A study on the effect of pH conditions on the properties of self-healing mortar cubes

SruthySubash¹, Poornima V² and Karingamanna Jayanarayanan³

¹Post Graduate Student, Center of Excellence in Advanced Materials & Green Technologies (CoE – AMGT), Amrita School of Engineering, Coimbatore, Amrita VishwaVidyapeetham, India.
²Assistant Professor, Department of Civil Engineering, Amrita School of Engineering, Coimbatore, Amrita VishwaVidyapeetham, India.
³Professor, Department of Chemical Engineering and Materials Science, Amrita School of Engineering, Coimbatore, Amrita VishwaVidyapeetham, India.

E-mail: ¹sruthysubash194@gmail.com, ²poorni.engg@gmail.com, ³kjnarayan@gmail.com

Abstract. This paper discusses the effect of pH on the various properties of the self-healing mortars (SHM). The bacteria induced structures have the ability to heal cracks generated on its surfaces by precipitating minerals inside the pores which bio mimics the natural healing processes occurring in nature, thus being an environment friendly technology that contributes in maintaining the structural durability. The microbiologically induced precipitation accelerates at different rates at different conditions. Hence an attempt has been made to analyse the changes in the properties in mortar cubes induced with 10⁵ cells/ml concentration of Bacillus megaterium solution and subjecting them to different curing media at different pH conditions. The healing efficiency of the cracks induced in the mortar cubes are evaluated visually for successive days. Through X-ray Diffraction analysis the white powder covering crack surface is confirmed to be calcite. The percentage reduction in the compressive strength of the samples after healing was found to be only 3% after 28 days of curing. The findings of the paper suggest promising application of self-healing mortar cubes at different geographical locations by varying the amount of bacterial solution.

1. Introduction
A variety of research activities are progressing on improving the durability of civil structures and finding out highly efficient repair materials which are environment-friendly. The approach using bacterial concrete for crack healing has received a lot of attention in the last few years [1,2,7]. From the time of understanding the relevance of calcium carbonate induced mineral precipitation by different soil bacteria, the application of bio-remediation materials has significantly increased [4]. The studies showed that the pores were filled by bio-deposited materials like calcium carbonate which is more environment friendly having a better compatibility with the construction materials like concrete and masonry when compared with traditional repair or healing materials like epoxy resin, self-healing polymers, pore blockers, mortar, cement grout etc. This self-repair or healing property could be gained by the addition of bacteria promoting microbial induced deposition [1].

³ To whom any correspondence should be addressed.
The cracks that get propagated on structures are due to drying shrinkage, thermal stress error in designing and detailing, chemical reaction, constant overload and external load etc. The ingress of water, salts, and impurities through the cracks initiates corrosion, thereby reducing the service life and structural integrity [10, 12]. When these cracks are formed in bacteria-induced surfaces, on contact with water and nutrients microbial action commences and conversion of urea into ammonia and carbonate occurs with the release of urease enzyme. The formation of ammonium hydroxide in the water-rich environment and the presence of calcium results in the precipitation of calcium carbonate (CaCO3) as a microbial sealant [10, 18, 23].

The precipitated CaCO3 crystals can fill the cracks and pores and can be healed by the technique i.e., via microbial healing called Microbiologically Enhanced Crack Remediation (MECR). Its efficiency depends on the types of microbes used for the healing process, the nutrient source provided, amount of calcite precipitated also the properties of concrete or mortar based structures [9]. Various studies with different Bacillus species like pasteurii, licheniformis, sphaericus, flexus, subtilis, cereus, megaterium etc. were conducted. Different bacteria favors different pH and temperature conditions and calcite precipitation rate varies, accordingly, [4, 6, 15].

However, very few reports were found regarding the change in bacteria-induced properties when the structures were exposed to different environmental conditions. Considering this, the present experimental research are taken up to conduct a study on the environmental effects of self-healing mortars by casting mortar cubes in the presence of Bacillus megaterium. The present investigation is aimed to answer the following queries: Effect of changing pH on the properties like strength and hardness of the bacterial mortar cubes, the effect on properties of mortar cubes for different curing media. Additionally, the crack healing process was monitored visually for successive periods of time.

2. Material and methods

2.1. Mortar Specimens
Ordinary Portland Cement of 53 grade conforming to IS 12269-1987 was used. The fine aggregate used is locally available clean, well-graded, natural river sand with a fineness modulus of 2.89 and it conforms to IS 383-1970. Along with cement and sand, locally available portable water having properties as per IS: 3025 – 1964 part 22, part 23 conforming to standards specified in IS 456-2000 is used for casting and are taken as per the mix calculation. The desired proportion of cement sand mix chosen is 1:3 which means for 1 part of cement 3 parts of sand is used with a water to cement (w/c) ratio of 0.4. This is confirmed by the flow test and mortar Cubes are cast using a mould of standard size 70.6mm x70.6mm made of mild steel. The mortar cubes with and without the addition of bacteria are cast and then tested to study their properties.

2.2. Micro-Organism
The bacteria selected for the study should be harmless to humans and should have self-healing property with the ability to resist high alkaline environment. Considering these factors the bacteria selected for crack remediation is of Bacillus species Bacillus megaterium cultured in Central Plantation Crops Research Institute (CPCRI) Kasaragod with a cell concentration of 10^5 cells/ml, isolated from the rhizosphere soils of healthy coconut palm growing in Tumkur region of Karnataka. The bacteria were isolated by taking 10g rhizosphere soil in 90 ml sterile water blank, by keeping the flask in a water bath at 80°C for 20 min after thorough mixing and serial dilution was done. From the 10^3 and 10^4 dilutions, the 100μl sample was incubated at 30°C until the colonies of the Bacillus species grew after spreading the sample and then plated it on a nutrient agar plate [24]. Polyphasic identification using BIOLOG and 16srRNA sequencing was carried out to identify the bacteria to species level [24].
The Bacillus megaterium culture maintained at CPCRI was then multiplied for the experiments. For the multiplication, nutrient broth medium (5 ml quantity) was initially sterilized, one loop full of bacterial culture was added, and it was shaken well and kept in BOD at 30°C. Intermediate shaking is done after 24 and 48 hours so that the bacterial clumps are broken down, and was left to multiply. This initial broth culture was then added to flasks containing 250 ml of sterilized nutrient broth and likewise, about 1000 ml culture was prepared. The broth used includes 5g/l of peptone and NaCl along with 1.5g/l of meat and yeast extract.

2.3. Calcium Carbonate Estimation
Two methods for estimating calcium carbonate were conducted. Initially, 50g/l of Calcium Chloride along with 20g/l of Urea were taken in a 500ml conical flask of 3 numbers to which 5ml, 10ml and 15ml of bacterial solution were added and further raised to 500ml with distilled water. Initial weight was noted and they were incubated at 37°C for successive days till a white precipitate was obtained at the bottom. Estimation was carried out by noting the weight of 3 flasks for 0thday, 2nd, 3rd, 6th, and 8th days. The second method of calcite estimation was more accurate and was carried out by adding 0.6g of Nutrient broth, 5.7g of CaCl$_2$; 0.424g of NaHCO$_3$; 2.0g of NH$_4$Cl; 3.0g of Agar, 190ml of distilled water was weighted and taken in a 200ml conical flask. They were autoclaved and urea was added to the flask. Inoculated broth culture was transferred to plate and incubated at 30°C for 6 days and checked for calcite precipitation [23] was shown in the figure 1.

![Figure 1. Calcium carbonate estimation: shows agar plate to which broth culture was inoculated and incubated.](image)

2.4. pH Study
Bacterial growth is immensely influenced by the hydrogen ion concentration of the organism’s environment. There is an optimum pH for each bacterium for its regeneration. So, it is necessary to identify the pH at different conditions. The prepared nutrient broth was autoclaved and taken in test
tubes labelled for different pH values keeping one as a control sample. The test tubes containing nutrient broth were again autoclaved and then cooled to room temperature. Further, all the test tubes were incubated for 24hrs at 37°C and they were observed for turbidity and % transmittance using photo calorimeter and nephelometer.

2.5. Preparation of Mortar Cubes
The mortar cubes were prepared using the same method of preparation of conventional mortar cubes except the difference was that the bacterial solution was also added into the mortar during mixing. Cement sand of 1:3 proportion commonly used in plastering was used and then dry mixed by adding required amount of water with a w/c ratio 0.4. Along with water bacterial solution of 5ml, 10ml and 15ml were added for different mixes during casting. After the wet mixing, the cement paste was directly placed into greased moulds of standard size 70.6mm. Each layer, after laying was followed by tamping with tamping rods and were allowed to set for 24 hours after which they were demoulded and subjected to curing process. Both the normal curing i.e., curing with water and nutrient curing i.e., curing media containing 20g/l of urea and 50g/l of calcium chloride were used for 7 and 28 days of curing. Once the curing was completed, the cubes were exposed to various environmental conditions by changing pH alone and after drying they were subjected to different tests.

2.6. Tests on mortar cubes
Mortar cubes with the addition of megaterium after subjecting to different environmental conditions were tested for identifying the changes in properties. NDT (includes UPV Test and Rebound Hammer) and compressive strength analyses were carried out. The cubes were loaded in UTM at a uniform rate of loading to initiate the crack and then allowed to heal by different curing media.

2.7. Characterization
Calcium Carbonate precipitated on the pores by bacteria Bacillus megaterium were analysed by XRD, which endorsed the white powder formation as calcite. The mineralogy of precipitates was determined by powder X-Ray diffraction (XRD) [13, 21].

3. Results and discussions
3.1. Calcium Carbonate Estimation
The test results show maximum calcite estimation in 5ml bacterial solution added flask in comparison with the other two samples. Accordingly, it is clear that Bacillus megaterium precipitates maximum calcium carbonate at an amount of 5ml/l of water.

The second method estimates white spots as shown in the figure 2, resembling the calcite precipitation whose mineralogy was ensured as calcium carbonate through XRD.
3.2. pH Study on bacterial solution

The pH study on the bacterial solution Bacillus megaterium is a preliminary study to identify the behaviour of the bacterial solution under different pH conditions before incorporating it into mortar cubes. Using photo calorimeter and turbidity measuring device optical density, % Transmittance and Turbidity measured in NTU are determined.

Table 1. Obtained values of pH study.

| Sample | NTU |
|--------|-----|
| Control | 7.6 |
| Acidic | 27 |
| Basic | 780 |
| Neutral | 439 |

The test result obtained from pH study shown in table 1 and 2 indicate that the megaterium sample has an alkaline nature. They thrive and generate in basic pH than at other pH levels. The % Transmittance is least under basic conditions and optical density is higher at basic pH. Furthermore, the turbidity is maximum at the basic level which is clear from figure 3 and 4. This is clear evidence of the capability of megaterium samples to grow and generate under basic conditions in comparison with normal conditions.
Table 2. Observations from photocalorimeter.

| Sample | OD  | %Transmittance |
|--------|-----|----------------|
| Control| 0.04| 089            |
| Acidic | 0.05| 089            |
| Basic  | 0.65| 022            |
| Neutral| 0.16| 069            |

Figure 3. Shows the Optical Density and %Transmittance curve obtained for different samples by photocalorimeter.

Figure 4. Turbidity Vs Samples with maximum turbidity at basic condition.

3.3. pH Study on mortar cubes
Mortar cubes which were cast with Bacillus megaterium are subjected to different pH condition after curing it for 7 and 28 days. Each mortar cubes with 5, 10 and 15 ml that were cured in water and nutrient were subjected to acidic, basic and neutral pH. Thus 72 cubes each of water cured and nutrient cured cubes for 7 and 28 days were cast covering the entire range of bacterial solution. The environmental effects on self-healing mortar cubes were studied by subjecting it for tests like NDT, Compressive strength and by creating cracks in the application of load and then comparing it with conventional ones. The test results obtained for 5ml, 10ml and 15ml bacteria induced samples are shown in table 3, 4 and 5. Those samples were compared with the properties of conventional mortar cubes cured in both water and nutrient media [11], [21].

3.4. Crack healing
On applying load uniformly on the mortar cubes cracks were created on their surface. To check the healing efficiency of cracks first they were immersed in the bacterial solution for 24hrs. Then they were immersed in a nutrient solution containing calcium chloride and urea. The healing process of the induced cracks were checked for successive days. Figure 5 and 6 show the mortar cubes before healing and after healing on the 8th day. The healing rate is different for different samples and it depends on the amount of bacterial solution it contains. For 5ml sample the healing begins on the 3rd day while for others mineral remediation process initiates after 3rd day.
Table 3. Test results of mortar cubes with 5ml bacterial solution cured for 7 & 28 days.

| Curing days | Sample | Curing media | Hardness value | Pulse velocity (km/s) | Compressive strength (MPa) | Crack Load (kN) | Compressive strength after healing (MPa) |
|-------------|--------|--------------|----------------|-----------------------|--------------------------|----------------|---------------------------------|
| 7 days      | 5.a.w  | Water curing | 10             | 3.349                 | 5.58                     | 10             | 4.8                             |
|             | 5.b.w  | Water        | 8              | 3.608                 | 8.98                     | 45.2           | 7.9                             |
|             | 5.n.w  | Water curing | 9.5            | 3.608                 | 8.98                     | 85             | 7.2                             |
|             | 5.a.N  | Nutrient curing | 8.5          | 3.608                 | 8.98                     | 12             | 8.2                             |
|             | 5.b.N  | Nutrient     | 9              | 3.608                 | 8.98                     | 24.8           | 8                              |
|             | 5.n.N  | Nutrient curing | 8             | 3.703                 | 10.6                    | 14.8           | 9.4                             |
| 28 days     | 5.a.w  | Water curing | 19.8           | 3.431                 | 6.5                      | 12             | 6.3                             |
|             | 5.b.w  | Water        | 16.8           | 3.365                 | 5.75                     | 36.8           | 5                               |
|             | 5.n.w  | Water curing | 18             | 3.626                 | 9.27                     | 18             | 9                               |
|             | 5.a.N  | Nutrient curing | 15            | 3.703                 | 10.6                    | 54             | 9.8                             |
|             | 5.b.N  | Nutrient     | 14.5           | 3608                  | 8.98                     | 41.6           | 8.4                             |
|             | 5.n.N  | Nutrient curing | 9             | 3.703                 | 10.6                    | 65.2           | 10                              |

5.a.w, 5.b.w, 5.n.w – 5ml water cured cubes subjected to acidic, basic and neutral pH
5.a.N, 5.b.N, 5.n.N – 5ml nutrient cured cubes subjected to acidic, basic and neutral pH
### Table 4. Test results of mortar cubes with 10ml bacterial solution cured for 7 & 28 days

| Curing days | Sample | Curing media | Hardness value | Pulse velocity (km/s) | Compressive strength (MPa) | Crack Load (kN) | Compressive strength after healing (MPa) |
|-------------|--------|--------------|----------------|-----------------------|---------------------------|----------------|------------------------------------------|
| 7 days      | 10.a.w | Water        | 6              | 3.589                 | 8.68                      | 10             | 7.62                                      |
|             | 10.b.w | Water curing | 9.5            | 3.608                 | 8.98                      | 55             | 7.9                                       |
|             | 10.n.w | Water        | 8              | 3.608                 | 8.98                      | 45.2           | 8.0                                       |
|             | 10.a.N | Nutrient     | 9.8            | 3.448                 | 6.72                      | 30             | 6.0                                       |
|             | 10.b.N | Nutrient     | 12             | 3.482                 | 7.158                     | 90             | 7.0                                       |
|             | 10.n.N | Nutrient     | 11.5           | 3.465                 | 6.938                     | 64.3           | 6.2                                       |
| 28 days     | 10.a.w | Water        | 8              | 3.703                 | 10.6                      | 42.4           | 9.41                                      |
|             | 10.b.w | Water curing | 13.5           | 3.225                 | 4.38                      | 19.2           | 4.0                                       |
|             | 10.n.w | Water        | 12.5           | 3.604                 | 8.91                      | 44.8           | 8.5                                       |
|             | 10.a.N | Nutrient     | 9              | 3.608                 | 8.98                      | 68.8           | 8.0                                       |
|             | 10.b.N | Nutrient     | 12.5           | 3.645                 | 9.58                      | 50             | 9.2                                       |
|             | 10.n.N | Nutrient     | 11.5           | 3.626                 | 9.27                      | 39.2           | 8.6                                       |

10.a.w, 10.b.w, 10.n.w – 10ml water cured cubes subjected to acidic, basic and neutral pH
10.a.N, 10.b.N, 10.n.N – 10ml nutrient cured cubes subjected to acidic, basic and neutral pH

### Table 5. Test results of mortar cubes with 15ml bacterial solution cured for 7 & 28 days

| Curing days | Sample | Curing media | Hardness value | Pulse velocity (km/s) | Compressive strength (MPa) | Crack Load (kN) | Compressive strength after healing (MPa) |
|-------------|--------|--------------|----------------|-----------------------|---------------------------|----------------|------------------------------------------|
| 7 days      | 15.a.w | Water        | 16.4           | 3.153                 | 3.8                       | 24             | 3.0                                      |
|             | 15.b.w | Water curing | 21.8           | 3.167                 | 3.9                       | 54.4           | 3.2                                      |
|             | 15.n.w | Water        | 22             | 3.181                 | 4.0                       | 59.2           | 3.2                                      |
|             | 15.a.N | Nutrient     | 17.3           | 3.349                 | 5.58                      | 4              | 5.0                                      |
|             | 15.b.N | Nutrient     | 19.3           | 3.500                 | 7.39                      | 23.2           | 7.0                                      |
|             | 15.n.N | Nutrient     | 14.8           | 3.365                 | 5.75                      | 32             | 4.5                                      |
| 28 days     | 15.a.w | Water        | 10.5           | 3.589                 | 8.68                      | 40.3           | 8.4                                      |
|             | 15.b.w | Water curing | 10.5           | 3.645                 | 9.58                      | 48.8           | 8.8                                      |
|             | 15.n.w | Water        | 8              | 3.645                 | 9.58                      | 48             | 9.0                                      |
|             | 15.a.N | Nutrient     | 10             | 3.349                 | 5.58                      | 10             | 5.0                                      |
|             | 15.b.N | Nutrient     | 20.8           | 3.286                 | 4.94                      | 36.8           | 3.6                                      |
|             | 15.n.N | Nutrient     | 15.5           | 3.365                 | 5.75                      | 24             | 5.2                                      |

15.a.w, 15.b.w, 15.n.w – 15ml water cured cubes subjected to acidic, basic and neutral pH
15.a.N, 15.b.N, 15.n.N – 15ml nutrient cured cubes subjected to acidic, basic and neutral pH
Figure 7. Comparative chart of compressive strength before and after healing.

Table 6. %Regain in strength values obtained from comparative study of compressive strength result.

| Sample | Compressive strength before cracking | Compressive strength after healing | %Regain in strength |
|--------|--------------------------------------|-----------------------------------|---------------------|
|        | 7days  | 28days  | 7 days  | 28 days  | 7days  | 28days  |
| 5.a.w  | 5.58   | 6.5     | 4.8     | 6.3      | 86     | 96.9    |
| 5.b.w  | 8.98   | 5.75    | 7.9     | 5        | 87.9   | 86.9    |
| 5.n.w  | 8.98   | 9.27    | 7.2     | 9        | 80.2   | 97.0    |
| 5.a.N  | 8.98   | 10.6    | 8.2     | 9.8      | 91.3   | 92.4    |
| 5.b.N  | 8.98   | 8.98    | 8       | 8.4      | 87.7   | 93.5    |
| 5.n.N  | 10.6   | 10.6    | 9.4     | 10       | 88.6   | 94.3    |
| 10.a.w | 8.68   | 10.6    | 7.6     | 9.4      | 87.7   | 88.7    |
| 10.b.w | 8.98   | 4.38    | 7.9     | 4.0      | 87.9   | 91.3    |
| 10.n.w | 8.98   | 8.91    | 8.0     | 8.5      | 89     | 95.3    |
| 10.a.N | 6.7    | 8.98    | 6.0     | 8.0      | 89.2   | 89.0    |
| 10.b.N | 7.15   | 9.58    | 7.0     | 9.2      | 97.9   | 96.0    |
| 10.n.N | 6.93   | 9.27    | 6.2     | 8.6      | 97.2   | 92.7    |
| 15.a.w | 3.8    | 8.68    | 3.0     | 8.4      | 78.9   | 96.7    |
| 15.b.w | 3.9    | 9.58    | 3.2     | 8.8      | 82     | 91.8    |
| 15.n.w | 4.0    | 9.58    | 3.2     | 9.0      | 80     | 93.9    |
| 15.a.N | 5.58   | 5.58    | 5.0     | 5.0      | 89.6   | 89.6    |
| 15.b.N | 7.39   | 4.94    | 7.0     | 3.6      | 94     | 72.8    |
| 15.n.N | 5.75   | 5.75    | 4.5     | 5.2      | 78.2   | 90.4    |

From table 3, 4 and 5 it is clear that for 5ml bacterial solution added mortar cubes, the strength increases with increase in curing days for the basic condition. It is indicative of the fact that the
samples which use 5ml solution in self-healing mortars are highly efficient under alkaline conditions. In the case of mortar cubes with a 10ml bacterial solution, an increase in strength is observed for 28 days curing when compared to 7 days. However, under alkaline conditions, there is a considerable decrease in hardness, strength and crack load. Hence they can be made use of under acidic and normal conditions.

Basic condition is favoured for mortar cubes with 15ml megaterium solution. It should be mentioned that a significant decrease in strength and hardness properties are in comparison with the other two. Therefore with the change in curing media and environmental conditions, there is considerable variation in relevant properties depending on the amount of bacterial solution added. As shown in figure 7 and table 6 the compressive strength of cubes before cracking were compared with the cubes after healing the cracks generated on it by the application of a suitable crack load. From, the studies conducted earlier there is a significant increase in properties of bacteria-induced mortar cubes cured in both media to that of control specimens.

3.5. Calcite Identification
An XRD analysis was employed for the identification and determination of the crystalline form of the CaCO3 crystals precipitated. From the results of XRD pattern obtained below shown in figure 8, it is clear and evident that the white precipitates obtained were calcite. The XRD spectra were obtained with a Cu anode (40kV and 40mA) with a scanning speed of 2°/min. The results from XRD analysis confirms maximum number of calcite peaks which is consistent with the previous reports [19], [20]. The peaks are observed in the range of an angle 2θ (Bragg's angle) 30°. Calcite peaks had even observed at an angle ranging above 35°.

![Figure 8. XRD (X-ray diffraction) pattern of the precipitates.](image)

4. Conclusion
From, the study of the effect of pH on self-healing mortar it is clear that with a change in pH condition the properties and healing mechanism of mortar cubes varies. With the use of Bacillus megaterium - a crack healer as an additive in mortar cube, there is a considerable improvement in the strength property though they were exposed to varying pH conditions. All the preliminary studies reveal the compatibility of bacterial species which survives in an alkaline environment to mortar rich
environment which adds alkalinity of the cement mortar bacterial mix as a whole. So when they are exposed to acidic and other neutral conditions the alkalinity still exists and a pH ratio is stabilized by accelerating the growth of bacterial species. The experimental results suggested that it’s better to use 5ml of the solution in self-healing mortars under alkaline conditions than 10ml and 15ml. Increase in strength with curing days varies with the amount of bacteria in the cubes, pH condition, curing media to which the cube is exposed etc. That is for 5ml bacterial solution added mortar cubes, strength increases with increase in curing days under basic condition. Whereas, for 10ml, strength increases for 28days curing, but under alkaline conditions, there is a considerable decrease in hardness, strength and crack load. Thus 10ml induced bacterial mortar cubes confirm its application more in acidic and normal conditions, while 5ml and 15ml more favoured in an alkaline environment. Also, there is no considerable reduction in the compressive strength of mortar cubes after healing compared to the one before cracking. Further the calcite confirmation made from the XRD analysis shows a maximum number of peaks at calcite range.

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