

Research Article

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Strengthening of structures with bacterial concrete for effective crack repair and durability enhancement

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Abstract: This study explores the impact of bacterial solutions on the performance and microstructural properties of concrete mixes. Incorporating up to 10% bacterial solution demonstrated significant improvements in the dynamic modulus of elasticity for both M40 and M20 grade concrete mixes, particularly when crushed stone sand was used in place of river sand. Microstructural analyses conducted after 28 days of curing confirmed the formation of calcite (calcium carbonate) in all bacterial concrete samples. Advanced investigations using X-ray diffraction and scanning electron microscopy revealed enhanced production of calcium silicate hydrate gel and non-expansive ettringite in bacterial concrete mixes containing 10% bacterial solution, leading to superior compressive strength. Energy-dispersive X-ray analysis further highlighted an increased calcium content, correlating with the presence of calcite. The findings underscore the potential of bacterial solutions to enhance the strength, durability, and longevity of concrete structures through improved microstructural composition. From the microstructural analysis, it has been derived that calcite contributed more to the improved strength and longevity of the bacterial concrete specimens.

Keywords: bacterial concrete, *Bacillus subtilis* bacteria, calcium lactate, SEM, XRD, EDAX

1 Introduction

Bacteria, microscopic single-celled organisms, inhabit a wide range of environments worldwide, including soil, water, hot springs, and even within living organisms. They are incredibly abundant, with a gram of soil containing around 40 million bacterial cells and a milliliter of water containing approximately one million bacterial cells. Collectively, bacteria represent a significant portion of the Earth's biomass, estimated at around five non-million ($5 \times 1,030$) bacteria. The study of bacteria dates back to the seventeenth century when Antoine van Leeuwenhoek first observed microorganisms using a single-lens microscope he designed. He referred to these organisms as "animalcules" and communicated his discoveries to the Royal Society through letters. Later, in 1838, Christian Gottfried Ehrenberg coined the term "bacterium." Louis Pasteur's demonstration in 1859 that microorganisms are responsible for fermentation, along with the work of Robert Koch and Pasteur, laid the groundwork for the disease germ hypothesis. Bacteria typically possess two types of cell walls: Gram-positive and Gram-negative, which react differently to the Gram stain test, a widely used method for classifying bacterial species. In laboratory settings, bacteria are cultured using liquid or solid media. Solid growth media, such as agar plates, are used to isolate pure cultures from bacterial strains, while liquid media are employed when large quantities of cells are needed or when growth quantification is necessary. The growth of bacteria follows three phases. The lag phase is the initial stage, during which bacteria adapt to their new environment in a high-nutrient setting, preparing for rapid growth. This phase is characterized by slow expansion and increased biosynthesis to support subsequent growth. The log phase, or the exponential phase, follows, marked by rapid and exponential proliferation as cells divide at their maximum rate. Nutrients are metabolized efficiently during this phase until depletion begins to hinder further growth. The stationary phase is the final stage, triggered by nutrient exhaustion, during

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which cells reduce metabolic activity and utilize non-essential cellular proteins.

Researchers have proposed various bacterial concretes utilizing different bacteria, as referenced in studies [1–4]. In this particular study, *Bacillus subtilis* was chosen as the bacterial agent. The key advantage of incorporating bacteria into concrete lies in their ability to consistently precipitate calcite, a process known as microbiologically induced calcite precipitation (MICP) [5]. Given the broad scientific and technological implications of calcium carbonate precipitation, which is common among bacteria, this study focused on *B. subtilis*, a soil bacterium grown in laboratory conditions, to assess its impact on concrete strength and physical durability. Observations of bacterial colonies on nutrient agar plates revealed round, convex, soft, and translucent characteristics. After 24 h of incubation under dark aerobic conditions at 37°C, colony sizes could reach up to 2–3 mm. Furthermore, the organism produced a bluish-green pigment that diffused into the surrounding medium. Stock cultures of *B. subtilis* are maintained on nutrient agar slants, where they are streaked using an inoculating loop and then incubated at 37°C. Once grown for 2–3 days, the slant cultures are refrigerated at 4°C until needed again, with subculturing taking place every 90 days [6]. Contamination from other microorganisms on nutrient agar plates is routinely checked by streaking. The concept of bacterial concrete was first introduced by Ramakrishnan *et al.* [7], where microbiologically generated calcite (CaCO_3) precipitation was utilized to repair cracks and fissures in concrete. This process, known as MICP, falls within the field of biomineralization. *B. subtilis*, a common soil bacterium, can induce calcite precipitation, demonstrating its potential as a microbial sealer for consolidating cracks in granites and sand. MICP has been utilized to improve the compressive strength and stiffness of fractured concrete specimens, offering promising results in concrete rehabilitation [8–11].

The MICP process, part of the broader category of biomineralization, involves the generation of inorganic solids by living organisms or bacteria. *B. subtilis*, when incorporated into concrete under suitable conditions, consistently precipitates a new, highly impermeable layer of calcite on the existing concrete surface. This calcite possesses a coarse crystalline structure, adhering to the concrete surface in scales. Being highly insoluble in water, it continuously grows, resisting the penetration of detrimental agents like chlorides, sulfates, and carbon dioxide into the concrete, thus mitigating their harmful effects. The resulting concrete, enriched with bacteria, can be considered a Smart Bio Material for concrete repair due to its ability to consistently precipitate calcite [12]. MICP involves

intricate biological reactions that depend on factors like medium porosity, bacterial cell concentration, and nutrient availability. The bacteria produce calcite in the presence of nutrients, with an ideal hydrogen ion concentration of around nine for *Bacillus pasteurii* growth. However, concrete's alkaline environment, with a hydrogen ion concentration of approximately twelve, inhibits bacterial growth. Nevertheless, *B. pasteurii* can form endospores capable of surviving harsh conditions, making it adaptable for concrete applications. Microbially altered concrete or mortar is an increasingly significant area of research, with studies examining the effects of bacterial addition on the microstructure of cement–sand mortar [13].

In the realm of global infrastructure projects, cement concrete plays a vital role in building construction. However, its brittleness and susceptibility to strain necessitate steel reinforcement, leading to maintenance challenges over time. Self-healing concrete, incorporating *B. subtilis* along with salt and nutritional broth, aims to address these issues by enabling the concrete to repair itself when cracks occur. This innovative approach holds promise for extending the lifespan of concrete structures and reducing maintenance costs [14]. Ongoing research in this field aims to further understand the mechanisms behind self-healing concrete and facilitate its practical implementation in real-world structures [15]. Understanding the diverse range of bacteria capable of thriving in concrete is paramount for enhancing the durability of public infrastructure. It involves delving into the catalysts that trigger chemical processes within these microorganisms, the ensuing reactions they undergo when exposed to these catalysts, and their collaborative efforts in not only preemptively healing cracks but also reinforcing the overall structure they inhabit. When exposed to air and nutrients, these bacteria initiate a hardening and fusion process, effectively filling formed cracks, fortifying the concrete structure, and sealing crack boundaries. This proactive healing mechanism not only extends the structure's lifespan but also addresses and rectifies existing cracks, often within just a few days [16,17].

Current concrete designs adhere to established standards, tolerating cracks up to 2 mm wide as they typically do not compromise structural safety. Many types of concrete possess inherent crack-healing capabilities, with autonomous healing closely linked to the unreacted capacity of cement particles in the concrete matrix. Water ingress reacting with these particles closes small cracks. However, in tunnels and underground structures with minimal crack formation, water ingress poses a challenge to independent crack healing. While standard specimens exhibit self-healing in 0.2 mm wide cracks, samples

incorporating bacteria achieve complete crack closure [18]. Our unique version of self-healing concrete centers on employing specific bacteria, notably *B. subtilis*, to prevent microscopic cracks from progressing into larger, enduring ones. This biocalcification process involves several elements. Bacteria enzymatically hydrolyze urea, generating ammonia and carbon dioxide. Urease then deposits a highly impermeable calcite layer (CaCO_3) onto the concrete surface. This thick layer obstructs crack development, reduces vulnerability to water ingress and corrosion, and enhances concrete structure strength and durability. Modern techniques like X-ray diffraction (XRD) and scanning electron microscopy (SEM) are utilized to quantify spar deposition phases on surfaces and within cracks [19].

The microstructure of bacterial concrete was examined using XRD, energy-dispersive X-ray analysis (EDAX), and SEM analysis. Additionally, SEM, energy-dispersive X-ray spectroscopy (EDX), and XRD analyses were conducted on both bacterial and conventional concrete samples. These analyses facilitated the establishment of empirical connections between various parameters. Previous studies predominantly utilized river sand as fine aggregate to explore the impact of bacteria on mechanical characteristics. Hence, an investigation was undertaken to assess how the strength properties of bacterial concrete are influenced by substituting river sand with crushed rock sand. SEM and XRD measurements were employed to visualize the microstructure of concrete with and without the addition of bacteria.

2 Review literature

In a study by Abo-El-Enin *et al.* [20], the influence of carbonate precipitation induced by microbes on the strength and water absorption of cement–sand mortar was investigated. Using mildly alkalophilic aerobic *Sporosarcina pasteurii* in varying cell concentrations, the researchers found that adding approximately one optical density (1 OD) of bacterial cells to the mixing water increased the compressive strength of cement mortar by 33% within twenty-eight days. SEM analysis revealed the expansion of spar crystals within the cement-sand matrix's pores, contributing to the strength gain and reduction in water absorption. However, adding 1.5 OD of bacterial cells led to less strength enhancement, suggesting that 1 OD was the optimal concentration for maximizing mortar improvement, resulting in reduced water absorption and enhanced compressive strength.

In another study led by Siddique *et al.* [16], the impact of microbes on the strength and permeability properties of concrete incorporating silica fume (SF) instead of cement

was investigated. Varying amounts of SF (5, 10, and 15%) replaced cement, and a constant concentration of bacterial culture (105 cfu/mL of water) was added. The results showed that concrete with bacterial incorporation into SF exhibited a 10–12% increase in compressive strength after 28 days, along with a significant decrease in water absorption, porosity, and capillary water hazards of 42–48, 52–56, and 54–78%, respectively, compared to non-bacterial samples. Moreover, bacterial concrete demonstrated reduced chloride permeability and increased load-bearing capacity compared to non-bacterial concrete after 56 days. SEM and XRD analyses confirmed the presence of spar precipitation on additional microorganisms, contributing to the improved characteristics of the concrete.

Furthermore, Balam *et al.* [21] investigated the simultaneous use of structural lightweight concrete mixtures (LWAC) and microbially induced carbonate precipitation to reduce concrete porosity and water absorption. By inoculating bacteria in the concrete mix water and treating aggregates, four different forms of LWAC were created. The experimental specimens exhibited an average 20% increase in compressive strength, a 10% decrease in chloride penetration, and a 10% reduction in water absorption compared to control samples. SEM research revealed that LWAC specimens with bacteria in both the concrete mix water and aggregates were denser and less porous than those with bacteria solely in the concrete mix water.

Moreover, Zhang *et al.* [22] explored the use of expanded perlite (EP) as a special microbe carrier for fracture healing in concrete by immobilizing *Bacilli cohnii*. Compared to the direct injection of microorganisms, specimens containing EP-immobilized microorganisms showed superior fracture repair after each healing interval, with significantly higher values of totally restored fracture widths. XRD and field emission SEM analyses confirmed the presence of spar crystals on the fracture surfaces as mineral precipitations.

Additionally, Kunal *et al.* [23] focused on the effects of treating cement kiln dust microorganisms (*Bacillus halodurans* strain KG1) on concrete characteristics. Adding 10% treated cement kiln dust improved the concrete's strength, reduced water absorption, and enhanced consistency. SEM and XRD analyses revealed the production of non-expansive ettringite in dense pores and an increase in calcium silicate hydrate (CSH), contributing to improved compressive strength.

Furthermore, Luo *et al.* [24] developed a self-healing substance based on biological carbonate precipitation to enhance concrete's capacity to mend cracks. This biochemical compound effectively increased the self-healing capability of cement paste specimens, significantly reducing water permeability after 80 days of restoration. SEM and XRD analysis demonstrated a spherical crystal shape in the

carbonate precipitation, highlighting its potential to improve concrete durability.

Lastly, Siddique *et al.* [15] investigated how *Bacillus aerius* affected rice husk ash-infused concrete. Bacterial addition increased compressive strength while decreasing porosity, chloride permeability, and water absorption. SEM and XRD analysis confirmed the formation of ettringite, CSH, and calcite in the concrete, contributing to densification and improved durability.

3 Materials and mix design

3.1 Materials

3.1.1 Cement

The study utilized Ordinary Portland cement of grade 53, thoroughly examined in compliance with IS: 4031-1996 requirements. Table 1 summarizes the physical properties of Portland cement (53 grade), including fineness, normal consistency, specific gravity, initial and final setting times, compressive strength at different ages, and soundness. The results demonstrate that the cement meets the specified requirements, ensuring its suitability for concrete compositions.

3.1.2 Fine aggregates

Fine aggregates, comprising locally available river sand and crushed stone sand, underwent a meticulous

examination of their gradation characteristics. Specific gravity values for river sand and crushed stone sand were determined as 2.68 and 2.77, providing insights into their relative density. The study ensured a comprehensive assessment of these materials' quality, crucial for influencing concrete performance.

3.1.3 Coarse aggregate

The coarse aggregate component consisted of crushed granite broken stone with a nominal size of 20 mm, chosen for durability and suitability for concrete applications. The grading characteristics of the coarse aggregate were systematically analyzed, ensuring compliance with prescribed standards.

3.1.4 Water

Fresh water, meeting accepted quality standards, served as the main mixing medium, ensuring the integrity of the concrete mixture. Adherence to IS: 456-2000 protocols for mixing and curing concrete enhanced the dependability and repeatability of experimental findings. The study's dedication to industry standards underscored scientific rigor during the testing and concrete manufacturing stages.

3.1.5 *B. subtilis* bacteria

B. subtilis bacteria, cultured at the DVS Biolife Pvt Ltd Laboratory in Hyderabad, India, played a crucial role in the study. The bacteria, along with nutritional broth and calcium lactate, contributed to innovative concrete formulations. Calcium lactate, in powdered form, facilitated easy integration into the mix, enhancing specific properties alongside *B. subtilis* bacteria. The synergistic interaction between calcium lactate and bacteria showcased the versatility of such additives in influencing concrete properties.

The study also employed sophisticated analytical techniques such as SEM, EDAX, and XRD to provide a thorough analysis of microstructural and elemental composition, abrasion resistance, and dynamic modulus of elasticity. Figure 1 illustrates the culturing process of *B. subtilis* bacteria, highlighting the meticulous approach adopted in the research.

3.2 Mixing

Mixing ingredients are prepared in a pan mixer of a capacity of 100 L.

Table 1: Physical properties of Portland cement (53 grade)

S. no.	Test property	Result	Requirements as per IS: 12269-1987 [83]
1	Fineness		
	(a) Sieve test	2.2%	Not more than 10%
	(b) Blaine	292 m ² /kg	Min 225 m ² /kg
2	Normal consistency	31.6%	—
3	Specific gravity	3.05	—
4	Initial setting time	90 min	Not less than 30 min
5	Final setting time	292 min	Not more than 600 min
6	Compressive strength		
	(a) 3 days	30 N/mm ²	27 N/mm ² (min)
	(b) 7 days	42 N/mm ²	37 N/mm ² (min)
	(c) 28 days	57 N/mm ²	53 N/mm ² (min)
7	Soundness (Le-Chatlier Exp.)	2 mm	Not more than 10 mm



Figure 1: Culturing of *B. subtilis* bacteria.

3.2.1 Casting of specimens

Before concrete is poured, the inside surfaces of the cast iron cube, cylinder, and prism molds are carefully cleaned and oil-treated. The molds are filled with evenly mixed bacterial concrete.

3.2.2 Design proportions

Concrete grades M40 and M20 are constructed with the mix proportions according to IS: 10262-2009. The materials needed for one cubic meter of concrete are listed in Tables 2 and 3.

4 Results and discussion

This section describes the microstructural analysis of bacterial concrete specimens using an EDX analyzer-equipped SEM and an X-ray diffractometer. It shows the SEM micrographs, XRD pattern, and EDX spectra of concrete samples obtained from compressive strength and fracture healing tests.

4.1 SEM and EDX analysis

Concrete specimens are subjected to examination using an SEM to identify the presence of calcite crystals precipitated by bacteria. Additionally, an X-ray technique known as EDX analysis is employed to determine the elemental

Table 2: Proportion of ingredients per one cum of M20 grade concrete

Mixture no	RBC00	RBC05	RBC10	RBC15	CBC00	CBC05	CBC10	CBC15
Cement (kg/m ³)	340	340	340	340	340	340	340	340
River sand (kg/m ³)	736	736	736	736	—	—	—	—
Crushed rock sand (kg/m ³)	—	—	—	—	736	736	736	736
Coarse aggregate (kg/m ³)	1,214	1,214	1,214	1,214	1,214	1,214	1,214	1,214
W/c ratio	0.48	0.48	0.48	0.48	0.48	0.48	0.48	0.48
Bacterial cells (cfu/mL)	10 ⁵	10 ⁵	10 ⁵	10 ⁵	10 ⁵	10 ⁵	10 ⁵	10 ⁵
Percent of bacterial solution	00	05	10	15	00	05	10	15

Table 3: Proportion of ingredients per one cum of M40 grade concrete

Mixture no	RBC00	RBC05	RBC10	RBC15	CBC00	CBC05	CBC10	CBC15
Cement (kg/m ³)	390	390	390	390	390	390	390	390
River sand (kg/m ³)	642	642	642	642	—	—	—	—
Crushed rock sand (kg/m ³)	—	—	—	—	642	642	642	642
Coarse aggregate (kg/m ³)	1,261	1,261	1,261	1,261	1,261	1,261	1,261	1,261
w/c ratio	0.42	0.42	0.42	0.42	0.42	0.42	0.42	0.42
Bacterial cells (cfu/mL)	10 ⁵	10 ⁵	10 ⁵	10 ⁵	10 ⁵	10 ⁵	10 ⁵	10 ⁵
Percent of bacterial solution	00	05	10	15	00	05	10	15

RBC: bacterial concrete with river sand. CBC: bacterial concrete with crushed stone sand.

composition of various materials. EDX analysis specifically focuses on determining the weight percentage and intensity of the calcium element (Ca) and its correlation with the presence of calcite (CaCO_3). During the SEM and XRD examination, bacterial concrete specimens are crushed into fine powder to facilitate better observation of the results. The XRD spectrum of the powdered concrete is analyzed within the range of $2\theta = 2^\circ$ to 500° . Different peaks appear at

various phases of the study, providing insights into the composition and structure of the concrete.

To prepare the powdered specimen for analysis, it is placed on brass stubs using carbon tape and then coated with gold before being analyzed at 20 kV. This preparation method ensures optimal conductivity and stability of the sample during analysis. The samples are evaluated after a standard 28-day curing period, ensuring consistency and

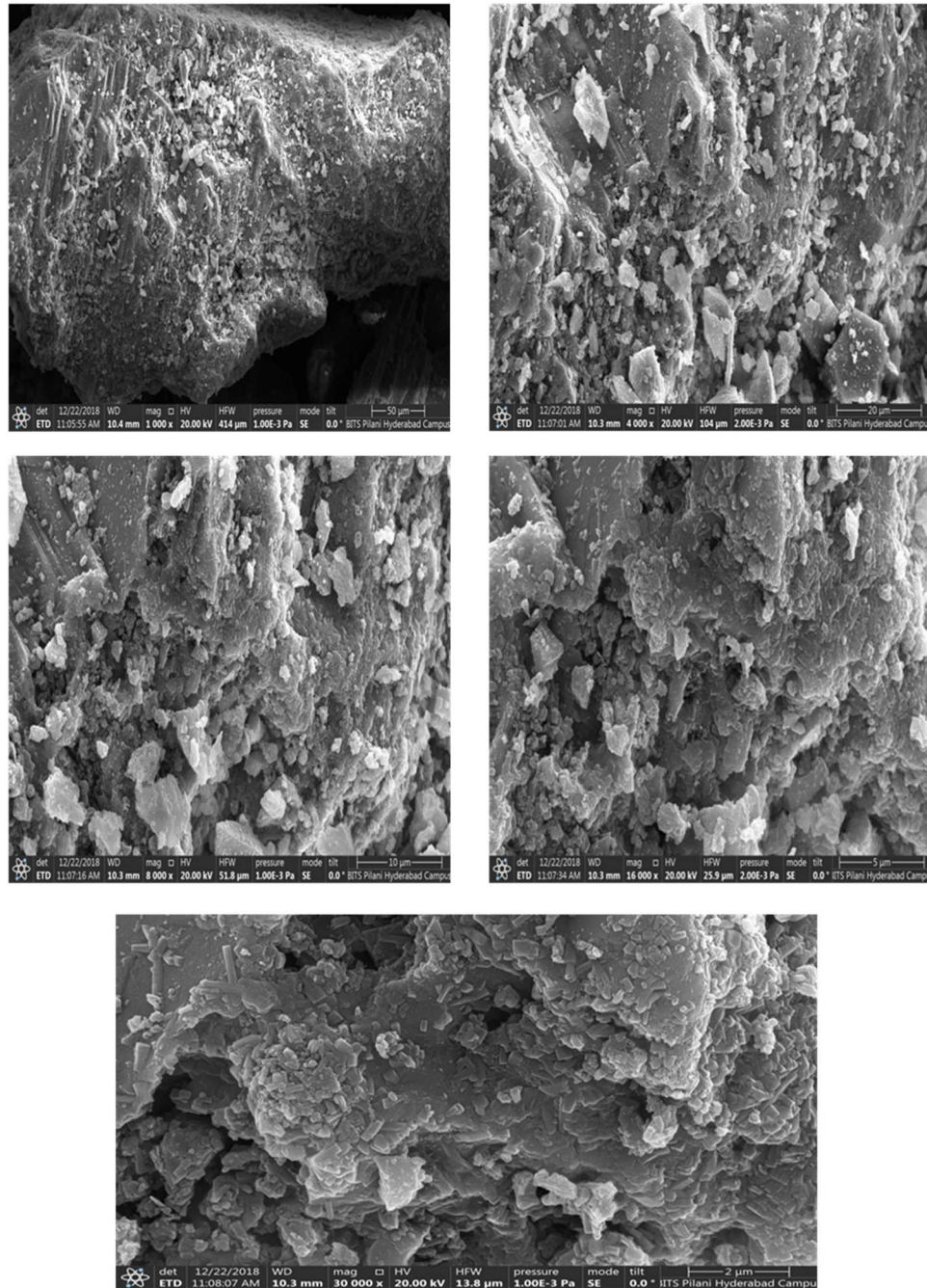


Figure 2: SEM micrograph for conventional concrete.

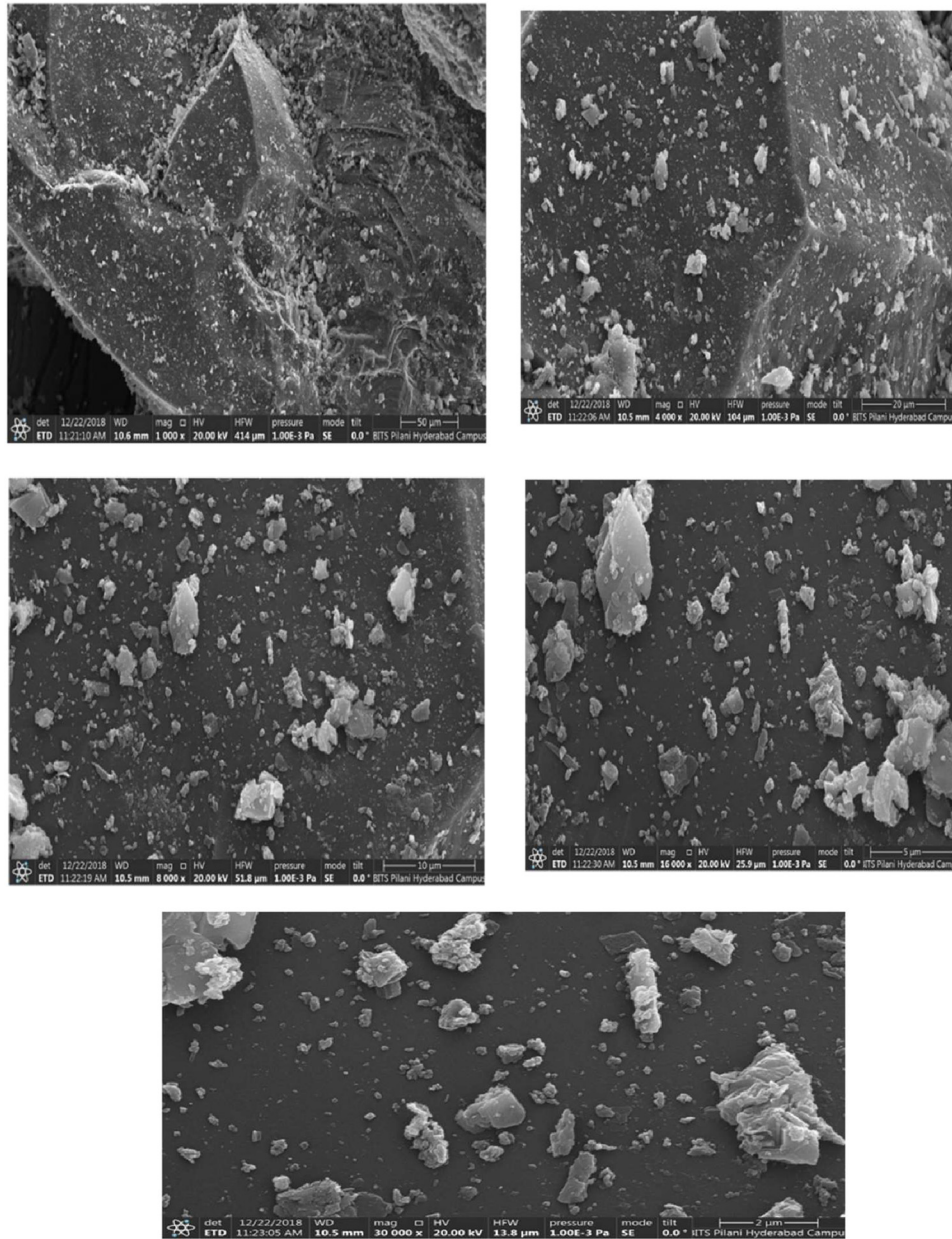


Figure 3: SEM micrograph for bacterial concrete.

reliability of the results. In addition to examining the concrete specimens, XRD analysis is performed on the healing compounds produced within the bacterial concrete. This analysis helps in understanding the concrete's healing process and confirming the formation of calcium carbonate compounds within the samples, further elucidating the mechanisms underlying the self-healing properties of bacterial concrete (Figures 2, 3 and Table 4).

The preceding pictures depict the microstructure of typical concrete and present the findings of the SEM investigation for conventional concrete at various amplification,

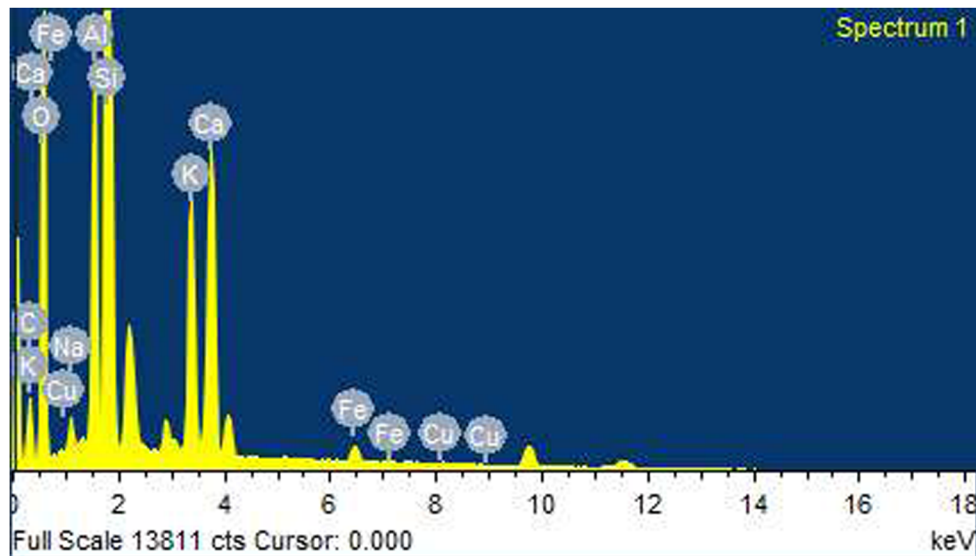
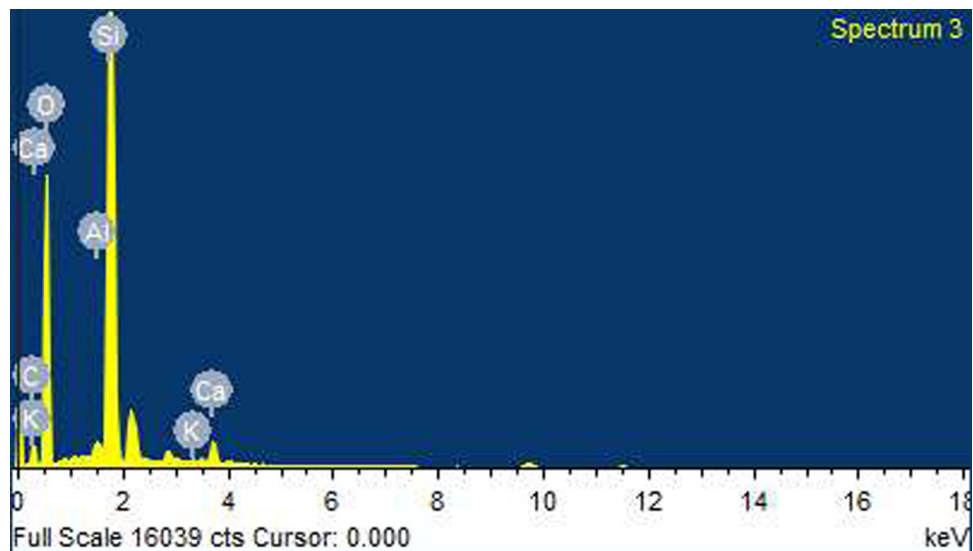
sub-acceleration voltage, and pixel sizes. An analogous setting is used for the investigation of bacterial concrete. In the bacterial concrete, SEM examination revealed the formation of calcite precipitation, whereas pores, CSH, and CH were present in nearly every sample. It demonstrates that the calcite development is responsible for the decrease in porosity as well as the enhancement in strength and fracture healing in concrete. This method was used to assess both the bacteria-treated and untreated concrete samples; SEM images are displayed in the figures. The concrete samples had identifiable calcite crystals, as shown by

Table 4: SEM samples for different samples

S. no	Mag.	HV (kV)	HFW (μm)	WD (mm)	Scale (μm)
1	1,000×	20	414	10.4	50
2	4,000×	20	104	10.3	20
3	8,000×	20	51.8	10.3	10
4	16,000×	20	25.9	10.3	5
5	30,000×	20	13.8	10.3	2
6	10,000×	10	—	8.7	5
7	2,500×	10	—	8.7	20
8	1,000×	20	414	10.5	50
9	4,000×	20	104	10.5	20
10	8,000×	20	51.8	10.5	10
11	16,000×	20	25.9	10.5	5
12	30,000×	20	13.8	10.5	2

the SEM study. All of the bacterial samples had high calcium concentrations, indicating the presence of calcite in the form of calcium carbonate. The fact that bacteria are linked to crystalline calcite suggests that the bacteria acted as nucleation sites for the mineralization process.

The development of calcite crystals was examined in both the treated and untreated bacterial samples. The concrete samples treated with the microbe have a crystalline matrix in which individual crystals are discernible, but the untreated (without bacteria) samples' matrix looks amorphous and displays no signs of crystal formation. There is some variation in the degree of crystal formation in the treated samples' matrix. Concentrations of comparatively big crystals can be found where the matrix and sand

**Figure 4:** EDAX analysis of conventional concrete.**Figure 5:** EDAX analysis of Bacterial concrete.

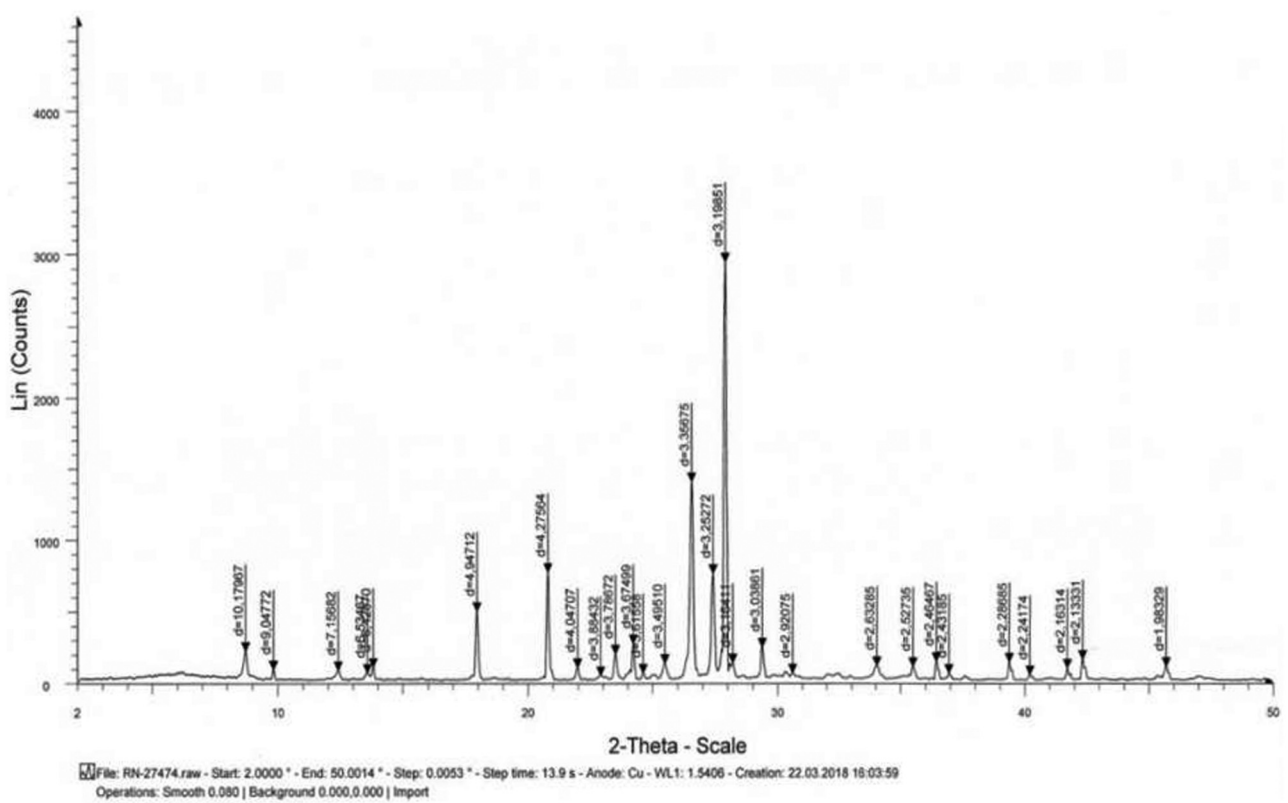


Figure 6: XRD of conventional concrete.

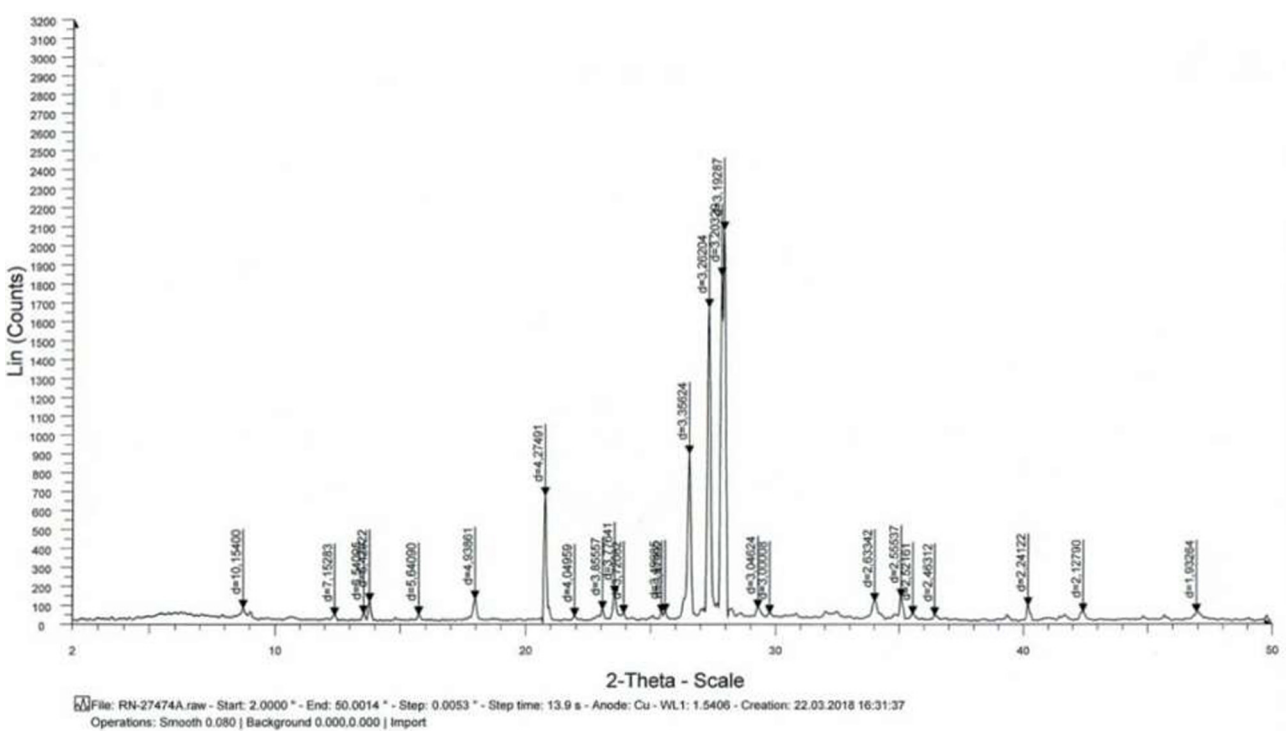


Figure 7: XRD of bacterial concrete.

particles meet. This kind of textural setting implies that preferential crystallization at the concrete–matrix interfaces is likely responsible for the micro-scale coherence between cement particles and the matrix (Figures 4 and 5).

As seen in Figure 7 when recordings are made at a wavelength of 1.54 Å, crests may be detected at different phases. Pure calcite is represented by the value 27.914, which is calculated from the observed maximum peak at the corresponding theta. These peaks are seen for a few further specimens that correspond to several different minerals, including ettringite (E), quartz (Q), calcite (C), CSH, larnite (L), and quartz (C). The findings are displayed in Figures 6 and 7. Following the quantitative examination, the composition of calcite for bacteria is visibly peaked in comparison to the non-bacterial samples. A hump in Figure 7 illustrates the presence of amorphous material combined with crystalline phases of calcite, portlandite, and larnite. XRD analysis was used to determine the presence of calcite and CSH in concrete samples containing *B. subtilis*. The presence of calcite peaks indicates that bacteria have precipitated calcite, which accounts for the concrete sample's improved strength and endurance.

The reason for the concrete specimens' strength growth is demonstrated by the existence of CSH peaks. XRD is also used to characterize fingerprints and determine the structure of crystalline minerals, such as CSH and calcite. An X-ray powder pattern that is specific to each crystalline solid can be utilized as a “fingerprint” to identify it. Samples of broken cubes from compressive strength tests and the precipitates found in the cracks of concrete beam specimens are utilized to measure the amount of crack healing. XRD analysis was performed on the fraction that passed through the 5 µm sieve.

5 Conclusion

The analysis of bacterial concrete mixes revealed several noteworthy findings. First, when incorporating up to 10% bacterial solution, the dynamic modulus of elasticity for both M40 and M20 grade mixes with crushed stone sand outperformed those with river sand. Additionally, micro-structure analysis conducted after 28 days of curing indicated the presence of calcite, or calcium carbonate, in all bacterial concrete mixes. Furthermore, detailed examinations using XRD and SEM revealed that bacterial concrete mixes with 10% bacterial solution exhibited higher production of CSH gel and non-expansive ettringite, thereby confirming enhanced compressive strength. EDX analysis spectra further supported these findings by demonstrating

a high intensity and weight percentage of calcium in the bacterial concrete samples, indicating a significant presence of calcite. This abundance of calcite contributed to the improved strength and longevity of the bacterial concrete specimens.

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