

# Bacteria based Self-Healing Cementitious Material - A Review

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**Abstract:-** The life of the healing in concrete is many years old and when the study was done on this, outcomes were coinciding with the auto-genus healing process which was capable to fill the micro-cracks in the cementitious based materials. And hereafter research led to the study of autonomous healing. In the autonomous healing process, the main aim was to fill the cracks and heal the fracture at the macro level. This was possible with the help of bacteria that were embedded in the cementitious based material with different technologies and methods. This research is all around the study of various bacteria used in the healing process of the cementitious based materials. Also, the effects of the various bacteria on the strength of materials are included in this work. Various bacteria have shown varied results when crack healing was studied with SEM analysis. Mainly various bacteria studied in this study are as *Bacillus Cohnii*, *Bacillus Hulodurans*, *Bacillus pseudofirmus*, *Bacillus sphaericus*, *Bacillus Subtilis*, *Sporosarcina pasteurii*, *Bacillus megaterium*, and *Escherichia Coli*. These bacteria have been proved a positive approach to the healing process in cementitious based material. Also, the important criterion has been studied which is essential when dealing with the autonomous healing process. An important criterion consists of the surface application and encapsulation method to use healing bacteria.

**Keywords:** Self-healing concrete; Healing bacteria; Genus Bacillus; Bacillus Subtilis; Bacterial concrete.

## 1. Introduction

Bacterial concrete concept highlighted by the researchers since it was found out that it is beneficial in calcium precipitation and an economical approach to reduce the maintenance cost in the concrete structures. The bacteria used in bacterial concrete is preferred for cracks healing in concrete structures and regaining the lost strength that helps to reduce the maintenance cost and to make the structure more durable without failure. The prime purpose of putting the bacteria in concrete is to utilize the bacteria that precipitate calcium after coming in contact with moisture and air. This study will be putting light on various bacteria that were used for healing purposes and given trial to make the bacterial concrete more economical.

Many researchers have worked on bio-concrete with various bacteria and defined the main concept of bacteria helping for healing in cementitious based materials e.g. concrete and mortar. Concrete is the most abundant, versatile and sustainable material for construction but being quasi-brittle, and is liable to cracks due to tension or shear. The main reason for cracks appearing in the construction component is faulty design, drying shrinkage and thermal contraction effect. In this paper *Bacillus psychrophilic* bacterium was prepared and mixed with concrete to evaluate the effect on the compressive and flexural strength of the concrete. Also, other important properties like permeability and corrosion-resistant of rebars were studied by the researcher [1]. These cracks make a pathway for the water penetration and other harmful chemicals that may corrode the steel and degrade the concrete component with the time. Micro cracks are the prime reason for the degradation of concrete structures. The encapsulation process was introduced in this paper by using microcapsules containing sodium silicate as a healing agent and polyurethane as shell material is used at different content. Although this is a chemical process that is helpful to fill the cracks up to 122 $\mu$ m that is lesser than microorganisms. XRD analysis was performed in this experiment to evaluate the chemical composition [2]. One of the researchers worked on the effects of various atmospheric curing conditions on the mechanical strength of the bio cement mortar. Main parameter that were focused in this paper are as different temperature, relative humidity, sunlight exposure time and wind speed. Microorganism was used in this paper was *Bacillus subtilis* and these bacteria was directly mixed with the mortar to evaluate the effect with various parameters. Except for the sunlight exposure, all the other three parameters (different temperature, relative humidity, and wind speed) were showing positive results in the compressive strength improvement [3].

## 2. Fundamentals & Classification of Genus Bacillus Bacteria

Many researchers have been worked on various bacteria that comes in the category of genus bacillus that have been used in concrete and mortar form to analyse the effects of these bacteria on the cementitious based materials (see Table 1).

Table 1. Various bacteria used in the bacterial concrete.

S. No.	Name of bacteria	References	Year
1	Bacillus Subtilis	[6] [3] [7]	2019 2019 2017
2	Bacillus Sphaericus	[8] [9]	2014 2010
3	Bacillus megaterium	[6]	2019
4	Bacillus mucilaginosus	[4]	2019
5	Bacillus pasteurii	[10]	2018

### 2.1 Bacillus subtilis

In a research work, have been introduced bacteria Bacillus subtilis to evaluate the effects on the mechanical properties of concrete and to observe the formation of calcium precipitation. Bacillus subtilis is a gram-positive bacteria from the genus 'Bacillus' [6]. According to [6] it was found out that Bacillus Subtilis is capable of forming highly resistant dormant endospores in response to nutrient deprivation and other environmental stresses. Subtilis Bacteria is that kind of bacteria that is commonly found in gut commensal in humans and as well, are easily found in an upper layer of soil.

For the culturing purpose, the culture media was prepared that includes water, nitrogen source, carbon source, trace elements, and other growth.

For the media, a nutrient broth, 13g of nutrient broth for every 1000ml of water, was used with compositions as provided in Table 2.

Table 2. Compositions of nutrient broth [6].

Compositions	Content
Peptone	5 g/l
Sodium chloride	5 g/l
Yeast extracts	2 g/l
Meat extracts	1 g/l
Water	1000 ml

In reference to the compressive strength, after mixing the bacteria Bacillus subtilis, a test was conducted on the compressive strength of the concrete in the compressive testing machine. In reference to the tensile strength, a split tensile strength test was conducted to see the effect of bacteria on the tensile strength of concrete. Tests have proved that adding this bacteria is a useful mechanism for the enhancement of compressive and tensile strength of the concrete [6].

Similarly, scanning electron microscope test have shown the presence of calcite crystal on the concrete surface as shown in Fig. 1.

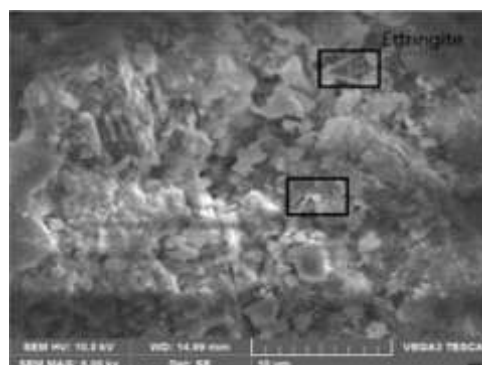


Fig. 1. The rectangular marked portion shows the presence of calcite in Bacillus subtilis Concrete [6].

EDAX (Energy dispersive spectroscopy) was carried out to observe the presence of CaCO<sub>3</sub> in the sample. The amount of CaCO<sub>3</sub> was examined with elemental constitutions examination of the sample and weight ratio was determined to examine the results (see Table 3)

Table 3. Results of Energy dispersive spectroscopy test of Bacillus subtilis [6].

Concrete specimen	Percentage of CaO in the specimen by weight for 28 days curing
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Table 4. Compositions of the media prepared for the culturing [3].

Compositions	Content
Tryptone	10 g
Yeast Extract	5.0 g
NaCl	10 g
H <sub>2</sub> O	950 ml

[7], in their study, have been evaluated the effects of bacillus Subtilis on healing and mechanical properties of shotcrete. Two main properties of the Bacillus Subtilis has been defined in this paper as this is a gram-positive bacterium with high spore formation capability and it can survive harsh environmental conditions. It was defined that bacteria spores are specialized cells that can endure high mechanical and chemical stresses and alkaline environment that makes it an ideal choice for shotcrete.

Table 5. Compositions of liquid nutrient agar [7].

Compositions	Content
Peptone	5.0 g
Meat Extract	3.0 g
MnSO <sub>4</sub> .H <sub>2</sub> O	0.01 g
H <sub>2</sub> O	950 ml

Further process is given by [7] is presented below:-

- The inoculation process was done under laminar flow hood and culture was incubated at 35°C in a shaker incubator at 130 rpm for 24 hours.
- The serial dilution technique was used to measure the bacterial cell concentration.
- The optical density was measured to obtain the cell concentration curve and equation. For this objective, a sample quantity of culture was extracted after 24 hours of incubation.
- To isolate the bacterial cells, the sample was washed by centrifugation at 5000 rpm for 10 minutes at 4°C.
- The specific volume of the isolated cells was serially diluted to distilled water.
- After that diluted solution were incubated into a solid medium in colonies of bacterial cells were quantified.
- The spore concentration was placed equal to 2.2×10<sup>6</sup> cells/cm<sup>3</sup> of the shotcrete mixture.

## 2.2 Bacillus sphaericus

[8] have been presented the mechanism of the Bacillus sphaericus in concrete and that are used with bacterial carriers microcapsules. Researchers suggested these microcapsules as beneficial resistant to the high pH of concrete and humidity sensitive. This study highlighted that these capsules are flexible under high humidity and easily become brittle at low humidity. That supports that the capsule shall withstand the process of mixing and shall easily break down when crack formation starts. Upon crack formation, microcapsules that comes in the crack zones will break down. After coming in the contact with the water, spores inside the capsules can germinate and as a result, precipitation of CaCO<sub>3</sub> will take place which will help to heal the crack. During the mixing process, the yeast extract(food) and deposition agent including urea and ca-nitrate were incorporated together with microcapsules.

Keypoints considered by the researchers in this report are as below [8]:-

- The liquid minimal basal salts medium was used to produce the Bacillus sphaericus spores.
- Before use, the medium was autoclaved at 120°C for 420 minutes.
- The incubation process of the cultures was done for 28 days at 28°C and 100 rpm.

- To minimize the vegetative cells cultures were subjected to pasteurization at 80°C for 20 minutes then 5 minutes in ice-cold water.
- Centrifuging of the culture was done to harvest the spores at 7000 rpm at 4°C for 7 minutes.
- After removing the supernatant, the spore's paste was subjected to vacuum drying under room temperature for 3 days.
- In the end, dry paste of the spores was ground in cement mill for 5 seconds to obtain fine powder that is used in encapsulation.

Researchers have presented the work of microcapsules after broken under tensile force. It was depicted from the Fig. 2 that at the fracture surface, microcapsules are broken and cells of the capsule remain in the matrix that indicated a good bonding between matrix and microcapsules. The size of the microcapsules was observed in the range of 2-5 μm [8].

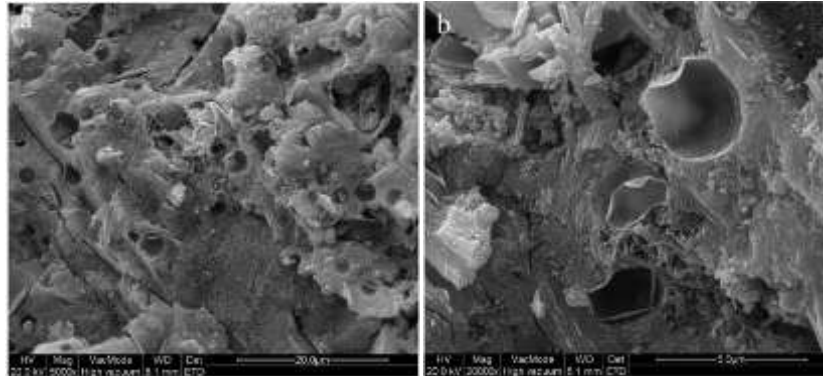


Fig. 2. Fracture surface of the mortar specimen with microcapsules (3%) embedded (a: 5000×; b: 20,000×) [8].

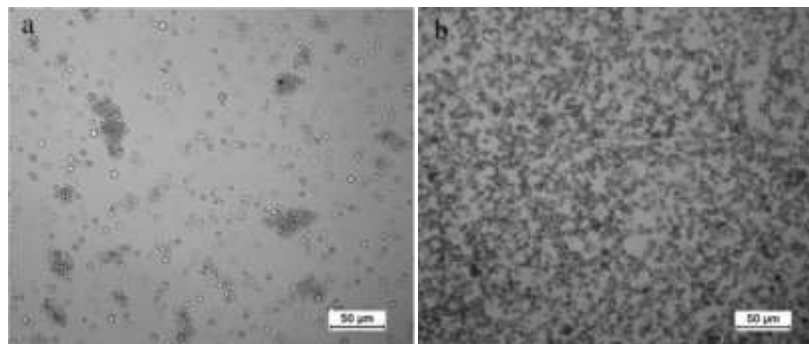


Fig. 3. Microcapsules (without spores) in the emulsion (a) and mixed with mortar paste (b) [8].

To examine the survival of microcapsule during mortar mixing, the microcapsule emulsion was mixed with water and microcapsules distribution can be seen in Fig. 3 (a) that depicts that the microcapsules are of round shape. Afterward, the microcapsule solution has been mixed with mortar and that is shown in Fig. 3 (b). It can be noticed that some light round dots adhered to cement or sand particles and distributed in the mortar paste [8].

In their research work, [9] have stated that a lot of research work has been presented on bacterial  $\text{CaCO}_3$  precipitation with  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$  but chloride ion can be hazardous to the steel reinforcement of the concrete. This investigation was around the use of calcium nitrate  $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$  and Calcium acetate  $(\text{Ca}(\text{CH}_3\text{COO})_2 \cdot \text{H}_2\text{O})$  at the place of calcium sources.

To repair the cracks,  $\text{CaCO}_3$  precipitation bacteria was used in the paper [9]. The isolation of the *Bacillus sphaericus* strains was done from calcareous sludge coming from a bio catalytic ureolytic calcification reactor. The protection of the bacteria from the strong alkaline environment in the concrete was essential, therefore the bacteria was mobilized in silica gel. In this study, Levasil® 200/30 % sol, with a specific surface area of 200 m<sup>2</sup>/g and solid content 30% has been used.

Keypoints considered by the researchers are given below [9]:-

- The bacterial treatment process was started by adding 1.2g NaCl to 10ml demineralized water.
- Mixture was incubated for 30seconds.
- Centrifugation of 50 ml of an overnight grown culture was done for 5 minutes at 4°C and 7000 rpm.
- NaCl solution was used to suspend the resulting pellet and incubated for 30 seconds.

- Furthermore, Levasil sol was added and complete solution incubated again then final obtained suspension was inserted into the crack using the syringe.

### 2.3 *Bacillus megaterium*

[6] have been introduced the bacteria *Bacillus megaterium* in their study and observed the effect of this bacteria on the compressive strength and tensile strength of the concrete. *Bacillus megaterium* is defined as gram-positive bacteria that are descendent of genus “*Bacillus*”. *Bacillus megaterium* is a common soil bacterium, that is also found out in certain food items including honey or on surfaces of various objects e.g. clinical specimens, paper, stone, etc. For this study, the *Bacillus megaterium* bacteria was obtained from microbial type culture collection and gene bank (MTCC) Chandigarh.

Keypoints considered by the researchers are given below [6]:-

- The cultivation of the bacteria starts with the process of preparation of culture medium.
- This culture medium includes water, carbon source, nitrogen source, trace elements, and growth factors.
- The composition of essential nutrient broth that is being used for the media is shown in Table 2.
- The *Bacillus megaterium* forms irregular dry white colonies on the petri dish.
- The platinum inoculating loop was used to transfer the few colonies of the bacteria into the culture medium in a conical flask, which is a part of inoculation.
- The incubation was done of the inoculated media at 37°C temperature, 150 rpm with the orbital shaker for overnight. Optical density test was performed to determine the concentration.
- The bacterial concentration is kept 10<sup>8</sup> cells/ml [6].

SEM (Scanning Electron Microscope) test was performed to inspect the specimen incubated in bacterial water. Fig. 4 shows the presence of carbonate crystal which was observed with the SEM test. It can be seen that rhombohedra crystals are visible on the surface that proved the presence of calcite ion [6].

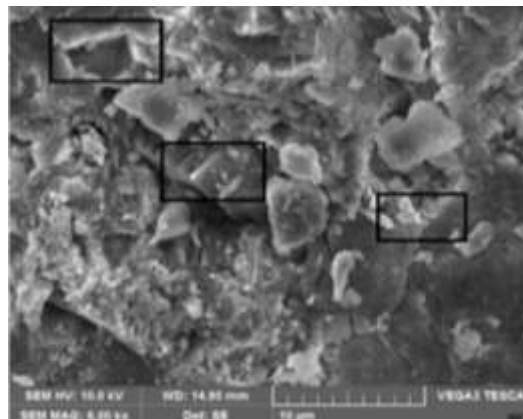


Fig. 4. Presence of Calcite precipitate in *Bacillus megaterium* Concrete [6].

EDAX (Energy Dispersive Spectroscopy) test is performed to examine the quantity of CaCO<sub>3</sub> present in the sample treated with *Bacillus Megaterium*. The weight ratio was determined with the help of EDAX test by elemental constitutions examination of the sample and the results are shown in Table 6.

Table 6. Results of Energy dispersive spectroscopy of *Bacillus megaterium* [6]

Concrete specimen	Percentage of CaO in the specimen by weight for 28 days curing
<i>Bacillus megaterium</i>	80.65

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