strength by 5.2% and 6.4% at 14 and 28 days, respectively, when juxtaposed with conventional control concrete. This observation aligns with prior research findings of normal-strength concrete (Durga et al., 2020; Rao et al., 2015).

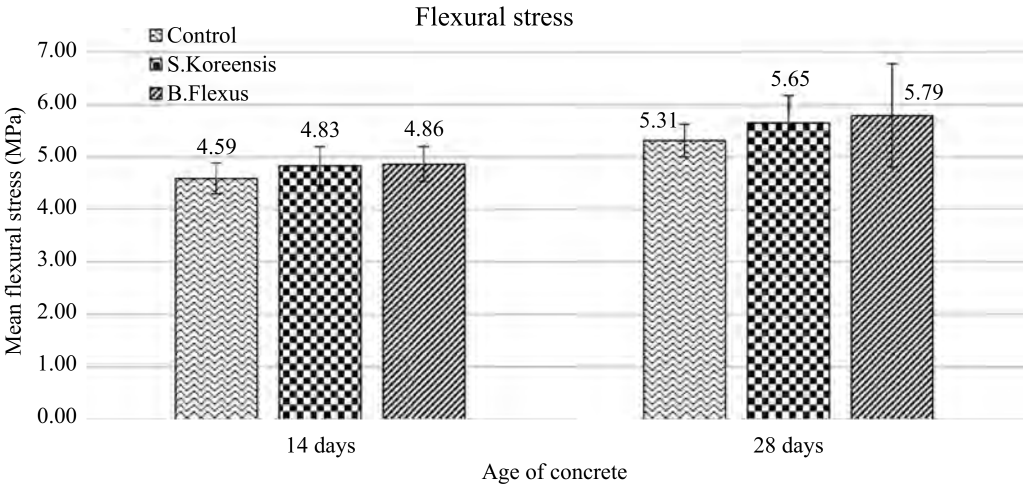


Fig. 4 Flexural strength test result

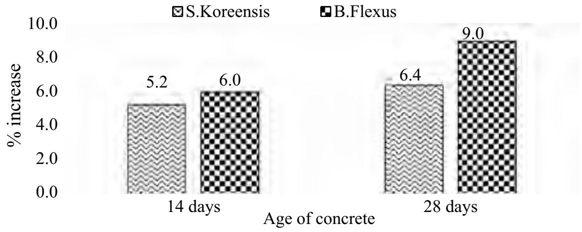


Fig. 5 Percentage increase in flexural strength of bacterial concrete compared to control

3.3 Ultrasonic Pulse Velocity Test

Conducted in accordance with ASTM C-597 standards (ASTM, 2003), the ultrasonic pulse velocity test was administered at varying concrete ages. The outcomes of the ultrasonic pulse velocity test for this study are depicted in Fig. 6.

Fig. 6 showcases that all concrete structure specimens attained nearly identical ultrasonic pulse velocities at their respective ages. A higher ultrasonic pulse velocity signifies superior concrete quality (ASTM, 2003). The test results revealed ultrasonic pulse velocities ranging from 4.01 km/s at 7 days to 4.71 km/s at 56 days. This evidence suggests commendable quality grading of the concrete at earlier ages (7 days) and exemplary quality grading at later stages (56 days), consistent with the concrete quality grading (Saint-Pierre et al., 2016). An observable trend emerges whereby the ultrasonic pulse velocity value heightens with the progression of age, indicating an advancement in concrete quality. This enhancement is attributed to the ongoing hydration of previously unhydrated cement within the concrete, coupled with the precipitation and deposition of calcite facilitated by bacterial action.

In accordance with the UPV findings of this study, a fitting curve was established to depict the relationship

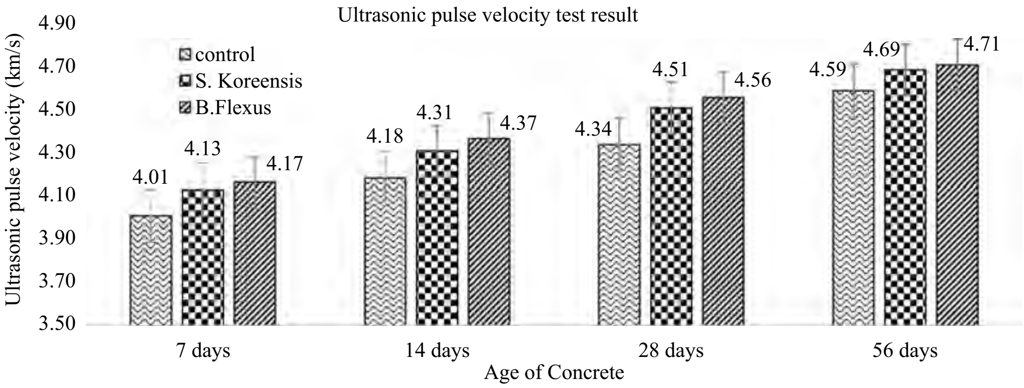


Fig. 6 Ultrasonic pulse velocity test result

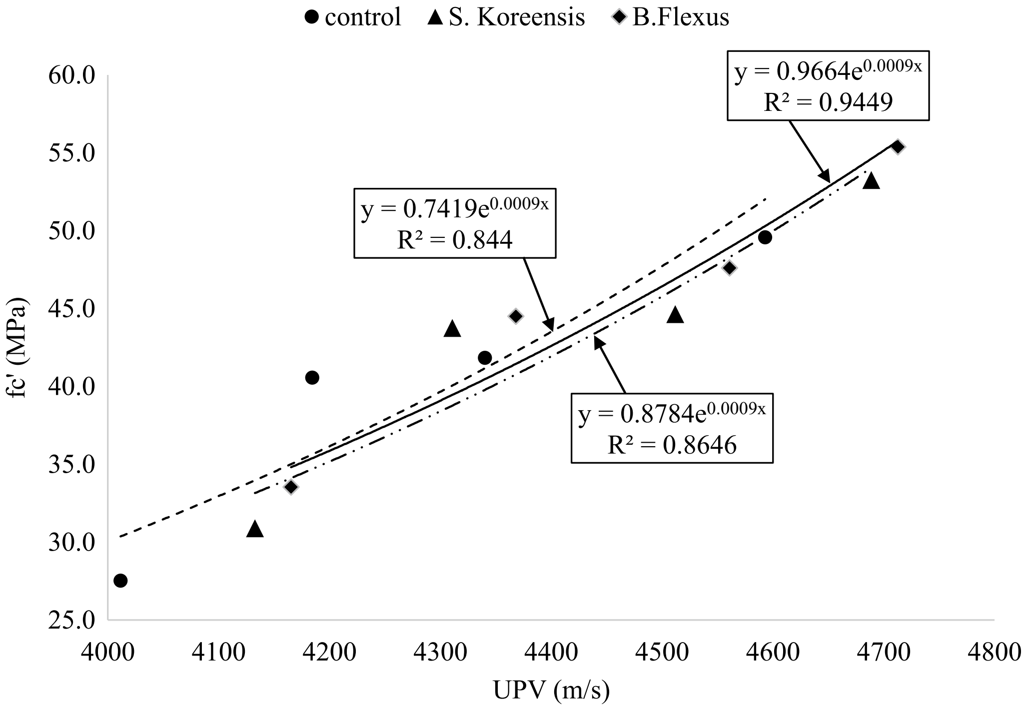


Fig. 7 Fitting curve for the compressive strength associated with UPV

between UPV and cylindrical compressive strength (fc′), encompassing conventional concrete as well as bio-concrete. Fig. 7 presents curve fitting formulae for conventional concrete, bio-concrete produced with *B. flexus*, and *S. koreensis*. The R^2 values for the fitting curve formulas were 0.844, 0.9449, and 0.8646 for conventional concrete, bio-concrete made with *B. flexus*, and *S. koreensis* bacteria, respectively. These values signify a high level of accuracy in the relationship between UPV and cylindrical compressive strength for the aforementioned materials.

3.4 Acid Resistance Test Result

The effect of acid concentration on concrete was explored by immersing 28-day cured concrete cube samples in a 1% concentration sulfuric acid solution for 28 days. The evaluation encompassed weight loss and compressive strength reduction assessments. Percentage of strength loss.

Figs. 8 and 9 depict outcomes pertaining to strength loss and weight loss resulting from acid exposure.

The loss of compressive strength attributed to sulfuric acid attack proved to be more pronounced in conventional concrete compared to bacterial concrete. While control concrete experienced a 10.5% decrease from its 28-day original strength, concrete containing *B. flexus* and *S. koreensis* incurred reductions of 4.8% and 5.3%, respectively. This disparity underscores the elevated acid resistance of bacterial concrete.

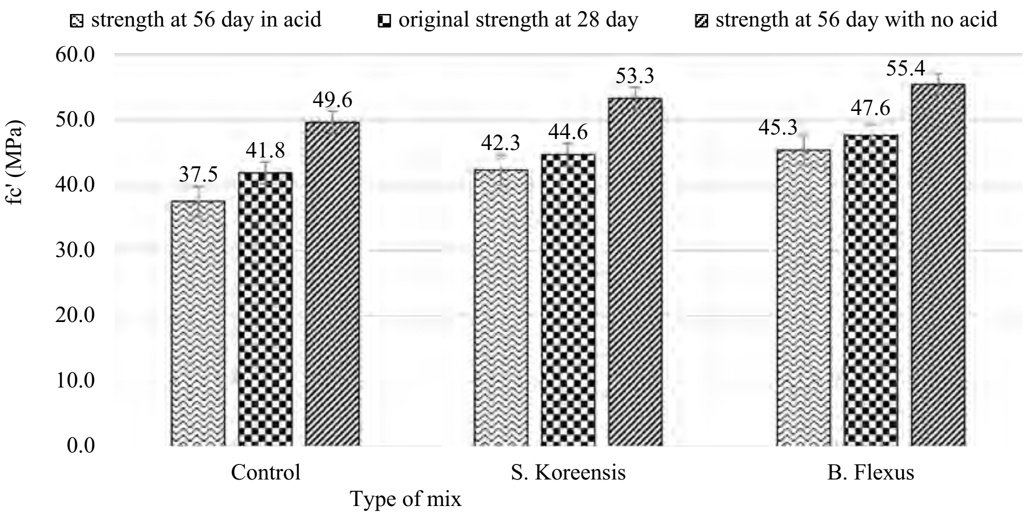
3.5 Salt Resistance Test Result

The salt resistance test was conducted in a manner analogous to the acid resistance examination, except that magnesium sulfate was employed as the corrosive agent. Weight loss and compressive strength deterioration were assessed across all specimen types in accordance with (Acharya & Patro, 2016), with the outcomes depicted in Figs. 9 and 10.

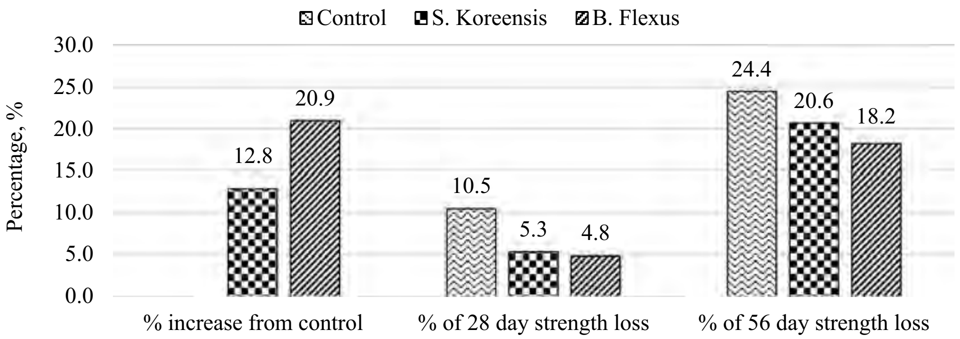
In the context of salt resistance, control concrete underwent a 4.3% reduction from its 28-day original compressive strength, while concrete containing *B. flexus* and *S. koreensis* experienced strength reductions of 1.8% and 2.1%, respectively. These values further decreased to 19.3%, 18%, and 15.6% for the respective categories at 56 days. This exploration underscores the superior resistance of bacterial concrete to both salt and acid attacks, signifying its heightened durability in comparison to conventional concrete.

3.6 Compressive Strength Test for Cracked Section after Healing to the Age of 28 days

The compressive strength test was conducted on cracked specimens that underwent 28 days of healing. The initiation of cracks was achieved by applying a



(a) Comparison for compressive strength of concrete exposed to acid



(b) Percentage of strength loss

Fig. 8 Comparison of compressive strength of concrete exposed to acid

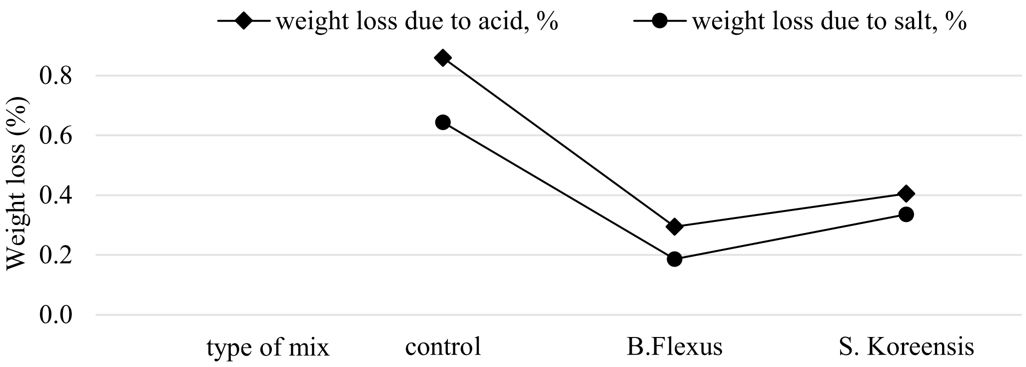


Fig. 9 Percentage of weight loss due to acid and salt attack

load equivalent to 55% of the concrete's compressive strength (MacGregor et al., 1997). Subsequently, these crack-initiated concrete specimens were subjected to 28 days of immersion in water to promote a self-healing process. Fig. 11 illustrates the outcome of the compressive strength test after the 28-day healing period, compared against specimens that did not undergo crack initiation.

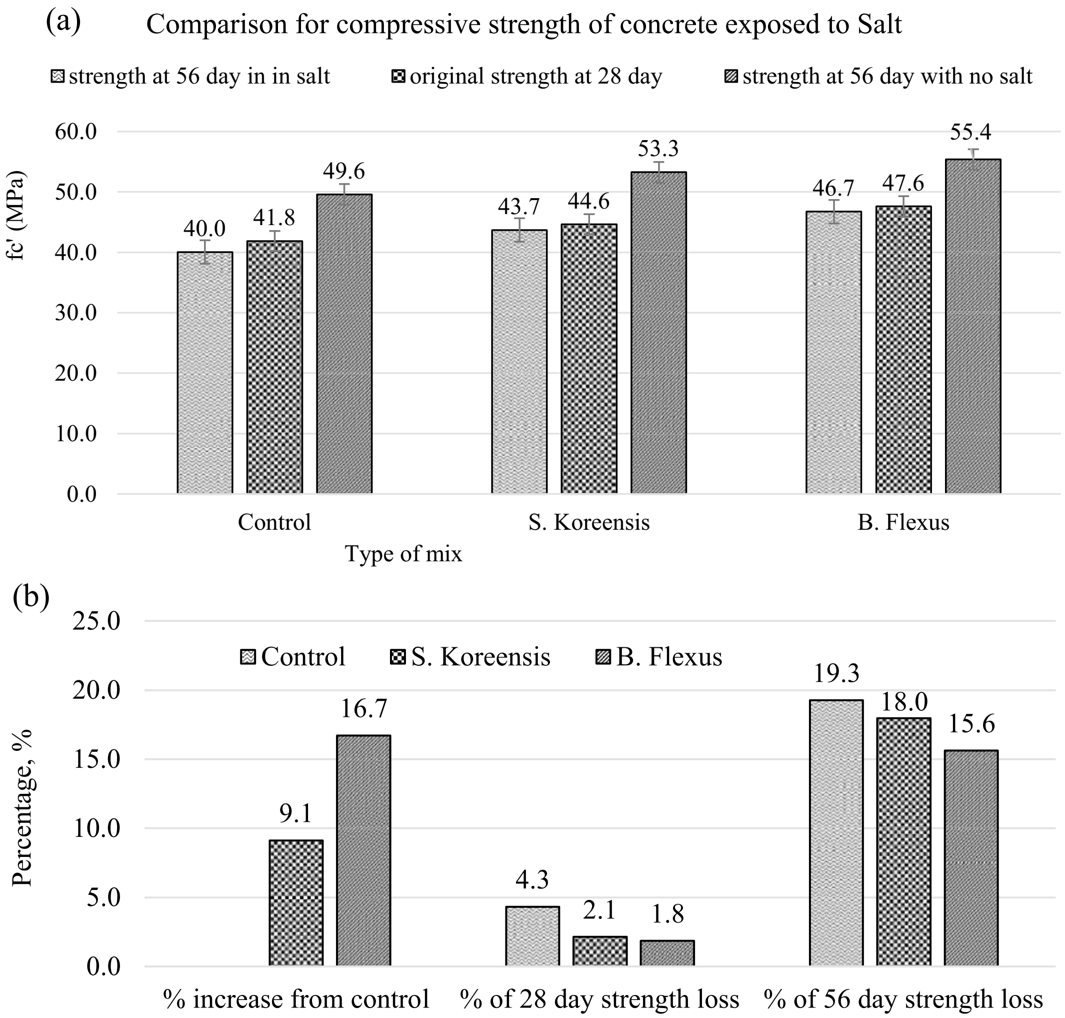


Fig. 10 Comparison of compressive strength of concrete exposed to salt

Following the restoration process triggered by the 55% stress-level crack initiation, standard concrete regained only 64.0% of its original 28-day compressive strength. Conversely, concrete containing *B. flexus* and *S. koreensis* displayed recovery rates of 96.1% and 94.1%, respectively, in relation to their original 28-day compressive strength. This observation underscores the superior restoration of strength observed in bacterial concrete, attributed to the microbial-induced precipitation of calcite. The presence of calcium carbonate fills the cracks, progressively replenishing the concrete matrix's pores and thereby restoring the original strength (Andalib et al., 2016; Durga et al., 2020).

3.7 Water Absorption Test

At the 56-day mark, a water absorption test was administered on both standard and crack-initiated concrete samples. For the crack-initiated specimens, cracks were induced at the age of 28 days with a stress level equating to 55% of their strength at that juncture. Both types of samples, whether crack-initiated or standard, were subjected to immersion in water to facilitate healing and further curing. Water absorption post-immersion was evaluated in accordance with ASTM C 642-13 standards (ASTM, 2013), employing Eq. (1):

$$w\% = \frac{\text{SSD weight} - \text{oven dry weight}}{\text{oven dry weight}} \times 100. \tag{1}$$

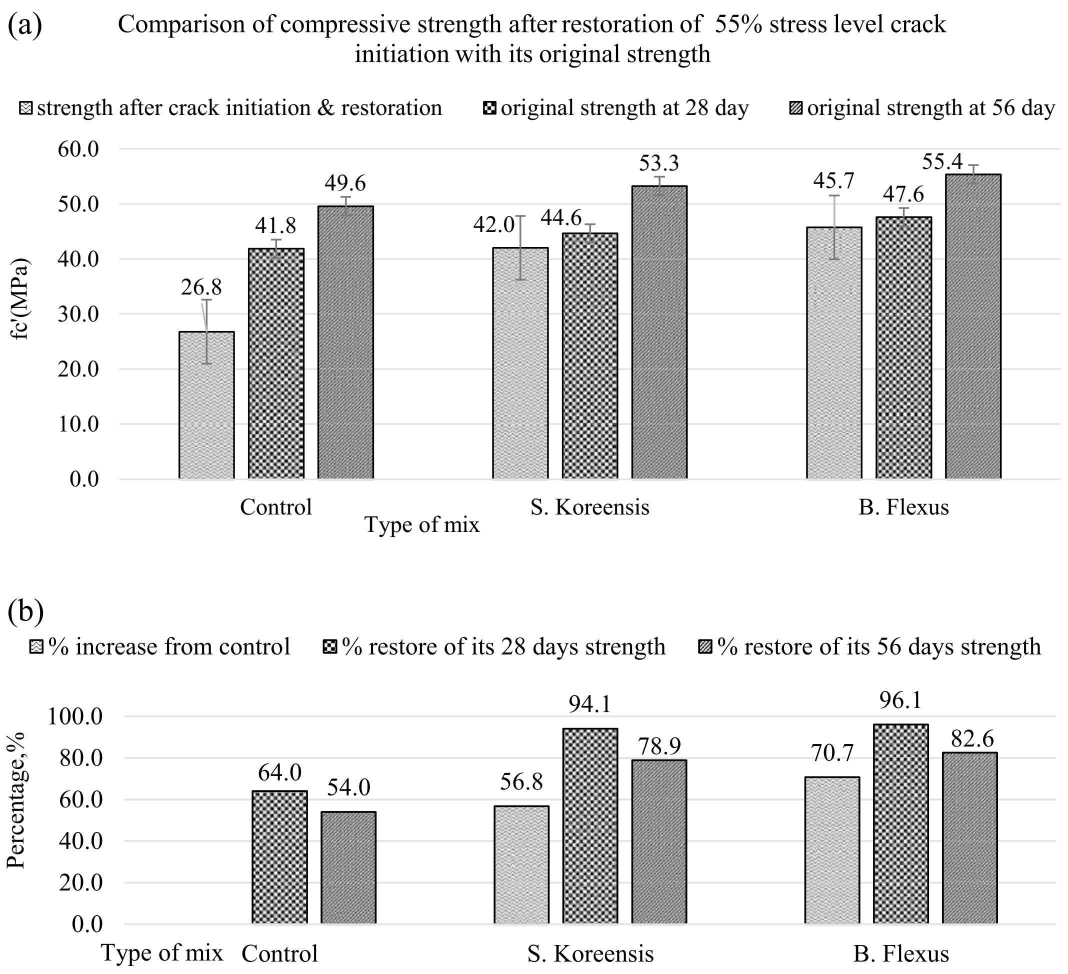


Fig. 11 Comparison of compressive strength for ordinary and crack-initiated concrete

Table 4 presents the percentage decrease in water absorption relative to the control specimens. Notably, both the 55% stress-level crack-initiated and standard microbial concrete exhibited superior outcomes in the water absorption test when compared to the control concrete mixture. Concrete containing *B. flexus* recorded reductions of 15.7% and 13.1% in water absorption for standard and crack-initiated specimens, respectively. Likewise, concrete containing *S. koreensis* exhibited reductions of 4.3% and 2.6% in water absorption for standard and crack-initiated specimens, respectively, in comparison to normal concrete. Relatively, concrete with *B. flexus* demonstrated a more pronounced reduction in water absorption. It is noteworthy that water absorption and compressive strength exhibit an inverse relationship: a decrease in water absorption corresponds to an increase in compressive strength, as fewer pores are present within the concrete matrix (Durga et al., 2021; Mondal & Ghosh, 2018).

Table 4 Percentage decrease in water absorption as compared to the control

Concrete specimen made with	% Decrease as compared to the control (55% stress-level crack-initiated and healed specimen)	% Decrease as compared to the control (ordinary specimen)
S. koreensis	2.6	4.3
B. flexus	13.1	15.7

3.8 Micro-structural Investigation

3.8.1 3-D Optical Surface Microscope Investigation

Utilizing a 3-D optical surface morphology microscope, the investigation delved into the morphological alterations within cracked concrete sections. Employing a compressive strength testing machine, cracks were induced in 25 mm × 50 mm × 50 mm concrete samples. The samples were subjected to loading at a rate of 0.8

KN/sec following BS EN 12390-3 guidelines until visible cracks formed on the surface (Standard, 2009). The progression of self-healing in both control and bacterial specimens was observed at different concrete curing ages through zeta analysis, with the instrument's field of view set at 1743 μm × 1308 μm. Two samples of each bacterial concrete type and one sample of standard concrete were scrutinized to monitor the fissure section's restoration progress. The instrument gauged both crack width and rift area.

Concrete healing ability was monitored at 7-day intervals up to the age of 42 days. The results demonstrated more substantial self-healing in bacterial concrete compared to the control. Table 5 presents self-healing progress of cracked concrete, whereas Fig. 12 depict the percentage progressive rift area change of cracked sections, summarizing zeta analysis outcomes from the 3-dimensional optical surface morphology microscope. Fig. 12 showcases the alteration in a 288.7 μm crack width section of a *S. koreensis* 2 concrete specimen filled with the precipitated and deposited calcite, detected via the 3-D optical surface morphology microscope.

As evident from Table 5 and Fig. 13, *B. flexus* bacterial concrete recuperated a crack with a width of 218.8μm (corresponding to a crack sectional area of 114,531.4 μm^2) to 100.5% of its original state by the 28th day. Similarly, concrete containing *S. koreensis* restored a crack with a width of 288.7 μm (crack sectional area of 144,700.2 μm^2) to 84.1% of its original state by the 28th day. In stark contrast, the control specimen exhibited a considerably lower healing rate, achieving a mere 3.7% of area repair for a crack width of 467.6 μm (crack sectional area of 376,719.6 μm^2) by the 28th day. By the 42nd day, almost all microbial concrete specimens involving *B. flexus* and *S. koreensis* had restored the cracked sections. The filling of the cracked sections is attributed to the formation and deposition of calcite via microbial activity. The mechanism of calcium carbonate precipitation by these bacteria finds support in multiple findings for normal-strength concrete (Feng et al., 2021; Islam et al., 2022; Mondal & Ghosh, 2018; Xu & Wang, 2018). Consequently, results of this research affirm that bacterial concrete containing these two types of bacteria effectively repairs cracks within a short timeframe for high-strength concrete as well.

3.8.2 XRD and SEM Analyses of Microbiologically Induced Calcium Carbonate

SEM, an electron microscope, produces images by scanning specimens with focused emitted electron beams. These electrons interact with the sample's electrons, generating detectable signals that convey surface topography and structure. Conversely, XRD employs X-rays for mineral quantification and identification. An X-ray beam targets samples at an angle of incidence, with diffracted X-rays reflecting crystal arrangement. Recorded intensities and angles offer insights. Compounds possess distinct diffraction patterns aiding substance identification via comparison to stored library data. Valuable mineral data are extracted from peak shapes and intensities (Andalib et al., 2016).

In this study, bacterial-induced $CaCO_3$ precipitation was investigated using XRD and SEM analyses. Samples for both methods were concrete powder passing through a 75 μm sieve, obtained by grinding cured concrete from rehabilitated crack regions. XRD analysis revealed a higher intensity of calcite in precipitated bacterial concrete. Fig. 14 illustrates XRD results based on the generated powder diffraction by the X-Ray Diffractometer (XRD).

The XRD analysis of *B. flexus* and *S. koreensis* concrete displayed increased calcite intensity, indicating microbial-induced calcite precipitation. Three polymorphs of crystalline CaCO3—vaterite, aragonite, and calcite, in increasing order of stability—exist. The pristine crystalline form, calcite, possesses superior intermolecular forces, rendering it stiffer and less compressible, thereby contributing to the enhancement of bio-concrete's mechanical strength (Rauf et al., 2020). Calcium oxide (CaO) was also present in bio-concrete but with lower

Table 5 Progress of self-healing in cracked concrete

Specimen	Crack width (μm)	Area (μm^2)					
		0 day	7 days	14 days	21 days	28 days	42 days
Control	467.6	– 376,719.6	– 370,158	– 368,673	– 363,601.6	– 362,732	– 360,154
B. flexus 1	218.8	– 114,531.4	– 26,152.7	– 13,790.2	– 8404.5	521.83	2191.54
B. flexus 2	259.69	– 126,747.6	– 84,519.2	– 55,767.4	– 32,763.8	– 20,593	– 4992
S. koreensis 1	203.4	– 111,859.6	– 78,860.3	– 63,204.6	– 39,970.7	– 19,661	– 201.82
S. koreensis 2	288.7	– 144,700.2	– 108,689	– 81,792.1	– 54,179.7	– 23,027	– 3595.1

It should be noted that the negative sign (–) indicates the area is a valley area in the cracked section of concrete

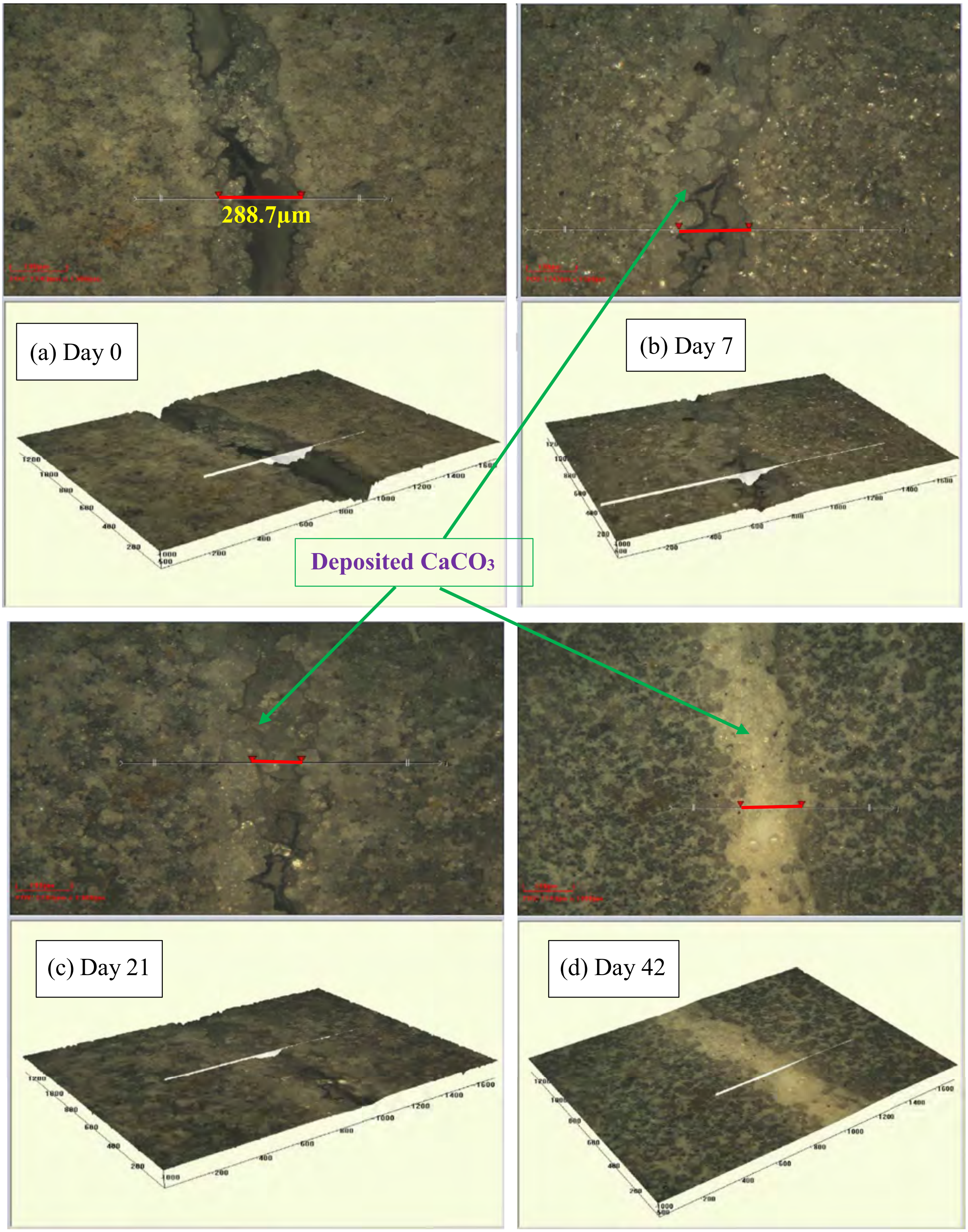


Fig. 12 Self-healing progress of concrete specimen *S. koreensis* 2 at the age of **a** 0 day, **b** 7 days, **c** 21 days **d** 42 days

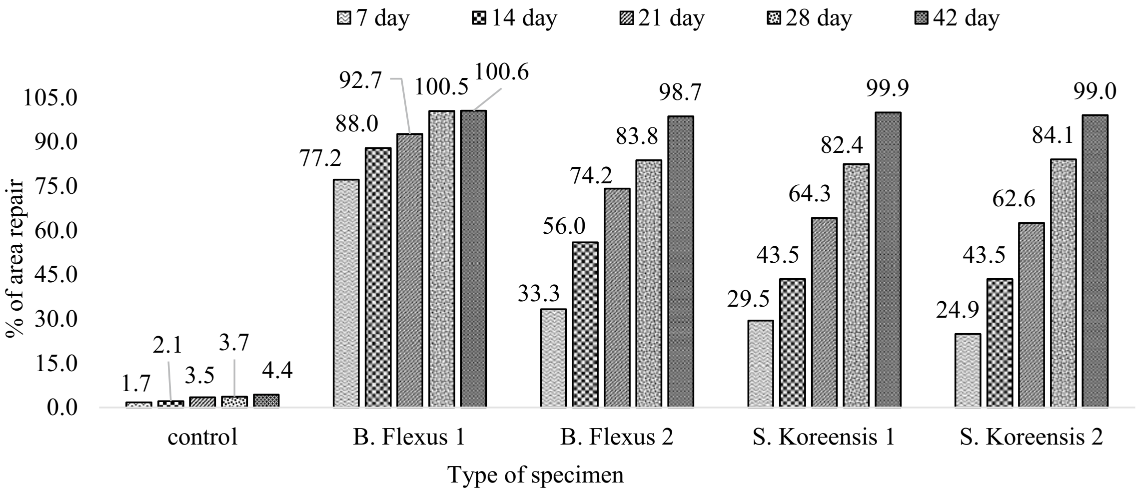


Fig. 13 Percent of area repair in cracked concrete

intensity than calcite. Less stable $CaCO_3$ polymorphs (vaterite, aragonite) decompose into CaO and CO_2. The presence of CaO in bacterial concrete's XRD analysis stems from the decomposition of less stable $CaCO_3$ polymorphs. In contrast, control concrete's XRD analysis revealed byproducts of cement hydration, aligning with previous reports. Pristine crystalline $CaCO_3$ (calcite) was observed in healing product made from bacterial specimens, contributing to the mechanical strength of bacterial concrete due to its crystalline nature.

SEM analysis was employed to assess the physical surface morphology of precipitated calcite in bacterial concrete powder. Analysis of concrete powder passing through a 75 µm sieve revealed the presence of precipitated calcium carbonate in bacterial concrete made with *B. flexus* and *S. koreensis*, as indicated in Fig. 15. Hexagonal calcite crystals were identified in *B. flexus* and *S. koreensis* concrete. This induced calcite filled pores in conventional concrete, enhancing its strength. Conversely, control concrete's SEM analysis showed no calcium carbonate crystals.

Both XRD and SEM analyses substantiate the presence of precipitated calcite in bacterial concrete due to microbial metabolic activity, significantly improving concrete strength by filling voids and fissures in the matrix.

4 Conclusions

This study investigated the impact of microbes on high-strength concrete properties, utilizing *B. flexus* and *S. koreensis*. The research encompassed their effect on mechanical strengths (compressive, flexural), ultrasonic pulse velocity, acid and salt resistance, and microbial healing. Key findings are presented as follows:

- High-strength bacterial concrete, employing *B. flexus* and *S. koreensis*, exhibited improved macro-physical properties compared to conventional concrete. *B. flexus* concrete showed 21.8% and 11.7% higher compressive strength at 7 and 56 days, while *S. koreensis* concrete exhibited 12.2% and 7.4% increases. Flexural strength for *B. flexus* concrete was 6.0% and 9.0% higher at 14 and 28 days, and for *S. koreensis* concrete, it increased by 5.2% and 6.4%.
- Microbial-induced calcite deposition significantly enhanced the healing capacity of cracked bacterial concrete. Within 42 days, *B. flexus* and *S. koreensis* concrete restored cracked sections by 259.7 µm and 288.7 µm crack widths, respectively.
- Bacterial concrete exhibited greater strength restoration following 55% stress-level crack initiation. While normal concrete regained only 64.0% of its 28-day compressive strength, *B. flexus* and *S. koreensis* concrete achieved 96.1% and 94.1% restoration, respectively.
- Enhanced durability against acid and salt attacks was observed in bacterial concrete, with reduced weight and strength loss compared to conventional concrete. *B. flexus* and *S. koreensis* concrete experienced lower strength losses (4.8% and 5.3% for sulfuric acid, 1.8% and 2.1% for magnesium sulfate) than conventional concrete.
- Microbial concrete showed decreased water absorption due to calcite filling voids. *Bacillus flexus* concrete displayed 15.7% and 13.1% reduc-

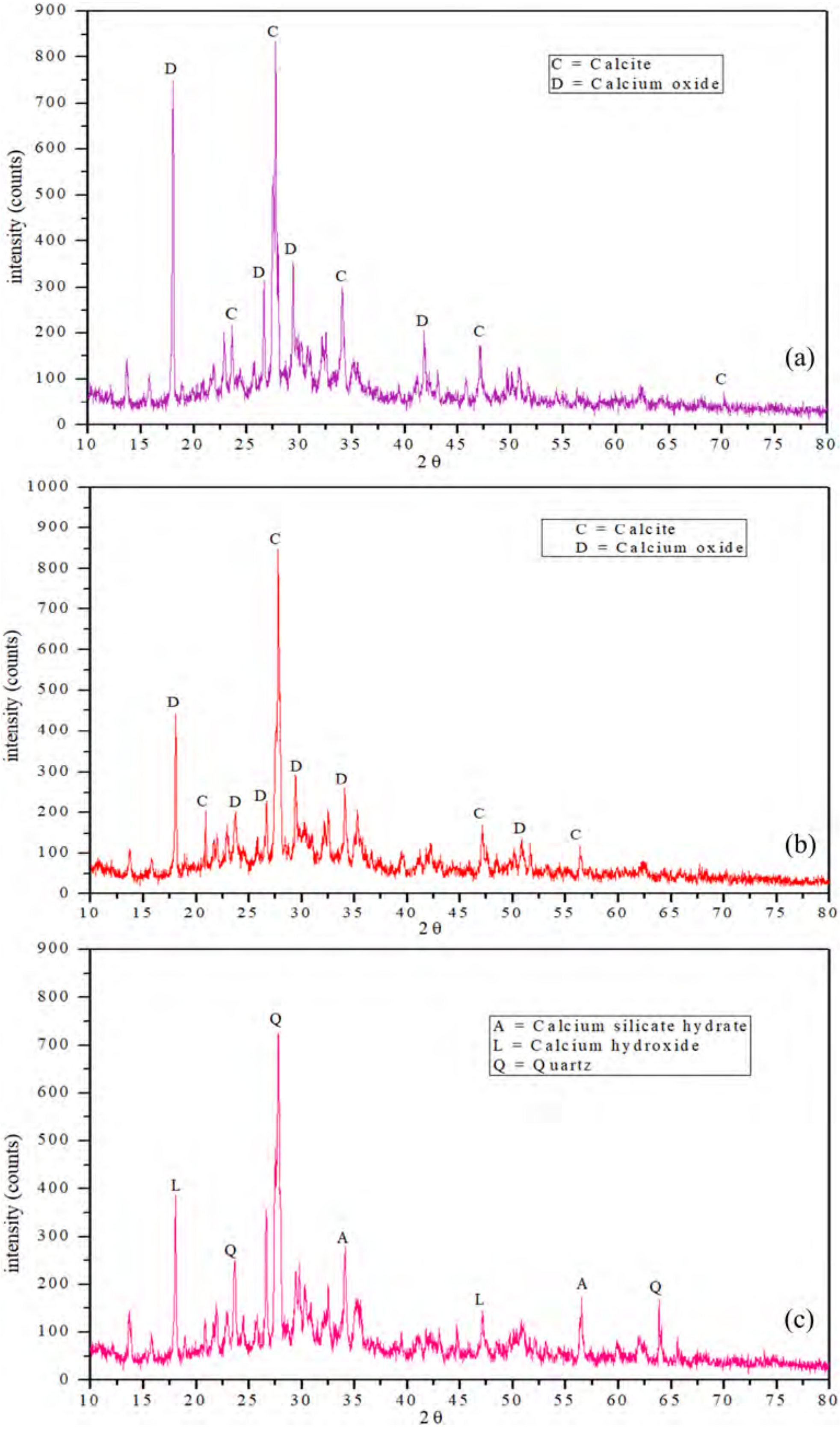


Fig. 14 XRD analysis of concrete made with **a** *B. flexus*, **b** *S. koreensis*, **c** no bacteria

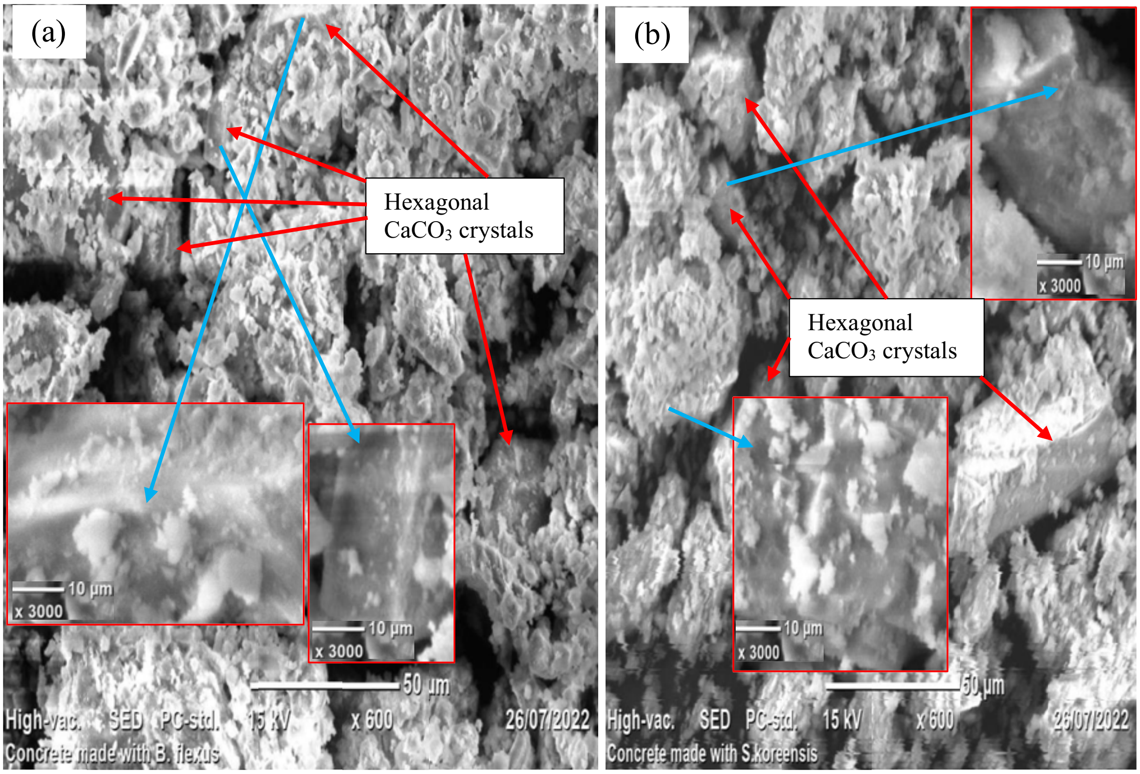


Fig. 15 SEM image analysis of concrete made with **a** *B. flexus*, **b** *S. koreensis*

tion in water absorption, while *S. koreensis* concrete exhibited 4.3% and 2.6% reductions.

- Ultrasonic pulse velocity increased in microbial concrete, particularly *B. flexus*.
- *Bacillus flexus* demonstrated superior enhancement across measurements compared to *S. koreensis*.
- Improved strength, UPV, and acid/salt resistance stem from calcite deposition by microbial activity, filling voids, and fissures in the concrete matrix. The presence of calcium carbonate in these voids bolsters strength and durability of bacterial concrete relative to conventional concrete.

5 Recommendations for Future Study

- Further investigation of high-strength concrete, produced by combining microbial self-healing agents with waste materials for partial aggregate replacement, is recommended for sustainable construction industries.
- Additional research is recommended to promote the utilization of bio-concrete in practical construction projects and assess its self-healing performance under real environmental conditions, including maritime settings, continuous loading, and freeze–thaw cycles.

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Author contributions

Conceptualization and methodology were performed by YMK, TAM, EA, YMG, and DHT; data analysis and interpretation were performed by YMK, TAM and EA; supervision was performed by TAM and EA; and reviewing and editing were performed by YMK, TAM and EA. The first draft of the manuscript was written by YMK, and all authors commented, edited, and revised the manuscript. All the authors have read and approved the final manuscript.

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References

Achal, V., Mukerjee, A., & Reddy, M. S. (2013). Biogenic treatment improves the durability and remediates the cracks of concrete structures. *Construction & Building Materials, 48*, 1–5.

Acharya, P. K., & Patro, S. K. (2016). Acid resistance, sulphate resistance and strength properties of concrete containing ferrochrome ash (FA) and lime. *Construction and Building Materials, 120*, 241–250.

ACI, 2008. *Guide for selecting proportions for high-strength concrete using portland cement and other cementitious materials.* s.l., s.n.

Algaifi, H. A., Bakar, S. A., Alyousef, R., Sam, A. R., Ibrahim, M. W., Shahidan, S., Ibrahim, M., & Salami, B. A. (2021). Bio-inspired self-healing of concrete cracks using new *B. pseudomycoides* species. *Journal of Materials Research and Technology, 12*, 967–981.

Andalib, R., Abd Majid, M. Z., Hussin, M. W., Ponraj, M., Keyvanfar, A., Mirza, J., & Lee, H. S. (2016). Optimum concentration of *Bacillus megaterium* for strengthening structural concrete. *Construction and Building Materials, 118*, 180–193.

ASTM 33. (2016). *Specification for concrete aggregates*. ASTM International.

ASTM C. (2003). *597–02; Pulse velocity through concrete*. American Society for Testing and Material.

ASTM C. (2013). *Standard test method for density, absorption, and voids in hardened concrete*. C642-13.

ASTM C-1. (2020). *Standard practice for making and curing concrete test specimens in the laboratory*. Annual Book of ASTM Standards.

ASTM C184-94e1. (1994). *Standard test method for fineness of hydraulic cement by the 150-µm (No. 100) and 75-µm (No. 200) Sieves (Withdrawn 2002)*. s.l.:s.n. https://www.astm.org/Standards/C184. Accessed Feb 2023.

ASTM C187, 1. (2016). *Standard test method for amount of water required for normal consistency of hydraulic cement paste*. s.l.:s.n. https://www.astm.org/Standards/C187. Accessed Feb 2023.

ASTM C188, 1. (2017). *Standard test method for density of hydraulic cement, ASTM C188-17 standard test method for density of..* s.l.:s.n. https://www.astm.org/Standards/C188. Accessed Feb 2023.

ASTM C191, 1. (2019). *Standard test methods for time of setting of hydraulic cement by vicat needle*. s.l.:s.n. https://www.astm.org/Standards/C191. Accessed Feb 2023.

ASTM International. (2018). *Test method for flexural strength of concrete (using simple beam with third-point loading*. Annual Book of ASTM Standards.

Bhutange, S. P., & Latkar, M. V. (2020). Microbially induced calcium carbonate precipitation in construction materials. *Journal of Materials in Civil Engineering, 32*, 03120001.

Chen, H.-J., Peng, C.-F., Tang, C.-W., & Chen, Y.-T. (2019). Self-healing concrete by biological substrate. *Materials, 12*, 4099.

Durga, C. S. S., Ruben, N., Chand, M. S., Indira, M., & Venkatesh, C. (2021). Comprehensive microbiological studies on screening bacteria for self-healing concrete. *Materialia, 15*, 101051.

Durga, C. S. S., Ruben, N., Chand, M. S. R., & Venkatesh, C. (2020). Performance studies on rate of self healing in bio concrete. *Materials Today: Proceedings, 27*, 158–162.

Feng, J., Chen, B., Sun, W., & Wang, Y. (2021). Microbial induced calcium carbonate precipitation study using Bacillus subtilis with application to self-healing concrete preparation and characterization. *Construction and Building Materials, 280*, 122460.

Gupta, S., Dai Pang, S., & Kua, H. W. (2017). Autonomous healing in concrete by bio-based healing agents. *Construction and Building Materials, 146*, 419–428.

Huynh, N. N., Phuong, N. M., Toan, N. P., & Son, N. K. (2017). *Bacillus subtilis* HU58 immobilized in micropores of diatomite for using in self-healing concrete. *Procedia Engineering, 171*, 598–605.

Iheanyichukwu, C. G., Umar, S. A., & Ekwueme, P. C. (2018). A review on self-healing concrete using bacteria. *Sustainable Structures and Materials, an International Journal, 1*, 12–20.

Islam, M. M., Hoque, N., Islam, M., & Gias, I. I. (2022). An experimental study on the strength and crack healing performance of *E. coli* bacteria-Induced microbial concrete. *Advances in Civil Engineering, 2022*, 1–13.

Jahami, A., & Issa, C. A. (2023). Exploring the use of mixed waste materials (MWM) in concrete for sustainable construction: A review. *Construction and Building Materials, 398*, 132476.

Jahami, A., Khatib, J., & Raydan, R. (2022). Production of low-cost, high-strength concrete with waste glass as fine aggregates replacement. *Buildings, 12*, 2168.

Jonkers, H. M., et al. (2010). Application of bacteria as self-healing agent for the development of sustainable concrete. *Ecological Engineering, 36*, 230–235.

Khaliq, W., & Ehsan, M. B. (2016). Crack healing in concrete using various bio influenced self-healing techniques. *Construction and Building Materials, 102*, 349–357.

Khan, M. B., Dias-da-Costa, D., & Shen, L. (2023). Factors affecting the self-healing performance of bacteria-based cementitious composites: A review. *Construction and Building Materials, 384*, 131271.

Krishnapriya, S., Babu, D. L. V., & Prince Arulraj, G. (2015). Isolation and identification of bacteria to improve the strength of concrete. *Microbiological Research, 174*, 48–55.

Kwon, S.-W., Kim, B. Y., Song, J., Weon, H. Y., Schumann, P., Tindall, B. J., Stackebrandt, E., & Fritze, D. (2007). *Sporosarcina koreensis* sp. nov. and *Sporosarcina soli* sp. nov., isolated from soil in Korea. *International Journal of Systematic and Evolutionary Microbiology, 57*, 1694–1698.

Luo, M., Qian, C.-X., & Li, R.-Y. (2015). Factors affecting crack repairing capacity of bacteria-based self-healing concrete. *Construction and Building Materials, 87*, 1–7.

MacGregor, J. G., Wight, J. K., Teng, S., & Irawan, P. (1997). *Reinforced concrete: Mechanics and design*. Prentice Hall.

Mondal, S., & Ghosh, A. (2018). Investigation into the optimal bacterial concentration for compressive strength enhancement of microbial concrete. *Construction and Building Materials, 183*, 202–214.

Osman, K. M., Taher, F. M., EL-Tawab, A. A., & Faried, A. S. (2021). Role of different microorganisms on the mechanical characteristics, self-healing efficiency, and corrosion protection of concrete under different curing conditions. *Journal of Building Engineering, 41*, 102414.

Rao, R., Kumar, U., Vokunnaya, S., Paul, P., Orestis, I., & Grade, S. (2015). Effect of *Bacillus flexus* in healing concrete structures. *International Journal of Innovative Research in Science, Engineering and Technology, 4*, 7273–7280.

Rauf, M., Khaliq, W., Khushnood, R. A., & Ahmed, I. (2020). Comparative performance of different bacteria immobilized in natural fibers for self-healing in concrete. *Construction and Building Materials, 258*, 119578.

Rohini, I., & Padmapriya, R. (2021). Effect of bacteria subtilis on e-waste concrete. *Materials Today: Proceedings, 42*, 465–474.

Rosewitz, J. A., Wang, S., Scarlata, S. F., & Rahbar, N. (2021). An enzymatic self-healing cementitious material. *Applied Materials Today, 23*, 101035.

Ryparová, P., Prošek, Z., Schreiberova, H., Bílý, P., & Tesarek, P. (2021). The role of bacterially induced calcite precipitation in self-healing of cement paste. *Journal of Building Engineering, 39*, 102299.

Saint-Pierre, F., Philibert, A., Giroux, B., & Rivard, P. (2016). Concrete quality designation based on ultrasonic pulse velocity. *Construction and Building Materials, 125*, 1022–1027.

Sathanandam, T., Awoyera, P. O., Vijayan, V., & Sathishkumar, K. (2017). Low carbonbuilding : Experimental insight on the use of fly ash and glass fi bre for making geopolymer concrete. *Sustainable Environment Research, 27*(3), 146–153.

Schaefer, G. E. (1995). How mid-range water reducers enhance concrete performance. *Concrete Construction, 40*, 5599–5602.

Singh, H., & Gupta, R. (2020). Influence of cellulose fiber addition on self-healing and water permeability of concrete. *Case Studies in Construction Materials, 12*, e00324.

Standard, B., 2009. Testing hardened concrete. *Compressive strength of test specimens, BS EN,* p. 12390–3.

Vijay, K., Murmu, M., & Deo, S. V. (2017). Bacteria based self healing concrete. *Construction and Building Materials, 152*, 1008–1014.

Xu, J., & Wang, X. (2018). Self-healing of concrete cracks by use of bacteria-containing low alkali cementitious material. *Construction and Building Materials, 167*, 1–14.

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