Effect of Bacillus Cohnii Bacteria on the Properties of Concrete

S. Jena, B. Basa, and K. C. Panda

Abstract Concrete plays an important role in this era of rapid urbanization. But concrete is quite prone to crack formation, which affects its durability. If immediate precaution is not taken then cracks tend to expand further and require costly repair. Due to the cracks, an easy path is developed in the structure, through which water, oxygen and carbon dioxide, etc. from air penetrates into the concrete which leads to the decrement in durability of concrete. To overcome this disadvantage, various crack healing techniques have come into limelight; one of them is self-healing bacterial concrete. This paper is aimed at finding out the influence of *Bacillus Cohnii* bacterium on the properties of concrete. Bacterial cell count, i.e. colony-forming unit (CFU) of 10^5 and 10^{10} cells/ml were selected for the present work. Then 10^3 and 10^8 cells/cm^3 bacterial concentrations have been added to concrete for 10^5 and 10^{10} cells/ml, respectively. Specimens were tested after different intervals of curing period. When compared to control mix (without the addition of bacteria), it is noticed that concrete with *Bacillus Cohnii* bacteria shows increment in compressive, flexural and split tensile strength in all curing periods for both cell concentrations 10^3 and 10^8 cells/cm^3. The highest strength is achieved when 10^3 cells/cm^3 bacterial concentration have been added to concrete for 10^5 cells/ml.

Keywords *Bacillus cohnii* · Compressive strength · Split tensile strength · Flexural strength

1 Introduction

After knowing the fact that crystal formation is quite a typical behaviour in bacterial species, it has been utilized vastly in various fields, i.e. oil industries, civil engineering, geological engineering, etc [1]. A few examples of the applications are: plugging

S. Jena (✉) · B. Basa
Institute of Technical Education and Research, SOA (Deemed to be University), Bhubaneswar, India
e-mail: sjena4203@gmail.com

K. C. Panda
Government College of Engineering, Kalahandi, Bhawanipatna, India

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of rock system for oil recovery enhancement and protection of ornamental stones [2–8]. Bacteria species could come in use in these applications as these applications require the use of calcium carbonate precipitate which are available in bacteria [9, 10]. Usage of bacterial concrete solves one of the most important vulnerabilities of concrete which is crack formation [11–15]. Crack formation not only decreases the life span of concrete, but also affects reinforcement of concrete as it results in corrosion. Through the cracks, water, oxygen and chloride enter into the concrete, which results in chemical reaction causing a shortening of concrete activity life. Usage of microbial concrete is also economic because, as the cracks remain unhealed for more days, they require more and more money to heal which is not in the case of bacterial concrete, since the healing process of cracks begin from the very moment they are formed.

The saviour of infrastructure industries is the cutting-edge technology of self-healing concrete. Self-healing concrete uses a completely new and unconventional method of dealing with the problem of crack formation [16]. This concrete can heal itself. Conventional concrete, to some extent is self-healing, since it can block the formation of further cracks in concrete by the method of hydration of un-hydrated microparticles. It can also be done by including some external agents in the concrete which can autogenously quicken the process of healing of concrete [17]. Since self-healing concrete is the newest and most promising solution to many concrete related problems, we need to use economic and environment-friendly method to achieve this. Usage of microbial concrete is hence taken into consideration. Till today, three most important bacterial metabolism processes have been found to be very useful for calcium carbonate precipitation. First one is hydrolysis of urea using enzymes [18–21]. The alternate mechanism is the oxidation of organic carbon [22–25]. The third pathway is the denitrification process under anoxic condition [26]. Out of the three mechanisms, the hydrolysis of urea is the most effective and the easiest one to perform.

The objective of this work is to observe the effects of bacteria and bacterial calcite precipitation on the various properties of concrete. For this purpose, *Bacillus cohnii* bacterium was chosen and its effect on compressive, flexure and split tensile strength were observed.

2 Experimental Details

2.1 Materials

For this work Ordinary Portland Cement (OPC), Natural Fine Aggregate (NFA), Natural Coarse Aggregate (NCA), *Bacillus cohnii* bacterium and potable water were taken. OPC-43 grade was utilized which is grey in colour and acquired in fine powdered form. NFA available in zone II was utilized for the current study. NCA supplied

Table 1 Physical characteristics of NFA and NCA

Characteristics	Results (IS: 383-1970) [27]	
	NFA	NCA
Fineness modulus	2.73	6.92
Specific gravity	2.69	2.78
Water absorption	0.85	0.24

Table 2 Characteristics of *Bacillus cohnii*

NCMR accession no.	MCC 2819
Taxonomic designation	*Bacillus* Cohnii
Strain Designation	LAP217
Source of isolation	Lonar Lake water sample
Location	Village: Lonar, Dist.: Buldhana, State: Maharashtra, India
Medium name and no.	34c (Alkaline Nutrient Agar)
Growth conditions (pH/Temp. °C)	10/28–30 °C
Incubation (days/h)	24–48 h
Sub culturing period (days)	1 month
Reference	Int J Syst Bacteriol (1980) 30:225

from Khurda, Odisha was used which is having size in between 10 and 20 mm. Different properties of fine aggregate such as specific gravity, water absorption and bulk density results are shown in Table 1. Bacterial samples were ordered from MCC, Pune which was in a freeze-dried condition. The detail description of pure culture for *Bacillus cohnii* is given in Table 2.

2.2 Mix Proportion

M30 grade of concrete was outlined according to standard specification IS: 10262-2009 [28]. The mix proportion was 1: 1.491: 2.69. Two kinds of concrete mixes were prepared, first mix is concrete without bacteria, second mix is concrete added with *Bacillus cohnii* bacterium. Bacterial cell count, i.e. colony-forming unit (CFU) of 10^5 cells/ml and 10^{10} cells/ml were selected for the present work and the bacterial cell count were added with concrete by referring to Jonkers et al. [23]. Two sets of concrete with bacteria were prepared, i.e. Bacterial cell concentration was added in concrete as 10^3 and 10^8 cells/cm^3 for 10^5 and 10^{10} cell/ml, respectively. Tables 3 and 4 show the mix identity and mix calculation of test sample, respectively.

Table 3 Mix identity of test sample

Mix identity	Bacterial cell count	Bacterial cell concentration in concrete (Cell/cm^3)
MSC0	0	0
MSC5	10^5 cells/ml	10^3 cells/cm^3
MSC10	10^{10} cells/ml	10^8 cells/cm^3

Table 4 Mix quantity per m^3 of concrete

Mix identity	MSC0	MSC5	MSC10
Cement (Kg)	442.85	442.85	442.85
CA (Kg)	1191	1191	1191
NFA (Kg)	660	660	660
Water required (Kg)	186	186	186
Bacteria (Kg)	0	10	10
Water added (Kg)	186	176	176

2.3 Bacterial Culture

For this experimental work, bacterial sample of *Bacillus cohnii* was taken and maintained in agar Petri plate. After that to grow the bacteria, a readymade Nutrient Hi Veg broth (Yeast Extract of 2.0 g, Beef Extract of 1.0 g, 5.0 g of Peptone, NaCl of 5.0 g, Agar of 15.0 g) was used. It was grown at 37 °C in a shaker incubator. To calculate the cell concentration with the help of spectrophotometer, Optical density test was carried out. Bacterial culture concentration of 10^5 cells/ml and 10^{10} cells/ml were maintained in the samples.

2.4 Casting and Testing of Specimen

OPC with NCA, NFA and bacteria were weighed and put in the concrete mixer and it was altogether mixed in dry condition until the point when the mixture becomes homogeneous. Then the required measure of water for each mix was included. Immediately after mixing for deciding workability of fresh concrete, slump test was done. Prior to casting of specimens in steel moulds, they were vibrated with the assistance of table vibrator. Then concrete specimen was casted and remoulded in the following 24 h. From that point, the specimens were permitted to cure in potable water for a time period of 7, 14 and 28 days.

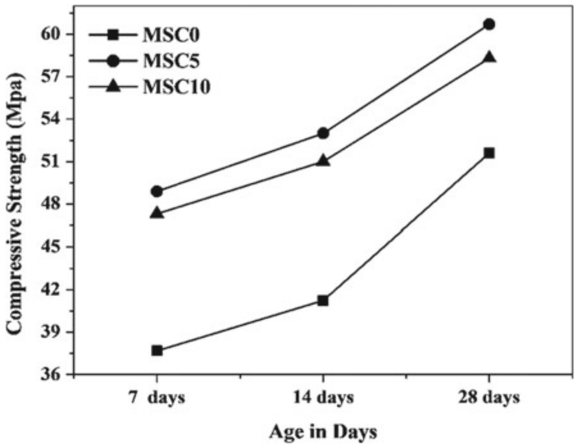


Fig. 1 Comparison of compressive strength of control concrete and bacterial concrete

3 Hardened Concrete Test Results

3.1 Compressive Strength

The compressive strength of specimen is tested after 7, 14, 28 days. Figure 1 shows the comparison of compressive strength between control concrete and bacterial concrete, i.e. concrete added with *Bacillus cohnii.*

It is observed that the compressive strength of concrete mix with *Bacillus cohnii* with cell concentration 10^3 cells/cm^3, increases up to 29.81, 28.54 and 17.61% at 7, 14 and 28 days, respectively, in comparison to concrete without bacteria. While, in concrete mixture having *Bacillus cohnii* cell concentration 10^8 cells/cm^3, the compressive strength increases up to 25.59, 23.69, 12.98% at 7, 14 and 28 days, respectively, in comparison to concrete without bacteria. The measured compressive strength of concrete mixes containing bacteria in different concentrations gives higher value in comparison to control specimen, i.e. concrete without bacteria. The increase in early strength is more in comparison to 28 days strength.

3.2 Split Tensile Strength

A test is conducted to measure split tensile strength of concrete specimen after 7, 14, 28 days. Figure 2 shows the comparison of split tensile strength between control concrete and bacterial concrete, i.e. concrete added with *Bacillus cohnii.*

It is noticed that the split tensile strength of concrete mix having *Bacillus cohnii* cell concentration 10^3 cells/cm^3, increases up to 26.13, 25.13 and 24.39% at 7, 14 and 28 days, respectively, in comparison to concrete without bacteria. While in concrete mix with *Bacillus cohnii* cell concentration 10^8 cells/cm^3, the split tensile

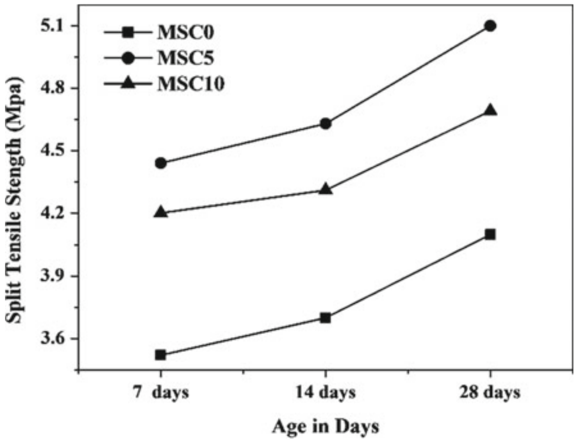


Fig. 2 Comparison of split tensile strength of control concrete and bacterial concrete

strength increases up to 19.31, 16.48, 14.39% at 7, 14 and 28 days, respectively, in comparison to concrete without bacteria. The highest percentage change is observed after 7 days curing period, i.e. 27.42% in case of cell concentration 10^3 cells/cm^3. The measured split tensile strength of almost all concrete mixes with bacteria in different concentrations gives comparatively higher value than control specimen, i.e. concrete without bacteria.

3.3 Flexural Strength

There is a test conducted to measure the flexural strength of specimen after 7, 14, and 28 days. Figure 3 shows the comparison of flexural strength between control

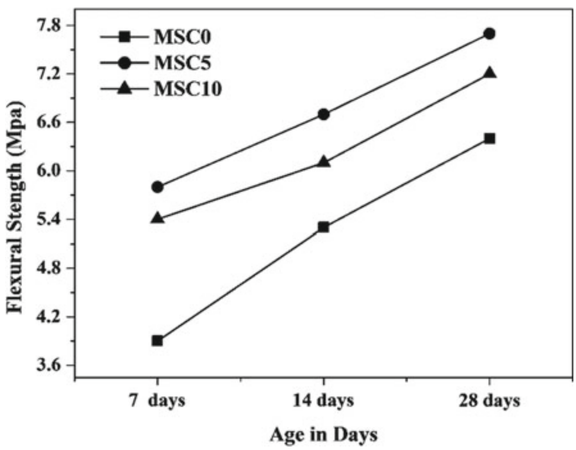


Fig. 3 Comparison of flexural strength of control concrete and bacterial concrete

concrete and bacterial concrete, i.e. concrete added with *Bacillus cohnii.*

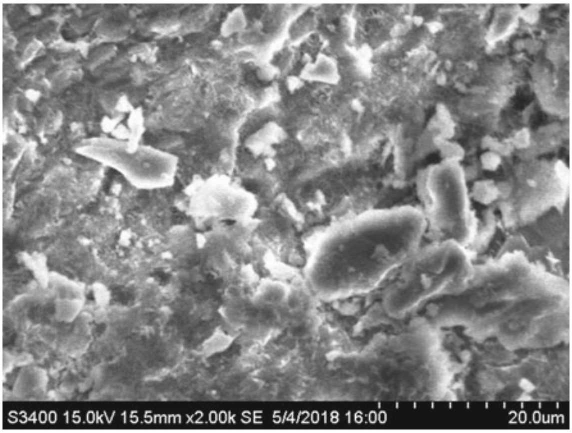
The flexural strength of concrete mix containing *Bacillus cohnii* with cell concentration 10^3 cells/cm^3, increases up to 48.71, 26.41 and 20.31% at an interval of 7, 14 and 28 days, respectively, in comparison to concrete without bacteria. While on the contrary, *Bacillus cohnii* cell concentration 10^8 cells/cm^3, the flexural strength increases up to 38.46, 15.09, 12.5% at 7, 14 and 28 days, respectively, in comparison to concrete without bacteria. The measured flexural strength of concrete mixes containing bacteria in different concentrations gives comparatively better value than control specimen, i.e. concrete without bacteria.

4 Microscopical Study

Figure 4 shows the SEM of control concrete and Fig. 5a, b shows SEM of bacterial concrete.

Rod-shaped bacteria of different sizes are observed in Fig. 5a and precipitation of calcite on the surface of concrete is observed in Fig. 5b. A comparison of the control and bacterial concrete specimens after a span of 28 days of curing has shown that bacterial concrete is more compact and denser due to $CaCO_3$ precipitation by bacteria and has more compressive strength than normal concrete.

Fig. 4 Microscopical observation of control concrete



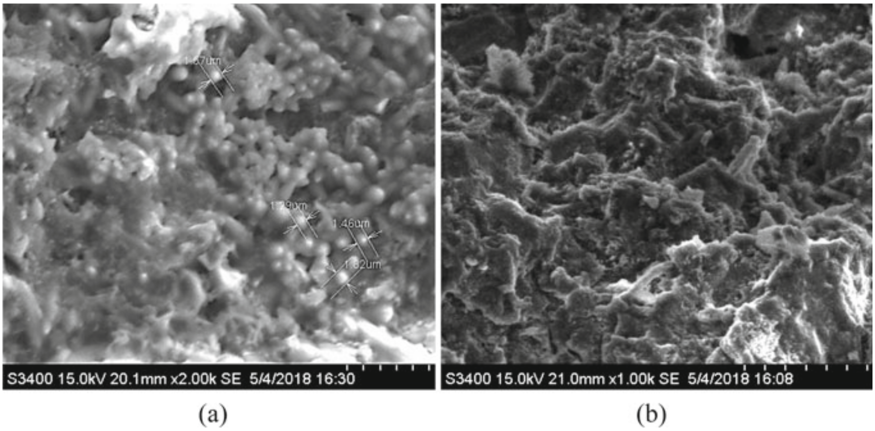


Fig. 5 Microscopical observation of bacterial concrete

5 Conclusions

The above-shown results lead to the following conclusions.

- In contrast to control mix, concrete having *Bacillus cohnii* bacteria shows increment in compressive, flexural strength and split tensile strength in all curing period for both cell concentration 10^3 and 10^8 cells/cm^3.
- At 28 days curing period, concrete with cell concentration 10^3 cells/cm^3 gives highest compressive strength, i.e. 60.7 Mpa and with cell concentration 10^8 cells/cm^3 gives 58.31 Mpa compressive strength which is lowest.
- Concrete with cell concentration 10^3 cells/cm^3 gives highest split strength, i.e. 5.1 Mpa and with cell concentration 10^8 cells/cm^3 gives 4.69 Mpa split tensile strength which is the lowest.
- Concrete with cell concentration 10^3 cells/cm^3 gives highest flexural strength, i.e. 7.7 Mpa and with cell concentration 10^8 cells/cm^3 gives 7.2 Mpa flexural strength which is the lowest.
- The highest strength is achieved when cell concentration of 10^3 cells/cm^3 have been added to concrete for 10^5 cells/ml.
- Strength increases with addition of bacteria up to certain cell concentration but after that level of cell concentration strength of the structure decreases.
- From SEM it is confirmed that *Bacillus cohnii* bacterium successfully precipitates calcite. Due to the deposition of calcite, pores of the concrete are getting plugged, which is the main reason for increase in strength.

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