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“EXPERIMENTAL INVESTIGATION OF MECHANICAL PROPERTIES OF MORTAR UNDER INFLUENCE OF BACTERIA USING SEM ANALYSIS”

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ABSTRACT

In the present scenario development in every direction is expanding which needs to be located with an advantageous material. This will improve the solidarity and have less ecological impacts, which is of incredible criticalness. Bacillus or ureolytic organisms are the ones which improves the quality of bond in a mortar by the precipitating calcium carbonate with the help of urea and a calcium source. In the present work, Bacillus sphaericus is utilized to check its quality as a material, for this manner. A number of different tests such as consistency and setting time are carried out to discover the impact of bacterial arrangement on mortar. The tests such as, pressure quality and sorptivity test are carried out in the present research work for studying the variation of the mechanical properties of concrete mortar. The mineralogy and morphology of the calcium carbonate which is encouraged by the microscopic organisms are studied through XRD and FESEM analysis. The compressive strength of mortar at 7 days and 28 days in 3D shape is studied to increase with an expansion of microbes up to 107 cells/ml. The ideal dosages of microscopic organisms is found as 58% (at 7-day) and 23% (at 28-day) over the controlled condition for expanding the normal compressive strength. The more increase in strength following the multi day relieving might be because of the nearness of supplementary medium and it getting exhausted as it achieves 28 days and causing passing of microorganisms. The base aggregate water ingestion is acquired for a cell centralization of 109 cells/ml. The mineralogy and morphology of the calcium carbonate encouraged by the microscopic organisms test had the capacity to affirm that the bacterially accelerated calcium carbonate is calcite and is having lamellar rhombohedra or hexagon shape.

Keyword: SEM, bacterial concrete, compressive strength, calcite precipitation, cement mortar, microstructure

I. INTRODUCTION

Concrete is one of the most important building materials, which is used in the construction sector all over the world. Advancement in concrete technology can be achieved to a great extent through its strength improvement and its enhancement in durability using pollution-free and natural methods. With the progress of construction industry, the usage of cement is also increased rapidly as stronger and durable structure is of prime importance. This increases the productivity of cement globally which in turn increases the emission of carbon dioxide to the atmosphere. We need to find a technique which can increase the strength and durability of structures without increasing the use of cement for a better future.

Supplementary cementing materials (SCMs) are mostly used in concrete mixes for reducing the cement content, improve the workability, increase the strength and enhance its durability through pozzolanic activity. The most commonly incorporations in concrete for cement replacement are silica fume and fly ash, which has gained a lot of popularity in past two decades. All building materials are porous, which allow penetration of moisture and other harmful chemicals such as acids, chlorides and sulphates. These chemicals adversely affect the concrete and reduce the strength and life of structures. An admixture that would seal these pores and cracks and eventually reduces the permeability of the structure results in immense improvement the life of structure. Conventionally, various forms of sealing agents such as latex emulsions have been incorporated in concrete. But these agents suffered from significant limitations of incompatible interfaces, susceptibility to UV radiations, instability in molecular structure and high cost. One of the progressing bio-mimetic processes in nature is done by soil-thriving bacteria, which converts sand to sandstone.

Bacteria are also known as prokaryotic microorganisms. They are short in length with various numbers of shapes from sphere to rods and even spirals. Bacteria are present in different habitats in whole world. They can inhabit in any soil or water or in acidic hot springs, radioactive substance, and the deep inside earth's crust. They live in symbiotic and parasitic relationships with plants and animals.

Bacteria have a wide diversity in shapes and sizes, known as morphologies. The general size of Bacterial cells ranges between 0.5–5.0 micrometers in length and the shape is spherical, known as cocci, or rod-shaped, known as bacilli. Some of the bacteria are shaped like slightly curved rods or comma-shaped known as vibrio; and the remaining may be of spiral-shaped, known as spirilla. In the recent searches, it has been discovered that deep under Earth's crust some bacteria is growing as a branching filamentous types with a star-shaped cross-section. The large surface area to volume ratio of this morphology gives these bacteria an advantage in nutrient-poor environments.

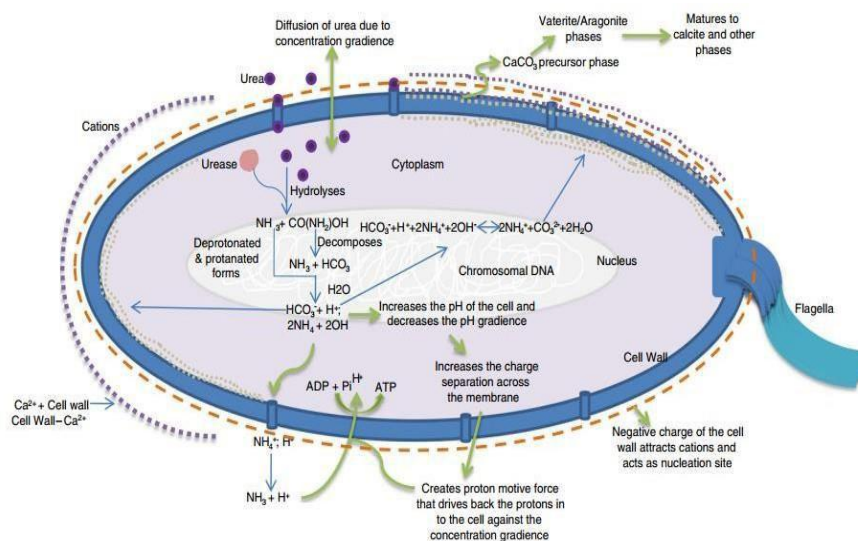


Figure 1: Mechanism of calcite precipitation by bacteria

II. LITERATURE REVIEW

Bang et al. (2001) Bacillus pasteurii immobilized in polyurethane is used in the study. The polyurethane provide protection to the bacterial cells from the extremely alkaline environment of concrete, while serving as nucleation sites for calcite crystals. The variation in the amount of precipitation of calcite by free cells and immobilized cells is little. The compressive strength of the mortar cubes are increased considerably after 7 days of curing in urea calcium chloride medium. Calcite in polyurethane showed little effect on the improvement of elastic modulus and tensile strength of the polymer. This can be accounted for the deterioration of chemical interaction between the calcite and the form after immersion on the medium.

Bachmeier et al. (2002) uses urease enzyme produced by *Bacillus pasteurii* immobilized polyurethane (PU) for calcium carbonate production. Here also recombinant *E.coli* is also used for calcite production. It is suggested that use of immobilized enzyme is more recommended than using bacterial cells. The viability of enzyme immobilized in the PU is to be studied further. SEM images show well organized calcite crystals

De Muynck et al. (2007) the improvement of resistance of mortar specimens to degradation processes is studied here. *Bacillus sphaericus* is used here. The durability of the treated substrate has been studied by measuring the resistance to carbonation and chloride ingress. Precipitation of calcite was quantified by X-ray diffraction (XRD) analysis and morphology observed by SEM. The presence of a newly formed layer of calcite on the surface of the mortar specimens is observed. Mortar cubes were used for the accelerated carbonation tests and capillary water suction experiments. Cylindrical specimens were drilled out of mortar slabs for chloride migration and for water vapor diffusion tests. Bacterial calcite on the surface of the specimens resulted in a decrease of capillary water uptake and permeability towards gas. This bacterial treatment resulted in a limited change of the chromatic aspect of mortar and concrete surfaces.

Achal et al. (a) (2011) the durability of concrete or mortar is examined in the study. The effect of calcite precipitation induced by *Sporosarcina pasteurii* is analyzed. An inexpensive industrial waste, corn steep liquor (CSL), from starch industry is used as nutrient source for the growth of bacteria and calcite production, and the results obtained compared with those of the standard commercial medium. Cement mortar cubes of 70.6 mm is used, as per IS4031-1988. Water uptake, permeability, and chloride permeability tests are done with bacteria cultured in CSL medium and it is found out that the results are compact table with those bacterial cultured using commercial medium and thus indicating the economization of the bio calcification process.

Majumdar et al. (2012) bacterial protein produce by bacteria closely related with the Thermo anaero bactor formicates is used in the study. This is directly mixed with the mortar and cubes of size 70.6 x 70.6 x 70.6 mm are casted. Crack repair test, sulphate resistance test, and water absorption test, flexural (The dimension of the standard beam was 200mm × 50mm × 50 mm. The beams were cured for 28 days under water and their flexural strength was determined in 4-point condition) tests are also conducted in addition to the compression tests. Maximum increase in compressive strength was observed in bacterial concentration of 105 cells/ml and increment was 42.4% after 120 days of water curing. The maximum flexural strength increment was 33% with 3 µg/g bio remedies protein incorporated samples.

Achal et al. (2015) the bio cementation ability of a *Bacillus sphaericus* to seal cracks is demonstrated. The reduction in porosity and chloride permeability was studied with bacterial strain. The crack healing capability is also checked and visualization of amount of calcite deposits is done by using scanning electron microscope. Cubes of size 70.6x70.6x70.6mm are used to evaluate the improvement in compressive strength by the application of bacterial strain. Cubes are cured in nutrient broth-urea medium. 40% increase in compressive strength is observed in microbial remediated cubes and significant crack healing of 13.4mm wide ones are also seen. It results in the decrease in water and chloride ion.

Maheswaran et al. (2018) comparison of ureolytic activity of *Bacillus cereus* and *Bacillus pasteurii* is done here. The bacterial mortar cubes are casted by replacing entire volume of water was replaced with phosphate buffered saline (PBS) suspended bacteria. Curing of bacterial cubes where carried out in nutrient solution. The test results shows an increase of 38% compressive strength using *Bacillus cereus* at bacterial cell concentration of 106 cells/ml and 29% increase in the case of *Bacillus pasteurii* over the control cement mortar specimen at bacterial concentration of 105 cells/ml. *Bacillus cereus* incorporated mortar cubes show significant decrease in chloride permeability. X-ray diffraction, scanning electron microscope, thermo gravimetric analysis and Fourier transform- infrared spectroscopy are used to confirm the bacterial calcite precipitation.

III. MATERIALS AND EXPERIMENTAL WORK

3.1. Selection of Appropriate Bacterial Species

To study the effect of bacteria on the different engineering properties of cement mortar an experimental program was planned. This experimental program consists of four major parts:

1. Selection of the appropriate bacterial species which is suitable for cement mortar and the culture of bacteria.
2. Casting of specimens (cement mortar) and curing of specimens.
3. Assessing the mechanical properties of hardened cement mortar.
4. Characterization studies of bacteria on hardened cement mortar.

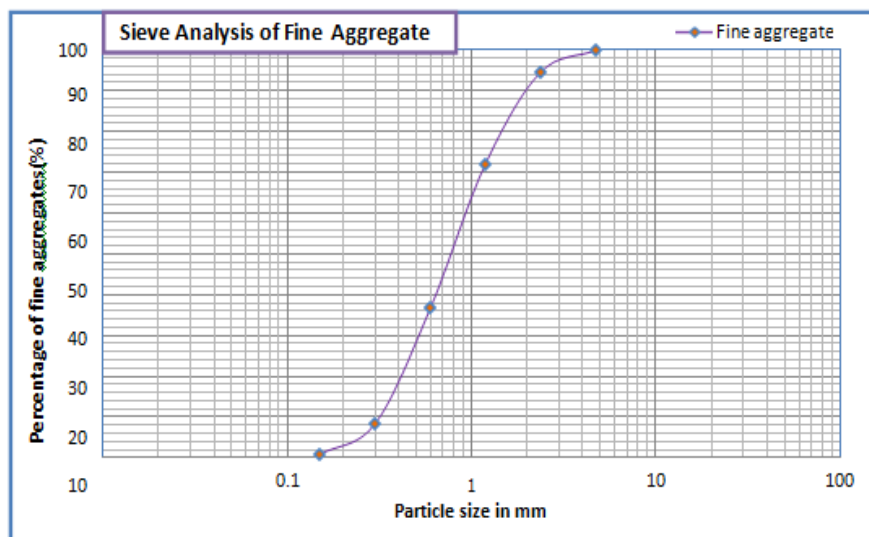
The ureolytic bacteria used in this work should be in living state in these alkaline environments and should have temperature tolerance. Two different non-contagious ureolytic bacteria (bacillus), such as, *Bacillus cereus* and *Bacillus sphaericus* are tested in this research work to check their survival in a concrete-like environment. The tests for both the bacterial species is carried out in concrete like environment and found that *Bacillus cereus* could survived only in the cultures with pH 8 and 9. Whereas *Bacillus sphaericus* is able to survive the temperature in concrete (or cement mortar) arising out of the heat of hydration. The materials are then mixed into a mix of cement mortar and the tests for consistency, compressive strength are carried out.

3.2. Objectives

- To study the compressive strength variation of cement mortar with bacteria.
- To study the setting time of cement with the presence of bacteria.
- To study the capillary water absorption of cement mortar using bacteria.

3.3 Casting and Curing of specimens

- **Cement:** The cement used for the experimental program was having following properties; consistency 29%, IST 45minutes, FST 265 minutes, and specific gravity 3.19. the compressive strength for the conventional cement paste is found out to be 48 n/mm².
- **SAND:** The fine aggregate used was locally available river sand from Hosangabad Bhopal. This passed through 4.75 mm sieve.



Graph 3.1 Sieve Analysis of Fine Aggregate

3.4 Mix Design of concrete

As one of the objectives is to study the variation of compressive strength of mortar cubes with various concentrations of bacteria, cement to sand ratio of 1: 6 and water cement ratio of 0.55 are considered to prepare the mortar cubes. Accordingly, the amount of cement, sand and water is calculated as shown in the table below.

Table 3.1: Details of test specimen for mechanical properties of mortar

Mortar cube ID	Bacteria concentration (cells per ml)	Number of specimen for			Mix proportion			Curing Soln.
		7 day comp strength	28 day comp. strength	Capillary water absorption	Cement (kg)	Sand (kg)	Water (ml)	
Control	0	3	3	3	0.13	0.77	72ml	†
Control	0	0	3	0	0.13	0.77	72ml	Ø
B1	10 ⁵	3	3	3	0.13	0.77	72ml*	Ø
B2	10 ⁶	3	3	3	0.13	0.77	72ml*	Ø
B3	10 ⁷	3	3	3	0.13	0.77	72ml*	Ø
B4	10 ⁸	3	3	3	0.13	0.77	72ml*	Ø
B5	10 ⁹	3	3	3	0.13	0.77	72ml*	Ø

* indicates that the volume of water includes bacteria and culture medium
† indicates tap water as curing solution
Ø indicates a mix of tap water, urea and calcium chloride as curing solution

The cement mortar cubes mixed with the bacterial species are then tested for consistency, initial and final setting times and compressive strength.

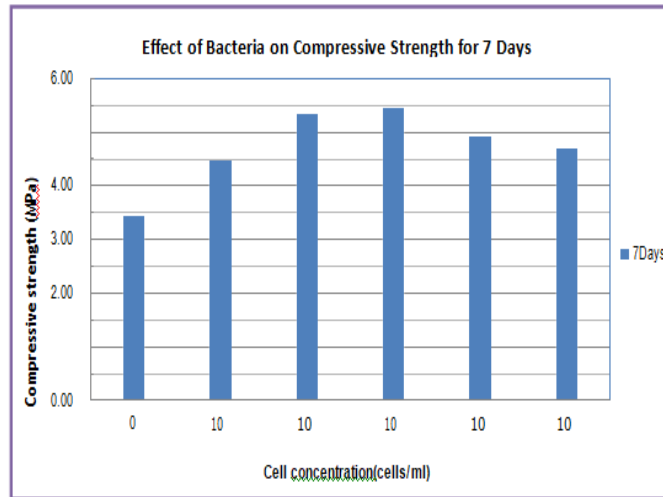
IV. RESULTS AND DISCUSSION

4.1 Compressive Strength Test on Cement Mortar

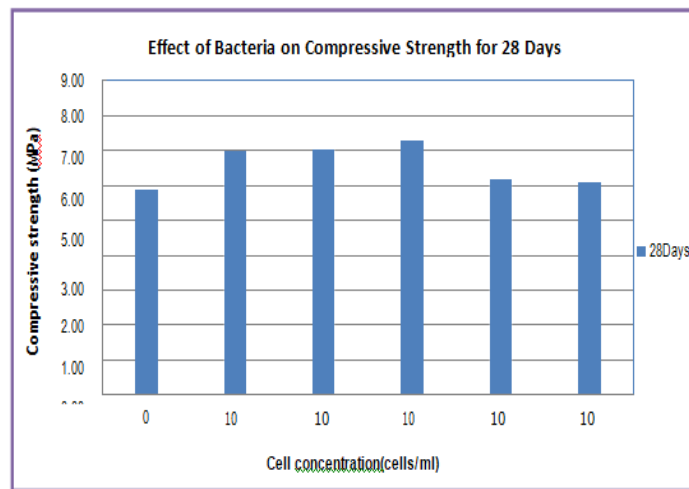
All the mortar cubes are tested in a load controlled Compressive testing machine (CTM) to obtain the unidirectional compressive strength obtained at 7 days and 28 days. It can be observed that as the cell concentration increase the compressive strengths at both 7 days and 28 days increases initially and then decreases. The maximum strength occurs at a cell concentration about 10⁷ cells/ml and hence this cell concentration can be treated as optimum dosage.

Table 4.1 Effect of bacteria on compressive strength

Cell concentration (cells/ml)	Mean compressive strength at 7 days (MPa)	Percentage increase (%)	Mean compressive strength at 28 days (MPa)	Percentage increase (%)
0 (Control)	3.44	-	5.90	-
10 ⁵	4.46	29.65	6.98	18.30
10 ⁶	5.34	55.23	7.02	18.98
10 ⁷	5.44	58.23	7.28	23.38
10 ⁸	4.91	42.73	6.19	4.90
10 ⁹	4.71	36.90	6.10	3.38



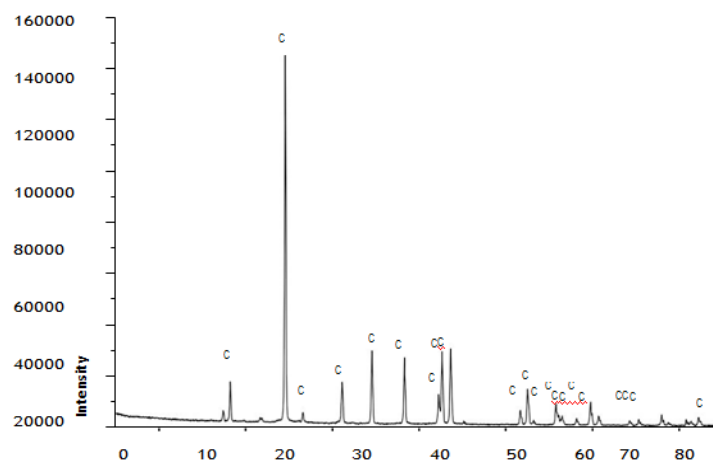
Graph 4.1: Compressive strength – cell/ml graph for 7 days curing



Graph 4.2: Compressive strength – cell/ml graph for 28 days curing

4.2 X-Ray diffraction spectrometry on the layer formed over the curing water

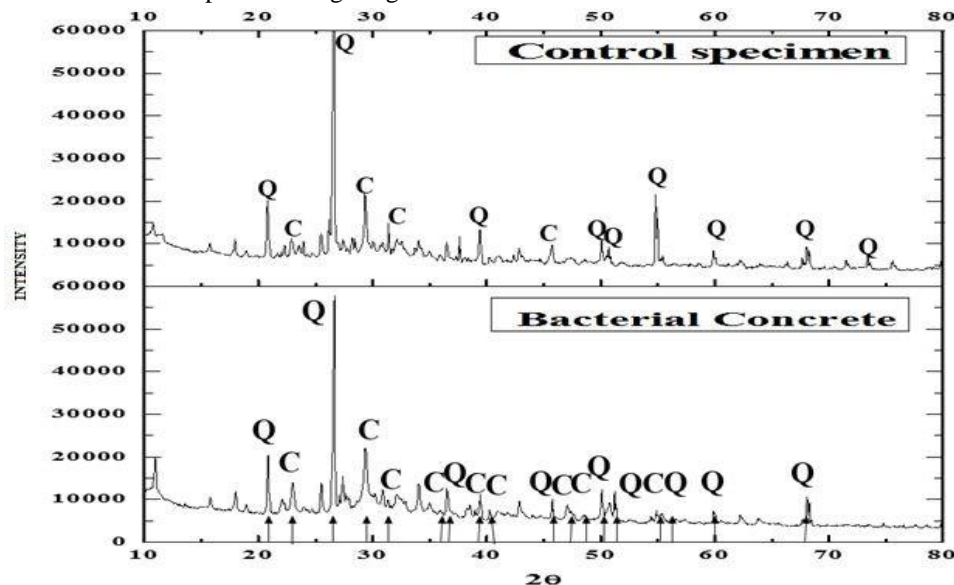
It can be observed that a layer is formed over the surface of water. When this layer was dried it gave a white colour powder. The layer was calcite which was produced by bacteria. This layer was not observed on the curing solution with control specimen in absence of bacteria. This result was helpful to gain confidence on the precipitation of calcite by the metabolism of bacteria.



Graph 4.3: XRD for the surface layer found over water for curing (C represents calcite)

4.3 X-Ray diffraction spectrometry on mortar cubes

From the specimens used for compressive strength testing small amount of sample was collected from the core of the cube and sieved through 100 μ IS sieve and was tested by XRD. The result obtained was analyzed using XPert High Score software and the result was plotted using Origin Pro software.



Graph 4.5 XRD for the mortar cube sample with bacteria and control cubes ('Q' represents quartz or silica and 'C' represents calcite)

V. CONCLUSION & RECOMMENDATIONS

On the basis of above experimental investigation and analysis of the results, the important conclusions drawn from the above study are listed as follows:

- i. To improve the properties of cement mortar or concrete the appropriate bacteria should be selected judiciously. For example, *Bacillus cereus* could not survive in the given environment whereas another *Bacillus* species *Bacillus sphaericus* survived.
- ii. Addition of bacteria alone cannot improve the properties of concrete/cement mortar. An Ureolytic bacterium requires urea and a source of calcium to produce CaCO_3 .
- iii. *Bacillus sphaericus* found to be not altering the normal consistency and setting time of the cement paste.
- iv. Compressive strength (at 7-day and at 28-day) of mortar cube found to be increasing with the increase of bacteria concentration up to 107 cells/ml. However, further increase of bacteria concentration found to reduce the compressive strength of cement mortar.
- v. The optimum doses of bacteria found to increase the average compressive strength by 58% (at 7-day) and 23% (at 28-day) over the control specimen.
- vi. The more increase in strength after 7 day curing may be due to the presence of nutrient medium and it getting depleted as it reaches 28 days and causing death of bacteria.

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