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Article

Performance Evaluation of Rice Husk Ash Incorporated Multi-Bacterial Self-Healing Concrete for Enhanced Mechanical Properties, Durability, and Crack-Healing Efficiency

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Abstract: Shrinkage, mechanical loading and environmental exposure are common causes of cracking in concrete structures, leading to increased permeability, corrosion of the reinforcement, and loss of service life. The previous studies on the self-healing were primarily conducted with a single bacterial species or additional cementitious materials, and the synergistic effect of multi-bacterial consortia and rice husk ash (RHA) has not been well investigated. This research suggests a sustainable approach to improve the mechanical properties, durability and self-healing ability of cracks through the synthesis of a multi-bacterial self-healing concrete using the Microbially Induced Calcium Carbonate Precipitation (MICP) technique through incorporation of rice husk ash (RHA). Three binary bacterial consortia and bacterial dosages (10, 50 and 100 mL/L) were investigated in 28 concrete mixes along with 10, 20 and 30% RHA using compressive strength, flexural strength, ultrasonic pulse velocity (UPV), water penetration, crack-healing efficiency, SEM analyses. The optimum BC-100-RHA20 mixture had a 28-day compressive strength of 32.9 MPa, flexural strength of 5.31 MPa, UPV of 4.78 km/s, minimum water penetration of 11.9 mm and crack-healing efficiency of 96%, which was significantly higher than the control concrete. SEM showed high degree of calcium carbonate precipitation and fine pore structures. The results show that the addition of binary bacterial consortium to RHA is an effective and sustainable method for building concrete with long life infrastructure applications and low maintenance.
Keywords: Bacterial self-healing concrete; Rice husk ash; Microbially induced calcium carbonate precipitation; Bacillus subtilis; Bacillus megaterium; Pseudomonas aeruginosa; Crack healing; Durability; Sustainable concrete.

1. Introduction

1.1 Background

The rising interest in infrastructure deterioration, rising maintenance costs and the environment impact of cement production have driven the research in sustainable and intelligent construction materials. The latest research study completed and published in Scopus indicates that using both self-healing and additional cementitious materials can greatly improve structural durability, while also lowering carbon emissions. The latter technologies have gained significant interest, especially the bacterial self-healing concrete, which enhances the mechanical, durability and sustainability properties. Recent studies have shown that multi-bacterial systems survive better, promote greater calcium carbonate precipitation, and heal cracks better than single bacterial systems. The developments demonstrate the feasibility of achieving the creation of a resilient, eco-friendly concrete that can self-repair cracks and perform for an extended service life, contributing to next-generation resilient infrastructure (Rajesh et al., 2024; Vishal et al., 2025; Sunantha et al., 2025; Akhtar et al., 2025; Elgendy et al., 2025; Gupta & Agarwal, 2026).

1.2 Bacteria Self-Healing Concrete

Among the most promising bio-inspired technologies to autonomously repair cracks, bacterial self-healing concrete has appeared. Among them, the most promising technology for autonomous crack repair based on Microbially Induced Calcium Carbonate Precipitation (MICP) is bacterial self-healing concrete. Bacterial spores enter dormant when the water comes in contact with cracks, and upon activation, they utilize available nutrients to produce calcium carbonate ($CaCO_3$) that fills cracks, refines the pore structure, increases the strength of the interfacial transition zone, and increases matrix densification. Some previous studies have shown that the compressive strength has improved by 10–40% and the water absorption and permeability have been greatly reduced. Some bacterial species, *including Bacillus subtilis, Bacillus megaterium, Bacillus sphaericus* and *Sporosarcina pasteurii*, have been shown to be efficient biomineralizers, but most of the current studies are limited to the use of a single bacterial strain. Recent studies have shown that multi-bacterial consortia are more durable, more efficient in nutrient assimilation, higher in microbial survivability and better at calcium carbonate deposition compared to single species, because of synergistic microbial interaction.

Despite the advantages of multi-bacterial systems, research in the field is limited to specific investigations under distinct experimental conditions, thereby presenting an important research gap (Feng et al., 2021; Sunantha et al., 2025; Akhtar et al., 2025; Elgendy et al., 2025; Gupta & Agarwal, 2026).

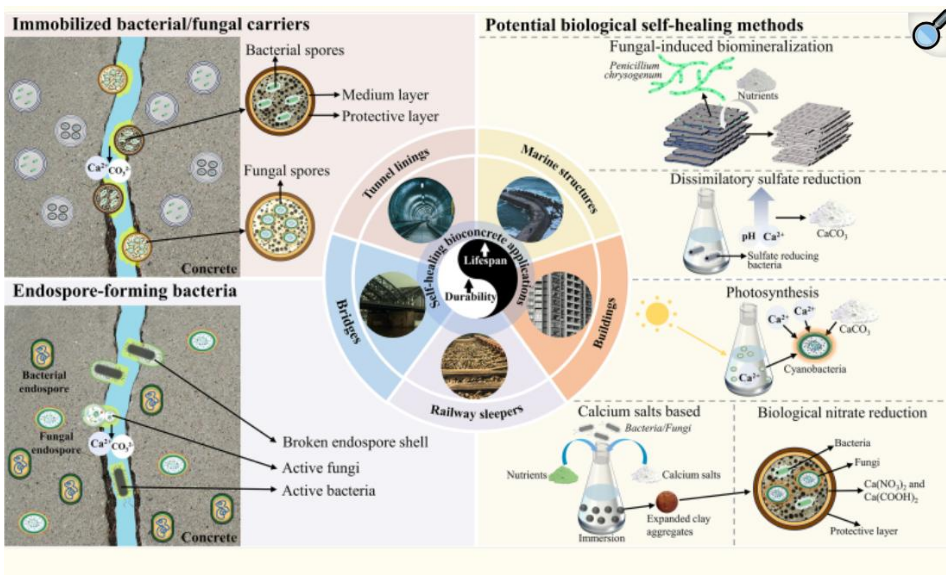

Figure 1: Microbial Induced Calcium Carbonate Precipitation (MICP) Mechanism of Bacteria Self-Healing Concrete (Wong et al., 2024)

1.3 Rice Husk Ash for Sustainable Concrete

The use of supplementary cementitious material is an effective approach to reduce the cement use, minimize the greenhouse gas emission and enhance the durability of concrete. Rice husk ash (RHA), a large amount of pozzolanic by-product of agriculture, is rich in amorphous silica, has high pozzolanic reactivity, can form more calcium silicate hydrate (C–S–H) gel by consuming calcium hydroxide, and can form more C–S–H gel than the amount of amorphous silica in the concrete, which can also enhance the long-term strength. Rice husk ash (RHA) is a plentiful pozzolanic by-product of agriculture which has high pozzolanic activity and can form additional calcium silicate hydrate (C–S–H) gel using calcium hydroxide, can densify the concrete matrix, reduce the permeability of the concrete, and can form more C–S–H gel than the amount of amorphous silica in the concrete, can enhance the long-term strength. The synergistic effect of RHA and bacterial self-healing technology has been proved in recent studies, which have shown that the hybrid of microbial calcium carbonate precipitation and secondary C–S–H formation is found to be more effective in crack healing.

Though these developments are encouraging, few studies have compared the performance of RHA incorporated multi-bacterial self-healing concrete under similar experimental conditions. This is why this study was motivated to create a sustainable concrete system that can enhance mechanical properties, durability and self-healing ability of the concrete, as well as minimizing the maintenance cost and encouraging the utilization of agricultural waste. It has wide industry applications in bridges, marine structures, tunnels, pavement, water-retaining structures, and other critical infrastructures which require long service life and low maintenance (Kokate & Kumar, 2022; Rajesh et al., 2024; Vishal et al., 2025; Akhtar et al., 2025; Elgendy et al., 2025; Gupta & Agarwal, 2026).

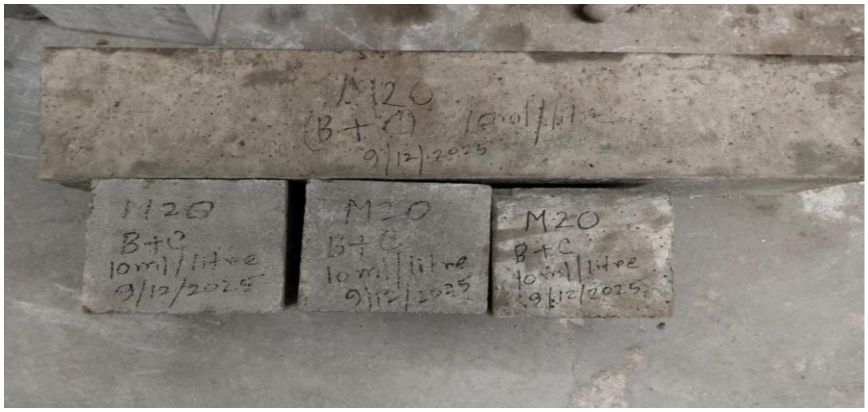

Figure 2: Rice Husk Ash Incorporated Multi-Bacterial Self-Healing Concrete

1.4 Objectives of the Present Study

The specific objectives are

- To evaluate the effect of bacterial dosage and rice husk ash content on compressive and flexural strength,
- To study the durability characteristics by ultrasonic pulse velocity and water penetration tests,
- To assess the crack-healing efficiency under moist curing conditions,
- To validate the healing mechanism by SEM analyses, and
- To identify an optimum sustainable bacterial concrete mixture for durable infrastructure applications.

2. Literature Review

2.1 Development of Bacterial Self-Healing Concrete

One of the most promising bio-based technologies to improve durability and extend the service life of concrete structures is bacterial self-healing concrete, through Microbially Induced Calcium Carbonate Precipitation (MICP). In this procedure metabolically active bacteria precipitate calcium carbonate (CaCO3) which seals microcracks, refines the pore structure and restores the integrity of the cementitious matrix (Achal et al., 2015; Feng et al., 2021). The bacterial biomineralization has shown to significantly reduce permeability, increase crack-healing ability and promote long-term sustainability of concrete structures (; Chen et al., 2019; Gupta & Agarwal, 2026).

Bacillus subtilis, *Bacillus megaterium*, *Bacillus sphaericus* and *Sporosarcina pasteurii* have been the best performers among the other bacterial species studied due to their high urease activity, good survivability under alkaline conditions and efficient calcium carbonate precipitation (Vishal et al., 2025; Chaitanya et al., 2023; Kumar et al., 2024; Javeed et al., 2024; Vedrtnam et al., 2025). Bacterial consortia have recently attracted considerable attention due to the synergistic interactions between different bacterial species, which enhance bacterial survivability, nutrient utilization and biomineralization efficiency, thereby improving mechanical properties and crack-healing performance (Sunantha et al., 2025; Li et al., 2025; Elgendy et al., 2025).

2.2 Mechanical Performance of Bacterial Concrete

The mechanical properties of bacterial concrete are mainly enhanced by the filling of calcium carbonate in the pores, micro-cracks and interfacial transition zone (ITZ), which leads to the densification of the matrix and enhanced load transfer (Feng et al., 2021). Previous studies have reported an improvement in compressive strength of between 10 and 40% depending on the bacterial species, bacterial concentration, curing conditions and nutrient availability (Vishal et al., 2025; Kayam & Bindu, 2024; Kumar et al., 2024; Gupta & Agarwal, 2026). Riad et al. (2025) reported an increase of 26 % in the compressive strength of ultra-high-performance concrete using *Bacillus sphaericus*. Damodaran and Mayilsamy (2025) have reported further enhancement in the compressive strength of ultra-high-performance concrete with the combined incorporation of bacteria, nano-silica and steel fibres. Likewise, Sunantha et al. (2025) reported that bacterial consortia had higher compressive strength and lower porosity compared to single bacterial cultures due to increased bio mineralization.

2.3 Durability and Crack-Healing Performance

In addition to strength, bacterial self-healing concrete also improves durability significantly by decreasing water absorption, permeability, chloride ingress and sorptivity by pore refinement and crack sealing (Rais & Khan, 2021; Singh et al., 2024; Sukumaran et al., 2025). Arora et al. (2025) reported crack healing efficiencies of >85% in fly ash concrete. Carter (2025) found that encapsulated bacterial systems were able to completely heal cracks smaller than 0.4 mm. A comparable study by Elgendy et al. (2025) demonstrated the huge potential of encapsulation techniques in enhancing the survival and long-term healing efficacy of bacteria in harsh environmental conditions. The results show that the bacterial calcium carbonate precipitation has a significant effect on improving the transport resistance and durability of cementitious composites.

2.4 Rice Husk Ash in Bacterial Concrete

Rice husk ash (RHA) has received considerable attention as a sustainable supplementary cementitious material owing to its high amorphous silica content and good pozzolanic reactivity (Amran et al., 2021; Kokate & Kumar, 2022). Amorphous silica reacts with calcium hydroxide, forming more calcium silicate hydrate (C–S–H) gel, which increases the matrix density, reduces permeability and increases durability (Rajesh et al., 2024; Ogundiran et al., 2025). Additionally, recent studies have demonstrated that the integration of bacterial self-healing technology with RHA has resulted in synergistic enhancements in mechanical performance and crack-healing efficiency, attributed to the combination of the formation of secondary C–S–H and bacterial calcium carbonate precipitation (Rajesh et al., 2024; Gupta & Agarwal, 2026).

2.5 Integrated Use of Rice Husk Ash and Multi-Bacterial Systems

In recent years, the importance of microbial biotechnology and use of agricultural waste for sustainable material development has been proved. Kumar et al. (2026) emphasized that industrial biotechnology is a tool for following principles of circular economy by microbial bioprocesses for carbon neutral manufacturing and valorization of biomass. In the synergistic fermentation, crude fibre was reduced by 55.3% and soluble protein was increased by 214% (Zeng et al. 2025), and the amount of reducing sugars was increased by 13.8-fold (Zeng et al. 2025), while the protein digestibility was enhanced by 17% (Zeng et al. 2025). Soponpongpipat et al. (2025) found that the bulk density of biomass briquettes containing 10-20% torrefied cassava rhizome (TCH) showed an increase of about 30% with a heating value of 17.6–20.7 MJ kg-1. Likewise, Li et al. (2023) showed that phenol was completely removed from 300 mg L^{-1} within 24 h with 249.45 nmol P g^{-1} biochar, and it showed up to six reuses. All these studies show that using microbial systems along with agricultural residues can greatly improve the material performance, resource efficiency, environmental sustainability and circular bioeconomy applications, thus creating a solid basis for the development of rice husk ash incorporated multi-bacterial self-healing concrete.

Table 1: Summary of Literature on Bacterial Self-Healing Concrete and Rice Husk Ash

Ref.	Author(s) & Year	Research Focus	Materials/Technique	Major Findings
1	Achal et al. (2015)	Bacterial self-healing concrete	MICP using bacteria	Demonstrated effective calcium carbonate precipitation for autonomous crack healing and improved durability.
2	Feng et al. (2021)	Bio-mineralization in concrete	Bacterial $CaCO_3$ precipitation	Reduced permeability, refined pore structure, and enhanced mechanical properties.
3	Chen et al. (2019)	Durability enhancement	Bacterial concrete	Improved long-term durability through pore refinement and crack sealing.
4	Gupta & Agarwal (2026)	Sustainable bacterial concrete	Multi-bacterial system	Reported significant improvement in crack healing and compressive strength with bacterial biomineralization.

5	Chaitanya et al. (2023)	Bacillus species performance	*Bacillus subtilis*	High survivability under alkaline conditions with efficient $CaCO_3$ precipitation.
6	Kumar et al. (2024)	Self-healing concrete	*Bacillus megaterium*	Improved compressive strength and reduced water permeability.
7	Javeed et al. (2024)	Bacterial concrete	*Bacillus sphaericus*	Enhanced crack healing efficiency and durability.
8	Vishal et al. (2025)	Mechanical performance	Bacterial concrete	Reported 10–40% increase in compressive strength depending on bacterial dosage.
9	Sunantha et al. (2025)	Multi-bacterial consortium	Mixed bacterial cultures	Higher compressive strength and lower porosity than single bacterial cultures due to synergistic biomineralization.
10	Li et al. (2025)	Multi-bacterial interactions	Bacterial consortium	Improved nutrient utilization, bacterial survivability, and calcium carbonate formation.
11	Elgendy et al. (2025)	Encapsulation technique	Encapsulated bacteria	Increased bacterial survival and long-term crack-healing performance.
12	Riad et al. (2025)	UHPC mechanical properties	*Bacillus sphaericus*	Increased compressive strength by approximately 26%.
13	Damodaran & Mayilsamy (2025)	Hybrid bacterial concrete	Bacteria + Nano-silica + Steel fibres	Achieved superior compressive strength through combined reinforcement mechanisms.
14	Rais & Khan (2021)	Durability	Bacterial concrete	Reduced water absorption and chloride penetration.
15	Singh et al. (2024)	Transport properties	Self-healing concrete	Improved sorptivity and permeability resistance.
16	Sukumaran et al. (2025)	Durability assessment	Bacterial concrete	Enhanced long-term durability by effective pore sealing.
17	Arora et al. (2025)	Crack healing	Fly ash bacterial concrete	Achieved crack-healing efficiency greater than 85%.
18	Carter (2025)	Encapsulated bacterial system	Bacterial capsules	Completely healed cracks smaller than 0.4 mm.
19	Amran et al. (2021)	Rice husk ash	Supplementary cementitious material	High amorphous silica content improved pozzolanic activity and sustainability.
20	Kokate & Kumar (2022)	Rice husk ash concrete	RHA incorporation	Enhanced strength and durability through pozzolanic reactions.
21	Rajesh et al. (2024)	RHA + bacterial concrete	Combined RHA and bacteria	Secondary C–S–H and $CaCO_3$ formation significantly improved mechanical properties and crack healing.
22	Ogundiran et al. (2025)	Pozzolanic activity	Rice husk ash	Increased matrix density and reduced permeability.

23	Kumar et al. (2026)	Industrial biotechnology	Biomass valorization	Highlighted microbial biotechnology for circular economy and sustainable material development.
24	Zeng et al. (2025)	Synergistic fermentation	Agricultural biomass	Reduced crude fibre by **55.3%**, increased soluble protein by **214%**, reducing sugars by **13.8-fold**, and protein digestibility by **17%**.
25	Soponpongpipat et al. (2025)	Biomass briquettes	Torrefied cassava rhizome	Improved bulk density by approximately **30%** with heating value of **17.6–20.7 MJ/kg**.
26	Li et al. (2023)	Biochar application	Microbial biochar	Achieved complete removal of **300 mg/L phenol within 24 h** with excellent reusability.

3. Materials and Methods

3.1 Materials

The main binder used in the experimental program was 43 grade Ordinary Portland Cement (OPC) as per IS 8112:2013. Fine aggregate used was river sand confirming to Zone II of IS 383:2016. Coarse aggregate was crushed granite aggregate of nominal size 10mm and 20mm. Potable water was used for mixing and curing of concrete as per requirement of IS 456:2000. Rice husk ash (RHA) is a supplementary cementitious material because of its high amorphous silica content and pozzolanic activity from controlled combustion of rice husk ash. It was observed that optimum addition of RHA resulted in effective refinement of pores, encouraged the formation of secondary calcium silicate hydrate (C–S–H) and enhanced the long-term durability through reduction in cement consumption (Amran et al., 2021; Kokate & Kumar, 2022; Rajesh et al., 2024). 2% weight of cement of Calcium lactate was added as a source of nutrients for bacterial metabolism and calcium carbonate precipitation (Siddique et al. 2017 and Gupta & Agarwal 2026).

Figure 3: Rice Husk Ash, 43 grade Ordinary Portland Cement (OPC), Fine Aggregates (Zone II), Coarse Aggregate (10 mm and 20 mm size), Potable Water, Calcium Lactate (2%) as Nutrient

3.2 Bacterial Culture and Preparation

The three bacterial species, *Bacillus subtilis* (A), *Bacillus megaterium* (B) and *Pseudomonas aeruginosa* (C) were selected due to their high calcium carbonate precipitation potential, good survivability in alkaline environment and proved effectiveness in self-healing concrete (Vishal et al., 2025; Javeed et al., 2024; Sunantha et al., 2025). Binary bacterial consortia A+B, A+C and B+C were prepared and added in a concrete mixture at 10, 50 and 100 mL/L of mixing water. Previous studies have shown that bacterial concentrations between 10^5 and 10^8 cells/ml are optimal for crack-healing efficiency and do not adversely affect cement hydration (Adil et al., 2024; Osta & Mukhtar, 2024; Gupta & Agarwal, 2026).

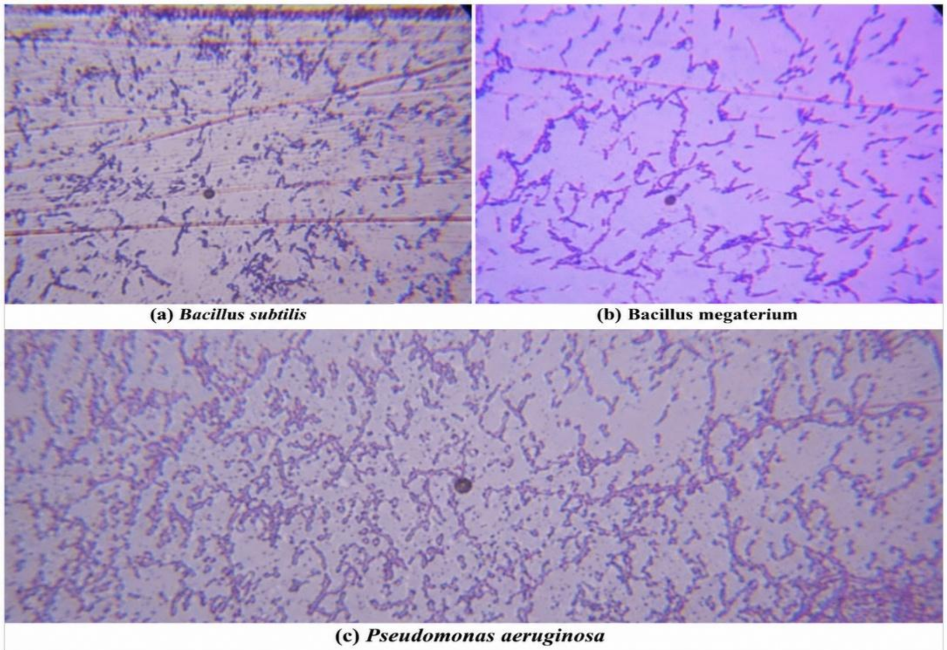

Figure 4: Microscopic View of *Bacillus subtilis*, *Bacillus megaterium and Pseudomonas aeruginosa*

3.3 Mix Proportions and Experimental Programme

The M20 grade concrete was designed as per IS 10262:2019 with water cement ratio of 0.55. The rice husk ash was used in 10%, 20% and 30% of cement content and the bacterial solutions replaced equal amounts of mixing water. A total of 28 concrete mixtures were prepared which include one control mixture and twenty seven bacterial-RHA combinations. The experimental programme studied the combined effect of type of bacterial consortium, bacterial dosage and rice husk ash content on mechanical, durability and self-healing properties of concrete (Gupta & Agarwal, 2026).

- Experimental Procedure

The experimental investigation was carried out in a systematic manner including material characterization, bacterial preparation of culture, mixing of concrete, casting of specimens, curing, mechanical testing, durability assessment, crack-healing evaluation and microstructural characterization. Initially Ordinary Portland Cement (OPC), rice husk ash (RHA), fine aggregate, coarse aggregate and calcium lactate were characterized as per Indian standards. Bacterial cultures (*Bacillus subtilis*, *Bacillus megaterium and Pseudomonas aeruginosa*) were cultured independently to approximately 10^7 colony-forming unit (CFU/mL) and then binary bacterial consortia (A+B, A+C, B+C) were made. The laboratory pan mixer was used to produce concrete mixtures with RHA content of 0%, 10%, 20%, with bacterial dosage of 10mL/L, 50mL/L and 100mL/L. The fresh concrete was poured into moulds, compacted through a vibrating table, demoulded after 24 h and water cured for 28 days. Mechanical, durability, SEM, crack-healing tests were then carried out following an experimental procedure.

Table 2: Concrete Mix Design

Mix ID	Bacterial Consortium	Bacterial Dosage (mL/L)	Rice Husk Ash (%)	Calcium Lactate (% by Cement Weight)	Cement (kg/m³)	Fine Aggregate (kg/m³)	Coarse Aggregate (kg/m³)	Water (L)	Water – Cement Ratio

CM (Control)	None	0	0	0	320	826	1122	176	0.55
AB-10-RH10	*B. subtilis + B. megaterium*	10	10	2	320	826	1122	176	0.55
AB-50-RH10	*B. subtilis + B. megaterium*	50	10	2	320	826	1122	176	0.55
AB-100-RH10	*B. subtilis + B. megaterium*	100	10	2	320	826	1122	176	0.55
AB-10-RH20	*B. subtilis + B. megaterium*	10	20	2	320	826	1122	176	0.55
AB-50-RH20	*B. subtilis + B. megaterium*	50	20	2	320	826	1122	176	0.55
AB-100-RH20	*B. subtilis + B. megaterium*	100	20	2	320	826	1122	176	0.55
AB-10-RH30	*B. subtilis + B. megaterium*	10	30	2	320	826	1122	176	0.55
AB-50-RH30	*B. subtilis + B. megaterium*	50	30	2	320	826	1122	176	0.55
AB-100-RH30	*B. subtilis + B. megaterium*	100	30	2	320	826	1122	176	0.55
AC-10-RH10	*B. subtilis + P. aeruginosa*	10	10	2	320	826	1122	176	0.55
AC-50-RH10	*B. subtilis + P.*	50	10	2	320	826	1122	176	0.55

	aeruginosa								
AC-100-RH10	*B. subtilis + P. aeruginosa*	100	10	2	320	826	1122	176	0.55
AC-10-RH20	*B. subtilis + P. aeruginosa*	10	20	2	320	826	1122	176	0.55
AC-50-RH20	*B. subtilis + P. aeruginosa*	50	20	2	320	826	1122	176	0.55
AC-100-RH20	*B. subtilis + P. aeruginosa*	100	20	2	320	826	1122	176	0.55
AC-10-RH30	*B. subtilis + P. aeruginosa*	10	30	2	320	826	1122	176	0.55
AC-50-RH30	*B. subtilis + P. aeruginosa*	50	30	2	320	826	1122	176	0.55
AC-100-RH30	*B. subtilis + P. aeruginosa*	100	30	2	320	826	1122	176	0.55
BC-10-RH10	*B. megaterium + P. aeruginosa*	10	10	2	320	826	1122	176	0.55
BC-50-RH10	*B. megaterium + P. aeruginosa*	50	10	2	320	826	1122	176	0.55
BC-100-RH10	*B. megaterium + P. aeruginosa*	100	10	2	320	826	1122	176	0.55

BC-10-RH20	*B. megaterium + P. aeruginosa*	10	20	2	320	826	1122	176	0.55
BC-50-RH20	*B. megaterium + P. aeruginosa*	50	20	2	320	826	1122	176	0.55
BC-100-RH20	*B. megaterium + P. aeruginosa*	100	20	2	320	826	1122	176	0.55
BC-10-RH30	*B. megaterium + P. aeruginosa*	10	30	2	320	826	1122	176	0.55
BC-50-RH30	*B. megaterium + P. aeruginosa*	50	30	2	320	826	1122	176	0.55
BC-100-RH30	*B. megaterium + P. aeruginosa*	100	30	2	320	826	1122	176	0.55

3.4 Specimen Preparation and Curing

The dry ingredients such as cement, rice husk ash, fine aggregate and coarse aggregate were initially mixed for around 2 min to get uniform distribution of the ingredients. The bacterial solution with calcium lactate was then added gradually with the rest of the mixing water and the concrete was wet mixed for 3–5 min to get a homogenous concrete. The concrete was poured into the moulds three times, with each layer compacted by vibrating it in the laboratory with a vibrating table to remove entrained air. For compressive strength tests, cube specimens (150 x 150 x 150 mm) were prepared and for flexural strength tests, beam specimens (100 x 100 x 500 mm) were cast. All samples were demoulded and cured in potable water at 27 ± 2°C for 24 h before the testing ages established. To assess the ability of the material to heal cracks in isolation under moist curing conditions, artificial surface cracks were created, ranging between 0.20 and 0.50 mm in width, after curing.

Figure 5: Specimen Preparation and Curing

3.5 Experimental Investigation

The mechanical performance was checked for the compressive strength and flexural strength test according to IS 516 (Part 1):2021. Ultrasonic Pulse Velocity (UPV) as per IS 13311 (Part 1):1992 and water penetration measurements were used to test the durability performance. The efficiency of crack-healing was assessed by measuring the reduction of crack width after 7, 14 and 28 days of moist curing. Some specimens were characterized using Scanning Electron Microscopy (SEM) for the detection of calcium carbonate precipitation, pore refinement and matrix densification. These techniques are well known to validate bacterial biomineralization and to correlate microstructural refinement to improved mechanical and durability performance (Chen et al., 2019; Li et al., 2025).

Figure 6: Compressive Strength Test, Flexural Strength Test, Ultrasonic Pulse Velocity Test, Water Penetration Test

- Replication of Experimental Tests

Three identical specimens were made and tested for every concrete mix and at every testing age to ensure repeatability and reliability of experimental results. The compressive strength, flexural strength, ultrasonic pulse velocity, water penetration depth and crack-healing efficiency are the average value of three independent measurements. A total of 84 cube specimens (28 mixtures x 3 replicates) and 84 beam specimens were cast for mechanical evaluation as well as additional specimens were cast for durability and microstructural investigations. Replicate measurements showed coefficient of variance less than 5%, which is acceptable for the experiment.

- Statistical Analysis

Data from the experiments were analysed by IBM SPSS Statistics 29 or OriginPro 2024 (based on software) All the data is given as mean ± SD (standard deviation) from three independent specimens. One-way Analysis of Variance (ANOVA) was used to compare the significance of different bacterial dosages and rice husk ash replacement levels in mechanical properties, durability and crack healing performance. Differences among the concrete mixtures were found to be significant at the 5% level. If applicable, Tukey's post hoc test was conducted to determine if there were any statistically significant differences between the individual mixtures.

3.6 Experimental Reliability and Safety Considerations

All laboratory work was conducted according to standard concrete testing practices to achieve uniformity in specimen preparation, curing and testing. Bacterial cultures were handled according to standard microbiological safety procedures including sterile preparation, appropriate personal protective equipment (PPE) and controlled disposal of bacterial waste to prevent environmental contamination. Bacillus species are generally considered safe laboratory organisms, but appropriate handling procedures were followed during the investigation. Since this study was conducted in laboratory conditions, it is suggested by recent studies that further long term field studies are required to validate the performance of bacterial concrete under different environmental exposure conditions (Mitikie & Elsaigh, 2025; Carter, 2025; Gupta & Agarwal, 2026).

4. Results and Discussion

4.1 Compressive Strength

The results of the 28-day compressive strength test of all concrete mixtures are shown in Figure -. The improvement in compressive strength compared to control concrete was significant with the addition of binary bacterial consortia and rice husk ash. The control mix (CM) had compressive strength of 22.3 MPa while all bacterial mixtures showed better performance irrespective of bacterial consortium and dosage. The improvement increased progressively with increasing bacterial dosage from 10 to 100 mL/L, which indicated that higher bacterial concentrations promoted higher calcium carbonate precipitation and matrix densification.

The BC-100-RH20 specimen with *Bacillus megaterium–Pseudomonas aeruginosa* consortium with 20% rice husk ash exhibited the highest compressive strength of 32.9 MPa among the mixtures studied, which was an increase of about 47.5% compared to the control concrete. Similarly, AC-100-RH20 and AB-100-RH20 achieved compressive strengths of 32.4 MPa and 31.8 MPa respectively, thereby confirming that binary bacterial consortia greatly enhanced the concrete performance. However, the increase of rice husk ash content to 30% caused a slight decrease in the compressive strength, which indicates that the excess of replacement caused a dilution of the cementitious matrix and a decrease of the availability of hydration products despite the continued activity of bacteria in table 3.

Table 3: Compressive Strength Test Result After 28 Days

Sl. No.	**Mix ID**	**Rice Husk Ash Addition (%)**	**Bacterial Dosage (mL/L)**	**28-Day Compressive Strength (MPa)**
1	CM	0	0	22.3
2	AB-10-RHA10	10	10	25.5
3	AB-10-RHA20	20	10	26.8

4	AB-10-RHA30	30	10	25.9
5	AB-50-RHA10	10	50	27.4
6	AB-50-RHA20	20	50	29.1
7	AB-50-RHA30	30	50	28.3
8	AB-100-RHA10	10	100	29.6
9	AB-100-RHA20	20	100	31.8
10	AB-100-RHA30	30	100	30.7
11	AC-10-RHA10	10	10	25.3
12	AC-10-RHA20	20	10	26.5
13	AC-10-RHA30	30	10	25.8
14	AC-50-RHA10	10	50	28.0
15	AC-50-RHA20	20	50	30.0
16	AC-50-RHA30	30	50	29.1
17	AC-100-RHA10	10	100	30.2
18	AC-100-RHA20	20	100	32.4
19	AC-100-RHA30	30	100	31.1
20	BC-10-RHA10	10	10	25.7
21	BC-10-RHA20	20	10	27.0
22	BC-10-RHA30	30	10	26.1
23	BC-50-RHA10	10	50	28.2

24	BC-50-RHA20	20	50	29.3
25	BC-50-RHA30	30	50	30.4
26	BC-100-RHA10	10	100	30.4
27	BC-100-RHA20	20	100	**32.9**
28	BC-100-RHA30	30	100	31.7

The compressive strength over a 28-day period for all concrete mixtures is shown in Figure 7. The compressive strength of the control mix was the lowest at 22.3 MPa. The study revealed the significant improvement in compressive strength, from 25.3 to 32.9 MPa, when bacterial consortia and rice husk ash were incorporated. As the dosage of bacteria increased, from 10 to 100 mL/L, strength increased successively for all bacterial series. The compressive strength of 20% RHA replacement was found to be always higher than 10% and 30% RHA. The BC-100-RHA20 mixture (*B. megaterium* + *P. aeruginosa*) obtained the highest compressive strength (32.9 MPa) which is about 47.5% better than the control concrete, proving the synergistic effect of multi-bacterial biomineralization and RHA.

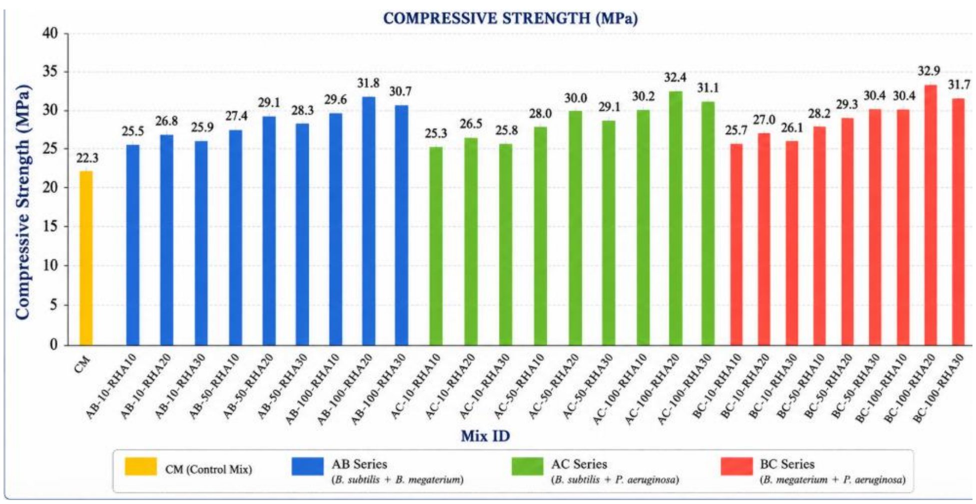

Figure 7: 28-day compressive strength of concrete mixes

4.2 Flexural Strength

All bacterial concrete mixtures were better than the control mix (3.42 MPa) as shown in table 4. The flexural strength was raised with the increase in bacterial dosages, from 3.88-4.21 MPa at 10 mL/L to 4.61-5.31 MPa at 100 mL/L. 20% RHA consistently resulted in the highest strength among the replacement levels of RHA while 30% RHA had a slight drop in strength. The BC-100-RHA20 mixture (*B. megaterium* + *P. aeruginosa*) with the highest flexural strength of 5.31 MPa, which is about 55.3% higher than the control concrete shows the synergistic benefits of using multiple-bacterial consortia and rice husk ash (RHA).

Table 4: Flexural Strength Test Result After 28 Days

Sl. No.	Mix ID	Rice Husk Ash Addition (%)	Bacterial Dosage (mL/L)	28-Day Flexural Strength (MPa)
1	CM	0	0	3.42
2	AB-10-RHA10	10	10	3.92

3	AB-10-RHA20	20	10	4.11
4	AB-10-RHA30	30	10	3.98
5	AB-50-RHA10	10	50	4.23
6	AB-50-RHA20	20	50	4.45
7	AB-50-RHA30	30	50	4.12
8	AB-100-RHA10	10	100	4.61
9	AB-100-RHA20	20	100	5.02
10	AB-100-RHA30	30	100	4.74
11	AC-10-RHA10	10	10	3.88
12	AC-10-RHA20	20	10	4.08
13	AC-10-RHA30	30	10	3.94
14	AC-50-RHA10	10	50	4.31
15	AC-50-RHA20	20	50	4.63
16	AC-50-RHA30	30	50	4.28
17	AC-100-RHA10	10	100	4.72
18	AC-100-RHA20	20	100	5.14
19	AC-100-RHA30	30	100	4.82
20	BC-10-RHA10	10	10	4.01
21	BC-10-RHA20	20	10	4.21
22	BC-10-RHA30	30	10	4.03

23	BC-50-RHA10	10	50	4.42
24	BC-50-RHA20	20	50	4.75
25	BC-50-RHA30	30	50	4.36
26	BC-100-RHA10	10	100	4.81
27	BC-100-RHA20	20	100	**5.31**
28	BC-100-RHA30	30	100	4.95

4.3 Ultrasonic Pulse Velocity (UPV)

Table 5 suggest that the use of rice husk ash and multi-bacterial consortia led to a substantial enhancement in the quality of concrete. The lowest UPV value of 3.82 km/s was obtained for the control mix (CM) which showed comparatively lower matrix density. The UPV values for all bacterial concrete mixtures were found to be from 4.01 km/s to 4.78 km/s, which proved that there is an improvement in internal compactness and reduction in the voids. The highest bacterial dose (100 mL/L) was always better for UPV and 20% RHA delivered the best results for all bacterial consortia. The BC-100-RHA20 mixture had the highest UPV of 4.78 km/s which was about 25.1 % higher than the control mix, indicating better matrix densification and concrete quality.

Table 5: Ultrasonic Pulse Velocity Test Result After 28 Days

Mix ID	**UPV (km/s)**
CM	3.82
AB-10-RHA10	4.01
AB-10-RHA20	4.12
AB-10-RHA30	4.05
AB-50-RHA10	4.18
AB-50-RHA20	4.32
AB-50-RHA30	4.25
AB-100-RHA10	4.41
AB-100-RHA20	4.58
AB-100-RHA30	4.49
AC-10-RHA10	4.03
AC-10-RHA20	4.16
AC-10-RHA30	4.08
AC-50-RHA10	4.27
AC-50-RHA20	4.39

AC-50-RHA30	4.31
AC-100-RHA10	4.50
AC-100-RHA20	4.64
AC-100-RHA30	4.55
BC-10-RHA10	4.08
BC-10-RHA20	4.20
BC-10-RHA30	4.12
BC-50-RHA10	4.33
BC-50-RHA20	4.46
BC-50-RHA30	4.37
BC-100-RHA10	4.61
BC-100-RHA20	**4.78**
BC-100-RHA30	4.69

The minor reduction in UPV at 30% rice husk ash indicates that the excessive replacement of cementitious material contributed slightly to the increase in internal porosity due to the reduction in the availability of cementitious hydration products even though the bacteria remained active. But all mixtures with bacteria had higher UPV values than concrete of control, which confirms positive effect of bacterial biomineralization on the quality of concrete.

4.4 Water Penetration

Table 6 shows that the durability of concrete with rice husk ash containing multi-bacterial self-healing is significantly higher than that of control concrete. The concrete with control admixture (CM) had the highest water penetration depth of 28.4 mm, which showed its high permeability. Unlike the bacterial concrete mixtures, all of the bacterial mixture have lower penetration depths of 11.9 to 23.8 mm that indicate better water resistance. Water penetration values progressively decreased with increasing bacterial dosage from 10 to 100 mL/L, and 20% RHA always showed the lowest water penetration values. The BC-100-RHA20 mixture had the lowest water penetration depth of 11.9 mm, which is about 58.1% less than the water penetration depth of the control concrete, verifying that the mixture has a significant pore refinement and matrix densification, as a result of the combined effect of precipitation of calcium carbonate by the bacteria and pozzolanic reaction of the RHA.

Table 6: Water Penetration Test Result After 28 Days

Mix ID	Water Penetration (mm)
CM	28.4
AB-10-RHA10	23.8
AB-10-RHA20	21.7
AB-10-RHA30	22.8
AB-50-RHA10	19.8
AB-50-RHA20	18.6
AB-50-RHA30	19.2
AB-100-RHA10	16.5

AB-100-RHA20	14.8
AB-100-RHA30	15.6
AC-10-RHA10	23.2
AC-10-RHA20	21.2
AC-10-RHA30	22.1
AC-50-RHA10	18.9
AC-50-RHA20	17.5
AC-50-RHA30	18.4
AC-100-RHA10	15.4
AC-100-RHA20	13.6
AC-100-RHA30	14.4
BC-10-RHA10	22.6
BC-10-RHA20	20.4
BC-10-RHA30	21.3
BC-50-RHA10	18.1
BC-50-RHA20	16.7
BC-50-RHA30	17.5
BC-100-RHA10	13.8
BC-100-RHA20	**11.9**
BC-100-RHA30	12.8

4.5 Crack-Healing Efficiency

According to the results of the experiments carried out in this study, the self-healing ability of the rice husk ash (RHA) incorporated multi-bacterial concrete showed the progressive behavior under moist curing conditions as shown in figure 8. The healing efficiency of control mix (CM) was marginal with 8% after 7 days and 18% after 28 days. All bacterial concrete mixtures, on the other hand, experienced significant crack closure over a period of curing. After 28 days of testing, the mixtures of AB-100-RHA20 and AC-100-RHA20 had healing efficiency of 89% and 92%, respectively. The mixture with BC-100-RHA20 had the best crack-healing efficiency of 96%, which increased from 56% at 7 days to 82% at 14 days. The results are consistent and indicate that calcium carbonate precipitation is significantly enhanced by both multi-bacterial consortia and 20% rice husk ash, thus providing the effective sealing of cracks and good long-term durability.

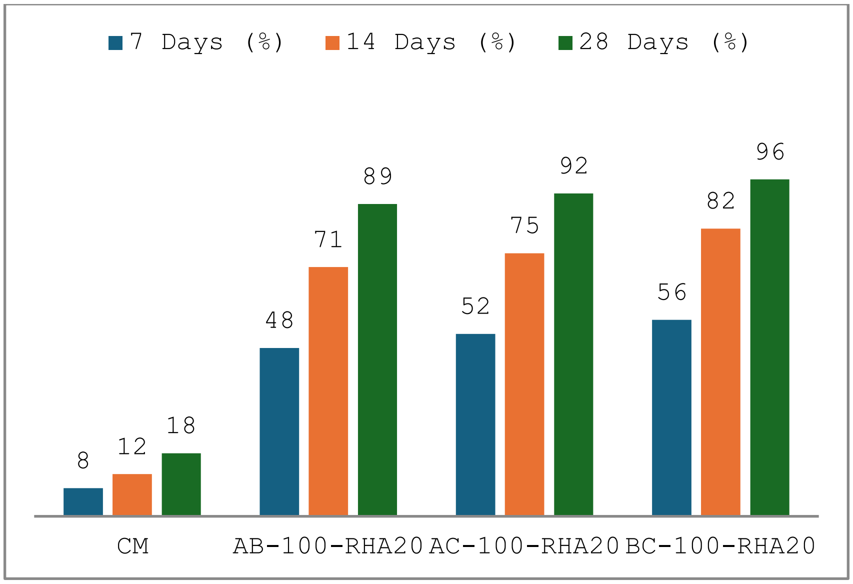

Figure 8: Crack-Healing Efficiency

4.6 Correlation Between Mechanical, Durability, and Microstructural Properties

The results for the cracking efficiency of selected mixtures of concrete under moist curing is shown in Figure 9. Narrow healing occurred in the control mix (CM): There was only 8% healing after 7 days and 18% healing after 28 days. A bacterial concrete mix, on the other hand, exhibited outstanding autonomous crack-healing properties. The healing efficiency for AB-100-RHA20 and AC-100-RHA20 was 89% and 92% respectively, after 28 days. The highest crack healing efficiency was achieved for the BC-100-RHA20 mixture which achieved 96% crack healing at 14 days with progressive healing from 56% at 7 days. The results show that the synergistic effect of multi-bacterial consortium with 20% rice husk ash resulted in high rate of calcium carbonate precipitation, which results in the effective crack closure and better durability.

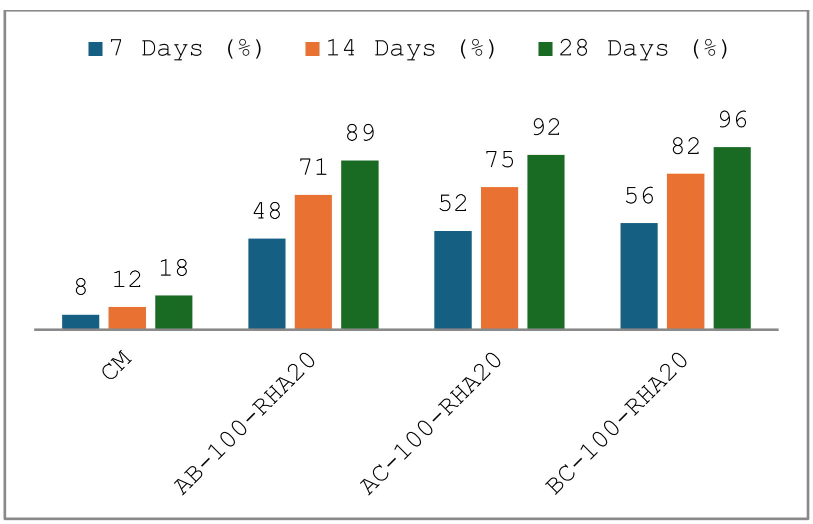

Figure 9: Crack-Healing Efficiency Under Moist Curing

The relation between Compressive Strength (CS) and ultrasonic pulse velocity (UPV) of the selected concrete mixes is shown in figure 10. There is a clear and positive relationship between the compressive strength and UPV values. The compressive strength of the control mix was the lowest (22.3 MPa) while the UPV was the lowest (3.82 km/s) for the same mixture. The highest compressive strength (32.9 MPa) and UPV (4.78 km/s) were obtained for BC-100-RHA20 mixture. The same was found to apply for the AB-100-RHA20 and AC-100-RHA20 mixtures.

Enhanced matrix densification, reduced internal voids and improved quality of concrete due to bacterial calcium carbonate precipitation and pozzolanic reaction of rice husk ash confirmed by increased UPV.

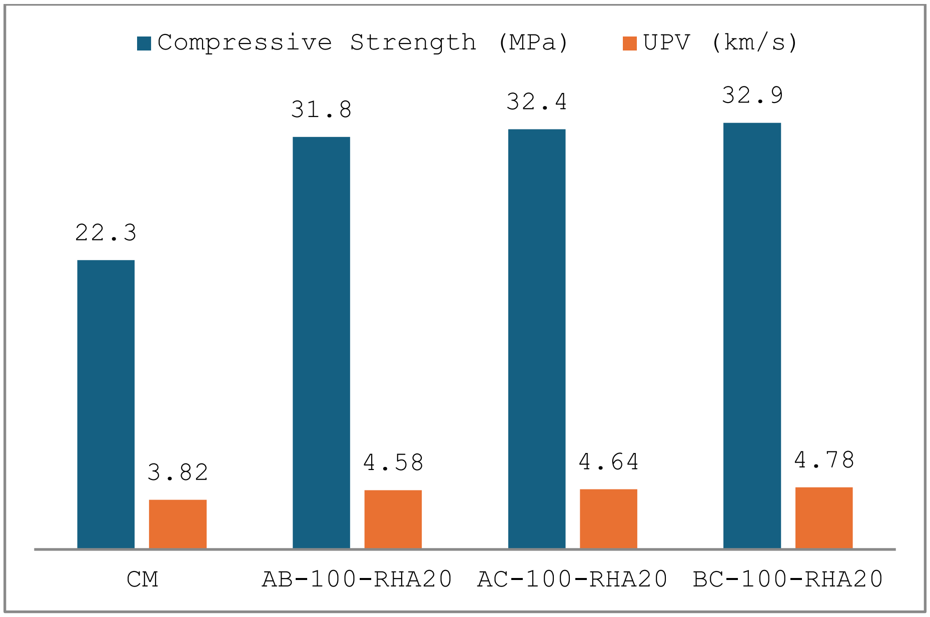

Figure 10: Correlation between Compressive Strength and UPV

The compressive strength has a very negative correlation with water penetration depth as shown in Figure 11. The lowest compressive strength (22.3 MPa) and the highest water penetration (28.4 mm) occurred in the control mix, suggesting that it had the weakest resistance to water penetration. The results, on the other hand, indicated significantly lower depths of penetration for the bacterial concrete mixtures of higher compressive strength. The compressive strength of 32.9 MPa and the minimum water penetration of 11.9 mm were obtained with the BC-100-RHA20 mix followed by the AC-100-RHA20 and AB-100-RHA20 mixes. The results obtained also validate that the permeability of the concrete decreases significantly with the increase of the matrix densification process obtained by the addition of the bacterial biomineralization and rice husk ash, and the mechanical performance is improved.

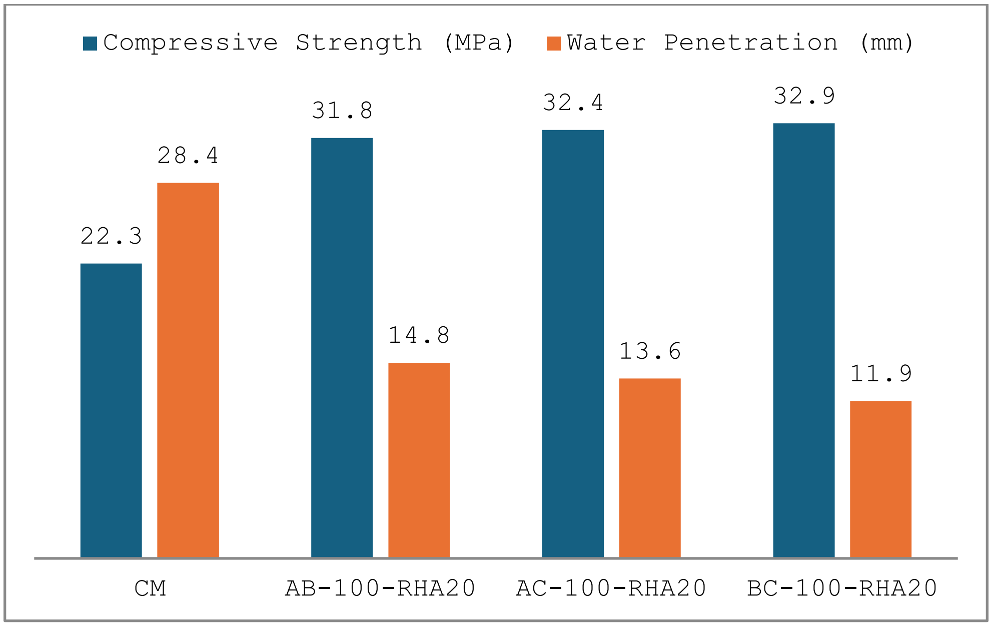

Figure 11: Correlation between Compressive Strength and Water Penetration

The effect of rice husk ash (RHA) dosage on the compressive strength of 100 mL/L dosage of bacteria concrete is presented in Figure 12. The RHA replacement rate was increased from 10% to 20% to boost compressive strength of all bacterial consortia that was achieved by increasing pozzolanic activity and densification of matrix. The BC mixture with the highest RHA content of 20% had the highest strength of 32.9 MPa, followed by the AC (32.4 MPa) and AB (31.8 MPa). The strength, however, declined slightly with RHA content levels of 30%, indicating that too much replacement of cement had a negative effect on hydration. So 20% RHA is the best replacement to maximize the compressive strength.

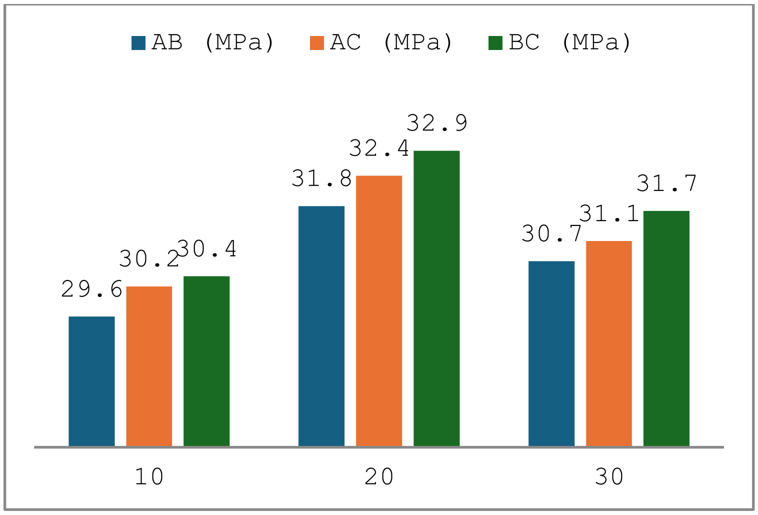

Figure 12: Effect of Rice Husk Ash on Compressive Strength (100 mL/L)

The effect of different dosages of bacteria on the compressive strength with the same RHA (20%) replacement is presented in figure 13. For all of the bacterial consortia, the compressive strength had a consistent trend of rise with increasing bacterial dosage from 10 to 100 mL/L. The AB mixture went from 26.8 MPa to 31.8 MPa and the AC mixture went from 26.5 MPa to 32.4 MPa. The BC consortium showed the greatest improvement from a performance of 27.0 MPa to 32.9 MPa. The enhanced strength is related to increased bacterial activity, increased calcium carbonate precipitation, improved filling of pores and enhanced transition zones between the concrete and the bacteria.

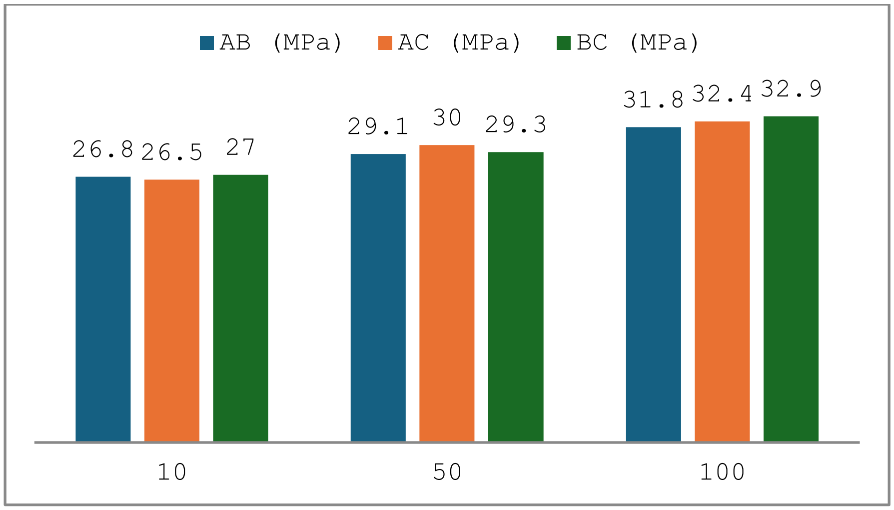

Figure 13:Effect of Bacterial Dosage on Compressive Strength (20% RHA)

4.7 SEM

The SEM micro-graphs showing the comparison of control concrete (CM) and the bacterial concrete (BC-100-RHA20) have been shown in figure (14). The matrix of the control has many interconnected micro-cracks, large pores and less cement hydration products, which means it has a porous microstructure that results in a lower compressive strength (22.3 MPa) and UPV (3.82 km/s). The BC-100-RHA20 specimen has a more compact and dense matrix with significantly fewer voids, a significant number of well-developed calcium carbonate ($CaCO_3$) crystals along crack faces and a clearer and more refined pore structure. The above-mentioned microstructural changes were in good agreement with the highest compressive strength (32.9 MPa), flexural strength (5.31 MPa), UPV (4.78 km/s) and lowest water penetration (11.9 mm), indicating that bacterial biomineralization and 20% rice husk ash could significantly improve the matrix densification, crack sealing and overall durability of concrete.

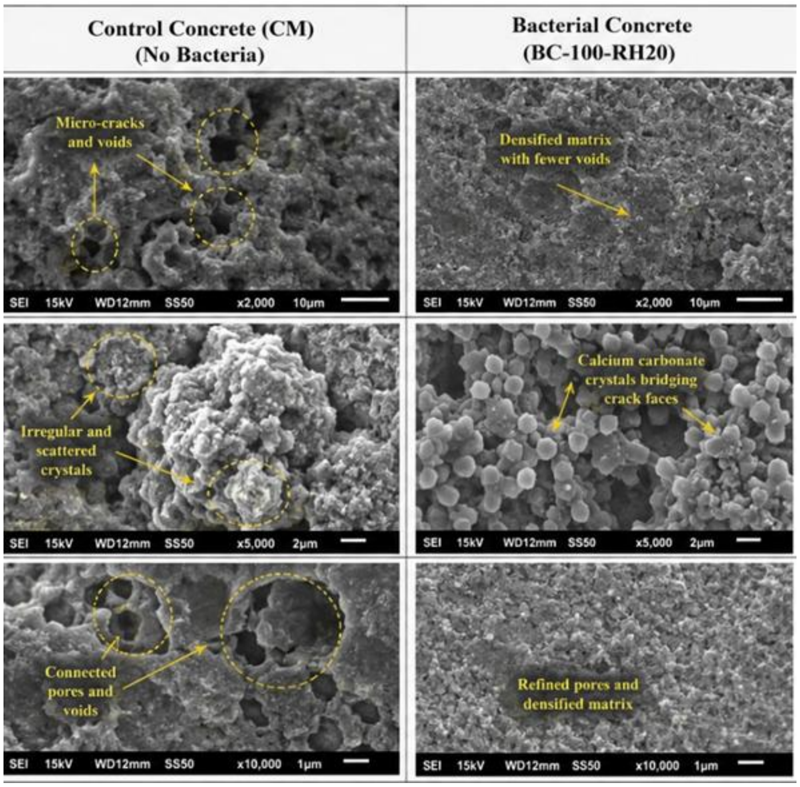

Figure 14: Scanning Electron Microscopy Analysis (SEM)

5. Conclusions

In this study, a multi-bacterial self-healing concrete using binary bacterial consortia together with the Microbially Induced Calcium Carbonate Precipitation (MICP) mechanism was successfully developed and tested for sustainable use of rice husk ash (RHA). In a single experimental programme, the research presents a novel framework by systematically investigating the combined effect of the bacterial consortium type, bacterial dosage and RHA replacement on mechanical properties, durability, crack-healing efficiency and microstructural characteristics. BC-100-RHA20 (*Bacillus megaterium + Pseudomonas aeruginosa*, 100 mL/L bacterial proportion, 20% RHA) proved to be the best performing mixture with compressive strength of 32.9 MPa, flexural strength of 5.31 MPa, UPV of 4.78 km/s, minimum water penetration of 11.9 mm, and crack healing efficiency of 96% after 28 days. The dense calcium carbonate deposition and the refined pore structures were confirmed by SEM observations, which lead to the better engineering performance.

The scientific contribution of this work is the discovery of synergistic effect of the bacterial biomineralization and the pozzolanic effect of rice husk ash in the self-healing of concrete. Applications of the developed material include the use of bridges, tunnels, marine structures, underground constructions, which can benefit from the material's applications in reducing maintenance requirements, improving long-term durability, lowering cement usage and promoting sustainable use of agricultural waste. Long-term field tests, further development of the bacterial encapsulations, life-cycle assessment, techno-economic analysis and embedding with smart structural health monitoring systems are recommended for future research into large-scale infrastructure applications.

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