

Evaluation of Biocalcification and Strength Aspects in Bacterial Concrete with *Bacillus Subtilis*

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Abstract - Everyone wants to break the monotony of life in some way. Yes, Concrete's crack formation due to brittling should be changed, thus our project "self healing of bacterial concrete using *BACILLUS SUBTILIS*", just as our skin heals the wound. This Project deals with the novel technique of self healing of cracks in concrete by the microbial induced calcite precipitation in concrete by the bacteria *BACILLUS SUBTILIS*. In our Project , the non pathogenic spore forming soil bacterium

"*BACILLUS SUBTILIS*" is added in the making of concrete along with water in the concentration of 10^5 per ml. During the crack formation, this bacteria gets activated due to its exposure to air and water. The bacteria taking its food calcium lactate and reacting with cement particles emits CaCO_3 by the process of BIOCALCIFICATION. Our Project deals with the experimental finding of its compressive, tensile, corrosion and healing properties.

Key Words: SELF HEALING CONCRETE, Microbial Induced Calcite Precipitation (MICP), *BACILLUS SUBTILIS*, Biocalcification, , Corrosion.

I. INTRODUCTION

Concrete is the most commonly used building material, but the cracks in concrete create problem. Cracks in concrete occur due to the various mechanisms such as shrinkage, freeze-thaw reaction and mechanical compressive and low tensile strength^[4]. High tensile stresses can result from external loads. Imposed deformations (due to temperature gradients, confined shrinkage, differential settlement), plastic shrinkage, plastic settlements, expansive reactions(e.g. due to reinforcement corrosion, alkali silica reaction, sulphate attack). Without immediate and proper treatment, cracks tend to expand further and eventually require costly repair^[5]. Durability of concrete is also impaired by these cracks, since it provide an easy path for transportation of liquids and gases that potentially harmful substances. If microcracks grow and reach the reinforcement, not only the concrete itself may be attacked (direct degradation) but also the reinforcement may corrode when it is exposed to water and oxygen and possibly carbon dioxide and chlorides (indirect degradation). Microcracks are therefore precursors to structural failure. Therefore a novel technique has been developed by using a selective microbial metabolic activities promote calcium carbonate (calcite) precipitation.,

this technique is referred as Microbiologically Enhanced Cracks Remediation (MECR).In this technique, Urolytic bacteria are used hence the concrete is called Bacterial concrete " can be prepared by adding spore forming bacteria in the concrete that are able to continuously precipitate calcite, this process of production of calcite precipitation is called Microbiologically Induced Calcite Precipitation (MICP).Therefore bacterial induced calcium carbonate deposition has been proposed as an alternative and environmental friendly crack repair technique.

II. OBJECTIVE

- To induce the self healing property of concrete.
- To know the corrosive effect of this concrete
- To find the percentage of increase in compressive strength and tensile strength in bacterial concrete
- To reduce the management cost.

III. REVIEW OF LITERATURE

Srinivasa reddy V et al, suggested that,concrete by its nature is very prone to deformations that expose its reinforcements, corroding them. Self healing concrete offers a approach on which studies were carried out to investigate the crack healing mechanism in enhancing the strength and durability of concrete.^[1]

The crack healing capacity of a specific biochemical additive, consisting of a mixture of viable but dormant bacteria and organic compounds packed in porous expanded clay particles was investigated by H.M.JONES^[2]. And he continued that further development of this new type of self healing concrete will result in more durable and more ever sustainable concrete which will be particularly suitable for applications in wet environments where reinforcement corrosion tends to impede durability of traditional concrete construction.^[2]

N DE BELIE & W DE MUYNK, experimenatally said that the crack healing potential of a bio depositon treatment, standardized cracks of 0.3mm were produced in concrete specimens by introducing thin copper plates in fresh

concrete and removing them after 1 day or by performing splitting tensile test on concrete coarse wrapped in fibre reinforced polymer sheets. The bacteria which were protected in a silica sol resulting in the formation of a bio ceramic material (sol-gel or biocer) which was able to bridge the cracks completely.

IV. BACTERIA TO BE USED

It is important to cover what kinds of bacteria will live in the concrete, how they work to improve the longevity of public infrastructure, what the catalyst will be that causes the chemical reaction in the bacteria, what happens to the specific kinds of specialized bacteria when exposed to the catalyst, and how they work together to not only heal cracks before they form, but also strengthen the overall structure they are incorporated into. When the bacteria are exposed to the air and the "food", the bacteria go through a chemical process that causes them to harden and fuse, filling in the crack that has formed, strengthening the structure of the concrete, and adhering to the sides of the crack to seal the damage site. This process extends the lifespan of the structure while also fixing the damage caused. The process of healing a crack can take as little as a few days^[4].

Bacteria used in our project is *BACILLUS SUBTILLIS*

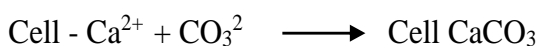
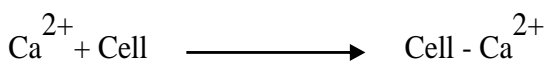
V. BACILLUS SUBTILIS

Bacillus subtilis, known also as the hay bacillus or grass bacillus, is a Gram-positive, catalase-positive bacterium. ^[3] A member of the genus *Bacillus*, *B. subtilis* is rod-shaped, and has the ability to form a tough, protective endospore, allowing the organism to tolerate extreme environmental conditions. Unlike several other well-known species, *B. subtilis* has historically been classified as an obligate aerobe, though recent research has demonstrated that this is not strictly correct.

Although this species is commonly found in soil, more evidence suggests that *B. subtilis* is a normal gut commensal in humans. A 2009 study compared the density of spores found in soil (~10⁶ spores per gram) to that found in human feces (~10⁴ spores per gram). The number of spores found in the human gut is too high to be attributed solely to consumption through food contamination. Soil simply serves as a reservoir, suggesting that *B. subtilis* inhabits the gut and should be considered as a normal gut commensal.

VI. CHEMICAL PROCESS IN THE BIOLOGICAL HEALING OF BACTERIA

Microorganisms (cell surface charge is negative) draw cations including Ca²⁺ from the environment to deposit on the cell surface. The following equations summarize the role of bacterial cell as a nucleation site.



The bacteria can thus act as a nucleation site which facilitates in the precipitation of calcite which can eventually plug the pores and cracks in the concrete. This microbiologically induced calcium carbonate precipitation (MICCP) comprises of a series of complex biochemical reactions. As part of metabolism, *B. Subtilis* produces urease, which catalyzes urea to produce CO₂ and ammonia, resulting in an increase of pH in the surroundings where ions Ca²⁺ and CO₃²⁻ precipitate as CaCO₃. These create calcium carbonate crystals that further expand and grow as the bacteria devour the calcium lactate food. The crystals expand until the entire gap is filled.

VII. MATERIALS AND METHODOLOGY

The following are the details of the materials used in the investigation:

Cement

Ordinary Portland cement of 53 grade of ultra tech cement in local market is used in the investigation. The cement used has been tested for various properties having specific gravity of 3.0.

Fine Aggregate

Locally available clean, well-graded, natural river sand having fineness modulus of 2.69 was used as fine aggregate.

Coarse Aggregate

Crushed granite angular aggregate of size less than 20 mm nominal size from local source with specific gravity of 2.7 was used as coarse aggregate.

Water

Locally available potable water is used.

Microorganisms

Bacillus subtilis, a soil bacterium which is cultured in our microbiological laboratory is used.

Mix Design

The mix proportions for ordinary grade concrete is designed using IS:10262-1982. The mix proportion is 1:1.42:3.09 with 0.50 water cement ratio.

VIII. METHODOLOGY

STEP 1: Culture and growth of bacteria .

(*Bacillus subtilis*)

STEP 2: Preparation of specimens .

(cubes and cylinders)

STEP 3: Evaluation of various tests for strength

and other property assessments.

**IX. ISOLATION AND CULTURE OF BACTERIA
 BACILLUS SUBTILIS**

First the bacteria *BACILLUS SUBTILIS* which is isolated from the soil is brought from the INSTITUTE OF MICROBIAL TECHNOLOGY, CHANDIGARH. The strain is at first made for broth subculture for mass cultivation of bacteria. First the medium of culture is prepared. The culture is prepared with 2 gram each of peptone, glucose, beef extract and 0.8 gram of sodium chloride in 200ml of distilled water in a 500ml conical flask and sterilized in the autoclave.

After the sterilization is done a single loop of bacteria is taken from the original culture and is introduced into the medium in the laminar air flow chamber. The culture medium with the bacteria is kept inside the incubator at 34°C for 24 hrs. Then the same type of medium is prepared in 34 conical flasks of 250 ml and sterilized in the autoclave. After sterilization a single loop of bacteria from the subculture is introduced into the flask containing the culture medium. this is kept inside the shaker for three days at 34°C with 100 rotations per minute for mass cultivation. Thus, the bacteria to be used in the bacterial concrete is prepared in the microbiological laboratory. The bacteria of optimum cell concentration of 10⁵ cells per ml of mixing water was used in the investigation.

X. PREPARATION OF SPECIMENS (CUBES AND CYLINDERS)

The cubes and cylinders were prepared for concrete mix with and without addition of microorganisms. The size of the cubes and cylinders were taken as 150mm x 150mm and 300mm height and 150mm diameter respectively. Cubes and cylinders were prepared in a standard manner according to INDIAN SPECIFICATIONS. The cubes and cylinders were demoulded after 24 hours and subsequently cured in a water bath for 28 days.

XI. RESULTS COMPRESSIVE STRENGTH

Table 1. Results of the compressive test with and without addition of microorganisms.

SL.NO	SPECIMEN TYPE	COMPRESSIVE STRENGTH	PERCENTAGE INCREASED
1	WITHOUT BACTERIA	23.94	+ 62%
2	WITH BACTERIA	38.8	

Table 2. Results of the compressive tests with and without addition of *B. Subtilis*

NO.OF DAYS	Compressive Strength Of Conventional Conc. Cubes N/mm ²	Compressive Strength Of Bacterial Conc. Cubes N/mm ²	% increase in strength
7	19.24	24	24.7
14	23.66	34	43.7
28	34.52	47	36.15

SPLIT TENSILE STRENGTH

Table 3. Results of the split tensile test with and without addition of *B. subtilis*

NO.OF DAYS	Split Tensile Strength Of Conventional Conc. Cylinders N/mm ²	Split Tensile Strength Of Bacterial Conc. Cylinders N/mm ²	% increase in strength
7	3.5	4.00	14.29
14	4.5	5.30	17.78
28	4.9	5.80	18.37

**SURFACE HARDNESS BY REBOUND
 HAMMER TESTS FOR BACTERIAL SPECIMEN**

NO.OF DAYS	GRADE OF CONCRETE	REBOUND NUMBER FOR BACTERIAL SPECIMEN
7	M20	24
14	M20	34
28	M20	40

HALF CELL POTENTIAL FOR CORROSION

NO.OF DAYS	GRADE OF CONCRETE	POTENTIAL AT 2V
28	M20	- 210

XII. CREATION OF CRACKS

Standardised cracks were made in concrete samples of 150mm x 150mm x 150mm by introducing copper plates of 0.3mm thickness upto a depth of 10 or 20mm into the fresh concrete. These plates were removed after 24 hr. the crack width were varied between 0.01mm and 0.60mm (mean value: 0.20mm).

XIII. VISUAL EVALUATION OF CRACK REPAIR

BS + CaCO₃ treatments were able to bridge cracks. CaCO₃ deposition was visible at the crack edges on the samples surface .

XIV. CONCLUSION

Thus Based on the present experimental investigations, the following conclusions are drawn:

The addition of *Bacillus subtilis* bacteria increases the compressive strength of concrete upto 32% and the tensile strength of concrete is found to be increased upto 18%. Moreover surface hardness is experimentally found to be very high and has very low corrosive nature. It can be concluded that *Bacillus subtilis* can be safely used in crack remediation of concrete structure and environmental friendly crack repair technique.

XV. REFERENCES

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MASS CULTIVATION IN ROTATION CHAMBER WITH 100 RPM FOR 3 DAYS



CASTED BACTERIAL CUBES

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BACILLUS SUBTILLIS BACTERIA



STAINING PROCESS TO FIND GRAM POSITIVE OR GRAM NEGATIVE BACTERIA



SUB CULTURED BACTERIA