Journal of Applied Science and Engineering, Vol. 23, No. 1, pp. 1–8 (2020) *DOI: 10.6180/jase.202003_23(1).0001*

An Experimental Study on Performance of *Bacillus pumilus* KC845305 and *Bacillus flexus* KC845306 in Bacterial Concrete

Anand Elumalai[1], G. Mohan Ganesh[2]* and Kalaichelvan Gurumurthy[3]

[1]*School of Bio-Sciences and Technology, Vellore Institute of Technology, Vellore-632 014, TN, India*
[2]*Department of Structural and Geotechnical Engineering, Vellore Institute of Technology, Vellore-632014, TN, India*
[3]*VIT School of Agricultural Innovations and Advance Learning (VAIAL), Vellore Institute of Technology, Vellore-632014, TN, India*

Abstract

In recent years, concrete has become an important versatile construction material. This paper evaluates the strength obtained by concrete with the influence of bacteria. The bacterial strains were isolated from calcareous sludge and urea ware house. The bacterial strains were identified through 16srRNA gene sequencing as *Bacillus pumilus* and *Bacillus flexus*. Using these strains, an experiment on cylinder and prisms cast was performed. Compressive strength, split tensile and flexural tests were conducted at the age of 7, 28 and 56 days with ultrasonic pulse velocity and rebound hammer. The results were compared with *Bacillus cohnii* MTCC 3616 obtained from microbial type culture collection and gene bank, Chandigarh, India. Based on the experimental results, the improvement in the mechanical strength is due to the deposition of calcite crystals on the bacterial cell surfaces within the pores which enhanced the strength of concrete and reduced porosity and permeability.

***Key Words*:** Biomineralization, Bacterial Concrete, Ultra Sonic Pulse Velocity, Scanning Electron Microscope (SEM), X-ray Diffraction (XRD)

1. Introduction

Concrete is a made up of cement, aggregate (fine and coarse) and water. Concrete plays an indispensable role in many fields where cement is the major part of concrete material and are mainly used in the building construction, bridges, roads, tunnels, dams, subways, sea-ports, storage tanks and other infrastructures. For many application, it binds the aggregates and fills the voids between coarse aggregate and fine aggregate. The availability, higher compressive strength, durability and reinforcement compatibility, easy preparation, lower cost and possibility of casting concrete in desired sizes and shapes make concrete the material of choice [1–3]. The production of ordinary portland cement (OPC) releases the carbon dioxide and the cement industry globally produces around 2.8 billion Tons of the green gases emissions perennially, approximately 7% of the petrol emission to the earth's atmosphere. It is necessary to find alternatives for suitable concrete [4,5]. Bacteria is the chief agent of geochemical diversity due to their full superficiary scope to scroll proportion, large and abundant distribution, developmental adaptability and enzymatic, dietary and mineral difference [6].

Biomineralization refers to a process in which the formation of minerals is originated by living organisms. Through this process, minerals are liberated by the enzymatic and non-enzymatic action of microbes [7,8]. Microbially incorporated calcium carbonate precipitation (MICP) is a phenomenon through which precipitation of calcium carbonate occurs by bacteria. A commonly known bacteria found in soil, *Bacillus Megaterium* was involved

*Corresponding author. E-mail: gmohanganesh@vit.ac.in

in $CaCO_3$ precipitation. The principle behind the application is that the urea gets hydrolyzed to produce ammonia and CO_2. The released ammonia resulted in an increase in the pH of surrounding media which leads to insoluble $CaCO_3$ accumulation [9]. Microbiological $CaCO_3$ precipitation has been associated with various microbial forms in extremely dissimilar environments [10,11]. The precipitation occurs on the cell walls by the attachment of positive ions to the negative ions. The $CaCO_3$ precipitation occurs mainly in anaerobic condition. It also depends on pH, nucleation site, temperature and concentration of dissolved inorganic carbon [12,13].

Reports state that bacteria are capable of forming broad range of minerals namely phosphates, carbonates, silicates and sulphates. They are able to precipitate in the presence of calcium source [14]. This pilot study examines the effect of bacteria on concrete mortar properties with respect to strength, split, flexural, UPV and rebound hammer test in goal of long term durability. This is the innovative alternative method to increase the durability of the concrete demand in place of the current existing practice.

2. Materials and Methods

2.1 Isolation

The samples were collected from two different soils *viz*. calcareous sludge from the urea warehouse and activated sewage plant. The collected samples were placed in the sterile plastic bags and transported to the laboratory. Samples were stored at 4 °C and tested within 48 h. Urease potential strains were isolated and plated on nutrient agar. After 24 h of incubation at 37 °C, the isolated colonies were sub-cloned for purification identification.

2.2 Urease Activity

The isolates were tested for urease activity. This identification of purified culture was done by repeated streaking on urease agar (Christensen agar, Himedia) and inoculating test tube with viable liquid cultures. Christensen's agar containing g per litre: Peptic digest- 1; Dextrose 1; Sodium chloride 5; Disodium phosphate 1.2; Mono-potassium phosphate 0.8 Phenol red 0.012 and Agar 15. The plates were incubated at 30 °C for 3 days. A change in coloration following incubation indicates urease positive reaction shown in Figure 1. The potential strains were compared with positive control (*Bacillus cohnii* MTCC) and negative control *Escherichia coli*. All the experiments were done in triplicates.

2.3 Polymerase Chain Reaction Amplification of 16SrRNA

The template DNA for amplification of PCR from pure culture was prepared by extracting the total genomic DNA from the isolated strains according to CTAB method. The PCR master mix contained 2 µl DNA template, 1 µl forward primer, 1 µl reverse primer and the final volume is made with to 20 µl with RNase free filter sterile water. The 16SrRNA amplicons were obtained by amplifying the 16SrRNA region with primers UF (5'–AGAGTTTGATCATGGCTCAG–3') and UR (5-'TAC GGCTACCTTGTTACGAC-3'). PCR amplification was performed with initial denaturation at 94 °C for 10 min, denaturation at 94 °C for 1 min, annealing at temperature 49 °C for 1 min, final extension at 72 °C for 1 min, final extension at 72 °C for 7 min, with 30 PCR cycles [15].

2.3.1 DNA Sequencing and Sequence Analysis

PCR fragments from DNA sequencing was carried out by Eurofins. The DNA Sequence analysis and homology searches were completed by using BLAST algorithm of National centre for biotechnology information with standard sequencing programs of DNA [16]. Phylogenetic trees were constructed based on neighbor joining.

3. Experimental Program – Bacterial Concrete

3.1 Ordinary Portland Cement

Ordinary portland cement is one of the most widely

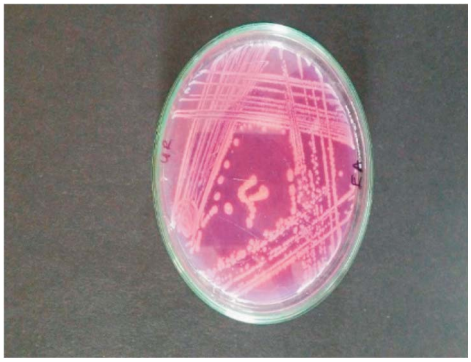

Figure 1. The isolated colonies of *Bacillus pumilus* KC845305 developed on Christensen agar.

used type of cement. Commercially obtainable Ordinary Portland Cement (OPC - 53 Grade) conformist to Indian Specifications IS: 8112-1989 was used [17,18].

3.2 Fine Aggregates

The natural river sand with 4.75 mm maximum size was used as fine aggregates and was tested as per IS Specifications IS: 383-1970. The values of specific gravity and fineness modulus of the fine aggregate were obtained in the laboratory [19].

3.3 Coarse Aggregates

Coarse aggregates are particles with size larger than 4.5 mm, which normally vary in diameter. Majority of the coarse aggregate constitute gravels were obtained by crushing the stones. The aggregates passing through 12.5 mm graded were used throughout the study.

3.4 Water

Water plays an important role in concrete preparation as it actively participates in chemical reactions with cement. Pure potable water was used for the preparation of concrete mixtures. The pH value of water is 7.1 in the laboratory. The prepared bacterial cultures in Christensen broth were added in indicated cell concentration.

4. Mix-proportion

Proportioning of the mix was done to create concrete having satisfactory workability and quality for the specific strength for different conditions. As per the IS: 10262: 2009 [20], the M30 grade concrete mixes were designed with cement, aggregate (fine and coarse). Concrete mix proportion was given in Table 1.

5. Experimental Testing Procedure

In this experimental work, 100 × 100 × 100 mm molds were used for casting. All materials were batched separately by weight. The materials were mixed thoroughly by hand mix and fresh concrete was poured into the mould, well compacted and top surface was finished with the help of trowel. The specimens were demolded after 24 hours and cured for 7, 28 and 56 days. The following tests were conducted with average values of 3 specimens was considered.

5.1 Compressive Strength

Mix design is the major factor controlling the strength of concrete and it is given in Table 1. The strength of a concrete specimen depends upon cement, aggregate, bond, water-cement ratio, curing temperature, and age. The compression test was used to determine the compressive strength of the concrete cube and cylindrical specimens. After the curing period the concrete specimens were examined for compressive strength with hydraulic digital compressive testing machine with a capacity of 2000 kN and pace rate of 2.5 kN/sec to find the compressive strength of the cubes IS 516:1959 [21]. The tests were carried out at 7, 28 and 56 days. Compressive strength is calculated using the following:

Compressive Strength (N/mm^2) = Ultimate load / Cross sectional area of specimen

5.2 Ultrasonic Measurements

The non-destructive testing (NDT) method such as ultrasonic measurement test was used to determine the strength of the concrete specimens. In hardened concrete the ultrasonic waves travel much easier, as they will travel freely around a fissure leading to an increase in transmission time (4000–5000 m/s) than in water (1480 m/s) or in air (350 m/s). The ultrasonic pulse velocity is measured after the curing periods as per IS 13311:1992 [22].

5.3 Rebound Hammer Test

The NDT method is widely used for the assessment of compressive strength of concrete specimens. The rebounded numbers were recorded from two sides of the concrete specimens [23].

5.4 Split Tensile Strength

Split-tensile strength is indirect way of finding the

Table 1. Composition of concrete mix

S.no	Material	Volume [kg/m³]
1	Fine aggregate	804
2	Coarse aggregate	1023
3	Cement	465
4	Christensen broth	186

tensile strength of concrete by subjecting the concrete cylinders to a compressive force. As per IS 5816:1999 [24], the tensile strength of concrete mix was evaluated by the split tensile strength after curing age of 7 and 28 days. 200 mm length with 100 mm diameter cylinder was placed horizontally, load was applied until the failure of vertical diameter of cylinder and noted. Average of three replicates was taken for each batch.

5.4 Flexural Strength

At 28 days the flexural strength results were carried out [25]. For each mix proportion an average of three prism of size (500 mm × 100 mm × 100 mm) were cast and tested. The concrete specimens placed in machine to that the load was applied to the top surface of the prism. The axis region of the concrete specimens is aligned carefully with the axis of loading device. The flexural strength of the concrete specimens was calculated by the code IS 516-1959 [26].

5.5 XRD

The concrete samples were pounded mechanically and subjected to X-beam diffraction. XRD is a procedure which used for distinguishing proof of shake or mineral substance of powder tests or assurance of the concoction structure of crystalline materials. The X-ray diffraction (XRD) investigation was performed on the samples of the concrete specimens and the investigation was utilized to decide the crystalline type of calcite crystals. XRD examination is completed to decide compound structure of the precipitation that happened because of bacterial mineralization and to analysis microstructure in order to confirm the presence of calcite in the form of $CaCO_3$ at the age of 28 days [27].

5.6 SEM Analysis

To confirm the formation of calcite by bacteria in concrete matrix, the tested concrete specimens were broken to acquire powdered form for SEM study. Cell concentration of 10^6 cells/ml were taken for analysis [28].

6. Result and Discussion

6.1 DNA Sequencing and Sequence Analysis

The DNA sequences analysis and homology searches was done using standard BLAST server of the National Center for Biotechnology Information with the BLAST algorithm and specially the 'blastn' program for comparison of a nucleotide query sequence with the nucleotide sequence database. The query sequence obtained showed similarity with *Bacillus* (AK1) and (AK2). The bacterial isolate AK1 was identified as *Bacillus pumilus* and AK2 was identified as *Bacillus flexus* the obtained accession number KC845305 and KC845306 respectively.

One of the main objectives of the bacterial concrete was the ability of bacteria incorporated specimens which survive in alkaline pH. The *Bacillus pumilus* and *Bacillus flexus* produced from the lab are non-pathogenic and cost effective. However direct incorporation of microorganism into construction materials such as concrete dramatically influences the microbial metabolism text.

6.2 Compressive Strength

The compressive strength results are given in Figure 3a. The concrete specimens were tested in saturated condition with no moisture, varying in curing age of 7, 28 and 56 days. The compressive strength of concrete with and without bacteria varying with different curing days were recorded and is given in the Figure 3. The strength is increased for potential bacterial strains of the incorporated concrete. From the results, it can be observed that there is an initial early strength gain for the first seven days of curing in bacteria incorporated specimens. The improvement could be attributed to bio-mineralization of $CaCo_3$ on the cell surface and also within the pores of cement-sand matrix. And it also noted that there is a significant increase in compressive strength for 28 and 56 days. This may be due to increase in pH level, provide good nourishment and buffering action to microbial cells with in the cement sand matrix initially. Due to the high pH, the cells were able to grow fast by calcite precipita-

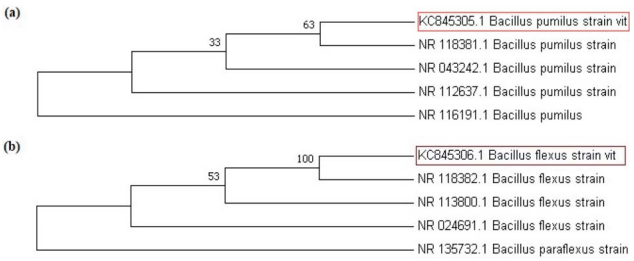

Figure 2. Phylogenetic tree for (a) *Bacillus pumilus* and (b) *Bacillus flexus*.

tion, then thereafter there could be pore-filling with calcite resulting in subsequent reduction in porosity. It was noted that in the bacterial concrete, the compressive strength was increased with bacterial cell concentration of 10^6 cells/ml. The result indicated that bacterial cells can be viable upto 56 days. Afterwards, the bio-mineralization of the microbial cells could be stopped due to the eventual death of micro-organisms.

6.3 Split Tensile Strength

The bacterial culture treated concrete specimens, AK1 and AK2 were tested. It is observed that the split-tensile strength is increased in the bacterial concrete specimens like compressive strength. The tensile strength was performed for each mix and tested for 28 days. The results of split tensile strength of concrete mixer with potential and conventional concrete were obtained at 28 days (Figure 3b). It shows the variation of tensile strength for conventional concrete and bacteria incorporated concrete. After 28 days of curing all the cylinders showed good behavior with increase in strength. These results review the efficacy of bacterial incorporated concrete.

6.4 Flexural Strength

The experimental result of the flexural tensile strength is given in Figure 3c. It is found that the flexural strength of the concrete specimen increases when the compressive strength with curing age. The maximum increase in the flexural tensile strength is obtained with the potential strain incorporated bacteria.

6.5 Ultrasonic Measurements

The ultrasonic pulse velocity results from the concrete mixtures were given in the Figure 3d. The ultrasonic pulse velocity was significantly found increased for the bacterial concrete at 7 days due to the presence of the $CaCO_3$ on the microorganisms, the porous decreased and the values were comparable at 28 and 56 days. The concrete incorporated with bacterial strain comes under "Excellent" category. In a comparative way, the higher speed in terms of velocity was achieved since concrete quality was high in terms of uniformity and density.

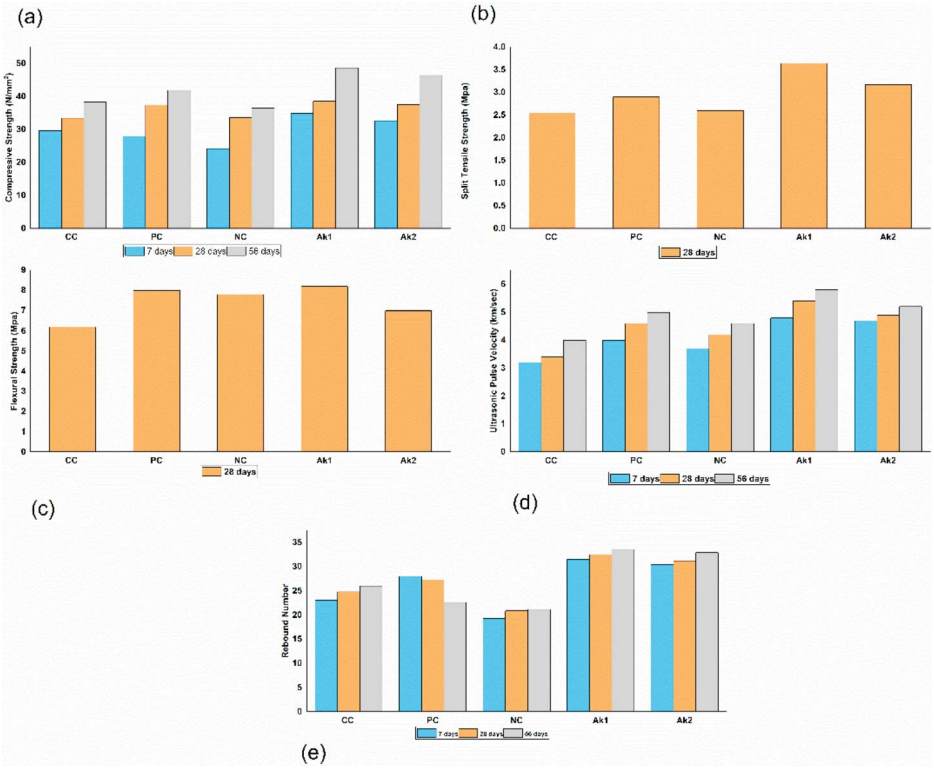

Figure 3. (a) Bacterial growth characteristics compressive strength results at 7, 28 and 56 days. (b) Split tensile strength 28 days. (c) Flexural strength for 28 days. (d) Ultrasonic pulse velocity test results at 7, 28 and 56 days. (e) Rebound hammer test values for 7, 28 and 56 days respectively.

6.6 Rebound Hammer Test

A significant increase in strength was seen for 28 and 56 days. From 7th day to 28th day of examination, it was noted that bacterial cement demonstrated noteworthy increase in extreme quality than the conventional concrete. The culture demonstrated to expand the quality of bacterial concrete due to bio-mineralization of $CaCO_3$ on the cell surfaces and inside the pores of the cement-sand lattice impacted the change in the quality of concrete. This upgraded variety in compressive strength affirms the artificially delivered urease as $CaCO_3$ precipitation amongst bond and sand lattices of the bond mortar example by the bacterial concrete. Due to steadiness of sustenance in curing process, the bacterial concrete demonstrated higher compressive strength than conventional concrete (Figure 3e).

6.7 XRD

Microstructure examination was done using XRD for affirmation of calcite which was available as calcium carbonate at age of 7, 28 and 56 days as shown in Figure 4. Calcite precipitation was more at 28 days and in concrete blends, calcite was available. The most extreme quantities of calcite crests were watched concrete containing *Bacillus pumilus* and *Bacillus flexus*. Hence, from the above outcomes, it can be reasoned that bacillus megaterium is more productive for calcite precipitation at 28 and 56 days. Calcium hydroxide if drained out causes sturdiness issues. Be that as it may, in our exploration work the nearness of calcium hydroxide ($Ca(OH)_2$) is unimportant in the solid blend which affirms the most extreme utilization in the hydration response and arrangement of calcium carbonate with the extra advancement of C-S-H gel which prompted enhance the quality and strength properties. The examples with potential strain did not build up any break, as they didn't extend much dissimilar to control and negative control examples when subjected to salt total reactivity. Metabolic exercises of *Bacillus pumilus* and *Bacillus flexus* in concrete is mindful to enhance the general conduct of cement and prompt gainful improvement. The higher pinnacle esteems demonstrates the higher nearness of calcite in the solid example.

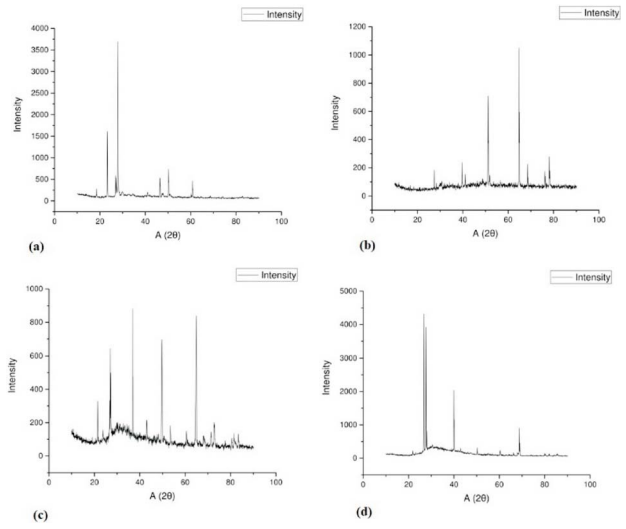

Figure 4. XRD for affirmation of calcite formation due to bio-mineralization. (a) Conventional concrete with the absence of $CaCO_3$, (b) Positive control with increased $CaCO_3$, (c) The isolated *Bacillus pumilus* KC845305 with increased $CaCO_3$ and decreased CaO, (d) The isolated *Bacillus flexus* KC845306 with increased $CaCO_3$ and decreased CaO.

6.8 SEM

The concrete samples with and without bacteria were analyzed using SEM and are represented in Figure 5. The bacteria and non-bacterial mixes were analyzed for the production of calcite crystal. The presence of high amounts of calcium in all the bacteria incorporated samples were identified. The calcium carbonate crystals associated with bacteria, indicated that the bacteria attend as nucleation situation during mineralization. More over inside the concrete complex the SEM analysis shows proper generation of adhesion due to chemical activity.

6.9 Effect of Calcium Carbonate Precipitation

The significant activity of bacterial culture in AK1 and AK2 concrete specimens, i.e., the biochemically induced calcium carbonate precipitation between cement sand matrix, increased the mechanical properties and the reduced the micropores of the concrete specimens. Immobilized bacterial spores in the concrete matrix would be metabolically active when revived by water and calcium media of concrete. The bacteria hydrolyze the urea to produce ammonia and carbon dioxide, resulting in an increase of pH in the surroundings where ions Ca^{2+} and CO_3 precipitate as $CaCO_3$. With significant increase in the calcite precipitation, these bacteria may be used commercially for the crack remediation process in construc-

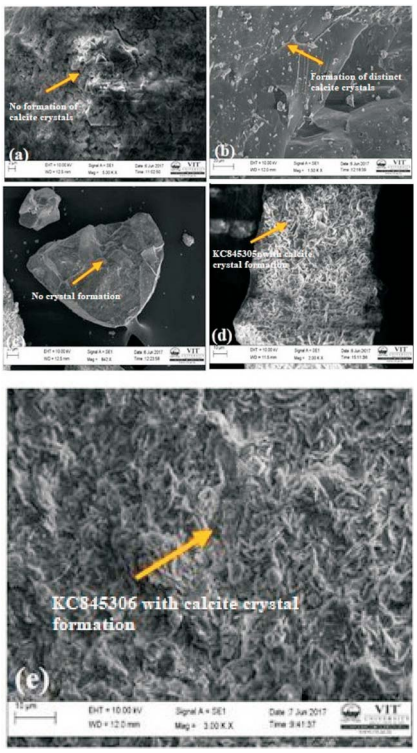

Figure 5. Calcite crystals formation in concrete specimens. (a) Conventional concrete with absence of crystal formation. (b) Positive control with distinct crystals. (c) Negative control with no crystal formation. (d) The isolated *Bacillus pumilus* KC845305 with calcite crystal formation. (e) The isolated *Bacillus flexus* KC845306 with calcite crystal formation.

tion industry. This bio-mineralization process by *Bacillus pumilus* and *Bacillus flexus* indicated that calcite formation is critical phase which needs bacterial presence.

7. Conclusion

In this study, the ability of bacterial culture that played a potential role in increasing the mechanical properties such as compressive strength and split tensile strength in concrete were investigated. The bacterial cultures used in the study were characterized as spore formers. It is found that bacterial incorporated concrete showed increase in compressive and split tensile strength than the conventional and negative control to a significant level. These spores produced inside is immobilized in the concrete matrix would be metabolically active when revived by water and calcium media of concrete. The chemistry behind the increase in strength was due to the hydrolyze urea to produce ammonia and carbon dioxide, resulting in increased pH in the surrounding medium. The conducted experiments provided valuable information about the mechanical properties of concrete specimens with or without bacteria and provides solid base for the future research in next generation building with a microbiology and civil engineering approach. Further the bacteria may be able to seal cracks by biominerals along with improving strength and durability of cement concrete. The characteristics of bacterial concrete therefore appeared to be promising in near future in the field of structural engineering. The outcome of this work is microbes in concrete play an important role in expansion quality, low support cost of the solid structure, protection from solidification and defrosting, high carbonation which results in reduced porosity and penetrability thus preventing chloride assault.

Acknowledgements

We are thankful to management of Vellore Institute of Technology for providing the infrastructure and space to carry out the research.

Conflict of Interest

The authors reveal there is no conflict of interest in this research work and for publication.

References

[1] Warscheid, T., and J. Braams (2000) Bio deterioration of stone: a review, *International Bio Deterioration & Biodegradation* 46(4), 343–368. doi: 10.1016/S0964-8305(00)00109-8

[2] Le Métayer-Levrel, G., et al. (1999) Applications of bacterial carbonatogenesis to the protection and regeneration of limestones in buildings and historic patrimony, *Sedimentary Geology* 126(1–4), 25–34. doi: 10.1016/S0037-0738(99)00029-9

[3] Samani, A. K., and M. M. Attard (2014) Lateral strain model for concrete under compression, *ACI Structural Journal* 111(2), 441–451.

[4] Malhotra, V. M. (2000) Introduction: sustainable development and concrete technology, *ACI Concrete*

Journal 1147–1165.

[5] Sumajouw, D. M., S. E. Hardjito D Wallah, and B. V. Rangan (2006) Fly ash based geopolymer concrete: study of slender reinforced columns, *Advances in Geopolymer Science and Technology* 3124–3130.

[6] Warren, L. A., and E. A. Hacck (2001) Biochemical controls on metal behaviour in freshwater environment, *Earth-science Reviews* 54, 261–320. doi: 10.1016/S0012-8252(01)00032-0

[7] Tebo, B. M., et al. (2005) Geomicrobiology of manganese (II) oxidation, *Trends in Microbiology*, 13(9), 421–428. doi: 10.1016/j.tim.2005.07.009

[8] Rivadeneyra, M. A., et al. (1994) Precipitation of calcium carbonate by Vibrio spp. from an inland saltern, *FEMS Microbiology Ecology* 13(3), 197–204. doi: 10.1016/0168-6496(94)90013-2

[9] Nagarajan, V., et al. (2012) A study on the strength of the bacterial concrete embedded with bacillus megaterium, *International Research Journal of Engineering and Technology (IRJET)* 4(12).

[10] Camoin, G. F. (1999) Microbial mediation in carbonate diagenesis (editorial), *Sedimentary Geology* 126, 1–4.

[11] Mcgenity, T. J., and B. W. Sellwood (1999) New approaches to studying the microbial precipitation of carbonate mineral, *Sedimentary Geology* 126, 5–8.

[12] Barton, L. L., and D. E. Northup (2011) *Microbes at Work in Nature: Bio Mineralization and Microbial Weathering*, in Microbial Ecology. John Wiley & Sons, Inc.: Hoboken, NJ, USA, 299–326. doi: 10.1002/9781118015841.ch11

[13] Hammes, F., and W. Verstraete (2002) Key roles of pH and calcium metabolism in microbial carbonate precipitation. doi: 10.1023/A:1015135629155

[14] Fortin, D., F. G. Ferris, and T. J. Beveridge (1997) Surface-mediated mineral development by bacteria, *Reviews in Mineralogy* 35, 176–180.

[15] Hammes, F. (2003) Strain specific ureolytic microbial calcium carbonate precipitation, *Applied and Environmental Microbiology* 69(8), 4902–4909.

[16] Altschul, S. F., T. L. Madden, A. A. Schaffer, J. Zhang, W. Miller, and D. J. Lipman (1997) Gapped BLAST and PSI-BLAST. A new generation of protein database search program, *Nucleic Acids Research* 25, 3389–3402.

[17] Ordinary Portland Cement, 43 Grade-Specification, IS 8112:2013, (2013) Bureau of Indian standard, New Delhi.

[18] Mohan Ganesh, G., A. S. Santhi, A. Elumalai, and K. Gurumurthy (2015) Experimental investigation of bacterial concrete to improve compressive strength: a microbiology and civil engineering approach, *The Indian Concrete Journal* 89(6), 24–30.

[19] IS 383: 1970. *Specification for coarse and fine aggregates from natural sources for concrete*, New Delhi, India: Bureau of Indian standards.

[20] IS 10262: 2009. *Method for concrete mix proportion sources for concrete*, New Delhi, India: Bureau of Indian standards.

[21] IS 516: 1959. *Indian standard code of practice - methods of test for strength of concrete*, New Delhi, India: Bureau of Indian standards.

[22] IS 13311: 1992. *Methods of non-destructive texting of concrete - methods of test*, part 2: rebound hammer [CED 2: cement and concrete].

[23] Brozovsky, J., L. Bodnarova, and J. Brozovsky Jr (2019) Rebound hammer tests of high-strength concrete: effects of internal stress and the shape of the impact area of the test specimens on the measurement results, *Periodica Polytechnica Civil Engineering* 63(1), 215–221.

[24] IS 5816 (1999). Method of Test Splitting Tensile Strength of Concrete [CED 2: Cement and Concrete].

[25] Pradeep, V., and K. Kumar (2013) The behaviour of concrete interms of flexural, tensile and compressive strength properties by using copper slag as an admixture, *International Journal of Engineering and Innovative Technology (IJEIT)* 3(4), ISSN: 2277-3754.

[26] IS 516: 1959. *Indian standard code of practice - methods of test for strength of concrete*, New Delhi, India: Bureau of Indian standards.

[27] Bashir, J., I. Kathwari, A. Tiwary, and K. Singh (2016) Bio concrete-the self-healing concrete, *Indian Journal of Science and Technology* 9(47).

[28] Ghosh, S., M. Biswas, B. D. Chattopadhyay, and S. Mandal (2009) Microbial activity on the microstructure of bacteria modified mortar, *Cem Concr Compos* 31, 93–98. doi: 10.1016/j.cemconcomp.2009.01.001

Manuscript Received: Aug. 21, 2019
Accepted: Dec. 25, 2019