



Effect of Calcite precipitation by microbes on strength of Concrete

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Abstract: During and after the treatment of concrete cracks, the phenomenon of microbially generated calcite precipitation occurs. *B. subtilis*, *B. pasteurii*, *B. megaterium*, and few *Bacillus* species have been found to help with the self-healing of concrete fractures caused by shrinkage enhancement and settling processes. The purpose of this research was to explore if particular bacteria may assist improve the compressive and tensile strength of concrete. The *Bacillus subtilis* microbial culture was dissolved in water and added in concrete mix. After casting, the blocks are cured for period of 7 and 28 days before being evaluated for compression and tensile strength. Compressive and tensile strength of *B. subtilis* species mix concrete found to be increase by 15% in 28 days compared to conventional concrete with M25 concrete. According to the findings, the microbe increased the compressive and split tensile strength of concrete. As a result, the bacterial treatment may be able to control micro cracks while simultaneously increasing concrete strength.

Keywords: Micro crack management, MICP, Calcium carbonate precipitate, ureolytic bacteria

I. INTRODUCTION

MICP is an old natural phenomenon that has altered the earth. Calcium carbonate precipitates are generated from calcium ions and carbonate ions in this process. MICP has been investigated as a solution for stone crack healing because CaCO_3 mineral is homogeneous substance that is compatible with concrete and is environmentally benign [1]. For healing fractured surfaces of ornamental stones, a method based on *Bacillus cereus*-induced CaCO_3 production has been commercialised [1,2]. MICP has also been proven to increase mechanical characteristics [3–5] and concrete self-healing [6] by adding live bacteria into concrete. Despite the benefits of utilising living bacteria to promote CaCO_3 precipitate in concrete, there have been a number of downsides. Various research on the effects of bacteria on concrete mechanical strength are conflicting, and organic nutrients added to concrete have sometimes had deleterious impacts on mechanical strength [3,7–9]. Furthermore, germs were not viable in concrete after 7 days of curing because the pores in the concrete contracted to a size where bacteria could not survive [9]. MICP is known to be dependent on bacterial cell walls, which make up the cell surface [1]. Bacterial cell walls, which are negatively charged in neutral or alkaline pH environments, attract Ca ion from extracellular environment, which react to CO_3 ions on the cell surface to form calcium carbonate minerals, which serve as nucleation centres for more mineralization [11]. No

research has been done on the impacts of directly putting cell walls to concrete. When *Bacillus subtilis* cell walls were applied to a broken stone surface, the creation of CaCO_3 crystals increased, lowering the stone's water permeability dramatically [2]. Using cell walls directly in concrete could avoid complexities and viability issues that come with employing live bacteria. Furthermore, because living bacterial cells actively pump H^+ ions across the cell membrane competes with Ca^{2+} ions for bonding with negative cell walls, cell walls have been demonstrated to be more successful than live bacteria in binding Ca^{2+} ions and generating CaCO_3 crystals [13,14]. Resulting *B. subtilis* with a faulty *etfA* gene that produced an excessive amount of H^+ ions on the cell surface were unable to create CaCO_3 crystals on the cell surface [13]. The impact on mechanical strength of concrete by bacterial cell are discussed, as well as the quantification of CaCO_3 production in cement mortar. Our findings show implying that bacterial cell walls could be a new biological additive for increasing concrete performance.

II. MATERIAL

A. Bacteria

B. subtilis is a Gram-positive rod-shaped bacteria that produces dormant spores that are heat resistant. It's not a disease-causing agent. It is a well-known manufacturer of business items. *Bacillus subtilis* is widely found in human gut commensals as well as the upper layer of soil. *Bacillus*



subtilis was submitted for testing by the MTCC Microbial Type Culture Collection and Gene Bank in Chandigarh.

B. Cement

Ordinary Portland Cement grade 43 is used for compressive and tensile strength as per IS: 4031-1988 (OPC 43) specifications, and it is determined to conform to IS:12269-1987 specifications with a specific gravity of 3.15.

C. Fine aggregate

Locally, fine aggregate that has been sieved through 4.75 microns is available. IS: 2720 is recommended for Specific Gravity. The matrix in concrete is filled with fine aggregate, resulting in a compacted structure joined by cement. 2.62 specific gravity was discovered.

D. Coarse Aggregate

Downsizing to 20 mm is employed, and local aggregate is utilized. IS: 2386-1963 is used to compute the Specific Gravity. The coarse aggregate in concrete not only gives strength and resistance to abrasion, but it also accounts for the majority of the volume. 2.65 specific gravity is attained.

E. Water

Water quantity is calculated according to the mix design. For every 800 ml of water, 20 ml of bacterial solution is added.

III. METHODOLOGY

A. Media formation

The media is made with 50 mL of water in two flasks. Then, by weight, we fill both flasks with the proper medium composition. The required medium composition is as follows: (a) Nutrient Agar growth medium. b) Beef Extract 1.0gm c) Yeast Extract 2.0gm d) Peptone 5.0gm e) NaCl 5.0gm f) Agar 15.0gm g) Distilled water 1.0 ltr Sterilized water added to both flasks after mixing the medium in the flasks to generate 200ml nutrient medium in one and 100ml nutrient medium in the other after mixing the medium in the flask. Instead, divide 200ml nutritional media into four sections, each of 50ml, and use 100ml nutrient medium to form plates with agar. The solution is then placed in an autoclave machine to be autoclaved (a process that kills any fungi or bacteria that may be present in the flask or elsewhere).

B. Bacterial Culture

To activate it, sterile water was mixed with freeze-dried microorganisms. Following the activation of the bacteria, 100ml of nutrient broth is added to the nutrient broth, and another 100ml is poured to each of the two 50ml nutrient agar plates. The active bacteria are introduced to the nutrient broth and nutrient agar plate, and they are incubated at 300°C for 24 hours in a 600rpm shaking incubator. After incubation, bacteria that have been incubated grow irregularly in colonies in dry white on nutrient agar. When one colony culture is needed, it must be injected into a 500ml conical flask containing 200ml nutrient broth, and the process must be repeated for bacteria culture. After 2 to 3 days of growth, slant culture must be cured in the refrigerator at 40 C before being used again. Sub culturing must be performed every 30 days. Other bacteria contamination was assessed by streaking over nutrient agar plates numerous times and determining the bacterial concentration of solution using an Optical Density test. The 10^9 cells per millilitre concentration is maintained. A spectrophotometer was used to determine the concentration. A concentration of 0.9×10^9 cells/ml is indicated by an optical density of 1.

C. Compression and split tensile test

Compression test cubes of M25 grade concrete and split tensile test cylinders of M25 grade concrete were cast according to IS 10262-2009 and injected with B.subtilis bacteria. Cubes measuring 15cm x 15cm x 15cm and cylinders measuring 15cm in diameter and 30cm in height were created. After 24 hours of casting, the cubes were taken from the mould and allowed to cure for 28 days. The specimen was cast and tested in accordance with IS 516-1959.

D. SEM analysis

The existence of calcium carbonate precipitation in the sample is investigated using SEM. A sample was taken from various separate layers of a cube that had been curing for 28 days and had undergone compression testing. This SEM experiment on gold-plated materials used a 15.0kV electron accelerating voltage and a magnification of 10m.

IV. RESULTS

A. Compressive strength Result

When the compressive strength of the bacteria-treated specimen was compared to conventional concrete with the same mix design parameters in Compression Testing Machine, bacteria-treated specimen showed much superior



strength, shown in Table 1. After 7 and 28th days after curing, conventional concrete showed compressive strength of 17.25 MPa and 27.46 MPa, respectively, whereas B. subtilis mix concrete had compressive strengths of 19.50 MPa and 32.40 MPa, representing a 13.04 and 17.98 percent increase in strength over conventional concrete, respectively.

TABLE I
 COMPRESSIVE STRENGTH OF MIX WITH AND WITHOUT B. SUBTILIS AT 7 AND 28 DAYS

Concrete mix	Compressive Strength (MPa)	
	7days	28 days
Conventional M25 grade Concrete	17.25	27.46
B.subtilis mixed M25 grade concrete	19.50	32.40

B. Split Tensile Strength

Tensile strength was determined indirectly using a Compression Testing Machine. Table shows conventional concrete developed tensile strength of 3.86 MPa, whereas B. subtilis developed tensile strength of 4.74 MPa, at 28 days suggesting a 23 percent improvement in tensile strength over conventional concrete.

TABLE I
 SPLIT TENSILE RESULT OF CONCRETE MIX WITH AND WITHOUT B.SUBTILIS FOR 28 DAYS

Concrete mix	Split tensile strength 28 days(MPa)
Conventional M25 Concrete	3.86
B.subtilis mixed M25 concrete	4.74

C. Scanning Electron Microscope (SEM) Test

The calcite ions presence is indicated by visible rhombohedra crystals, as shown in Fig.1, demonstrating the study's success based on previous investigations and discoveries.

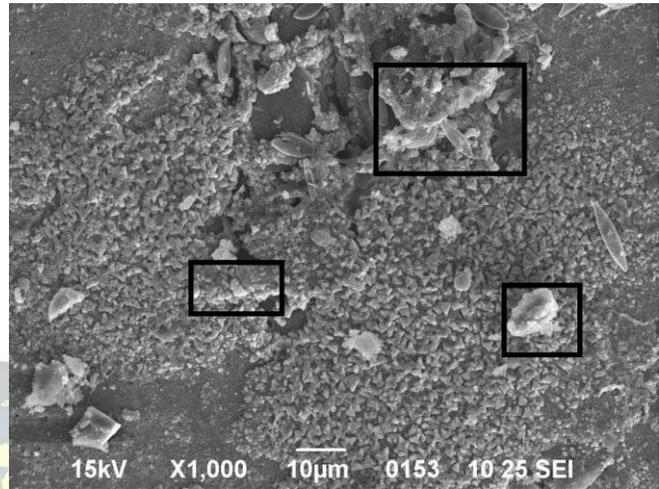


Fig. 1. Example of an image with acceptable resolution

V. CONCLUSION

According to the conclusions of this investigation, after curing plate count test supports the bacterial survivability in concrete and their capacity to withstand the harsh environment. B. subtilis exhibits a 17.98% increase in compressive strength and 23% tensile strength increment. comparing to normal specimen. In SEM images, the presence of calcite precipitation is clearly visible. The microorganism was found to perform a beneficial role in enhancing the strength of concrete by filling voids in the concrete specimen with calcite precipitation, according to the findings. As a result, micro fissures may be able to self-heal, albeit further research is needed in the marine and acidic environments.

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