ESCUELA DE CONSTRUCCIÓN CIVIL
FACULTAD DE INGENIERÍA
Revista de la Construcción
Journal of Construction

Research Article

Sustainable and strength-enhanced concrete using microbial consortia

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Received: 30.12.22; **Accepted:** 23.04.24; **Published:** 31.08.24

Citation: Subitha, T., Manju, R., Sasikumar, P. (2024). Sustainable and strength-enhanced concrete using microbial consortia. Revista de la Construcción. Journal of Construction, 23(2), 403-412. https://doi.org/10.7764/RDLC.23.2.403

Abstract: The mechanical properties of concrete were examined by using steel fibres and two types of bacteria (*Pseudomonas otitidis* and *Bacillus cereus*). These steel fibres and bacteria were incorporated into the concrete mix in proportion to the weight of the cement. To ensure the resilience of microbial consortia incorporated into bio-concrete against the mechanical and chemical stresses encountered during concrete production, these microorganisms should exhibit critical characteristics, including robust alkali resistance and the ability to produce endospores. By employing 16S rRNA gene sequencing, we verified the identity of a group of organisms isolated from fermented lime mortar and curing construction water. Microbiologically induced calcite precipitation (MICP) was confirmed through microstructural analyses using techniques such as scanning electron microscope (SEM) and x-ray diffraction (XRD). The concrete was prepared with optimal concentrations of cultured bacteria (ranging from 10^3, 10^5 and 10^7 cells/ml), and steel fibres were added to the concrete at varying percentages (0%, 1%, 1.5%, and 2%). The optimal steel fibre content was 1.5%, and its strength properties were compared to conventional concrete. Notably, when *Pseudomonas otitidis* and *Bacillus cereus* bacteria were added to the traditional and optimal concrete mix, the compressive strength was significantly enhanced compared to the conventional concrete mix.

Keywords: Alkali resistance, bio concrete, steel fibre, calcite precipitation, microbial consortia.

1. Introduction

A necessary component of practically all contemporary constructions is concrete. It is prone to developing micro-cracks as it ages. Concrete micro-cracks allow water to seep into them, causing them to enlarge and endangering the structural integrity of the area. Additionally, it hastens the corrosion of crucial structural reinforcements, lessening essential structural component's longevity and strength. Numerous methods exist to repair these fissures before the structural integrity is jeopardised. However, even if we exclude the possibility of harm to the environment and society, most of these are highly costly and dependent on hazardous chemicals (Abdelhamid et al., 2020; Zhang et al., 2020). Concrete is the most substantial component of the civil engineering world. Concrete structures are suitable in compressive strength and plasticity and have excellent environmental adaptability (Sharma et al., 2017; Wiktor & Jonkers 2016) but weak in tensile strength. Concrete is readily available and has good characteristics as a material. High loading conditions, freeze, climate changes, and cracks in concrete are inevitable, resulting in reduced structural elements' durability (Wiktor & Jonkers 2011). Microbiologically induced calcium precipitation (MICP) is thought to be a feasible substitute for conventional techniques for filling up the created micro-cracks in concrete (Subitha et al., 2023; Subitha & Manju 2023). The MICP technology is economical, bio-friendly, and does not lag behind traditional healing treatments. The urease enzyme is crucial to the precipitation of calcite because it catalyzes the conversion of urea to CO_2 and ammonia, raising pH levels, which is thought to be necessary for the microbial precipitation of calcite (Huynh et al., 2017; Yahya et al., 2019; Aullybux et al., 2019). To complete our job, we must separate and identify $CaCO_3$ bacteria from natural sources, analyze the concrete compressive strength CS and therapeutic qualities, and contrast the prepared bio concrete's toxicity with other concrete forms. The characteristics of the concrete are compressive strength (CS), split tensile strength (STS), flexural strength (FS), and impact resistance (Gümü & Arslan 2019). Even though the construction industries lower greenhouse gas emissions, tracks in concrete are unavoidable due to inherent weakness in concrete, and the reasons for cracking are shrinkage, salt crystallisation, and chemical expansion (Almutairi & Helal 2021; Konopacka-Łyskawa 2019).

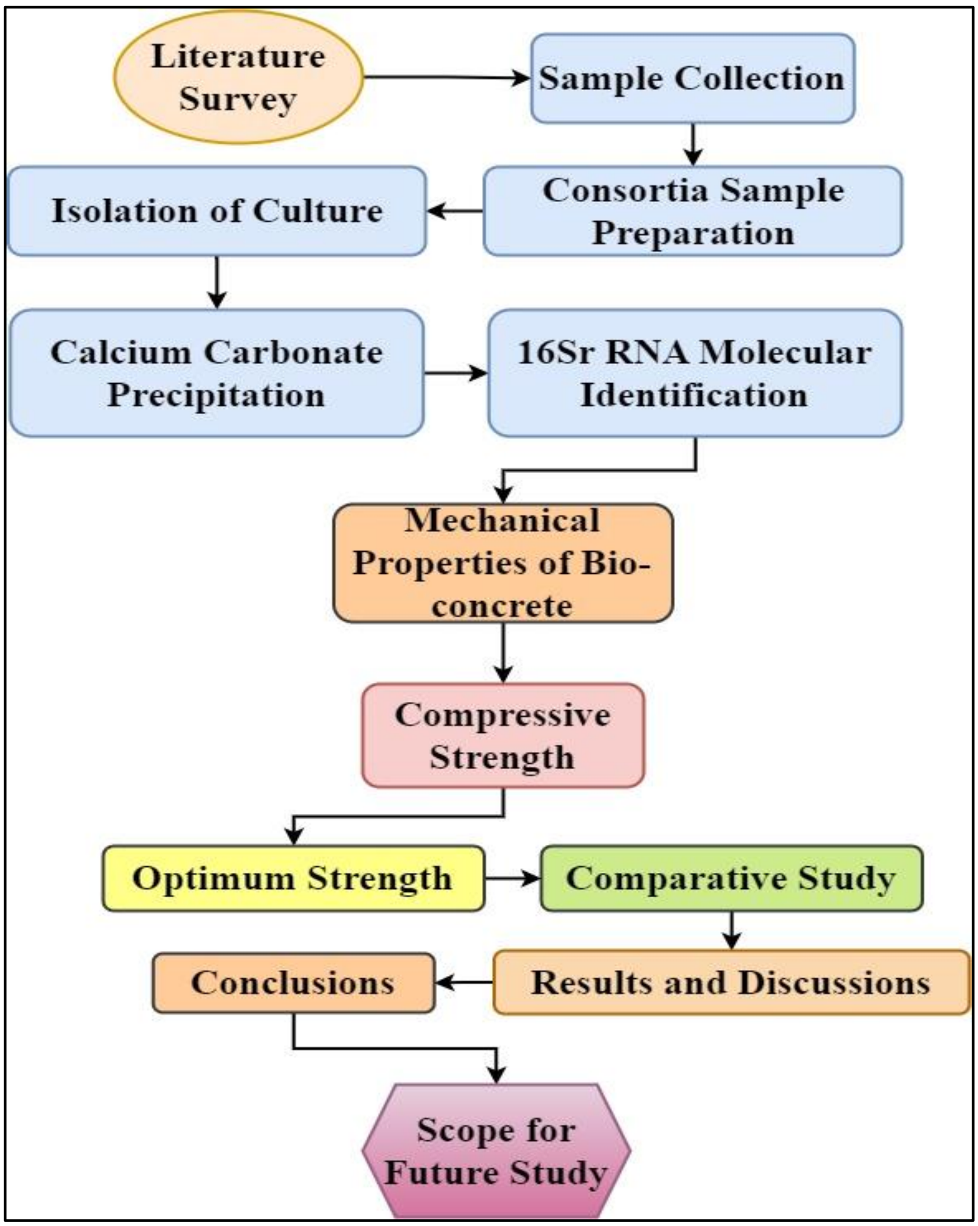


Figure 1. Flow of the research work.

Destructive elements are to migrate into fractures, which reduce the concrete's water resistance significantly. Chloride ions can cause concrete expansion and steel corrosion in coastal environments, accelerating the structure's corrosion (Andalib et al., 2016; Brykov et al., 2012). Concrete has an essential healing ability for cracks with widths below 200 µ. The CS of the bacterial mortar is achieved at 7,14,28,56 days compared to the conventional concrete. $CaCO_3$, which fills the pores and improves the concrete microstructure, was attributed to the growth in compressive strength (Krishnapriya & Babu 2015). Additionally, bridging the gap between the fibres reduces the width of the cracks (Borges et al., 2019). Furthermore, Polypropylene fibre shows virtuous results in the characteristics of the concrete. This literature review aimed to determine how to increase the nutrition sources and bacterial population in FRC concrete to enhance its durability. Nutrient sources and *Bacillus subtilis* are employed. *Bacillus subtilis* and nutrient sources are used (Qin et al., 2019; Eidan et al., 2019). FRCC exposes a growth in the capacity to absorb energy and a tensile force associated with the breakable response of control concrete (Vijay & Murmu 2019). Subsequently, adding fibre to concrete increases concrete's tensile strength and post-cracking behavior (Vijay & Murmu 2020). *Bacillus* bacteria reported healing micro-cracks in concrete, and adding bacteria increases compressive strength by 23% (Chalioris & Panagiotopoulos 2018; Sharma 2016). The effect of mechanical characteristics of polypropylene fibres on the concrete. These studies distinguished an increase in concrete traits (physical and chemical) by adding fibres (Bashir et al., 2016; Ahmed et al., 2006; Southararajan et al., 2013). Based on numerous examinations of the literature, it is recognized that bacteria or fibre are used to create bio concrete. The effectiveness of two bacteria from the same family, *Bacillus megaterium* and *Bacillus licheniforms*, were studied in healing the physical and chemical characteristics. The influence of bacteria on steel fibres was also evaluated when two bacteria were introduced into concrete. In addition, this research looks into the mechanical characteristics of concrete and the healing properties of blended bacteria in combination with steel fibres. Using XRD and SEM analysis techniques, it was determined that the calcite precipitates were in the form of vitriol crystals. The flow of research methodology is shown in Figure 1.

1.1. Novelty of the research work

According to the literature survey, the mechanical properties of concrete were examined using different types of bacteria. The present study investigated the mechanical properties of concrete by utilizing isolated bacteria (specifically, *Pseudomonas otitidis* and *Bacillus cereus*). Additionally, steel fibres were incorporated into the concrete mix at varying percentages: 0%, 0.5%, 1.0%, 1.5%, and 2.0%. Creation of microbial consortia using conventional lime mortar and identification of the bacteria that precipitate calcium carbonate. Using the 16S rRNA gene sequencing method, consortiums of organisms were identified from fermented lime mortar and curing construction water. The outcomes of tests on compressive strength were contrasted with conventional concrete and bio-concrete made from isolated cultures.

2. Materials and methods

2.1. *Collection of the samples*

For thirty days, a collaborative group was established to foster the development of building water, fine aggregate, and limestone through fermentation. We identified consortiums of naturally occurring organisms by examining building water and fermented lime mortar. Additionally, we procured stub cultures of *Pseudomonas otitidis and Bacillus cereus*.

2.2. Isolation of culture and screening of urease enzyme-producing bacteria

Two strains of bacteria were extracted from the fermented blend, while water samples from a nearby construction site were collected. Each sample, weighing one gram, was introduced into a nutrient-rich medium for enrichment and left to incubate at 37 °C for 2 to 3 days. Once the cultures displayed satisfactory growth, serial dilution was carried out, followed by injection onto Urea agar with an ideal pH of 9.4. After incubation, bacterial colonies exhibiting crystallized precipitation were recognized and moved to Urea broth. The vegetative cells will be red or pink, whereas the endospores will be green. Calcium chloride was introduced to nutritional broth containing 2% urea to maintain ideal conditions and used to study the $CaCO_3$ precipitation of selected isolates. 0.6 ml of an isolated inoculum was added to 30 ml of this broth, and it was then incubated for seven days at 30°C with 130 rpm. The spread plate method separated well-developed colonies and sub-cultured on nutrient agar slants. It is possible to cultivate bacteria in liquid or solid media.

The qualitative urease assay used agar tubes containing phenolic red (0.018 g/l) as a pH indicator. The discovered bacterial species were cultivated for three to five days at 37°C after being aseptically transferred into agar tubes. The test tubes were examined daily to see if the color changed, signifying that the urease enzyme was active. All the chosen isolates were cultivated in a culture consortium and centrifuged at 8000 g for 15 minutes when the broth was rich in nutrients. Urea was hydrolyzed into conductive ionic components by the urease process, which increased conductivity. For the enzyme experiment, 1.0 ml of bacterial broth culture was mixed with 9.0 ml of 1.11 M urea solution. After five minutes at 20°C, the final conductivity readings were obtained using an electrical conductivity meter (Yahya et al., 2019; Andalib et al., 2016).

2.3. Precipitation test for calcium carbonate ($CaCO_3$)

Bacterial samples were introduced to calcium chloride in urea broth to investigate calcite formation. The test tubes were placed in an incubator at 37°C and 130 rpm for three to five days (Aullybux et al., 2019). After this period, the broth underwent centrifugation at 8000g for fifteen minutes, forming pellets. These pellets were dried for a full day at 80°C and weighed. This step was crucial for controlling the bulk of the sediment. To confirm the presence of calcium carbonate precipitation by the bacteria, we analyzed the isolate species *Pseudomonas otitidis* and *Bacillus cereus* (Krishnapriya & Babu 2015). The cultures were also incubated for seven days, during which $CaCO_3$ was produced and measured (Konopacka-Łyskawa 2019; Xu et al., 2015; Li et al., 2015).

2.4. Fabrication of the bio-concrete samples

The trial and conventional mix design achieved twenty-eight days cube strength of 49.15 N/mm² without including fibre and bacteria culture. The materials were sourced from the local market. The mix proportion was determined to achieve the target design strength of 48.25 N/mm², even without adding fibre and bacteria. The quantities of materials used in the concrete were as follows: cement (348 kN/m³), fine aggregate (674 kN/m³), coarse aggregate (1186 kN/m³), water content (146 kN/m³), superplasticizer (2.15 kN/m³), and a concentrated water-cement ratio of 0.42. Steel fibres were added to the concrete mix based on the weight of the cement. Adding steel fibres and bacteria improved the strength properties of the concrete. The mechanical behaviour of bio-concrete and the mixes listed in Table 1 were studied. For this investigation, concrete of grade M40 was chosen, and the mix design followed the guidelines (IS: 10262 - 2019).

Table 1. Mix proportion of the bio-concrete in 1M³ (kg/m³).

Mix ID	Cement	Fine aggregate	Coarse aggregate	Water content	Superplasticizer	W/C	Steel fibre (%)
CC	348	674	1186	146	2.14	0.42	0
CC-SF0.5	348	674	1186	146	2.14	0.42	0.5
CC-SF1.0	348	674	1186	146	2.14	0.42	1.0
CC-SF1.5	348	674	1186	146	2.14	0.42	1.5
CC-SF2.0	348	674	1186	146	2.14	0.42	2.0

3. Results and discussions

3.1. Electrical conductivity assay

The electrical conductivity assay was conducted at various intervals and is reported in Table 2. The *Pseudomonas otitidis* and *Bacillus cereus* bacteria were used in the concrete to enhance its strength properties (Li et al., 2015). Specifically, *Pseudomonas otitidis* and *Bacillus cereus* produced 0.86 g, 0.84 g, 1.23 g, and 1.34 g of $CaCO_3$, respectively. Based on observations, *Bacillus cereus* exhibited lower calcite production, as shown in Figure 2.

Table 2. Electric conductivity assay for quantitative of urease.

Time intervals (ms/min)	Subculture	
	Pseudomonas otitidis	*Bacillus cereus*
0	138.6	122.8
350	140.5	136.4
3800	148.6	142.6
6688	152.6	147.2

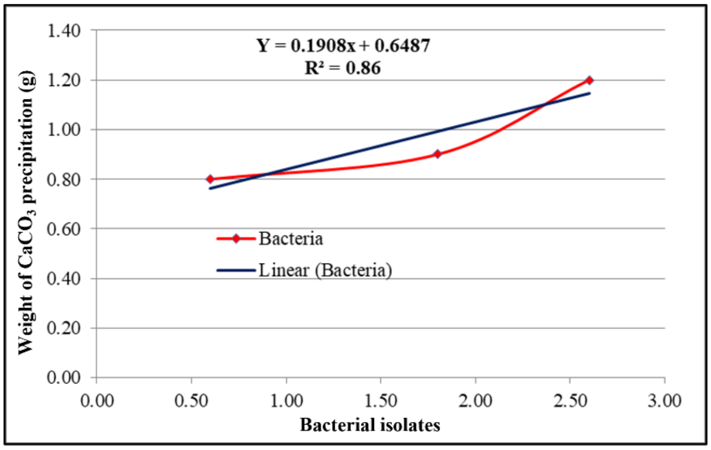


Figure 2. Comparison of different bacterial isolates.

3.2. Identification of molecular

The isolated cultures were identified by using the 16S rRNA sequencing method. The obtained DNA sequences were handed over to Blast software, which aided in identifying distinct species. Table 2 illustrates the Blast result of 16S rRNA sequencing of isolated species 1 and 2. From Table 3, solitary species such as *Pseudomonas otitidis* strain HR-2 scored 100 %, and *Bacillus cereus* strain HKG201 scored 100%.

Table 3. Blast result of 16S rRNA sequence from the Isolate I and II.

Description	Maximum	Total	Query cover	E value	%	Accession
Pseudomonas otitidis strain HR-2	2285	2285	100	0.0	100	MT645611.1
Bacillus cereus strain HKG201	1808	1808	100	0.0	100	KF947110.1

3.3. Compressive strength of concrete

This study investigated M40-grade concrete with steel fibre and microorganisms. The target strength of the concrete was designed to be 48.25 N/mm². Compressive strength was determined after curing for seven days, 14 days, and 28 days, using different percentages of steel fibre ranging from 0% to 2%. The compressive strength of concrete mixes for CC-SF0.5, CC-SF1.0, and CC-SF1.5 increased by 51.45 N/mm², 52.90 N/mm², 53.82 N/mm², and 53.16 N/mm², respectively, compared to the conventional concrete mix of CC-SF0, which has strength of 49.15 N/mm² at 28 days is illustrated in Figure 3. According to the experimental study, the optimum steel fibre content was 1.5%, and the concrete's strength properties enhanced the steel fibres addition (Sasikumar 2024; Sasikumar & Manju 2024; Sasikumar 2023; Sasikumar et al., 2022). Further investigation was conducted by adding the optimal concentration of 10^5 cells/ml bacteria, such as *Pseudomonas otitidis* and *Bacillus cereus*, to the optimum steel fibre content. Similarly, the compressive strength was investigated with the optimum steel fibre mix with bacteria, the concrete mixes of CC-SF0-BC1, CC-SF0-BC2, CC-SF1.5-BC1, and CC-SF1.5-BC2. The strength is improved by 50.96 N/mm², 51.45 N/mm², 52.96 N/mm² and 54.17 N/mm² compared to the conventional concrete strength of 49.15 N/mm² at 28 days as shown in Figure 4.

When *Pseudomonas otitidis* and *Bacillus cereus* bacteria were used at the optimum concentration level of 10^5 cells/ml with conventional concrete mix, the compressive strength also slightly increased to 50.96 N/mm² and 51.45 N/mm² at 28 days. Similarly, the *Pseudomonas otitidis* and *Bacillus cereus* bacteria were employed at an optimal concentration of 10^5 cells/ml in a concrete mix containing 1.5% steel fibre content. As a result, the compressive strength of the concrete exhibited a slight increase, reaching 52.96 N/mm² and 54.17 N/mm² at 28 days. Overall, observations from the experimental study demonstrated that *Pseudomonas otitidis* and *Bacillus cereus* contributed to the growth of concrete compressive strength.

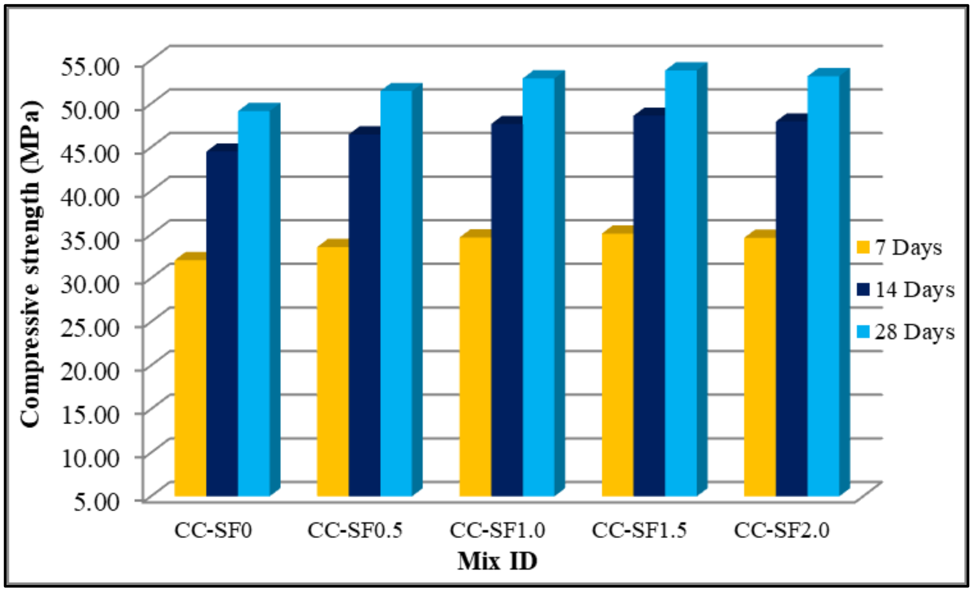


Figure 3. Compressive strength of steel fibre concrete at various ages.

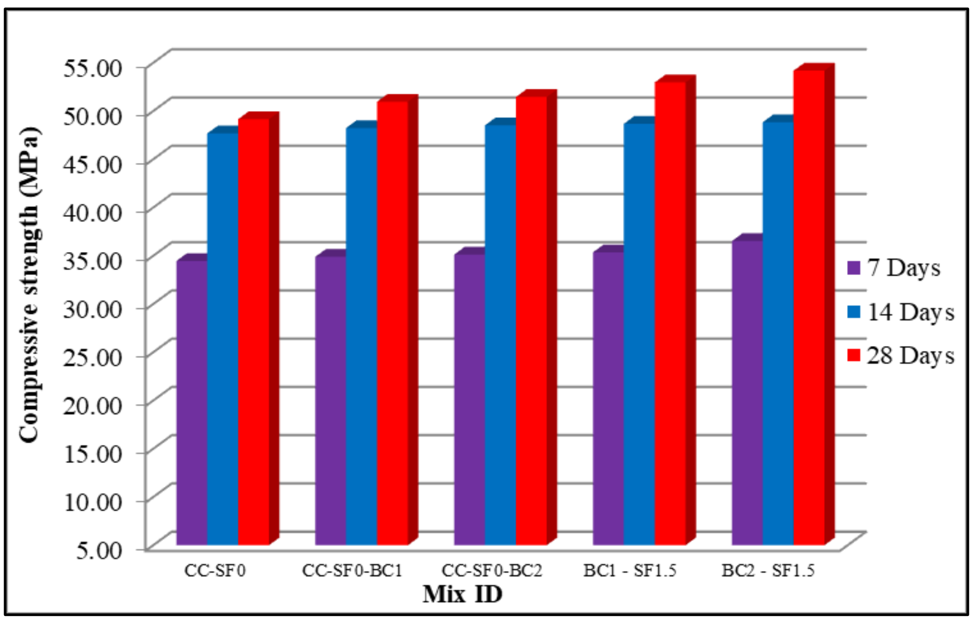
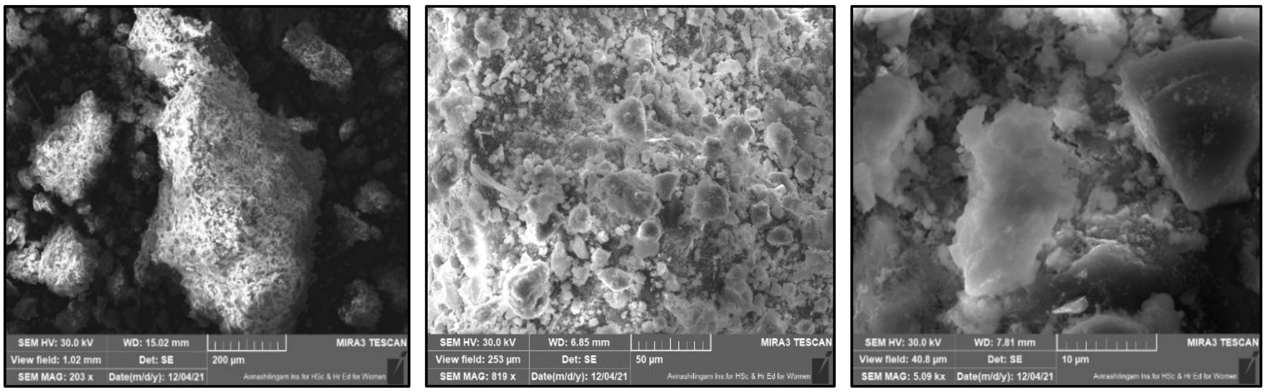


Figure 4. Comparison between conventional and bacterial concrete compressive strength at various ages.

3.4. Microstructural analysis

3.4.1. SEM analysis of bio-concrete

Calcite precipitation in concrete specimens by *Pseudomonas otitidis*, and *Bacillus cereus* was visualized. A growth of calcite crystals entrenched with bacterial cells was detected in the specimens, as shown in Figures 5 (a) to (c). Bacteria were found in close contact with the calcite crystals in both samples. *Pseudomonas otitidis* and *Bacillus cereus* cultures enhance the strength of the structures compared to conventional bio concrete and traditional concrete, where no crystals can be visualized.

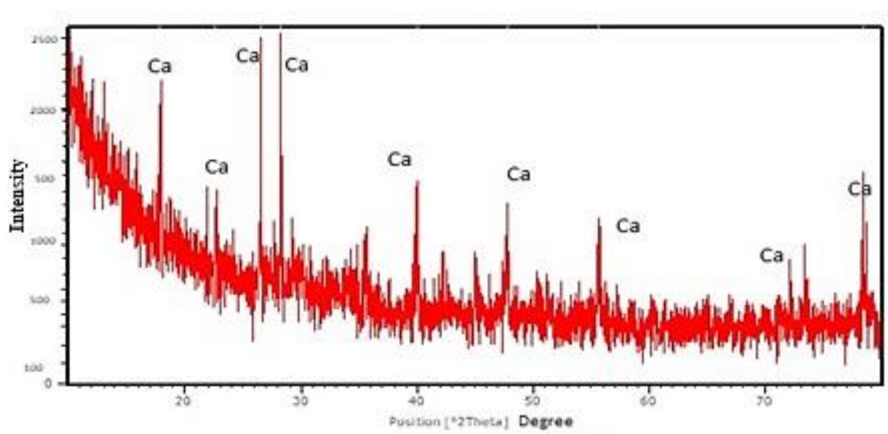


(a) (b) (c)

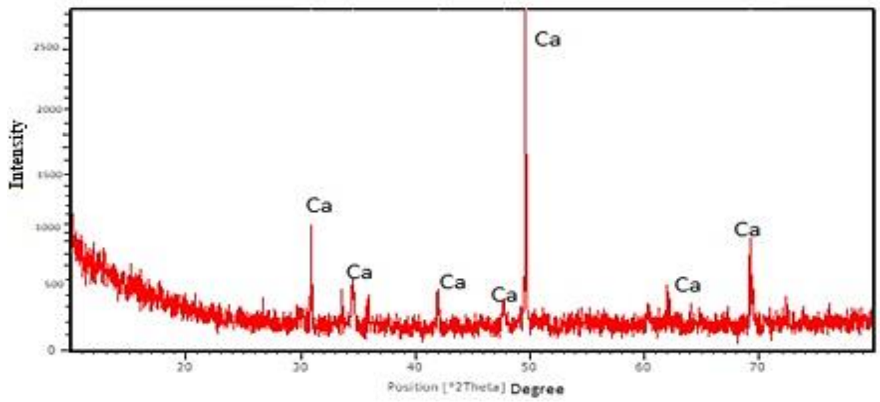
Figure 5. SEM analysis (a) Conventional concrete, (b) Conventional concrete with *Pseudomonas otitidis*, (c) Conventional concrete with *Bacillus cereus*.

3.4.2. XRD analysis of bio concrete

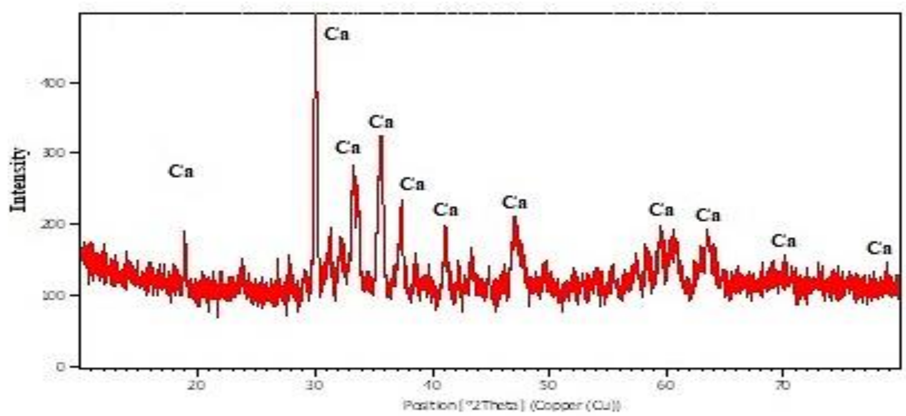
The XRD evaluation was conducted at 2 degrees, commencing at 10 and ending at 80 degrees. The scanning was carried out in a continuous mode with a fixed divergence slit at a temperature of 25°C, with Cu (Copper) as the anode material. The XRD data indicate distinct points, and Calcite ($CaCO_3$) was present throughout the points, as illustrated in Figure 6 (a) to (c).



(a)



(b)



(c)

Figure 6. XRD Analysis, (a) Conventional concrete, (b) Conventional concrete with *Pseudomonas otitidis*, (c) Conventional concrete with *Bacillus cereus*.

4. Conclusions and recommendation for the future works

These present research works were investigated using steel fibre and bacteria. Based on the experimental study observation, the following conclusion can be drawn:

1. The two bacterial isolates utilized for additional bio-concrete preparation were identified as *Pseudomonas otitidis* and *Bacillus cereus* using the 16S rRNA gene sequencing approach.
2. The mechanical characteristics of compressive strength in concrete increased with the addition of steel fibre by 51.45 N/mm², 52.90 N/mm², 53.82 N/mm², and 53.16 N/mm², respectively, compared to conventional concrete at 28 days. The optimum steel fibre content was found to be 1.5%.
3. Similarly, the compressive strength improved by 50.96 N/mm², 51.45 N/mm², 52.96 N/mm², and 52.96 N/mm², respectively, at 28 days when incorporating *Pseudomonas otitidis* and *Bacillus cereus* bacteria with the optimum steel fibre content.
4. It has been demonstrated that bacterial concrete exhibits higher compressive strength than conventional concrete with optimum steel fibre content.
5. SEM and XRD analyses were performed on both conventional and bacterial concrete, with the XRD analysis indicating the presence of calcite.

The present study examined the mechanical properties of concrete using steel fibre and subculture bacteria. Furthermore, the research may be studied for structural elements with different types of fibres.

Author contributions: Subitha T: Experimental investigation, writing a draft. Manju R: Supervise the experimental work. Sasikumar P: Methodology, preparation of graphical charts, copy editing, and review of the original manuscript.

Funding: The authors declared that no funding was received to complete this research.

Acknowledgments: The authors thank Kumaraguru College of Technology for the facilities and continuous support in this research work.

Conflicts of interest: The authors declare no conflict of interest.

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