Behaviour of Incorporation of Bacteria in Concrete

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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 heal the micro cracks inside 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. In this paper, Enterobacter species and Cohnii bacteria were incorporated into concrete. The behaviour of bacterial concrete was investigated in terms of compressive, tensile, flexural strength and ultrasonic pulse velocity. It observed that the compressive strength of concrete was increased by 11.5%, flexural strength increased by 11.9%, tensile strength increased by 12.8% with the replacement of Cohnii bacteria as compare to conventional concrete. 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. Both the bacteria generate the calcite that helps to fill the concrete crack and voids if water come in to contact.

Keywords: Bacterial concrete; e-species bacteria; cohnii bacteria; ultrasonic pulse velocity

1. Introduction

Indeed, concrete is being used, as a construction material, worldwide since ancient time because shelter has always been a fundamental requirement of human being. Although now it has been developed and is being used totally in a different way. Now it has a large consumption in construction of residential building, high rise building, bridges, tunnels, and dam etc. Concrete has long history and it has been advanced with the time with different techniques and innovations. The main reason for these improvements and advancement was to make concrete efficient for construction and to make it durable, light weighted, stronger, and sometimes to enhance the other properties of concrete as per need and requirement [1–3]. Out of these listed and unlisterd factors, strength and durability are two important factors which plays an important role in the business of concrete. With the time, many researchers have focused on the strength improving techniques and elements and on the techniques which can share hand in the durability of concrete. Because strength of concrete makes it cost
effective and possible to withstand the heavy loads [4–6]. Basically, high strength of concrete is easy to attain but it was found out that yielding of strength was always an issue in case of failure which is still required to be worked on. Because there are many more parameters and factors which leads the concrete to failure without achieving the yield strength of concrete. These parameters also affect the durability of concrete and decrease the life span of members and components. One of most known parameters/factors which affects the strength and durability of concrete is crack formation. Crack is a disease in concrete which do not let it survive in adverse conditions and make the concrete worthless by reducing its durability. Though crack formation is like an embedded part of concrete which cannot be stopped completely. Many advance techniques are there to repair the cracks and which try to support concrete life for little extension. With the time, researchers have worked hard and found a solution for this disease with the help of a bacteria. When small crack formation takes place in the concrete this bacterium fills the cracks when it comes in contact with air and water [4–9].

Another study on bacterial concrete found that it is a better alternative than many other traditional technologies because of its eco-friendly nature, self-healing ability, and other features. The compressive strength of bacterial concrete is improved by 19.41% on average when compared to regular concrete, according to this study. In addition, bacterial concrete has a 22.07% improvement in flexural strength when compared to regular concrete. They’ve helped us better grasp the potential and limitations of biotechnological uses in building materials. This study has proved that there is enhancement in the compressive strength, reduction in permeability, water absorption which are very important to be considered [10–12].

Research was performed on the coated pellet system which is useful for self-healing in cementitious material. Three different minerals are taken by author as healing agent. To use these minerals, pan palletisation was used to produce the pellets. Author has suggested the silica fume and bentonite the most suitable healing agent. Later, these pellets are covered with the capsulation. The coating of the capsulation was done with polyvinyl alcohol. Polyvinyl alcohol was examined for water solubility and in alkaline solution and for many other factors. These various pellets have shown positive results in respect of porosity but less crushing strength.

A study on self-healing agents revealed that the presence of the self-healing agent enhanced the consistency of the cement-based material. The microorganisms that are introduced aid in the production of hydration products by providing additional nucleation sites. The degree of hydration of cement particles increases as a result of this. Due to the presence of bacteria, there is a high quantity of calcium carbonate deposition and a thick pore structure, according to research. Because of the carbonic anhydrase produced by bacteria, the carbon dioxide hydration process is accelerated. In addition, self-healing agents might be used to improve nanopore structure by lowering capillary holes and increasing gel pore porosity [13–15].

The study looked at the effect of meteorological conditions on the strength of bio cement mortar. The bio cement mortar sample had a greater strength than the conventional cement mortar sample, according to the research. Other variables, such as a temperature of 40°C and a relative humidity of 95%, contribute to a 110% increase in the strength of the bio cement mortar sample. In addition, a wind speed of 6 m/s and 2 hours of sunshine exposure boosted the strength by 133%. It has been discovered that increasing the amount of bacterial spore solution improves the strength. Increased temperature, relative humidity, and wind speed are all favourable to sample strength, according to the findings. Only exposure to sunshine has a negative impact, as it reduces the sample's strength.

The necessity for this study stems from the fact that only a small amount of research has been done on the effects of the bacillus cohnii bacterial and Enterobacter species on the mechanical characteristics
of concrete M30 Grade and their comparison when employed in direct mixed concrete. The major goal of this research is to evaluate the compressive, tensile, and flexural strength of concrete infused with Bacillus cohnii and Enterobacter species bacteria to ordinary concrete. The concrete quality of bacterial concrete was also assessed using ultrasonic pulse velocity [16–18].

2. Materials

2.1 Bacteria

One of the bacteria was used for this research work was characterized as Enterobacter species as shown in Figure 1. This bacterial strain used in the present study was isolated and purified using Pikovskaya’s agar (Himedia, Mumbai) from the rhizospheric soil of Punjab. The pure colony of bacteria was used for mass cultivation of the strain. 500 ml of the Nutrient broth (Peptone 10 g/L, Beef extract 10 g/L, Sodium chloride 510 g/L, pH 7.0±0.1) was prepared and autoclaved at 120°C for 15 minutes. After the media reached to room temperature, 100°µl of pure inoculum was transferred to it in the sterile conditions. The inoculated media was incubated at 28°C±1 for 24 hrs and used for the study. The bacteria were characterised and identified using various biochemical tests that were performed according to the Bergey’s Manual of systematic bacteriology. Final characterization of bacteria was done by 16S ribosomal DNA (RNA) sequencing method [19–21].

![Bacteria cohnii sample](image1)

**Figure 1** Bacteria cohnii sample

The Bacillus cohnii (MTCC No. 3616) as shown in Figure 2 was purchased from Institute of Microbial Technology (IMTECH), Chandigarh. The freeze-dried bacterial culture from ampoules was revived as soon as it was received [22–24]. For this, the ampoule was disinfected with 70% ethanol. The neck of the ampoule was broken in the laminar air flow. 100 µl of sterilized Nutrient broth was added to the ampoule and mixed thoroughly using a sterile pipette. The culture was used to inoculate the sterile 100 mL nutrient broth. The inoculated media was incubated at 37°C±1 for 48 hrs and used for further experimentation.
2.2 Preparation of samples

To calculate the mechanical properties of concrete samples, the tests were performed i.e., compression test, flexural strength test, tensile strength test and ultrasonic pulse velocity. To perform the all the above tests, the bacterial sample mixed with water as 10ml of bacterial sample into the 1000ml of water. Three types of the samples were cast to compare the effect of the bacteria on the concrete mechanical strength i.e., conventional concrete (Sample-1) and bacterial concrete. To cast the conventional concrete (Sample-1), no bacteria was added and no other treatment was applied. Though when bacterial concrete was cast, bacteria sample was added to the water in ratio 1:100. Bacterial concrete is classified as Enterobacter species concrete (Sample-2) in which specimen Enterobacter species was added into the water during the casting and Cohnii bacteria concrete (Sample-3) in which cohnii bacteria was added into the water during casting. Concrete grade used for cast was M30 grade. Ordinary Portland cement was used in the study with sand and coarse aggregates after adding the bacteria in water, the concrete ingredients were mixed with good care. All the ingredients were taken as per required proportion.

3. Methodology

To test the specimen various concrete specimens are cast as M30 grade concrete, and water ratio was taken as 0.4. Testing was conducted after 7 days, 14 days and 28 days to evaluate the effect of bacteria in concrete.

3.1 Compressive strength test

To evaluate the compressive strength of specimens after 28 days, compressive strength tests were done on cubes of size 150 mm x 150 mm x 150 mm using a compressive testing machine at a loading rate of 13.7 N/mm²/m as per BIS: 516-1999. (1959). At the age of 24±1 h, all of the specimens were demolded and cured in the water tank at room temperature until the test age was reached. BIS: 516-1999 was used to evaluate the compressive strength of concrete specimens after 28 days (1959).

3.2 Flexural strength test

Flexural strength test was performed on beam of size 100 mm × 100 mm × 500 mm as per BIS: 516-1999. The specimen will be supported on the bed of the testing machine by two steel rollers with a
diameter of 38 mm, which have been positioned such that the gap between the rollers is 40 mm. Two comparable rollers positioned at the third places of the supporting span, spaced at 20 cm centre to centre, applied the load. The specimen was positioned in the machine so that the load would be applied to the highest surface as cast in the mould, along two lines 20 cm apart. At a rate of 0.68 N/mm²/mm, the load was applied.

3.3 Split Tensile test

Tensile strength tests were conducted on 150 mm × 300 mm cylinders using a compressive testing equipment at a loading rate of 13.7 N/mm²/m after 28 days according to BIS: 516-1999. At the age of 24±1 h, all of the specimens were demolded and cured in the water tank at room temperature until the test age was reached. A split tensile strength test is performed on concrete to determine its safety when subjected to a tensile force.

3.4 Ultrasonic Pulse Velocity

Each specimen containing Enterobacter species bacteria and Cohnii bacteria had their ultrasonic pulse velocity measured. Before completing the compressive strength test, cube specimens were subjected to an ultrasonic pulse velocity test in accordance with IS 13311 (Part 1): 1992.

4. Result and Discussion

4.1 Compressive strength

The compressive strength value of the conventional concrete (Sample-1), concrete containing Enterobacter species (Sample-2) and Cohnii bacteria (Sample-3) after 7, 14 and 28 days was obtained as listed in Table 1. In the sample-1, bacteria Enterobacter species has been used. Enterobacter species has increased the hardening of the concrete in the early time and hence advanced the 7 days compressive strength. At the other hand, Sample-3, bacillus cohnii bacteria has been used. In the 7 days compression test, bacillus cohnii has not shown much positive effect on the compressive strength but has increased the compressive strength as compare to sample-1 and 2 after 28 days of testing as shown in Figure 3. Bacillus cohnii take more time to react and in the production of the calcite as compared to bacteria Enterobacter species.

| Samples               | Compressive Strength (N/mm²) |
|-----------------------|------------------------------|
| Conventional concrete | 24.2                         |
|                       | 28.1                         |
|                       | 37.6                         |
| Enterobacter concrete | 26.4                         |
|                       | 32.4                         |
|                       | 39.7                         |
| Cohnii bacteria       | 24.3                         |
| Concrete              | 34.2                         |
|                       | 42.5                         |
After 7, 14, and 28 days, flexural strength values of ordinary concrete (Sample-1), concrete containing Enterobacter species (Sample-2) and concrete containing Cohnii bacteria (Sample-3) were obtained as listed in Table 2. After 28 days, the microbial influence on the concrete's flexural strength has improved the flexural strength as shown in Figure 4. Due to the microbiological action of bacteria in concrete, there was a small change in flexural strength for all three samples after 28 days of testing.

**Table 2** Concrete flexural strength

| Samples               | Flexural Strength (N/mm²) |
|-----------------------|---------------------------|
| Conventional concrete | 2.5 2.9 3.7              |
| Enterobacter concrete | 2.7 3.3 3.9              |
| Cohnii bacteria       | 2.5 3.4 4.2              |
4.3 Tensile strength

Tensile strength values of conventional concrete (Sample-1), concrete containing Enterobacter species (Sample-2) and Cohnii bacteria (Sample-3) after 7, 14 and 28 days was obtained as listed in Table 3. The tensile strength of concrete containing Bacillus cohnii (Sample-3) was obtained higher than Sample-1 and 2 after 28 days of testing. Bacteria Enterobacter species has improved the tensile strength after 28 days testing but shows less strength after 7 days testing as shown in Figure 5. Main reason behind this is the production of calcite after bacteria reaction. Bacillus cohnii bacteria start the reaction so its calcite precipitation starts soon after mixing in concrete.

Table 3 Concrete tensile strength

| Samples                  | Tensile Strength (N/mm²) |
|--------------------------|--------------------------|
| Conventional concrete    | 2.6 3.0 4.1             |
| Enterobacter concrete    | 3.1 3.5 4.4             |
| Cohnii bacteria concrete | 3.3 3.8 4.7             |
Bacillus cohnii bacteria has higher rate of calcite precipitation after reaction as compare to bacteria Enterobacter species so it shown early increase in strength of concrete but bacteria Enterobacter species has low rate of calcite precipitation but has more positive effect on the mechanical strength because of more capacity of calcite precipitation. Concrete containing Bacillus cohnii bacteria (Sample-3) has shown more improvement on the mechanical strength as compared to conventional concrete (Sample-1) and concrete containing Enterobacter species (Sample-2). Although both bacteria do not show much negative effect on the concrete when used with direct mixed method.

4.4 Ultrasonic Pulse Velocity

Ultrasonic pulse velocity of conventional concrete (sample-1), concrete containing Enterobacter species (Sample-2) and Cohnii bacteria (Sample-3) after 28 days was determined is listed in Table 4. The results of the tests show that, UPV of concrete mixes increases with adding Enterobacter species (Sample-2) and Cohnii bacteria (Sample-3) as compare to conventional concrete (Sample-1) after 28 days testing as shown in Figure 6. The reasons for increase in UPV are, Both the bacteria generate the calcite that helps to fill the concrete crack if water come in to contact. The UPV values are satisfactory for all concrete mixes and above the value of 3.5 km/s as prescribed by IS 13311 (Part 1): 1992.

| Samples                  | Ultrasonic Pulse Velocity (km/sec) |
|--------------------------|-----------------------------------|
| Conventional concrete    | 3.5                               |
| Enterobacter concrete    | 3.9                               |
| Cohnii bacteria concrete | 4.4                               |
5. Conclusions

The goal of this research was to see how bacteria (Enterobacter species and Cohnii bacteria) affected the compressive strength, flexural strength, tensile strength, and ultrasonic pulse velocity of conventional and bacterial concrete. Based on the test results for both concrete mixtures, the following conclusions may be drawn:

1. Concrete samples were evaluated for compressive strength in both conventional and bacterial concrete. In comparison to ordinary concrete, the compressive strength of Enterobacter species bacterial concrete rose by 5.5%, while the compressive strength of Cohnii bacteria increased by 11.5%. Bacillus cohnii take more time to react and in the production of the calcite as compared to bacteria Enterobacter species and due to which 28 days compressive strength of Bacillus cohnii bacterial concrete was observed high.

2. Concrete samples were evaluated for flexural strength in both conventional and bacterial concrete. In comparison to ordinary concrete, the flexural strength of Enterobacter species bacterial concrete rose by 5.4%, while the flexural strength of Cohnii bacteria increased by 11.9%. After 28 days of testing, there was a minor change in flexural strength for all three samples (conventional concrete, Enterobacter concrete, and Cohnii bacteria concrete) owing to the microbiological impact of bacteria in concrete.

3. The tensile strength of concrete samples was tested for conventional and bacterial concrete. The tensile strength was increased by 6.8% in case of Enterobacter species bacterial concrete and 12.8% in case of Cohnii bacteria as compare to conventional concrete. Bacteria Enterobacter species has improved the tensile strength after 28 days testing but shows less strength after 7 days testing. The main reason behind this is the production of calcite after bacteria reaction. Bacillus cohnii bacteria start the reaction so its calcite precipitation starts soon after mixing in concrete.

4. The results of the tests show that, ultrasonic pulse velocity of concrete mixes increased with adding Enterobacter species (10.3%) and Cohnii bacteria (20.5%) as compare to conventional concrete after 28 days testing. The reasons for increase in UPV are, both the bacteria generate the calcite that helps to fill the concrete crack and voids if water come in to contact.
According to the findings of this study, direct mixing of Enterobacter species bacteria and Cohnii bacteria in concrete enhances the concrete's compressive, flexural, and tensile properties. Furthermore, the ultrasonic pulse velocity of concrete indicated that bacterial concrete fills concrete fractures or cavities.

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