An Experimental Study on Self-remediating Bacterial Concrete

S. Girish, T. Soumya, and Sahana Girish

Abstract The bacterial concrete is a self-remediation biomaterial under favorable conditions. Bacteria can precipitate calcite in concrete or form a layer of calcite precipitation which plays an important role in remediation of the plastic shrinkage microcracks thereby increasing the long-term structural integrity and durability of concrete. This study investigates the impact on compressive strength of concrete by addition of aerobic microorganism such as *Bacillus subtilis* and *Bacillus megaterium*, which microbiologically induce the mineral precipitation. The bacteria were incorporated into the 100 mm concrete cube in different concentrations in two stages formerly by curing in distilled water and later by curing in peptone-based nutrient medium. The results show the positive impact on compressive strength of concrete cubes with an increase in the strength of 30% with *Bacillus megaterium.* The strength enhancement is due to the precipitation of calcite within the pores which in turn improves the pore structure of the concrete. The study also revealed the importance of culture media, type of microorganism and cell concentration on the strength properties of bioconcrete. However, there was no much improvement in strength by curing in nutrient medium.

Keywords Bacterial concrete · Remediation · Self-healing

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1 Introduction

1.1 Concrete

"Concrete" is one of the ideal construction materials which is relatively cheap and mostly used throughout the world. The setback for concrete is cracks which may form due to many reasons like low tensile strength, shrinkage, inferior materials, design gaps, construction practices and exposure conditions. Large cracks basically reduce the integrity and durability of structure, and it requires some major repair actions, whereas smaller cracks at early stage do not much affect the strength and durability of the structure, but with time it will affect due to porosity and permeability of water and other vulnerable agents which result in degradation of concrete and cause corrosion of steel reinforcement which in turn affects the durability of the structure. When the concrete mix not appropriately provide the supply of water to the cement particles by improper mixing or low water/cement ratio, it will make the cement particle to remain unhydrated. The concrete after the aging get cracks thereby through the moisture supply cement particles get hydrated and forms the hydration products to clog the cracks by autogenous healing and enhancing the microstructure of the concrete, which is explained well in 1999 by Edvardsen [1]. The concept of self-healing concrete from the stage of autogenous healing up to the applications was demonstrated by ven Breugel [2].

Attempts are made to imitate the ideas and processes of nature to find solutions to human problem stated as biomimicking as mentioned in article [3, 4] by Wang et al. in 2009, and bioconcrete is one such attempt. The concept of strengthening the intrinsic properties of concrete to enhance durability involved many investigations and methods as quoted by the article in 2013 by Schlangen [5]. The enhancement of compressive strength of concrete by bioremediation incorporating *Bacillus pasteurii* was highlighted in 2001 by Dr. V. Ramakrishnan in his article [6, 7]. Similarly, the role of anaerobic thermophilic bacteria in the enhancement of compressive strength of concrete was focused by Dr. Saroj Mondal [8].

There are many conventional methods to remediate cracks such as remediation by synthetic polymers, organic solvents, chemicals, etc. But these are not long-term solutions, and they impose a negative effect on the environment. There is a need for long-term as well as environmentally friendly solution into which the bacterial concrete fits in perfectly. Crack remediation is the secondary monitoring of the distress in concrete, whereas there is a need for primary monitoring and addressing the pore structure of the concrete by enhancing the strength properties of concrete. If a solution remediates the cracks, it is meanwhile demanded to play its role in strength enhancement also as a primary urge. In this perception, the study on strength properties of concrete by bacterial ingression is focused.

Durability is considered to be one of the important aspects for structures and is directly related to the degree and quality of consolidation efforts. Using conventional placing and vibration techniques, the resulting concrete can have considerable honey

combing due to development of voids. This problem occurs predominantly in reinforced structures with congested reinforcement. As a result, researches have been conducted in different parts of the world, which led to the development of a new type of concrete known as bacterial concrete. This could be a solution to most of the durability issues and also the strength-enhancing product. Without proper treatments, cracks in concrete structures tend to expand further and eventually requires repair. Hence, to achieve bioremediation of cracks, bacterial concrete was introduced. Cracks in concrete significantly influence the durability characteristics of structure.

Enormous research has been conducted by various methods as on self-healing concrete by Jonkers et al. [9–14], Ahn [15], Van Der Zwaag [16], Kathleen et al. [17] and the self healing fiber-reinforced composite by Homma [18]. Similar work with encapsulation of bacteria by superabsorbent polymers was focused by De Belie et al. [19, 20] and compiles the potential of bacterial concrete as a self-remediating biomaterial and the concept of self-healing of cracks achieved under aerobic condition. So that only when the crack propagates, it supplies oxygen, and the precipitation process is initiated and forms a layer of calcite. He also highlights the role of choice of nutrients and alkaliphilic bacteria in the remediation process in 2017 by articles [21, 22]. Shiwaki et al. in his study [23] experimented on method of heating around the crack to heal the cracks. Precipitation plays an important role, thereby remediating the plastic shrinkage microcracks, and increases the long-term structural integrity and durability of concrete. Effectiveness of the microbiologically induced calcite ($CaCo_3$) precipitation in remediation of cracks in concrete also increases the compressive strength, stiffness and modulus of rupture and also the durability characteristics. The industrial application bacterial concrete as an effective repair agent compared to traditional materials was highlighted by ven Breugel [24]. Similarly, the field application of bioconcrete was experimented by Jonker et al. [13]. Microbial-induced calcite precipitation (MICP) is a process in which ureolytic bacteria by metabolic activities promote $CaCO_3$ precipitation. In ureolytic activity when genus bacillus is subjected along with urea and calcium nutrients or phosphate in presence of oxygen, it will convert soluble calcium source to insoluble calcium carbonate, thereby plugging the cracks [25] by M. V. Sheshgiri Rao et al.

The two bacteria—*Bacillus subtilis* and *Bacillus megaterium*, being gram positive, endospore forming, alkaliphilic, soil bacterium—suit to most of the conditions of being a self-healing agent. A comparative study on performance of different bacteria was compiled in a review article [25] by M. V. Sheshgiri Rao et al., an overview article by Satinder Kaur in 2015 [26] and also a review by Wool [27]. Specifically, Bacillus species were experimented for their suitability one such is *Bacillus flexus* studied by Rao [28]. Hence, the performance of two bacteria in parametric study needs an intense focus by the researchers.

In the present research, the bacteria *Bacillus subtilis* and *Bacillus megaterium* were selected for achieving bacterial concrete and focused mainly on strength properties of bacterial concrete mix. Mix proportion for the concrete was taken as 1: 1.22:2.3 along with W/C ratio as 0.48. Cubes of size 100 mm and bacterial cell concentration of 10^1–10^8 cells/ml were adopted. Curing of concrete cubes was planned under two

phases, first with distilled water and second with peptone as nutrient solution. Tests on compressive strength scanning electron microscopy were conducted.

1.2 Bacteria

Bacteria are the most abundant of all organisms. They are ubiquitous in soil, water and as symbionts of other organisms. Most are microsized (0.5–5 μm) in their longest dimensions. Motile bacteria can move about using flagella, bacterial gliding or changes of buoyancy. Microorganisms can be cultivated in artificial media such as culture media, chemically defined media, complex media and anaerobic growth media.

2 Materials and Methods

2.1 Materials

Bacillus subtilis which is a gram positive, rod-shaped, catalyzes positive bacterium commonly found in soil and also has the ability to form protective endospore allowing the organism to tolerate extreme environmental condition and selected as one of the bacterial source. It has flagella which help for its motility. It multiplies binary times by 27 min at 37 °C. *Bacillus megaterium* which is a rod-shaped, gram positive, endospore forming species of bacteria having doubling time of about 1.6 h is also chosen for the investigation.

53 grade Ordinary Portland Cement is used for the mix and characterized as per IS: 4031-1988 [29] which found to be confirmed to the specification as per IS: 12269-1987 [30] indicating specific gravity of 3.15. Locally, available sand passing through 4.75 microsieves is used as fine aggregate. IS 2720-1980 [20] is referred to obtain specific gravity, and the specific gravity was found to be 2.70. A locally available coarse aggregate of 12.5 mm downsize is used. IS 2386-1963 [31] is referred to determine the specific gravity, and the specific gravity is found to be 2.74. The coarse aggregate occupies most of the volume in concrete, and it also adds on to the strength and resistance to abrasion. Distilled water was used in study. Even though it is practically unrealistic to cure the concrete by distilled water, the attempt is made to verify if there is any influence of impurities of normal water on the properties of concrete and also to make sure that the external supply of distilled water on distressed concrete can enhance the strength of concrete or not. The details of material characterization are shown in Table 1.

Table 1 Results of the material characterization

Results of material characterization		
Cement	Fine aggregates	Coarse aggregates
Normal consistency: **33**	Water absorption: **2.22**	Water absorption: **0.693**
Specific gravity: **3.15**	Specific gravity: **2.7**	Specific gravity: **2.74**
Specific surface (Blaine's in cm^2/kg): **296**	Sand zone: **zone I**	

2.2 Methods

2.2.1 Culturing of Bacteria

Prior to the culturing process, a nutrient medium was prepared using standard nutrient broth which was sterilized using incubator for the removal of contamination from it. Bacteria sample was inoculated using inoculation loop into the sterilized nutrient medium in an air flow UV chamber and kept in an incubated rotary shaker for 24 h to promote uniform cell growth in the nutrient medium. After 24 h, the main cultures were observed to have a turbid view indicating the growth of bacteria and in continuation the subcultures were prepared from the main cultures and adopted for bacterial concrete under cell concentrations 10^1–10^8 cells/ml which was obtained both by direct and indirect method. Indirectly, it is by optical density test by indirect interpretation of optical density with cell count.

2.2.2 Direct Method of Measurement of Microbial Growth is by Serial Dilution Method

Dilution blanks are arranged serially in a test tube stand and labeled with their dilution factor. 1 g of soil was taken added with 9 ml saline into the first test tube. Prior to that, subtilis bacterium was heated at 80 °C for 5 min and for *Bacillus megaterium* selective media was used. The sample was shaken well by keeping it was shaker for 10 to 15 min to achieve uniform mixing of sample or rotated the tube in between the palms. This uniform factor was taken as 10^{-1}. With the help of sterile pipette, 1 ml of mixed sample from 10^{-1} dilution was transferred into tube aseptically. This dilution becomes 10^{-2}.

Similarly, dilution up to 10–6 was increased every time. The sterilized Petri plates were arranged to avoid over growth and to make enumeration easy after the growth. The Petri plates are masked with their dilution factor, batch, media and data. 1 ml of well-mixed sample from each of the dilution tube was placed on to their respective plates. The sample was then mixed well by rotating the plates clockwise and anticlockwise. Petri dish was kept on the leveled surface, and when the medium is solidified, it was incubated at 37 °C in inverted position for 24-48 h for bacteria.

The number of colonies was counted and calculated for the number of bacteria per gram of original sample using 30–300 rules. The calculated plate counts were

recorded, and then, a small amount of sample was inserted into the peptone broth and again incubated it to obtain the desired concentration like 10–1 cells/ml etc.

2.2.3 Preparation of Concrete Cubes

The concrete cubes of 100 mm size were casted for M30 grade mix as per IS 10262-2009 [32] along with bacterial impregnation. The cubes were cast for varied cell concentrations from 10^1 to 10^8 to check the influence of cell concentration on the performance of bacterial concrete. The cubes were cured under distilled water for phase 1 and under nutrient peptone medium under phase 2 for 7, 14, 28 days.

2.2.4 Compressive Strength Test

The cubes after curing period were subjected to compression test using universal testing machine at the 28th day and tested for compressive strength as per IS 516-1959 [33].

2.2.5 Scanning Electron Microscopic Analysis

SEM analysis was used as a tool to study the morphological features of the bacterial concrete specimen and investigate the calcium carbonate crystal precipitation of the cured specimens. The concrete specimen sample is taken in a small quantity in powdered form under three different levels as required to be placed on the platform of SEM. Since the secondary electrons are the low-energy electrons, the surface absorption and reflection was made amplifiable by giving a conductive gold coating to the sample. The collision of this secondary electron beam with the concrete sample electrons creates the SEM image depicting the surface morphology of the particles of the sample under 10 μm magnification.

3 Experimental Results

3.1 Compressive Strength

By the tabulated compressive strength test results for both of the bacterial concrete specimen in Tables 2 and 3 and the graphical interpretation in Figs. 1 and 2, it is observed that the bacteria under distilled water curing indicate a superior compressive strength compared to peptone cured. Among the two bacteria *Bacillus megaterium* with the cell count 10^5 cells/ml shows top-notch performance with 57 MPa compressive strength under the 28 days of curing compared to normal concrete which shows

Table 2 Compressive strength test results for concrete cubes under phase 1 (*Bacillus subtilis* and *Bacillus megaterium*)

Bacteria	Compressive strength in MPa (28 days)								
	Normal concrete	Bacterial concrete (distilled water curing) concentration cells/ml							
Bacillus subtilis		10^1	10^2	10^3	10^4	10^5	10^6	10^7	10^8
	44	50	51	48	48	48	48	48	**52**
Bacillus megaterium	44	44	54	57	56	**57**	48	47	48

Table 3 Compressive strength test results for concrete cubes under phase 2 (*Bacillus subtilis* and *Bacillus megaterium*)

Bacteria	Compressive strength in MPa (28 days)								
	Normal concrete	Bacterial concrete (curing with nutrient peptone solution) concentration cells/ml							
Bacillus subtilis		10^1	10^2	10^3	10^4	10^5	10^6	10^7	10^8
	44	46	**49**	47	47	45	41	40	37
Bacillus megaterium	44	39	40	**44**	39	36	35	36	39

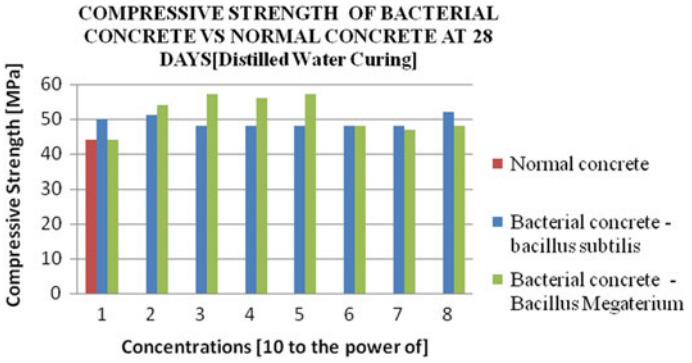


Fig. 1 Compressive strength of bacterial concrete versus normal concrete at 28 days under distilled water curing

44 MPa strength. By the results, one more observation has been highlighted that *Bacillus subtilis* when subjected to peptone nutrient medium perform better in the enhancement of compressive strength compared to *Bacillus megaterium.*

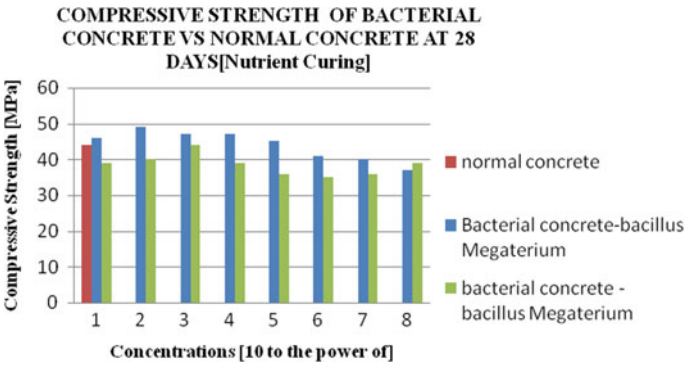


Fig. 2 Compressive strength of bacterial concrete versus normal concrete at 28 days under nutrient curing

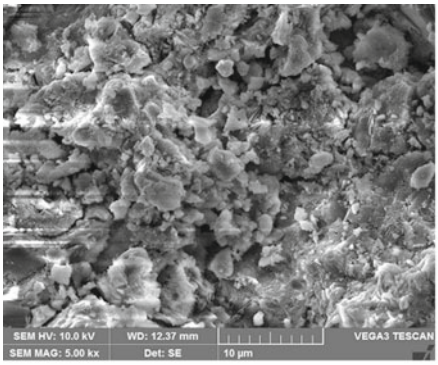
3.2 Scanning Electron Microscopic Analysis

The morphological observations made from SEM analysis as shown in Fig. 3 indicate the massiveness of the microstructure in the concrete treated with *Bacillus megaterium* by calcite crystals deposition which depicts the initiation of bacterial activity in concrete which in turn helps in strengthening the micropore structure of concrete and healing of microcracks also. The role of calcium carbonate crystals in concrete was stated by Matschei et al. [34].

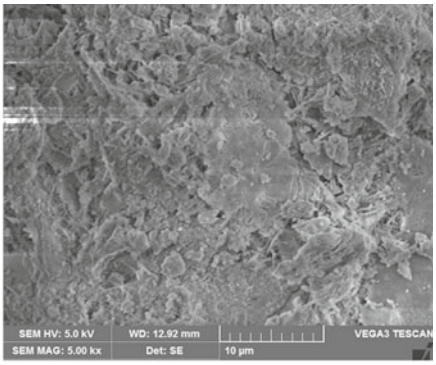
Observation: It is clearly observed from the morphology of three samples that the bacterial concrete specimens shown good amount of calcite precipitation than the normal concrete sample. This in turn says that role of the bacterial activity inside the alkaline concrete medium is crucial in generating the crack healing crystals to clog the cracks and enhance the compressive strength of the concrete.

4 Discussions

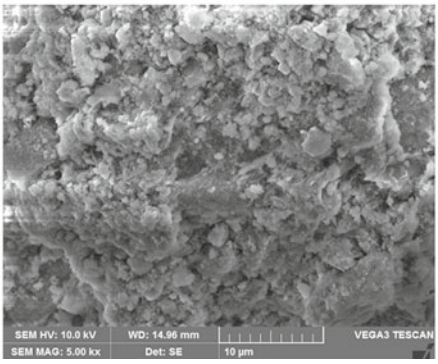
The role of bacteria in the calcite production even under high alkaline environment was highly influenced by the metabolic activity of bacteria. Especially the endospore-forming bacteria which have the capacity to get activated by the availability of suitable and undergo the metabolic activity to produce calcite precipitation and get dormant into endospore state instead of death seem to be a solid base for formulating the concept of self-healing bacterial concrete. Bacillus species being belongs to such category of spore forming has inspired researchers to choose them to study the performance of bacteria in concrete environment under various parameters. The *Bacillus subtilis* and megaterium being studied under this investigation exhibit following major observations.



a] Bacillus subtilis



b] Bacillus Megaterium



c] Normal concrete

Fig. 3 **a**, **b** Depicts the fully grown calcite under SEM with 10 µm magnification, **c** depicts the normal concrete morphology under 10 µm

- Concrete with distilled water curing and *Bacillus subtilis* with 10^8 cells/ml concentration has achieved 52 MPa compressive strength, whereas *Bacillus megaterium* with 10^5 cells/ml concentration has achieved 57 MPa compressive strength than normal concrete strength 44 MPa.
- Concrete with nutrient (peptone) solution curing with *Bacillus subtilis* of 10^2–10^4 cells/ml concentration compressive strength 49 MPa, whereas *Bacillus megaterium* with 10^3 has only 44 MPa as same as normal concrete.
- The SEM studies show the rhombohedra morphology of calcite crystals which prove to be the end product of bacterial activity in the concrete medium as a solid precipitation.

5 Conclusions

From the above results and discussions, following conclusions are drawn.

Addition of aerobic microorganisms has significant effects on the concrete compressive strength. The growth of fibrous filler materials within the pores alters the pore structure and seems to be beneficial from the strength point of view.

There is a significant increase in compressive strength due to the addition of bacteria, namely *Bacillus subtilis* and *Bacillus megaterium* which bring out the importance of choosing the beneficial microorganism.

The addition of nutrient peptone for the curing of bacterial concrete did not shown any significant improvement in the strength.

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