

Effect of *Bacillus Flexus* in Healing Concrete Structures

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ABSTRACT: Concrete is a widely used construction material in the field of civil engineering but still there are certain issues affecting its durability. Cracking is one of the several disadvantages affecting mechanical strength of the concrete structures. A biological repair technique is investigated in this paper to address the problem of cracking. This technique involves the use of ureolytic bacteria as a novel method of crack healing which is known as Bio cementation or microbiologically induced calcite precipitation (MICP). The process involves the microbial urease production which converts urea into ammonia and calcium carbonate (silica), the latter being responsible for filling the pores and cracks in the concrete leading to a more compact and resistant structure. The use of *Bacillus flexus* in this study showed a considerable increase in compressive (over 40%), flexural (over 30%) and split tensile (over 10%) strength. X-ray diffraction (XRD) results confirmed the impressive ureolytic properties of *B. flexus*.

KEYWORDS: *Bacillus flexus*, bacterial concrete and Bio calcification.

I. INTRODUCTION

Concrete is the most extensively used construction material. Cement is a binder that sets and hardens independently, holding the components together. It is generally graded according to certain sets of specifications [1, 2 and 3] which differentiate the various grades of cements. Concrete has a vast range of applications based on its strength, affordability and versatility. Despite its advantages though, it has several limitations. Some of the disadvantages include its vulnerability under external load, limited ductility and low resistance to cracking. Constant research has been going on for eliminating these disadvantages, leading to the development of special types of concrete, considering various parameters such as the speed of construction, the strength of concrete, the durability of concrete and the environmental friendliness.

This study deals with the application of bacteria *Bacillus flexus* in preventing cracking, as the durability of concrete is generally believed to be related to the characteristics of its pore structure [4, 5] and thus the porous media permeability has to be modified [6]. *B. flexus* are gram-variable, rod shaped aerobic bacteria having a width of 0.9 μm with opaque colonies. Bacterial growth occurs at pH in the range of 4.5-9.5 in 2-10% sodium chloride. This microorganism produces a fat-degrading compound that works under very alkaline conditions. A strain of this microbe, isolated from a Saudi lake, has been shown to degrade some important freshwater toxins [7]. Metabolic activities of favorable microorganisms like *Bacillus flexus* lead to the mineral precipitation in concrete and improve its overall behavior [8, 9, 10 and 11]. They precipitate inorganic crystals hence the healing of the cracks takes place in the concrete. They can withstand harsh temperature conditions and are cost effective.

Extensive research in the field of bio cementation has led to a new material called bacterial concrete. Microorganisms like *Sporosarcina pasteurii*, *Pseudomonas aeruginosa* [12, 13 and 14] and *Bacillus* sp. CT-5 [15] showed a substantial increase in the compressive strength of cement mortar. *Bacillus subtilis* JC3 [16] was reported to have shown 12.60% increase in

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concrete's split tensile strength. Even alternative methods were investigated for low cost bacteria to be used in crack healing [17]. Bacteria were reported to prevent leaching in channels [18]. Bacteria were also reported to be used as an admixture with waste by-products in concrete [19].

Microbiologically induced calcite precipitation (MICP)

Considerable research on bacterial calcite precipitation has been investigated [20].

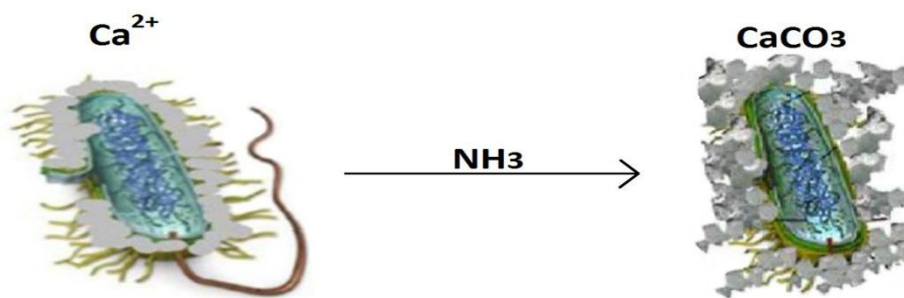
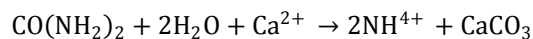


Figure 1- calcium carbonate precipitation

These bacteria induce precipitation of calcium carbonate by the production of a urease enzyme. This enzyme uses urea as the sole nitrogen source, catalysing the hydrolysis of urea into ammonium which increases the pH. This results in calcium carbonate precipitation as shown in Fig. (1).



This study was aimed at studying the ureolytic properties of *Bacillus flexus*.

II. MATERIALS AND METHODS

Bacillus flexus was procured from Microbial Type Culture Collection (MTCC Chandigarh, India). The laboratory tests were conducted on 43 grades Ordinary Portland Cement (OPC). River sand was used as fine aggregate. Quarried and crushed granite stones of maximum size 20mm were used as coarse aggregate. The adopted concrete was M30. The physical tests of the ingredients and the strength related tests, such as Compression, Split Tensile and Flexural tests on cubes, cylinders and beams on the hardened concrete were conducted according to IS specifications [21 and 22]. The composition of the hardened concrete was determined using X-Ray Diffractometer. Compressive strength tests were carried out on cubical specimens of size 150 mm X 150 mm X 150 mm. The wet concrete was poured into the moulds and for every layer (out of three layers) the mix was compacted with a vibrator. The test was done immediately upon removal of the specimen from the curing tank in damp condition. The surface water was wiped off and the specimen was placed into the machine. The loading was applied gradually and uniformly. Three cubes of 3rd day, 7th day and 28th day were tested for each mix. Split tensile tests were carried out on cylindrical specimens of 150 mm diameter and 300 mm length. The tests were carried out by mounting the test specimens horizontally on the testing platform of the compression testing machine (CTM) between two packing strips. Uniform load was applied along the vertical diameter until breakpoint. The determined strength in the splitting test is believed to be closer to the true tensile strength of concrete than the modulus of rupture. One cylinder of 28th day was tested for each mix. Flexural strength tests were carried out with prisms 100 mm length, 100 mm width and 500 mm height. The bed of the testing machine had two steel rollers, 38mm in diameter, which were mounted so that the distance between the centres would be 40cm for a 10cm specimen. The load was applied through two similar rollers mounted at the third points of the supporting span that was spaced at 13.30 cm between the centres. The load was divided equally between the two loading rollers and all rollers were mounted such that the load

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would be applied axially, without subjecting the specimen to any torsional stresses or restraints. One prism of 28th day was tested for each mix.

XRD test was carried out by obtaining a 1cm sample size from the specimen's macro sample which was less than 8 grams without moisture. It was, then, placed in the XRD test setup and it was subjected to X-ray. The graph was obtained for an angle of 2θ wavelength. The sample took exactly 40 minutes to be analyzed.

The procedure was the following:

1. Mix design for normal M30 grade concrete was tabulated using IS [22]. The bacterial solutions were prepared.
2. Normal concrete specimens were casted of OPC 43 Grade Cement. Then, the compressive strength, tensile strength and flexural strength tests were performed as per IS [21, 22] in order to compare the results with the *Bacillus flexus* mixes.
3. *Bacillus flexus* was grown in nutrient broth 30 °C.
4. Using the same mix design, the compressive, tensile and flexural strength was found for bacterial concrete with OPC as per IS [22, 21 and 24].
5. The test specimen's properties were analyzed. All the tests were carried out in the college laboratory as per relevant codes and equipment availability.

Table 1: Mix proportioning data

Grade Designation:	M30
Type of Cement:	OPC
Maximum coarse aggregate size:	20mm
Maximum water cement ratio:	0.45
Workability:	100mm Slump
Exposure Condition:	Severe
Maximum Cement Content:	438 Kg/m ³
Chemical Admixture Type:	None
Specific Gravity of Cement:	3.15
Specific Gravity of CA:	2.65
Specific Gravity of FA:	2.56

The data for mix proportioning was accordingly (Table 1) defined. Normal M30 grade OPC cement was prepared with coarse aggregate size of 20 mm. Based on the standard data obtained from [IS 516, 10262] 100 mm slump was generated with a maximum cement content of 438 kg/m³. The cement mix was exposed to severe conditions during its preparation.

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Figure 2- Confirmation of bacterial growth



Figure 3- Bacterial growth in rotary shaker

12.5 g of nutrient broth were added to a 500 mL conical flask containing 500 mL of distilled water; It was then covered with a thick cotton plug and was made air tight with paper and rubber band and sterilized in the autoclave at 121 °C, 15 psi for 15 minutes. Then a small amount of the lyophilized MTCC culture was inoculated in the sterilized broth and incubated overnight at 30 °C in a rotary shaker. The appearance of turbidity overnight confirmed bacterial growth as shown in Fig. (2 and 3). The bacterium was sub cultured in nutrient broth every 30 days. Concentration of bacterium obtained was at the end 10^5 cells/mL of culture obtained.

III. RESULTS AND DISCUSSION

XRD testing

XRD technique is used to determine the mineralogical analysis of crystalline solids. If a crystalline mineral is exposed to X-Rays of a particular wavelength, the layers of atoms diffract the rays and produce a pattern of peaks which is characteristic for every mineral. The horizontal scale (diffraction angle) of a typical XRD pattern gives the crystal lattice spacing and the vertical scale (peak height) gives the intensity of the diffracted ray (Fig. 4). When the specimen is

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subjected to X-rays and contains more than one mineral, the intensity of characteristic peaks from the individual minerals is proportional to their amount.

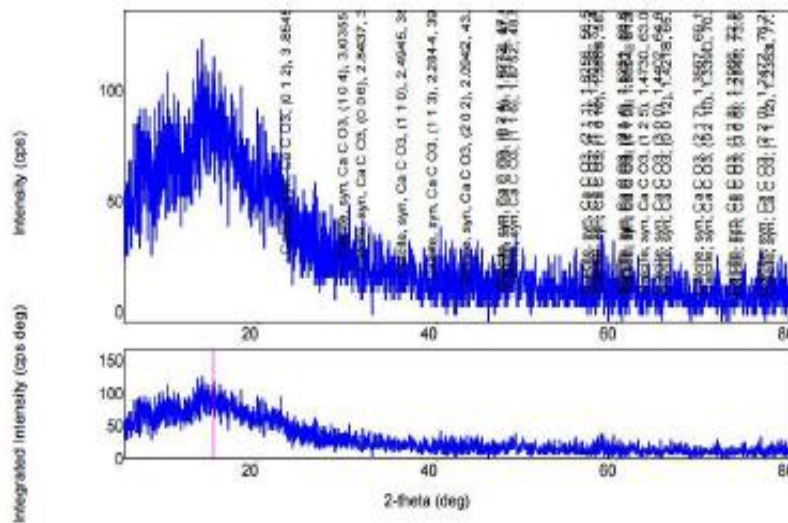


Figure 4- XRD Test results

The figure confirms calcite production which is subjected to XRD analysis. Calcite also enhances the quality of concrete by improving its strength. The growing peaks indicate bacterial presence which, improve the mechanical strength of concrete as well as heal the cracked structures.

Table 2: XRD concluding points

Name:	Calcite, syn.
Chemical Formula:	CaCO ₃
Formula:	CacO ₃
Z Value:	6.
Space Group:	R-3c (167).
Cell:	4.9890 4.9890. 17.0620 90.000 90.000 120.000
Volume:	367.780.
Crystal System:	Triagonal.

The sample appears to have an amorphous structure as shown in (Table 2). Urease activity is confirmed by the comparing the test data to the standard peak intensity range available for calcium carbonate formation. The crystallization patterns at specific points clearly explain the process.

Compressive strength testing

Compressive strength test was conducted in accordance with IS [22]. This test is not only important from structural point of view, but also for examining other properties of concrete such as fatigue, impact, shrinkage, creep, deformation and thermal sensitivity.

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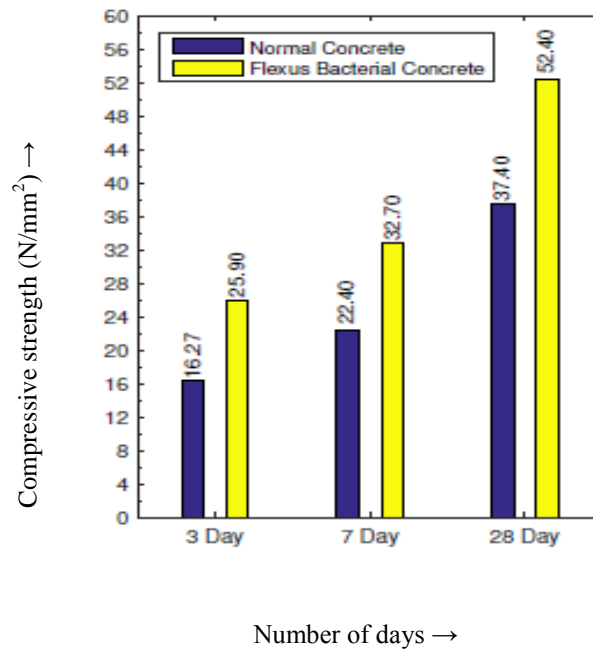


Figure 5: Compressive strength test results

There is a remarkable increase (Fig. 5) in bacterial concrete's compressive strength as compared to the ordinary concrete. The urease activity of *B. flexus* increased significantly when the concrete cubes were maintained for additional time. The bacterium produced better results for improving the compressive strength as compared to laboratory experiments [23].

Flexural strength testing

Flexural strength test was conducted in accordance with IS [21].

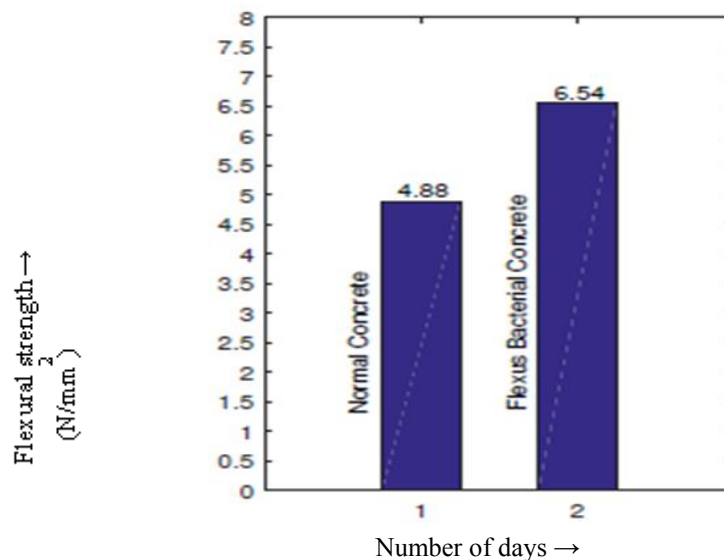


Figure 6- Flexural strength of cubes

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There was a significant increase in bacterial concrete's flexural strength as compared to the ordinary concrete as shown in (Fig. 6). This was due to bacterial urease activity. Flexural strength results proved that bacterial cement mix significantly improves the flexural, elastic characteristics of the concrete structure.

Split tensile testing

Split tensile test is the easiest test that can be conducted to determine the tensile strength of concrete. The test was conducted according to IS [21].

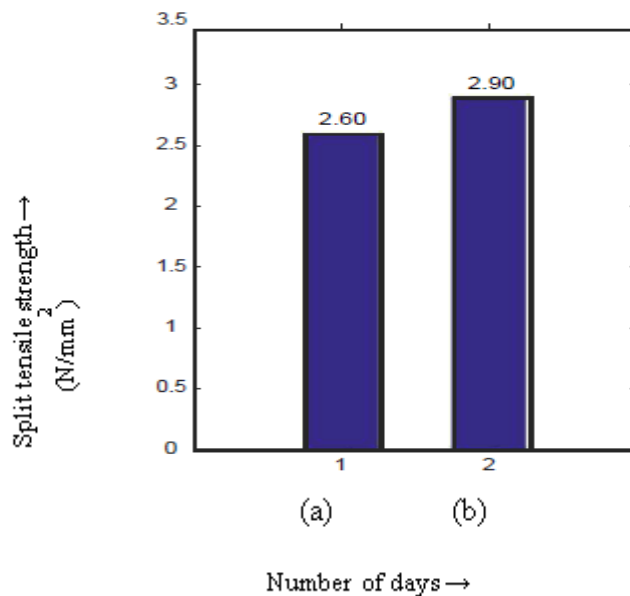


Figure 7- Split tensile strength of (a-normal concrete) and (b- *B. flexus* concrete)

There was a slight increase in bacterial concretes split tensile strength as compared to the ordinary concrete as shown in (Fig. 7). These tensile characteristics proved the efficiency of bacterial concrete in wound healing and improvement in mechanical properties of the concrete structure.

IV. CONCLUSIONS

Concrete obtained from *Bacillus flexus* produced significant results as compared to normal concrete. It showed an increase of over 40% in compressive, over 30% in flexural, and over 10% increase in the split tensile strength after 28 days of curing. Hence, the advantages of using *Bacillus flexus* bacteria as an admixture in bacterial concrete are:

1. Significantly higher strength compared to concrete made with OPC.
2. Steel reinforcement corrosion protection by crack bridging.
3. Better resistance to mild acidic water and aggressive chemicals.
4. Higher workability and reduced water-cement ratio improves concrete density.

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