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Research Article

Self-healing evaluation of bacteria grouted light weight aggregate concrete containing rice husk ash and steel fibers

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Abstract: Utilization of microbiologically induced calcite precipitation along with fiber composite have great influence on improving strength and durable properties of concrete. The concept of mechanical properties of grouted concrete added with bacteria, steel fibers (SF), rice husk ash (RHA) and light weight aggregate (LWA) has been focused on this work. In the fabrication of concrete specimens, concentration of bacteria, combination of steel fibers and LWA was placed in the formwork, and to fill the voids flowable grout was injected. The variables studied in this work are two different sizes of LWA viz., 10 mm and 12.5 mm with constant dosage of 2% hooked end steel fibers by volume of concrete, 10% RHA was used as cement replacement for preparation of grout and bacteria was incorporated in cement grout by direct application. The properties such as compressive strength (CS), compressive strength regain (CSR), crack width healing, impact strength for first crack and final failure, rate of healing was studied for pre-cracked specimens using visual and microscopic observation. In addition, microstructure was studied for grouted concrete without bacteria and with bacteria under immersed curing conditions. From the experimental results, performance of bacteria added grouted concrete properties such as CS, CSR, cracking healing capacity, and impact strength has improved with the addition of fibers.

Keywords: Grouted concrete, light weight aggregate, bacteria, steel fiber, strength properties, calcium carbonate precipitation.

1. Introduction

Infrastructure development in construction sectors majorly uses cement, aggregate and steel as primary materials. Estimated amount of cement produced worldwide is 4.1 billion tons in 2022 (Tkachenko et al. 2023) and CO_2 emission in cement production is shared by combustion process (40%) and calcination process (60%) (Bosoaga et al. 2009). It was found that for 1kg of cement production, 0.9 kg CO_2 is emitted (Fayomi et al. 2019) and globally 3.69 billion tons of greenhouse gas is let to atmosphere by cement production industries. Aggregates are non-renewable energy sources and recent survey estimates that 694 million metric tons construction aggregates are produced till 2020 (Pacheco and de Brito 2021). In crushing and excavation process though huge amount of petroleum energy is consumed, various researches are carried (Sastry and Pothala 2012; Sastry and Murthy 2015) to find an alternate sustainable petroleum product using natural raw source and still it's in progress for large scale productions. Cracks appear easily in concrete due to its brittle nature and mere tensile strength (Awad et al. 2022; Ogunbode et al. 2021). However, finding an alternative for primary materials (cement and aggregate) would be the acceptable solution for sustainable construction.

1.1 *Background of non-bacteria and bacteria based RHA, LWA and SF applications*

Uses of agro waste like RHA in civil engineering applications is common recent times and yearly thousand million tons of agro waste are generated every year (Akhter et al. 2021; Rajesh et al. 2023). Disposing such huge quantity as a landfill is not acceptable practice due to its less nitrogen composition and secondly incineration of RHA would lead to release of ashes, causing environmental hazard (Bushra and Remya 2020). Minimal optimum percentage of RHA in 10%, rendered higher compressive strength of 11.8% after 56 days and addition of *Bacillus aerius* in concrete with RHA reduced water absorption, chloride ion penetration, abrasion loss and porosity (Siddique et al. 2016). RHA is used as a substitute for fine aggregate in concrete at 0, 5, 10, and 15%. *Bacillus subtilis* added along with RHA in concrete rendered 10% increased compressive strength due to its ecofriendly and self-healing behaviors (Kokate and Kumar 2022).

In Self compacting concrete cement is replaced with RHA from 0% to 30% along with *Sporosarcina pasteurrii* bacteria. RHA of 15% rendered with bacterial concentration of 10^5 cells/ml rendered 21% of increased compressive strength and 10^7 cells/ml showed 80% reduced permeability (Ameri et al. 2019). LWA incorporated with *Sporosarcina pasteurii* (concentration of 10^6 cells/ml) showed reduction in water absorption (WA), increment in CS and decrement in chloride penetration of 10%, 10% and 20% respectively (Hosseini Balam et al. 2017). *Bacillus lentus* impregnated into LWA through vacuum techniques showed 22% increased compressive strength values. Even after 6 years the influence of added microbes (bacteria) to heal the cracks remains unchanged and 60-90% healing rates achieved with respect to crack width produced (Dembovska et al. 2019). The flexural residual strength of LWA concrete with micro-steel fiber is assessed and test results conclude that micro-steel fibers are potential replacement for conventional rebars to improve ductility properties in LWA concrete specimens (Kim et al. 2024). The effective use of waste expanded polystyrene beads in light weight screed mortar was evaluated. The test results depict that 60% replacement of fine aggregate with waste expanded polystyrene beads showed low thermal conductivity co-efficient, improved compressive strength and reduced unit weight (Serhat Çelikten et al. 2023).

Bacillus subtilis of 10^7 cells/ml concentration added with natural light weight aggregate and steel fiber decreased water absorption by 13.1%, carbonation depth by 27.2%, chloride penetration by 20.5% and water penetration by 44.3%. electrical resistance for the same combination is increased by 103.6% (Salmasi and Mostofinejad 2020). Soil bacteria *Bacillus subtilis* is incorporated in concrete along with steel fibers and glass fibers and concluded that there was increase in compressive strength of 30.05% and split tensile strength of 18.45% (Madhan Kumar et al. 2020). Incorporation of steel fibers up to 2% and RHA addition of 10% improved the structural and mechanical properties of high strength concrete (Sumathi et al. 2021; Sumathi and Raja 2021, 2018; Sumathi and Arthika 2022). The aim of this research is focused for three aspects, to reduce the use of cement in construction, to find alternate material for non-renewable aggregate source with steel fibers and bacteria to enhance strength and healing properties. Above aspects will be fulfilled by adding RHA, LWA, *Bacillus subtilis* and steel fiber in grouted concrete to investigate CS, CSR crack-healing capacity and impact resistance.

1.2. *Significance and novelty of research*

Bacteria-grouted lightweight concrete is a novel construction material that combines the advantages of lightweight concrete, steel fibers, RHA, and microbial activity remains unexplored. The inclusion of bacteria with concrete mixture allows for the self-healing of cracks and enhanced durability of the material. Bacteria-grouted lightweight concrete steel fiber concrete has the potential to contribute to sustainable construction practices. The self-healing capabilities of the material can extend its service life, reducing the need for frequent repairs and replacements. Therefore, novelty of this investigation is utilization of light weight aggregate in bacteria grouted concrete with 10% RHA as cement replacement and 2% steel fiber addition in volume to enhance the performance of strength regain, crack healing ability of concrete and impact resistance. The methodology of this study primarily involves in determining the mechanical properties, crack closure behavior and microstructure study on above said combinations. Figure 1 illustrates the methodology of this experimental study.

2. Materials and methods

2.1. *Raw materials used and its properties*

Ordinary Portland cement (OPC) of 53 grade was used with a specific gravity (SG) of 3.15, surface area of 245 m^2/kg, initial setting time of 35 minutes and final setting time of 355 minutes, conforming to IS12269 (Bureau of Indian Standards, 1997). FA was collected from locally sourced river sand with allowable size of 4.75 mm Zone II gradation in accordance with IS 383 (Bureau of Indian Standards, 1970). It had a fine modulus of 3.39, SG of 2.63, bulk density of 1696 kg/m^3, water absorption of 1.37%.

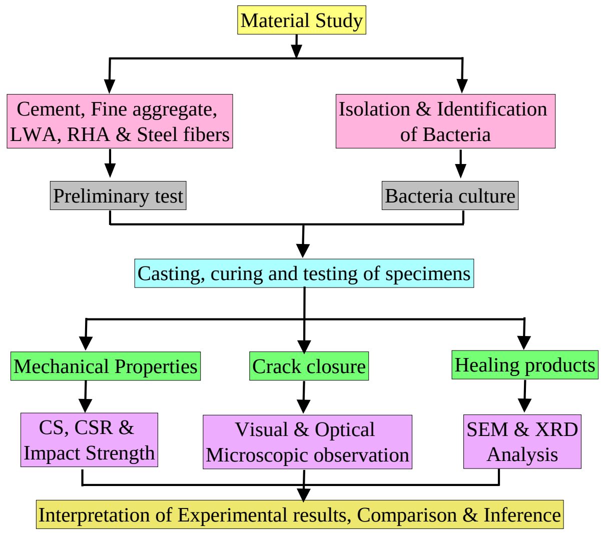


Figure 1. Methodology of experimental study.

Figure 2. RHA.

Figure 3. LWA.

Figure 4. Steel fibers.

Light weight aggregate (LWA) is used 100% replacement instead of normal angular aggregates. LWA is sieved with two different sizes by 12.5 mm and 10 mm has water absorption, impact value and SG of 18%, 49.86% and 0.65 respectively. Dramix hooked end steel fibers of 50 aspect ratio and density 7850 kg/m^3 was used. RHA, LWA and steel fiber are shown in Figure 2, 3 and 4. RHA was obtained from ASTRA Chemicals; Chennai was used in this work with specific surface area of 450 m^2/kg and SG of 2.25. Table 1 shows the chemical properties of RHA.

Table 1. Chemical properties of OPC and RHA.

Component	OPC cement (%)	RHA (%)
SiO_2	20.63	88.9
Al_2O_3	6.96	2.5
Fe_2O_3	3.4	2.19
CaO	61.35	0.69
SO_3	2.28	0.12
MgO	2.89	0.1%

2.2 Isolation of bacteria strain from soil

Formation of spores can be enhanced more by rhizobacteria, which have more survivability in extreme harsh exposure conditions in environment. Bacteria which can produce calcium carbonate can be isolated from soils around the roots of *Ocimum tenuiflorum* (Hesham et al. 2019). Samples of soil were collected from garden area of Nirman vihar block (10.27˚E 79.01˚N), SASTRA university, Thanjavur. 1 gram of soil is dissolved in 100 ml distilled water in a beaker stirred for 3 minutes using magnetic stirrer apparatus. Serial dilution carried out for stock solution from 10^{-1} to 10^{-6} (Navneet et al. 2011; Rajesh Anbazhagan and Arunachalam 2024). Nutrient agar medium is prepared (Kshitipati Padhan et al. 2023) with 0.1% sodium chloride, 0.5% peptone, 0.2% potassium hydrogen phosphate, 0.01% calcium chloride, 0.01% magnesium sulphate, 1.5% agar, 0.1% yeast extract and 1% glucose. Medium prepared in conical flask is autoclaved for 20 minutes in kept in chamber for temperature decrement. Later medium is poured in petri plates (30 ml/plate) and let out for solidification in laminar air-flow chamber. Using pipette 0.1ml solution from each test tube (serial dilution) is suspended in petri plates and spread using L-rod. Petri plates are kept in bacteriological incubator for 24 hours at 37.5˚C (Joshi et al. 2018). The complete isolation process is shown in Figure 5.

2.3 Morphological characterization of bacteria

Isolated bacteria from petri plates 10^{-5} and 10^{-6} is considered for morphological characterization. Further pure colonies are grown from plates for morphological characterization (Rajesh Anbazhagan et al. 2023). Gram staining technique was carried out with proper protocol and the isolated bacteria is gram positive and rod shape in its morphology. Visual examination and microscopic examination of bacteria is shown in Figure 6.

2.4 Molecular characterization of bacteria

Isolated colonies from plate were sent to Genurem Biosciences LLP, Trichy, Tamil Nadu, India, for molecular characterization by 16S rRNA sequencing. Bio-informatics analysis was carried, and BLAST tool is used to determine the similarity of sequence available in from National Centre for Biotechnology Information (NCBI). Based on phylogenetic tree evolved the isolated bacteria is found to be *Bacillus subtilis* and submitted to gene bank for accession.

2.5 Preparation of healing solution for concrete

Bacteria solution was prepared by inoculating *Bacillus subtilis* colony in broth medium. The inoculated broth medium is filled in falcon tubes of 50ml capacity and centrifuged for 15 minutes at 10000 rpm. The main purpose of centrifuge is to isolate bacteria as pellet from the broth solution. Using calorimeter cell concentration was adjusted to 10^5 cells/ml. Bacteria solution is added to nutrient solution (yeast 3 grams/l, urea 5 grams/l, calcium nitrate 5 grams/l) to desired water cement ratio chosen. The healing solution is mixed with the concrete and specimens were cast as usual procedure (Rajesh et al. 2023) shown in Figure 7.

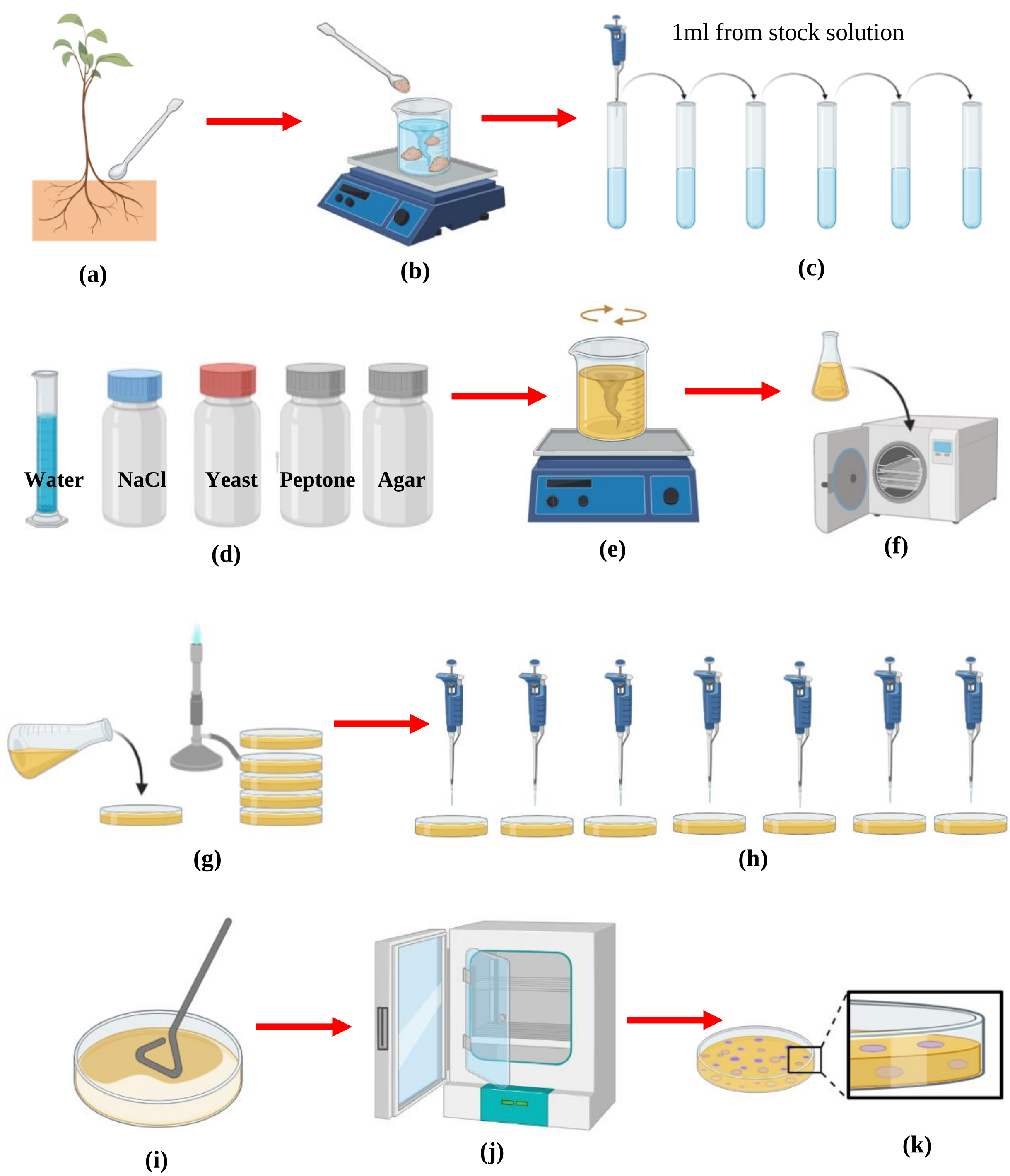


Figure 5. Bacteria isolation process. a) soil collection near roots of Tulsi plant, b) dissolving 1-gram soil in distilled water, c) serial dilution, d) ingredients for medium preparation, e) dissolving deserved quantity of medium, f) dissolved medium in Autoclave, g) medium poured in plates, h) 0.1ml stock solution suspended in plates, i) spreading stock solution using L-rod, j) plates in bacteriological incubator, and k) colonies grown after 24 hours.

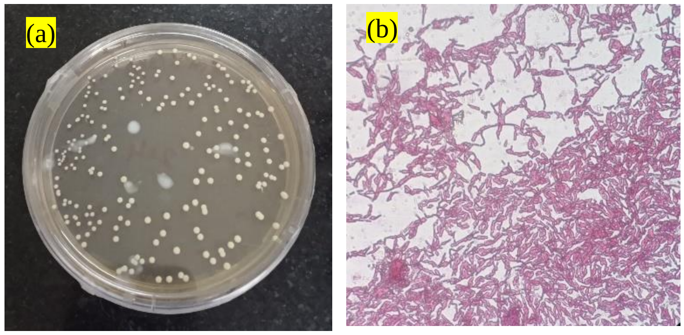


Figure 6. Morphological characterization, a) visual examination, and b) microscopic examination.

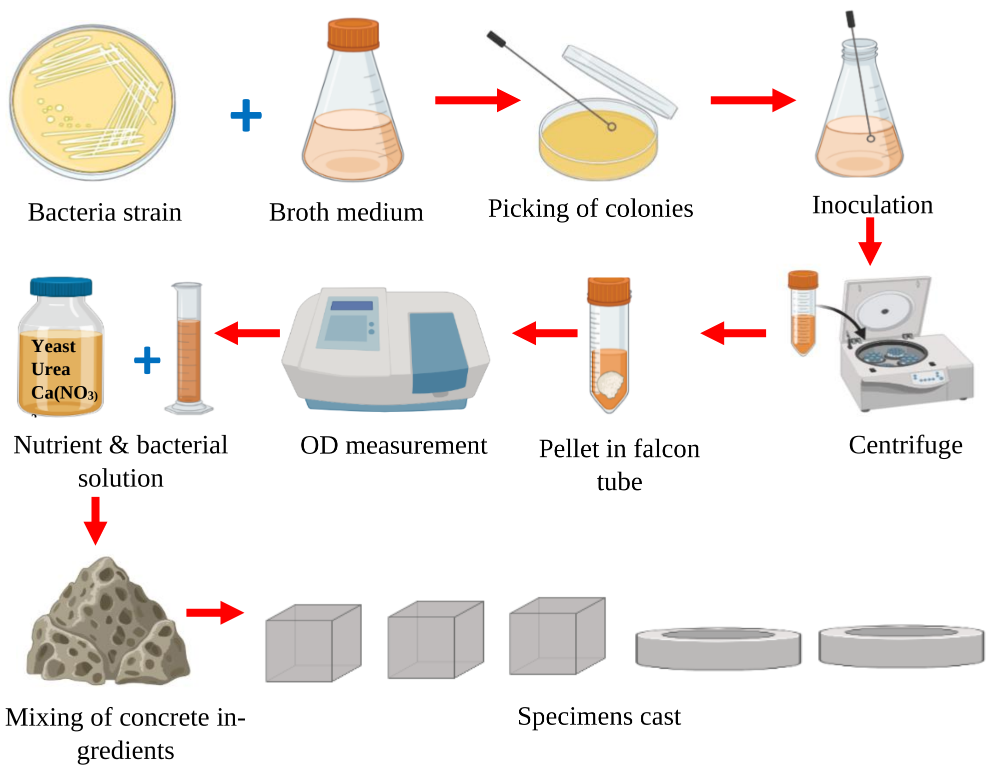


Figure 7. Preparation of healing solution and casting of specimens.

2.6 Details of mix and specimens

First, LWA and steel fibers was premixed and placed in the molds without any compaction. After that, the grout was prepared based on optimized cement to sand ratio (C/S) of 1:1, water to cement ratio (W/C) 0.42 to meet the flow and free from honeycombing and injected using the gravity method, which involved pouring the grout at the top surface of the LWA and fibers and allowing it to sink to the bottom of the mold due to gravity, as illustrated in Figure7. Three specimens of size 100 x 100 x 100 mm cubes were cast as per BS 1881(British Standards, 1983) for compressive strength, for impact strength three-cylinder specimens of size 150 mm diameter and 64 mm height was used. The step-by-step procedure carried for casting of impact specimen was shown in Figure 8.

There was a total of four different mixes used in the current work: a control mix made of grout and LWA was designated as (CC), a mix with grout, LWA, and bacteria added was designated as (CC+BS), a mix with grout, LWA, and SF was designated as (CC+SF), and a mix with grout, LWA, bacteria added and SF was designated as (CC+BS+SF). For evaluating the CS, CSR and crack healing was studied with 100 x 100 x 100 mm cube specimens with three specimens in each mixture and the average results were obtained for two different sizes of LWA in both uncracked and pre-cracked specimens for different days of curing. Similar mix was considered for impact test.

Using the falling weight device, a drop weight impact test was performed on cylinder specimens. ACI 544 (ACI Committee 544, 1999) was used as the basis for the falling weight test. The test consists of a 44.54 N steel ball lifted 0.457 m vertically from the top surface of the target object. The test is conducted by continuously dropping the ball onto the target. The cylinder specimen is held firmly and is prevented from moving by four positioning steel lugs. The number of strikes that result in visible cracking and failure in the target specimens gives a good indication of how the target specimens will react to impact. The instance at which the crack extends over the entire depth of the specimen section is known as the failure case. The formula specifies the streamlined approach that is used to determine the absorbed energy in cracking (U1) and failure (U2) cases utilizing the number of recorded impacts.

$$Impact\ energy\ (U1\ or\ U2) = N.m.g.H$$

where N is the number of hits, mg is the weight of the steel ball of 44.54 N, and H is the vertical fall distance.

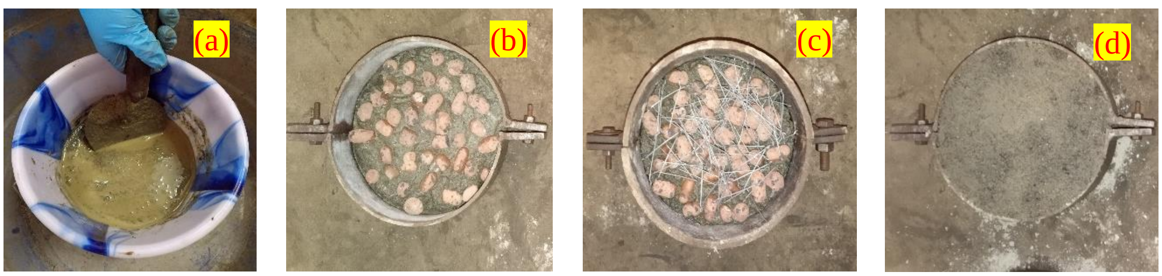


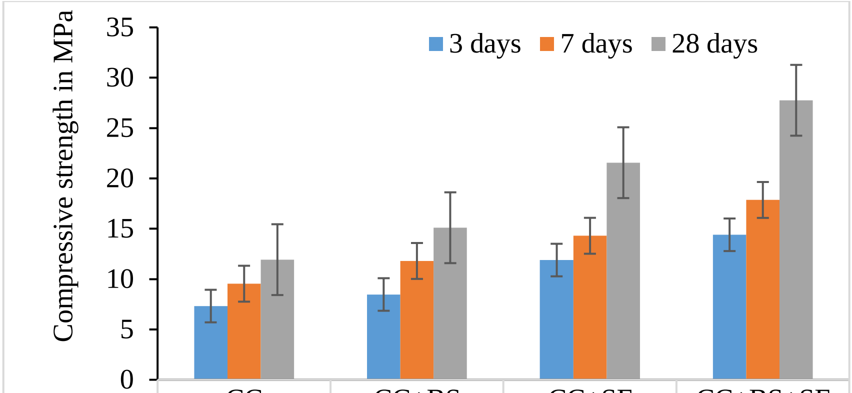
Figure 8. Casting procedure for grouted concrete, a) Preparation of grout, b) filling a layer of LWA, c) filling a layer of SF, and d) filling of grout.

3. Results and discussion

3.1. *Compressive strength*

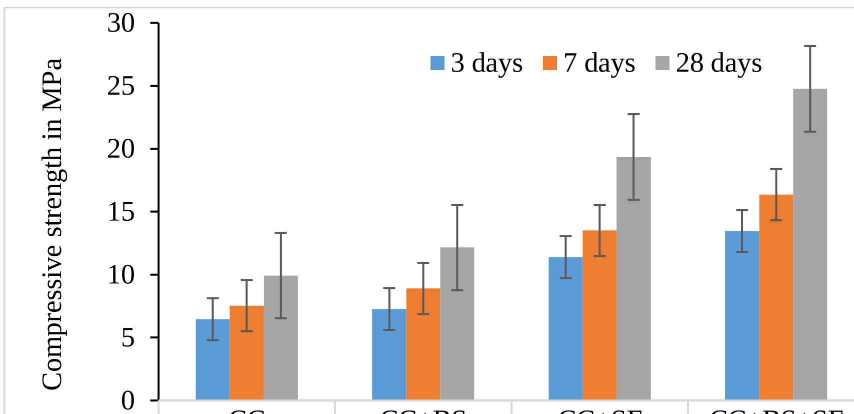
Figure 9 and 10 shows the CS results for mixes with and without bacteria and steel fiber in RHA admixed grout for 3, 7 and 28 days of water curing for two different sizes of LWA mix. Mix CC+BS enhanced the CS at all days of curing compared to mix CC. The maximum percentage improvement in CS of bacteria added mix (CC+BS) for 12.5 mm size aggregate was 15.71% at 3 days, 23.68% at 7 days and 26.57% at 28 days compared with CC. Similarly, the percentage improvement in CS was calculated for mix CC+BS+SF compared with mix CC+SF at all days of curing.

The percentage increase in CS for CC+BS+SF compared with CC+SF at 3 days was found to be 21.11%, 24.89% at 7 days and 28.76% at 28 days. Similarly, the percentage increase in strength was calculated for all mixes of 10 mm LWA and the results of bacteria mix was compared with the control mix at 3, 7 and 28 days of curing. Mix CC+BS was found to be increased in CS than mix with CC. The percentage improvement in CS was found to be 12.53%, 18.03% and 22.35% at 3, 7, 28 days respectively. The strength improvement in percentage was calculated for mix CC+SF was found to be 17,98%, 21.11% and 27.95% at 3, 7 and 28 days when compared with mix CC+BS+SF.



	CC	CC+BS	CC+SF	CC+BS+SF
3 days	7.32	8.47	11.89	14.4
7 days	9.54	11.8	14.3	17.86
28 days	11.93	15.1	21.56	27.76

Figure 9. Compressive strength results for 12.5 size aggregate.

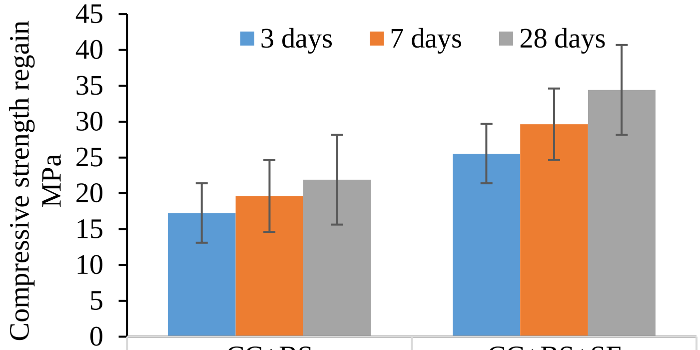


	CC	CC+BS	CC+SF	CC+BS+SF
3 days	6.46	7.27	11.4	13.45
7 days	7.54	8.9	13.5	16.35
28 days	9.93	12.15	19.35	24.76

Figure 10. Compressive strength results for 10 mm size aggregate.

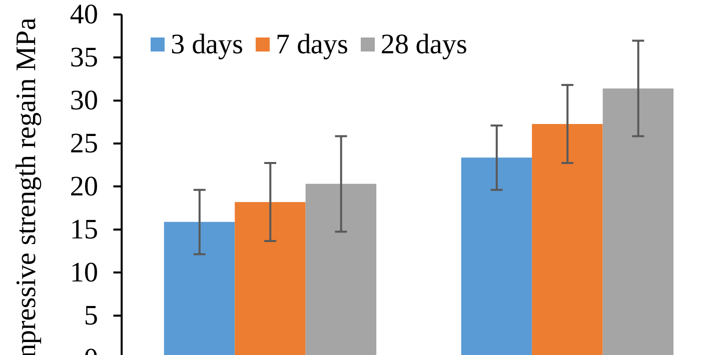
3.2 *Compressive strength regain (CSR)*

To determine CSR, mix with bacteria such as CC+BS and CC+BS+SF were pre-compressed at 3 days until the cracks were initiated in the specimens followed by curing for 3 days to determine the compressive strength of pre-cracked specimens at 3days. Similarly, to determine CSR for 7 and 28 days, specimens pre-compressed at 3 days and continued curing for 7 and 28 days. Figure 11 and 12 shows the experimental results of grouted concrete mix with 12.5 mm and 10 mm LWA incorporated with bacteria. The increase in CS for the bacterial concrete mix suggests that $CaCO_3$ was formed in the cracks, which enhanced the crack healing and strength (Rajesh et al. 2023).



	CC+BS	CC+BS+SF
3 days	17.25	25.54
7 days	19.62	29.62
28 days	21.9	34.43

Figure 11. CSR for 12.5 mm size aggregate.



	CC+BS	CC+BS+SF
3 days	15.87	23.35
7 days	18.2	27.27
28 days	20.3	31.4

Figure 12. CSR for 10 mm size aggregate.

3.3 *Impact strength*

Figure 13 and 14 show the impact energy of cylinder specimens at cracking and failure stages. Based on the observed outcomes, number of drops for first cracking (U1) and final failure (U2) for CC specimens. The number of hits for U1 of mix CC+BS, CC+SF and CC+BS+SF specimens with 12.5 mm LWA were 13, 65 and 72, respectively. For the CC+BS, CC+SF and CC+BS+SF specimens and comparing with CC, number of hits was enhanced by 160, 1200 and 1340% respectively. Similarly, number of hits for final failure was 17, 160 and 191 and it was compared with CC was higher by 41.66%, 1233.33, and 1491%. Similarly, U1 and U2 enhancement was observed for 10 mm size LWA mix with bacteria and steel fiber when compared with CC. It can be seen that the addition of hooked end fiber to the mix showed greater U1 and U2 values when compared to CC specimens.

Cracks initially begin on a microscopic scale and then develop to a massive scale. Steel fibers mix with CC+BS and interconnect at both the micro and macro levels, preventing cracks from advancing. Impact energy was calculated based on the number of hits at U1 for 12.5 mm was CC, CC+BS, CC+SF, and CC+BS+SF was 101.73, 264.968,1322.5 and 1464.9 N-m and for U2 was 244.15, 345.9, 3255.36 and 3886.1 N-m. Similarly, number of hits and impact energy was calculated for 10mm size LWA, number of hits at U1 was 3,8,55,68 and at U2 was found to be 9, 27, 116, 156. Impact energy at U1 and U2 was found to be 61.038, 162.768, 119.03,1353.83 N-m and 183.114, 549.342, 2360.136, 3173.98 N-m, respectively.

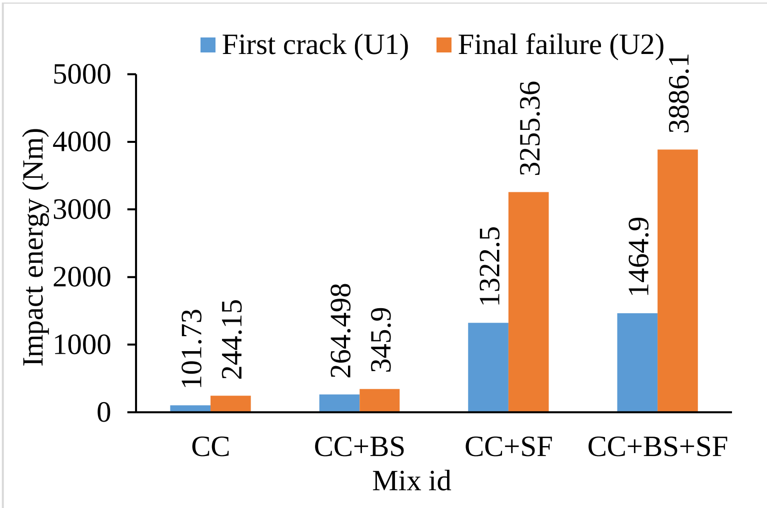


Figure 13. Impact energy results for 12.5 mm size aggregate.

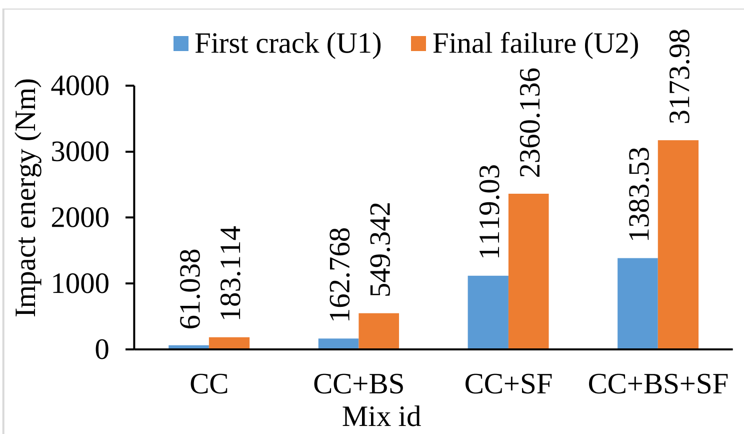


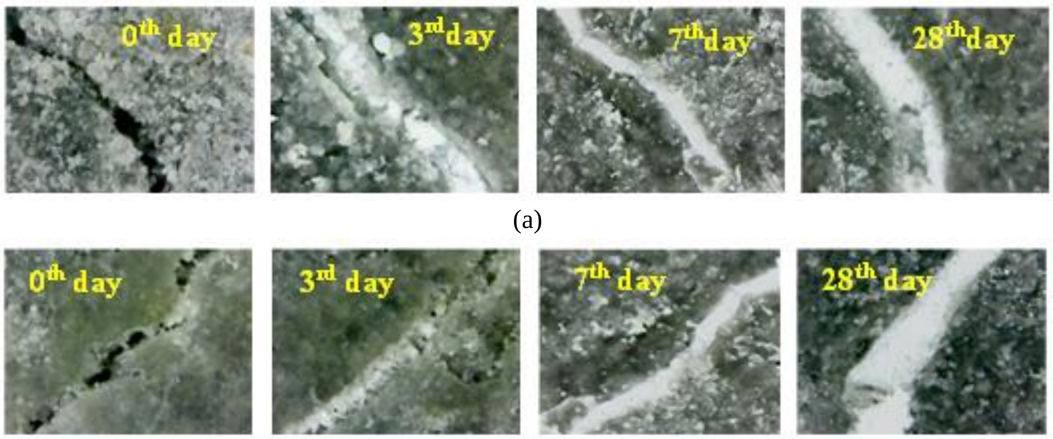
Figure 14. Impact energy results for 10 mm size aggregate.

3.4. *Microscopic observation of healing of cracks*

Figures 15 (a) and (b) make it abundantly evident that healing product with white color appeared in the fissures of bacterium specimens, demonstrating that throughout the curing process, the bacteria acquired the ability to heal themselves. On fractures, it was discovered that the white compound developed more after an increasing number of days from the day the crack was induced (Rajesh and Sumathi 2024). According to daily observations, bacterial concrete begins to precipitate calcium carbonate the day after a fracture is induced and eventually grows to partially or completely seal the crack. Non-bacterial specimens showed no signs of crack repair. From the images it is evident that rate of healing is increased as the number of days increases from 0 to 28 (Sumathi et al. 2020; Muddukrishna Padichetty et al. 2021 and Ganesh Vigneswaran et al., 2022).

Cracks width of 0.36 mm show faster healing capacity whereas wider cracks of 0.38 mm and 0.42 mm show slow healing rate.

Crack width healing % is determined from methods followed in our previous study (Sumathi et al. 2020; Muddukrishna Padichetty et al. 2021 and Ganesh Vigneswaran et al., 2022). As shown in Figure 16, subsequent increase in average crack width healing is noticed in mix CC+BS and CC+BS+SF. Average crack width healing % (at 28 days) of CC+BS and CC+BS+SF was 71% and 86.8% respectively. In CC+BS (minor crack width noticed), the white healing compound starts to develop at the inner depth and builds itself towards outside to seal the crack, the wider and deeper cracks may show slow healing rate. In CC+BS+SF, bacteria precipitates $CaCO_3$ in between crack zones of steel fiber and concrete matrix which is created during loading. Combined effect of bacterial healing product ($CaCO_3$) accumulations in steel fiber surface and internal cracks is found to be the major contributors for such higher healing rate. From this it is eventually clear that, *Bacillus subtilis* is a suitable bacterium in fiber reinforced concrete which renders higher healing in-line with mechanical properties.



(b)
Figure 15. (a) Crack healing of mix CC+BS, and (b) Crack healing of mix CC+BS+SF.

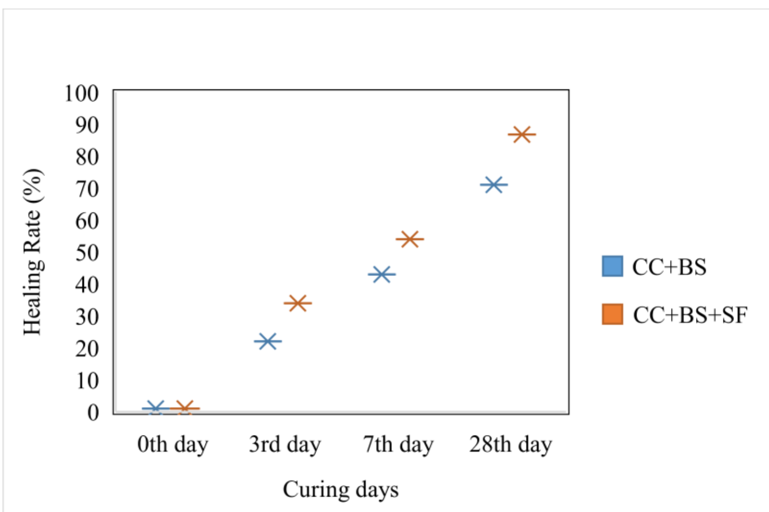


Figure 16. Crack healing % of mix CC+BS and CC+BS+SF.

3.4. *SEM analysis*

A portable microscope was used to first inspect the fracture under a microscope. After 28 days, a SEM analysis was conducted to look at the microstructure of the crushed sample was shown in Figure 17. The calcite crystals that are located on the pores of the concrete will reduce the number of pores while also boosting the concrete's strength. This is because germs have been added to the mixture. Therefore, it can be said that by adding bacterial solution to the treated cubes, the fissures will be partially filled by calcite production. Different crystalline diameters, hexagonal and rhombohedral formation can be seen in all of the samples.

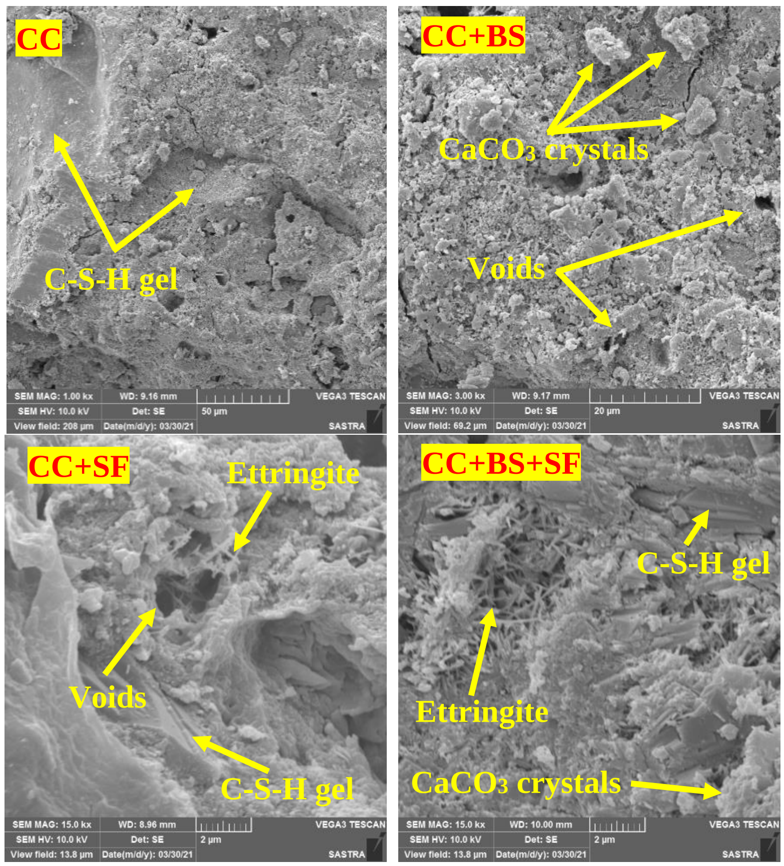


Figure 17. SEM images of different mixes.

3.5. *X-ray diffraction (XRD)*

The white powdery precipitate that was present in the precracked specimen was subjected to XRD examination. Calcite (Ca), aragonite (AR), and vaterite (Va) are among the mineral precipitations combined by bacteria in Figure 18. The fact that the bacterial test samples produced more calcite than expected, which is what caused the concrete mix to absorb less water, should have been highlighted. The XRD spectra of the microbiological samples and the magnitude of the peak location in the spectra of the bacteria confirmed the presence of calcium carbonate (Rajesh and Sumathi 2023).

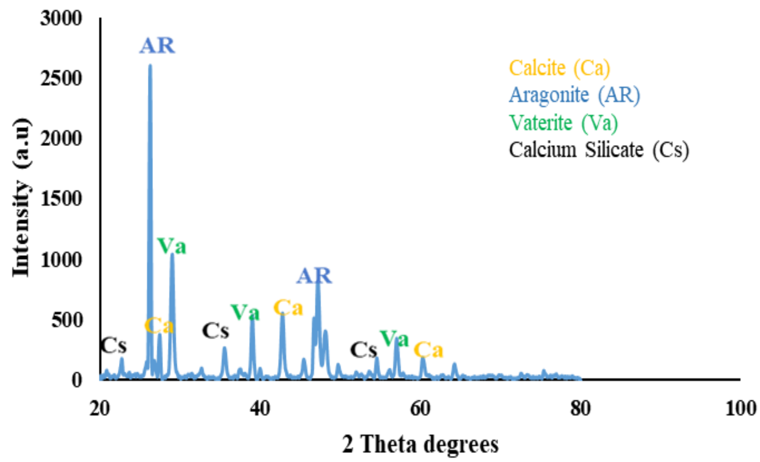


Figure 18. XRD analysis of bacterial concrete.

4. Conclusions

Based the experimental study the following conclusions were drawn:

1. When bacteria were added to grouted LWA concrete (CC+BS), its compressive strength was higher than CC on all days of water curing for two distinct aggregate sizes. In comparison to the other mixes, the percentage improvement in CS was better for the bacteria and steel fiber mix by facilitating the precipitation of calcium carbonate which leads to more compact matrix than CC.
2. For mixes with 10mm size aggregate, a comparable trend was seen with marginally lower values than mix with 12.5 mm. The pre-compressed concrete samples recovered their compressive strength and underwent for self-healing at different days. This suggests that $CaCO_3$ in fracture locations not only reinforces the crack but also aids in enhancing the CSR for CC+BS+SF with a maximum of 34.43 MPa for 12.5 mm size and 31.4 MPa for 10 mm size LWA concrete at 28 days.
3. When CC+BS is used instead of only CC, the amount of hits and impact energy are slightly enhanced at U1 and U2. The percentage enhancement in impact energy at U1 were found for CC+BS, CC+SF, CC+BS+SF as 160, 1200, 1339.98% respectively compared to CC. Similarly, percentage improvement at U2 were 41.675, 1233.34, 1491.68% compared to CC. However, CC+SF and CC+BS+SF have significantly better effect numbers and energy at U1 and U2 than CC. Steel fibers are added to grouted concrete to increase ductility and prevent unexpected failure.
4. Mixes CC+BS and CC+BS+SF shows maximum healing efficiency of about 71% and 87% at 28 days respectively. The improvement in CS of the concrete is explained by the more homogeneous structure and greater creation of a CSH gel, according to the examination of XRD and SEM. The densest structure, represented by mix CC, CC+BS, and CC+BS+SF, produced the highest CS.
5. Overall, the results indicated the potential use of bacteria in the grouted mix with RHA and steel fiber addition in enhancing the mechanical properties, especially the healing efficiency of the bacteria grouted mix.

5. Data availability statement

Author contributions: Rajesh. A: methodology, review, document writing and enhancement of manuscript. Karthikeyan. AR: conceptualization, data collection, results analysis, and document writing. Sumathi. A: methodology, conceptualization, data collection, review, supervision, results analysis, document writing and enhancement of manuscript.

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