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Potential Application of Bacterial Inoculum to Increase Different Mechanical Strength in the Prepared Concrete

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ABSTRACT: Cracking in the surface layer of concrete mainly reduces its durability, since cracks are responsible for the transport of liquids and gasses that could potentially contain deleterious substances. So the objective of the present investigation is to study the potential application of bacteria, *Bacillus sphaericus* to increase different mechanical strength of the concrete. Conventional and bacterial concrete was prepared and its strength was evaluated using standard Indian Specifications. A significant increase of compressive strength, split-tensile strength and flexural strength was observed for respective B1 (100ml) and B2 (150ml) cell concentrations after 28 days of bio-curing. The Ultra-sonic pulse velocity value of B2 concrete revealed the self-healing properties when compared with the conventional concrete. The present study concludes that inexpensive alternative for laboratory growth media would potentially bring down the cost of the bacteria based self-healing sustainable concrete.

KEYWORDS: Bacterial concrete, Self-healing, Flexural test, Rebound hammer, *Bacillus sphaericus*, Calcium carbonate precipitation.

I. INTRODUCTION

Conventional concrete is though mechanically quite strong, it suffers from significant drawbacks like low tensile strength, permeability to liquid, consequent corrosion of reinforcement, susceptibility to chemical attack and low durability [3]. Cracks in concrete occur due to various mechanisms such as shrinkage, freeze-thaw reactions and mechanical compressive and tensile forces [2]. Recently microbial remediation of concrete has been started to solve these drawbacks. Microorganism exists in the biosphere as geo-chemical agents, induces the formation of special minerals, the process called biomineralization. The concept of biomineralization contributes in the development of an inherent and self-repairing bacterial concrete that can remediate the cracks and fissures in concrete [8]. Microbiologically Enhanced Crack Remediation (MECR) is a novel technique involved in the self-repairing bacterial concrete [6]. *Bacillus pasteruii*, for remediating cracks and fissures in concrete utilizing microbiologically induced calcite ($CaCO_3$) precipitation. As a microbial sealant, $CaCO_3$ exhibited its positive potential in selectively consolidating simulated fractures and surface fissures in granites and in the consolidation of sand [8]. Willem et al. [10] highlighted the presence of bacteria in different media increased the resistance of concrete towards alkali, sulphate, freeze–thaw attack and drying shrinkage. They also reported that the effect of bio-deposition improves the durability of cement mortar/concrete specimens. Park et al. [7] studied the microbiological $CaCO_3$ precipitation ability to improve the compressive strength of concrete. A successful attempt has been made on the biomineralization process to enhance the compressive strength, indirect split tensile strength and durability of cement mortar/concrete by using the *Enterobacter sp.* in different calcium source and curing process [9].

The ultimate aim of the present study is to use a bacterial species in the deposition and/or precipitation of calcite minerals in the cement/concrete matrix so that the newly formed calcites may either remediate the cracks or fill

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up the pores in the concrete. CaCO3 so produced can be useful as a binding agent and also as a pore-filling medium to improve the strength of concrete. It improves the adhesive property within the concrete matrix, thereby increasing the strength of concrete, and also reduces the capillary pores thereby increasing both the durability and strength of concrete. Thus increase in compressive strength, split-tensile strength, flexural strength and self-healing properties was monitored for 28days in the present research.

II. MATERIALS AND METHODS

The cement used for mortar/concrete was 53 grade Ordinary Portland Cement (OPC) conforming to IS: 456-2003. Quarry waste (fineness modulus of crushed sand equal to 3.2) conforming to grading Zone III of IS – 383 – 1970 with the material size of less than 4.75mm was used as fine aggregates. The locally available coarse aggregate with equal proportion of 12.5 and 20 mm size conforming to IS: 383-1970 was used. Potable water has been used for casting concrete specimens. The water is free from oils, acids, and alkalis and has a water-soluble Chloride content of 140 mg/lit. As per IS 456 – 2000, the permissible limit for chloride is 500 mg/lit for reinforced concrete; hence the amount of chloride present is very less than the permissible limit.

Selection and cultivation of calcite producing bacteria

The strain *Bacillus sphaericus*, from Microbial Type Culture Collection centre CSIR–Institute of Microbial Technology (CSIR–IMT), Chandigarh, India. The strain was cultured to check their morphology on nutrient agar (NA), which contained peptic digest of animal tissue 5 g/l, sodium chloride 5 g/l, beef extract 1.5 g/l, yeast extract 1.5 g/l, and agar 15 g/l, and the final pH of the medium was found to be 7.4 ± 0.2 at 25°C. The culturing was done by spreading the stock culture of the bacteria onto the plates and allowing it to be incubated for 24 h at 37°C. The plates with pure culture of *Bacillus sphaericus* was stored at refrigeration temperature and used for further studies.

Potential for spore-formation and calcite production of these strains was tested by cultivation in specific media. Basic medium was composed of 0.2 g NH_4Cl, 0.02 g KH_2PO_4, 0.225 g $CaCl_2$, 0.2 g KCl, 0.2 g $MgCl_2 \cdot 6H_2O$ per liter of Milli-Q ultrapure water. For sporulation (spore-formation) experiments, 50mM $NaHCO_3$, 50mM Na_2CO_3 and 20mMsodium citrate was added to the basic medium. To investigate calcite production potential of these bacteria in liquid media, basic medium was amended with 50mM $NaHCO_3$, 100mM sodium citrate and 25mM $CaCl_2$. The high concentration of sodium citrate in the later medium was needed to inhibit abiotic calcite formation.

Preparation of specimen for compressive and split tensile strength test (Maheswaran et al., 2014)

The cubes and cylinders were prepared for concrete mix with (bacterial concrete) and without (conventional concrete) addition of calcite-producing *Bacillus sphaericus* as per Indian specifications. Thus prepared cubes and cylinders were tested for its compressive and split-tensile strength to differentiate the conventional concrete from the bacterial concrete.

Compressive strength test (Gavimath et al., 2012)

The compression test was used to determine the hardness of cubical and cylindrical specimens of the prepared concrete. The strength of a concrete specimen depends upon cement, aggregate, bond, water-cement ratio, curing temperature, and age and size of specimen. Mix design is the major factor controlling the strength of concrete. Cubes of size 15cm x 15cm x 15cm (IS: 10086-1982) were casted in the present study. All the specimens were provided with sufficient time for hardening and cured for 3, 7, 14 and 28 days. After the specified period (3, 7, 14 and 28 days) all the specimens were tested for its maximum load in the compression testing machine. Compressive strength of the test specimens were calculated by dividing maximum load by the cross- sectional area.

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

Split-tensile strength test **(**Senthilkumar et al., 2014)

Split- tensile strength is indirect way of finding the tensile strength of concrete by subjecting the concrete cylinders to a compressive force. Cylinders of size 150mm diameter and 300mm long were casted. After 24 hours the specimen were demoulded and subjected to water curing. After 3, 7, 14 and 28 days of curing the cylinders were taken allowed to dry and tested in compression testing machine by placing the specimen horizontal. The tensile strength is calculated from the formula as given below (IS: 5816-1970):

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$$\sigma Max = 2P/\Pi DL$$

where, P- is the maximum applied load to the specimen, D- is the diameter of the specimen, L- is the length of the specimen.

Flexural strength test

Flexural Strength tests were carried out on prisms of size 100×100×500 mm on flexure testing machine of capacity 100 kN as per IS 516:1959. The tests were carried out for all the conventional and bio-cured concretes. The strength was analysed for 7^{th} day, 14^{th} day and 28^{th} day.

II. RESULTS AND DISCUSSION

The cubes and cylinders have been tested as per IS specifications. The compressive strength test, split tensile strength test, flexural test, rebound hammer test and UPV test were carried out both on conventional and bacterial concrete specimens. The conventional and bacterial concrete cube specimens after casting were cured for 28 days in the water bath and were tested in compression testing machine.

Compressive strength test

The compressive strength test results revealed that there is an increase in strength for the bacterial concrete when compared to conventional concrete (Table-1). A significant increase of 20.5 N/mm^2 and 21.7 N/mm^2 was observed for respective B1 and B2 cell concentrations after 28 days. During 7^{th} day to 28^{th} day of analysis, it was observed that bacterial concrete showed significant increase in ultimate compressive strength than the conventional concrete. Among the two bacterial concentrations used, the higher concentration of *Bacillus sphaericus* (B2) culture proved to increase the compressive strength of prepared bacterial concrete. The improvement in compressive strength by B1 and B2 could be attributed to bio-mineralization of $CaCO_3$ on the cell surfaces and within the pores of the cement–sand matrix, i.e. pore filling effect within the mortar specimens. Increase in compressive strength after 28days may be due to phosphate buffered saline, which enabled high pH level to provide good nourishment and buffering action to microbial cells within the cement–sand matrix. Due to the high pH in the cement mortar, the microbial cells were able to grow fast by precipitating calcite, subsequently filling the pores; thereafter there could be pore-filling with calcite resulting in subsequent reduction in porosity [4].

Table-1: Compressive strength of conventional and bacterial concrete (N/mm^2)

S. No.	No of days	Compressive strength at first crack (N/mm^2)		
		CC	B1	B2
1	7	12.07	14.3	13.8
2	14	15.27	18.5	18.3
3	28	19.7	20.5	21.7

CC: Conventional concrete, B1: Addition of 100ml of bacterial culture, B2: Addition of 150ml of bacterial culture

Split-Tensile strength test

The bacterial culture treated concrete specimens, B1 and B2 are tested and split-tensile strength is given in Table-2. It can be observed that the split-tensile strength is increasing in the bio cured concrete than the conventional concrete specimens. A significant increase of 4.5N/mm^2 and 4.8N/mm^2 was observed for respective B1 and B2 cell concentrations after 28 days. From 7^{th} day to 28^{th} day like compressive strength, split-tensile strength also increased for bacterial concrete specimens when compared to conventional concrete specimens. Among the two bacterial concentrations used, the higher concentration of *Bacillus sphaericus* (B2) culture proved to increase the tensile strength of prepared bacterial concrete. The significant activity of bacterial culture in B1 and B2 concrete specimens, biochemically induced calcium carbonate precipitation between cement sand matrix, which in turn increased the load resisting capacity.

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Table-2: Split-Tensile strength of conventional and bacterial concrete (N/mm^2)

S. No.	No of days	Ultimate tensile strength (N/mm^2)		
		CC	B1	B2
1	7	3.92	4.2	4.3
2	14	4.23	4.3	4.7
3	28	4.31	4.5	4.8

CC: Conventional concrete, B1: Addition of 100ml of bacterial culture, B2: Addition of 150ml of bacterial culture

Flexural strength test

The flexural strength of the conventional and bacterial concrete prepared in two different concentrations was tabulated in Table-3. It was observed that the flexural strength increased in the bio-cured concrete than the conventional concrete specimens. A significant increase of 4.5N/mm^2 and 4.9N/mm^2 was observed for respective B1 and B2 cell concentrations after 28 days. From 7th day to 28th day like compressive strength and split-tensile strength, flexural strength also increased for bacterial concrete specimens when compared to conventional concrete specimens. Among the two bacterial concentrations used, the higher concentration (150ml) of *Bacillus sphaericus* (B2) culture proved to increase the strength of prepared bacterial concrete. The significant activity of bacterial culture in B1 and B2 concrete specimens, biochemically induced calcium carbonate precipitation between cement sand matrix, which in turn increased the load resisting capacity.

Table-3: Flexural strength of conventional and bacterial concrete (N/mm^2)

S. No.	No of days	Flexural strength (N/mm^2)		
		CC	B1	B2
1	7	3.96	3.5	4.3
2	14	4.33	4.0	4.7
3	28	4.41	4.5	4.9

CC: Conventional concrete, B1: Addition of 100ml of bacterial culture, B2: Addition of 150ml of bacterial culture

Rebound hammer test

The rebound hammer can provide a fairly accurate estimate of concrete compressive strength. When compared to the compressive strength of the concrete, the rebound hammer test results also revealed that there is an increase in strength for the bacterial concrete when compared to conventional concrete (Table-4). A significant increase of 18.6N/mm^2 and 20.1N/mm^2 was observed for respective B1 and B2 cell concentrations after 28 days. During 7th day to 28th day of analysis, it was observed that bacterial concrete showed significant increase in ultimate strength than the conventional concrete. Among the two bacterial concentrations used, the higher concentration (150ml) of *Bacillus sphaericus* (B2) culture proved to increase the strength of prepared bacterial concrete. Bio-mineralization of $CaCO_3$ on the cell surfaces and within the pores of the cement–sand matrix influenced the improvement in the strength of prepared bacterial concrete. This enhanced variation in compressive strength confirms the chemically produced urease in the form of $CaCO_3$ precipitation between cement and sand matrixes of the cement mortar specimen by the *Bacillus sphaericus*. Because of persistence of nutrition in bio curing process, the bacterial concrete specimen showed higher compressive strength than conventional concrete specimens [9].

Table-4: Rebound hammer test of conventional and bacterial concrete (N/mm^2)

S. No.	No of days	Rebound hammer (N/mm^2)		
		CC	B1	B2
1	7	11.8	13.7	13.8
2	14	13.7	16.3	17.3
3	28	18.4	18.6	20.1

CC: Conventional concrete, B1: Addition of 100ml of bacterial culture, B2: Addition of 150ml of bacterial culture

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Ultra-sonic pulse velocity

The NDT methods are candidates to be used for monitoring the self-healing of the bacterial concretes. The self-healing property of the bacterial concrete was recorded after 28days in two different set of concrete specimens (B1 and B2). The second set of specimen showed inevitably good UPV value interpreting the self-healing in the concretes. B2 concentration showed higher UPV value than the B1. Both the conventional concrete and B1 made concrete does not shoed much difference in their UPV value indicating absence of self-healing process. The microorganism used in the present study for manufacturing of microbial concrete should able to possess long-term effective crack sealing mechanism during its lifetime serviceability. The principle behind bacterial crack healing mechanism is that the bacteria should able to transform soluble organic nutrients into insoluble inorganic calcite crystals which seals the cracks. For effective crack healing, both bacteria and nutrients incorporated into concrete should not disturb the integrity of cement sand matrix and also should not negatively affect other important fresh and hardened properties of concrete. Only spore-forming gram positive strain bacteria can survive in high pH environment of concrete sustaining various stresses [1].

In the present study the bacterial culture used was one such organism which could able to thrive in stress conditions like high alkaline pH and high temperature; able to precipitate calcium carbonate. The involvement of microorganism in CaCO3 precipitation can be described in three type of mechanism, spontaneous mechanism, usually by photosynthetic microorganism; .through nitrogen cycle and through sulfur cycle [5]. The evidence of microorganism involvement in calcium carbonate precipitation, has lead the development of bioprocess technology in the field of construction material.

Table-5: Ultra-sonic pulse velocity test of conventional and bacterial concrete (N/mm^2)

S. No.	No of days	Ultra-sonic pulse velocity (N/mm^2)		
		CC	B1	B2
1	7	4.7	4.3	5.05
2	14	4.4	4.7	5.03
3	28	4.3	5.1	5.33

CC: Conventional concrete, B1: Addition of 100ml of bacterial culture, B2: Addition of 150ml of bacterial culture

III. CONCLUSION

In this study, the ability of bacterial cultures that act as a potential agent in increasing the compressive split tensile and flexural strength, and self-healing properties in concrete was investigated. Bacterial cultures used in this study were characterized as spore producers and urease producers. During this study, it was found that the bacterial concrete showed increase in compressive, split tensile and flexural strength than the conventional concrete to a significant level. Bacterial spores immobilized in the concrete matrix will become metabolically active when revived by water and calcium media of concrete. The bacteria hydrolyze urea to produce ammonia and carbon dioxide, resulting in an increase of pH in the surroundings where ions Ca^{2+} and CO_3^{2-} precipitate as $CaCO_3$. Due to these properties of bacterial cultures, we conclude that concrete-immobilized spores of such bacteria may be able to seal cracks by biomineral along with improving the strength and durability of cement concrete. The most expensive ingredient in developing bacterial concrete is nutrients. So any inexpensive alternative for laboratory growth media would potentially bring down the cost of the bacteria based self-healing sustainable concrete. Only factor need to be checked is the effect of nutrients media on the setting time of cement.

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