Experimental Study on Microbial Concentration Optimization in Cement Mortar using M-Sand as Fine Aggregate

Babitha Benjamin1*, Anisha Sabbavarapu2, J Sudhakumar3 and T V Suchithra4

1 Research Scholar, Department of Civil Engineering, National Institute of Technology, Calicut, India
2 PG Scholar, Department of Civil Engineering, National Institute of Technology, Calicut, India
3 Professor, Department of Civil Engineering, National Institute of Technology, Calicut, India
4 Professor, School of Biotechnology, National Institute of Technology, Calicut, India
5 babitha.babitha@gmail.com

Abstract. One of the major shortcomings of conventional concrete based constructions is its gradual strength deterioration over time due to its weak tensile strength. Without any effective measures to remedy this problem the alternatives we have now are periodic monitoring, retrofitting, repair & rehabilitation, etc. The above-mentioned methods are all highly unsustainable due to manpower, money and materials being spend in a cycle to upkeep. If a protective coating of microbial cement mortar is given to the structures like bridges, underground tunnels, etc. were a lot of dampness is observed, structural deterioration can be prevented. The study is aimed at optimizing the concentration of bacteria in self-healing cement mortar which uses microbiologically induced calcium carbonate precipitate to induce crack healing ability. For this purpose, bacteria Bacillus Subtilis MTCC441 (non-ureolytic) were added in concentrations $10^4$, $10^6$, $10^8$ cells/ml to a mix using M-sand as fine aggregate. The bacteria were cultured in a nutrient broth and were added to cement mortar by water medium during mixing. The addition to cement mortar was done when the microorganisms were still alive and active. In this study, a series of experiments like compressive strength, water absorption, and pour healing was done to optimise the concentration. The results showed that $10^6$ cells/ml was the optimum quantity of microorganisms required to enhance the physical properties of concrete.

1. Introduction
Concrete usually experience cracks as its tensile strength is much lower than its compressive strength. On cracking, the stiffness of the concrete is reduced and it allows the permeation of moisture causing corrosion of steel which further leads to the deterioration of the structure before the design period. This needs extra expenditure for regular inspection and maintenance. However, sometimes it is difficult to access the damaged site. An alternative method is to induce self-healing property to concrete that is whenever crack occurs the cracks gets healed within in a short duration thus preventing further structural deterioration. There are many methods to incorporate self-healing property to concrete but this paper focuses on self-healing consummated by bacterial action through Microbiologically Induced Calcium Carbonate Precipitate (MICCP) [1]. This partial method is chosen since it is the most sustainable and convenient approach than other self-healing methods since there is no additional consumption of raw materials or other chemical substances involved as in other self-healing methods. Bacterial induced Calcium Carbonate precipitate is environment friendly, heals micro-crack, closes pore, increase the density of concrete and improves the compressive strength thus increasing the durability of structures. The present study aims at optimizing the concentration for calcite precipitating non-ureolytic bacteria Bacillus Subtilis MTCC441 in cement mortar.
1.1. Significance of the study
As it is known, concrete is weak in tension but strong in compression which makes the material brittle. To compensate this problem in concrete we provide steel reinforcement for concrete structure, but still the inherent brittle nature of concrete causes micro-cracks which further deteriorate the structure and reduces its design lifespan. Hence it is necessary to come up with a solution for the micro-cracks created in concrete structures. There are several solutions available for this condition, but most of which are not sustainable since it consumes additional raw materials, use of chemical polymers, etc. Biomineralization is the most sustainable solution available so far for this problem, which is use of microorganisms in concrete to self-heal the cracks whenever it occurs just like a fracture in a human bone gets healed by osteoblasts cells. This crack healing by microorganisms in concrete does not require any additional resources or manpower for crack repair thus making it sustainable by reduction in manhours, money, and material. This study is conducted in cement mortar since there is no necessity to provide self-healing for the entire structure which increases the construction cost, rather a protective plastering for the structure can be given which eliminates the additional cost factor.

1.2. Need for this study
Traditionally, the river sand used as fine aggregate for cement mortar, which is a scarce material nowadays. The over mining of river sand causes environmental issues like disturbing aquatic ecosystem, land erosion, etc., it is high time we consider this problem and start using an alternative material like M-sand which is already available in markets as a substitute for river sand. In this study M-sand is used as fine aggregate for preparing cement mortar. This makes building construction more sustainable and makes us more responsible individuals to the society.

1.3. Concept of microbial action
MICCP or biomineralization is the basic concept behind bacterial based self-healing concrete. MICCP is the process in which bacteria produces calcium carbonate as a result of their existence under suitable environmental conductions. The cell wall is negatively charged which draws the cations from its surroundings, this way Ca$^{2+}$ reach the cell wall which acts as nucleation site where the reaction with CO$_3^{2-}$ ions occurs to form CaCO$_3$[2].MICCP via non ureolytic bacteria is simply through the extracellular activity as shown in equation below.

\[
Ca^{2+} + Cell \rightarrow Cell - Ca^{2+} \tag{1}
\]

\[
Cell - Ca^{2+} + CO_3^{2-} \rightarrow Cell - CaCO_3 \tag{2}
\]

2. Materials
Traditionally the materials involved in the preparation of cement mortar are cement, river sand and water. Ordinary Portland Cement (OPC) Grade-53 conforming to IS 12269-1987 were used having specific gravity 3.35 with initial and final settling time as 210 minutes and 300 minutes, respectively for preparing specimens. Well-graded Manufactured Sand (M-Sand) conforming to IS 383-1970 were used having specific gravity 2.56 and fineness modulus 2.65 as fine aggregate. The additional important constituents used in this study are microorganisms to impart self-healing property to cement mortar. The bacteria Bacillus Subtilis MTCC441were used in this present study, bought from gene bank MTCC (Microbial Type Culture Collection), Chandigarh. This particular bacterium is chosen from available literature since it is a spore forming non-ureolytic bacterium which can survive in high pH environment and sustain mechanical stresses faced during mixing of cement mortar.

2.1. Maintenance of stock culture
The pure culture of B.subtilis is maintained continuously on nutrient agar slants. The major components of the Nutrient Agar are peptone, beef extract, yeast extract, NaCl and Agar. The cultures were streaked on to agar slants with an inoculation loop and the slants are incubated at 37°C for 24-72 hrs. After sufficient growth on the agar slants, cultures were preserved on refrigerator at 4°C. For addition of bacterial culture into the concrete, a single colony from the nutrient agar plate is inoculated into 200ml of nutrient broth and the culture flasks are maintained at 37°C in a 125 rpm orbital shaker [3].
2.2. Concentration Control for bacteria
The concentration of bacteria in the nutrient broth is identified by comparing with McFarland Standards [4]. It standardises the concentration of bacterial suspension by comparing the turbidity of the test suspension with that of the McFarland Standard. The chemical constituents of McFarland Standard are barium chloride and sulphuric acid and the reaction between these two components results in the formation of a fine precipitate i.e. barium sulphate. McFarland standard tubes were shaken well prior to usage in order to visually compare a bacterial suspension of known concentration and the values were identified from the McFarland table.

2.3. Mix proportions and specimen preparation
The mix ratio adopted for the cement mortar study was 1:3 with a water cement ratio of 0.55. The water cement ratio was fixed by conducting the flow table test getting flow value 106.5. For preparing mixes using bacteria, the bacterial culture is used instead of distilled water, by maintaining bacterial culture cement ratio as 0.55. The test specimens for compressive strength, water absorption, and pore healing were done in a laboratory type mortar mixer of capacity 10 litres. The mixing continued until a uniform mix was obtained. The cement mortar was then placed into the moulds which were properly oiled. After placing of cement mortar in moulds, proper compaction was given to the cement mortar using the mortar vibrator. Specimens were de-moulded after 24 hours of casting and were kept in a curing tank for water curing until the age of test. Since the main objective is to find the optimum concentration of bacteria *Bacillus Subtilis*, different specimens were cast using water or equivalent amount of live bacterial culture with concentrations of $10^4$, $10^6$, $10^8$ cells/ml. Test specimens were prepared using standard cube moulds of the size 50mm [5]. The type of specimens prepared is detailed in table 1.

| Sl. No | Specimen Type | Specimen Name |
|-------|---------------|---------------|
| 1     | Normal Cement Mortar | NCM |
| 2     | Bacterial Cement Mortar $10^4$cells/ml | BCM4 |
| 3     | Bacterial Cement Mortar $10^6$ cells/ml | BCM6 |
| 4     | Bacterial Cement Mortar $10^8$ cells/ml | BCM8 |

2.4. Compressive strength
To study the compressive strength of concrete, 50mm cube specimens were cast and compacted using a vibration machine. After 24 h of casting, the cubes were demoulded and then cured in water at room temperature and compressive strength testing is done at the age of 3, 7, and 28 days as per IS 516:1959. The surface water is wiped from the cubes before testing. The tests were performed in sextuplets and average value was taken as compressive strength.

2.5. Water absorption test
Water absorption tests of the control mix and bacterial live mix were performed for 3, 7 and 28 days of curing. For this test, the mortar cubes were oven dried at 110°C for 24 h and dry weight $W_1$ is measured. The samples were then kept in saturated state in water at room temperature for 24 h and $W_2$ is weighed again. The water absorption was then calculated by the formula.

2.6. Pore healing test
The pore healing was done using Non-contact Radio 3D Optical Profiler. Optical profilers are interference microscopes, with great precision using the wavelength of light as the ruler and are used to measure height variations such as surface roughness on surfaces. The cube specimens of size 50 mm were kept under the optical profiler after marking a small area on surface, for repeat revaluation on 3, 7 and 28 days.

3. Results and discussions
3.1. Compressive Strength Development
The compressive strength for the respective days as per IS:4031 part 6 obtained by applying different bacterial concentrations to the cement mortar are compared with the control specimens. It is found that
the compressive strength of microbial tested specimens of all the concentrations is higher than the control specimens. The increase in compressive strength is due the filling of pores with the calcite precipitation of bacteria. It is noted that the compressive strength of cement mortar increases with increase in live bacterial concentrations up to $10^6$ cells/ml and there is a reduction at $10^8$ cells/ml. The 28th day compressive strength is increased by 20%, 31% and 3% for $10^4$, $10^6$ and $10^8$ cells/ml respectively. The figure 1 shows the bar chart for the compressive strength of different mix and table 2 shows the compressive strength values for the mixes.

| Sample | 3rd Day | 7th Day | 28th Day |
|--------|---------|---------|----------|
| NCM    | 10.36   | 15.93   | 23.88    |
| BCM4   | 11.99   | 22.61   | 28.60    |
| BCM6   | 12.35   | 23.23   | 31.17    |
| BCM8   | 15.14   | 17.19   | 24.53    |

3.2. Water absorption
The water absorption for 3, 7, and 28 days are measured for control and bacterial specimens. It is found that the water absorption obtained from different bacterial concentrations is less compared with control mix. It is noted that the water absorption decreases with increase in live bacterial concentrations up to $10^6$ cells/ml and there is an increase at $10^8$ cells/ml. The 28th day water absorption is decreased by 13%, 90% and 12% for $10^4$, $10^6$ and $10^8$ cells/ml respectively. Figure 2 shows the bar chart for water absorption for different specimens and table 3 shows the values for water absorption.

| Sample | 3rd Day | 7th Day | 28th Day |
|--------|---------|---------|----------|
| NCM    | 9.41    | 7.31    | 5.93     |
| BCM4   | 8.68    | 5.93    | 5.15     |
| BCM6   | 7.59    | 1.04    | 0.47     |
| BCM8   | 6.44    | 5.37    | 5.11     |
Figure 2. Graph for comparison between water absorption for specimens.

3.3. Pore healing
The table 3 below shows the surface measurement decreasing as curing days pass. The average surface measurements on 3, 7 and 28 days respectively are taken for all the specimens and concentration $10^8$ showed pore healing. As the concentration increases the pore healing is found to be increasing. Figure 3 shows the bar graph for pore healing and table 4 shows values for pore healing.

Table 4. Pore depth details for the specimens in microns.

| Sample | $3^{rd}$ Day | $7^{th}$ Day | $28^{th}$ Day |
|--------|--------------|--------------|---------------|
| NCM    | 0.45         | 0.3          | 0.26          |
| BCM4   | 0.22         | 0.14         | 0.09          |
| BCM6   | 0.22         | 0.12         | 0.09          |
| BCM8   | 0.24         | 0.22         | 0.1           |

Figure 3. Graph for pore depth comparison.
4. Conclusions
Based on the experimental studies conducted, the following conclusions are obtained. The optimal concentration of bacteria *Bacillus Subtilis MTCC441* for improving the mechanical properties and to impart self-healing to cement mortar is $10^6$ cells/ml. As the concentration of microorganisms increased beyond the optimal concentration of $10^6$ cells/ml, the compressive strength of cement mortar specimens decreased by 21%. The compressive strength of cement mortar specimens increased by 30% as the concentration of bacteria increased up to an optimum concentration. The water absorption for cement mortar specimens at optimal concentration decreased drastically than other concentrations specimens up to 80%, i.e. to a point much less than normal cement mortar specimens without bacteria. The water absorption for cement mortar specimens with concentration more than the optimal concentration showed 10% increase in water absorption than optimal concentration specimens but not greater than normal cement mortar specimens. The pours and surface roughness of cement mortar specimens decreased in diameter and depth as concentration of bacteria increased, maximum pore healing was achieved for BCM8. The decrease in surface roughness and pour size occurred beyond the optimal microbial concentration, i.e. as concentration of microorganisms increased the pour size and surface roughness decreased for the cement mortar specimens but compressive strength decreased.

5. References
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