Investigation of cement mortar incorporating *Bacillus sphaericus*

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**ABSTRACT**

Ureolytic-type bacteria has been used to improve the strength of cement mortar by the precipitation of calcium carbonate. In the present study *Bacillus sphaericus* has been used to improve the properties of cement mortar such as setting time, compressive strength and sorptivity. The setting time is found to be unaffected by the presence of bacteria. It is found that compressive strength at both 7-days and 28-days of mortar cube increases with the increase of bacteria concentration. At the optimum bacteria dosage of $10^7$ cells/ml, the average compressive strength increases by 58% (at 7 day) and 23% (at 28 day) over the control specimen. The sorptivity coefficient decreases as the concentration of bacterial cells increases. The mineralogy and morphology of the calcium carbonate precipitation have been tested by XRD and FESEM.

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bacteria; setting time; compressive strength; calcite precipitation; sorptivity

1. Introduction

The usage of cement is increasing exponentially and the requirement of stronger and durable structures is also increasing. Global demand for hydraulic cement is forecasted to increase 4.5% yearly to 5.2 billion metric tons by 2019 [1]. As the cement production increases, the emission of CO$_2$, a major greenhouse gas, increases. In such a scenario, any technology that would reduce the consumption of cement can contribute significantly to the health of the environment.

In the present study *Bacillus sphaericus* (*B. sphaericus*), a non-contagious ureolytic bacteria, is used to improve the strength and durability of cement mortar (thereby reducing the consumption of cement) through bio-mineralization. Bio-mineralization is defined as a biologically induced mineral precipitation in which an organism creates a local micro-environment with conditions that allow optimal extracellular chemical precipitation of mineral phases. The microbial precipitation of CaCO$_3$ is determined by several factors including: the concentration of dissolved inorganic carbon, the pH, the concentration of calcium ions and the presence of nucleation sites. The first three factors are provided by the metabolism of the bacteria while the cell wall of the bacteria will act as a nucleation site. The bacteria produces urease, which catalyzes the hydrolysis of urea (CO(NH$_2$)$_2$) into...
ammonium (NH$_4^+$) and carbonate (CO$_3^{2-}$). First, 1 mol of urea is hydrolyzed intracellular to 1 mol of carbamate and 1 mol of ammonia (Equation 1). Carbamate spontaneously hydrolyzes to form additionally 1 mol of ammonia and carbonic acid (Equation 2). These products subsequently form 1 mol of bicarbonate and 2 mol of ammonium and hydroxide ions (Equations 3 and 4). The last two reactions give rise to a pH increase, which in turn shifts the bicarbonate equilibrium, resulting in the formation of carbonate ions (Equation 5):

$$\text{CO(NH}_2\text{)}_2\text{H}_2\text{O} \rightarrow \text{NH}_2\text{COOH} + \text{NH}_3$$  \hspace{1cm} (1)

$$\text{NH}_2\text{COOH} + \text{H}_2\text{O} \rightarrow \text{NH}_3 + \text{H}_2\text{CO}_3$$  \hspace{1cm} (2)

$$\text{H}_2\text{CO}_3 \leftrightarrow \text{HCO}_3^- + \text{H}^+$$  \hspace{1cm} (3)

$$2\text{NH}_3 + 2\text{H}_2\text{O} \rightarrow 2\text{NH}_4^+ + 2\text{OH}^-$$  \hspace{1cm} (4)

$$\text{HCO}_3^- + \text{H}^+ + 2\text{NH}_4^+ + 2\text{OH}^- \rightarrow \text{CO}_3^{2-} + 2\text{NH}_4^+ + 2\text{H}_2\text{O}$$  \hspace{1cm} (5)

Since the cell wall of the bacteria is negatively charged, the bacteria draw cations from the environment, including Ca$^{2+}$, which are deposited on their cell surface. The Ca$^{2+}$ ions subsequently react with the CO$_3^{2-}$ ions, leading to the precipitation of CaCO$_3$ at the cell surface that serves as a nucleation site (Equations 6 and 7):

$$\text{Ca}^{2+} + \text{Cell} \rightarrow \text{Cell} - \text{Ca}^{2+}$$  \hspace{1cm} (6)

$$\text{Cell} - \text{Ca}^{2+} + \text{CO}_3^{2-} \rightarrow \text{Cell} - \text{CaCO}_3 \downarrow$$  \hspace{1cm} (7)

The schematic diagram showing the mechanism of calcite precipitation is given in Figure 1.

Microbial mineral precipitation using ureolytic bacteria is reported to improve the overall behavior of concrete including strength and durability [3–13]. Bacteria can be used externally as a healing agent on hardened concrete for sulfate treatment [14]. The microbial induced precipitation can resist the carbonation and chloride ingress in concrete [10].

Bio-mineralization has also been used as an alternative and environmental friendly crack repair technique [15–17]. Although most of the previous studies are focused on concrete, the effect of bio-mineralization can be understood more precisely in cement mortar. However, published literature on the effect of bacteria on the properties of cement mortar is limited [16].

The present study evaluates the effect of incorporating B. sphaericus on the setting time of fresh cement mortar, and on compressive strength and sorptivity of hardened cement mortar. The mineralogy and morphology of the hardened cement mortar have been investigated using XRD and FESEM.
2. Experimental program

2.1. Materials

Portland slag cement conforming to Indian Standard IS: 455[18] having a specific gravity of 2.92 and normal consistency of 32% has been used in this study. Locally available well-graded, clean river sand, with specific gravity 2.59 conforming to Indian Standard IS 383–1970, has been used as fine aggregate.

The behavior of bacteria is important as it must survive in the warm and alkaline-type environment of cement mortar. Fresh cement mortar has a pH of 11.5–13 and a higher temperature because of heat of hydration. A number of bacterial species have been reported in literature which improve different properties of concrete and cement mortar. However, the present study uses a non-contagious bacteria that can survive in the cement-like alkaline environment and is capable of producing calcium carbonate through metabolism. Bacillus cereus was selected at the beginning of this study, but it was discarded as it failed the test of pH and temperature tolerance. Nutrient medium (Luria Bertani broth) was prepared for the culture of bacteria. It was then transferred to 12 fresh clean test tubes and desired pH level (8, 9, 10, 11, 12 and 12.5) of the medium was obtained by adding NaOH. After the preparation of medium, the test tubes were sealed with cotton plug and sterilized using autoclave. After autoclaving, the bacteria specimen from the mother culture was scraped and added to the test tubes, mixed well, and incubated for 24 hours at 37ºC and 50ºC.

After incubation for 24 hours at different temperatures and pH, the growth of the bacteria was tested in each of the 12 test tubes by checking turbidity of the solution. Table 1 shows the temperature and pH tolerance of B. cereus and B. sphaericus in the cultures incubated. It can be seen from the table that B. Sphaericus could survive in the pH range of 8–12.5 at both 37ºC and 50ºC. Therefore, it can be concluded that
**Table 1. Temperature and pH tolerance of Bacillus cereus and Bacillus sphaericus bacteria.**

| pH  | Presence of Bacillus cereus | Presence of Bacillus sphaericus |
|-----|----------------------------|--------------------------------|
|     | Cultures incubated at      | Cultures incubated at           |
|     | 37°C                      | 50°C                           |
| 8   | +                         | −                              |
| 9   | +                         | −                              |
| 10  | −                         | +                              |
| 11  | −                         | +                              |
| 12  | −                         | +                              |
| 12.5| −                         | +                              |

+ presence of bacteria; – absence of bacteria.

*B. sphaericus* is suitable for fresh cement mortar (or concrete) with pH of about 11.5–12.5 and temperature of 37–50°C.

### 2.2. Analytical techniques

#### 2.2.1. CPA test for CaCO₃ precipitation in agar plate state

In order to confirm that the *B. sphaericus* is capable of producing calcium carbonate, a calcite precipitation agar (CPA) test [19] was undertaken. CPA is a solid medium for screening of bacterial precipitation of calcium carbonate. 0.6 g of nutrient broth, 5.7 g of CaCl₂, 0.424 g of NaHCO₃, 2.0 g of NH₄Cl, 3.0 g of agar, and 190 ml of distilled water were taken in a 200 ml conical flask. All media components were autoclaved. After autoclaving, urea was added to the medium. 20 µl of broth culture was inoculated in the center of a plate, and then incubated at 30°C for 6 days. After 6 days, calcium carbonate was found in the plate as white patches and thus it is inferred that *B. Sphaericus* is capable of producing calcium carbonate in the conditions described above.

#### 2.2.2. Preparation of cell culture for bacterial mortar cubes

Having selected a *Bacillus* bacteria and confirming its capability to precipitate calcium carbonate, the following procedure was used for cell culture for further studies.

1. 500 ml of Luria Bertani broth was prepared in two fresh clean conical flasks of 1 l.
2. After autoclaving the nutrient medium, bacteria was inoculated into it and incubated for 24 hours.

The above bacteria culture was tested for cell concentrations using optical density test, which is based on the light scattering principle. The cell concentration at 600 nm was determined as per McFarland’s standards. The cell culture was diluted to the required concentrations and added to the water used for making cement mortar.

#### 2.2.3. Mortar specimens for compressive strength

As one of the objectives of the study is to observe the variation of compressive strength of mortar cubes with various concentrations of *Bacillus* bacteria, a cement-to-sand ratio of 1:6 and water-to-cement ratio of 0.55 were considered to prepare the mortar cubes. Accordingly, the amount of cement, sand, and water were calculated as shown in Table 2. Mortar cubes were prepared by mixing the water containing the cell culture
in selected concentrations, with the cement and sand. These mortar cubes are referred to in this study as bacterial mortar cubes. The bacterial mortar cubes are represented by the prefix ‘B’, as shown in Table 2. The bacterial cubes were cured in 2% urea (in water) and 25 mM CaCl$_2$ per ml of curing water. Mortar cubes without bacteria are referred to as control cube specimens. Control cubes, denoted by CT (see Table 2), are cured with normal tap water. In order to study the effect of curing medium on control specimen, a set of control cubes were cured using 2% urea and 25 mM CaCl$_2$ per ml of curing water. This set is referred to as CUC. Cubes were cast in triplicates and compacted in a vibration machine. After demolding, all specimens are cured until the testing.

### 2.2.4. Mortar specimens for sorptivity test

Sorptivity ($S$) is a property that characterizes the tendency of a porous material to absorb and transmit water by capillarity. The capillary water absorption is called sorptivity, which can be considered as a measure of the durability of cement mortar. This can be determined by the measurement of the capillary rise absorption rate on reasonably homogeneous material. Sorptivity can be measured by various methods – the two main methods are mass method and volumetric method. In the present study mass method is used to measure the sorptivity of mortar. The cumulative water absorption (per unit area of the inflow surface), increases proportionally with the square root of elapsed time ($t$) as follows [20]:

$$ I = S \sqrt{t} $$

(1)

$$ S = \frac{I}{\sqrt{t}} $$

(2)

where $I$ is cumulative water absorption per unit area (mm$^2$) of inflow surface; $S$ is sorptivity in (mm), $t$ is elapsed time in minutes.

The cumulative water absorption per unit area ($I$) of the inflow surface can be calculated as follows:

$$ I = \frac{\Delta w}{A \times d} $$

(3)
where $\Delta w$ represents change in weight (g) of the cube after the elapse time $= w_2 - w_1$; $w_1$ is the oven dry weight of cube in grams; $w_2$ is the weight of cubes after capillary suction of water during the time period ‘t’ in minutes, $A$ is the surface area (mm$^2$) of the specimen through which water penetrates; and $d$ is the density of water in g/cm$^3$. The specimens were cured for 7 days and then dried in oven at a temperature of 100°C for a period of 24 hours. After drying in oven, the flow from the peripheral surface of the cubes was prevented by sealing it properly with non-absorbent coating (knife putty filler). The cubes were immersed in the water (Figure 2), with water level not more than 5 mm from the bottom of the cube after the filler dried out. The quantity of water absorbed in 0.5, 1, 2, 4, 6, 12, 24, 36, and 48 hours was measured by weighing the specimen using a weighing balance with a precision of 0.1 g. Surface water on the specimens was wiped with a dampened tissue and each weighing operation was performed within 30 seconds.

2.2.5. Characterization studies
In order to understand the effect of bacteria on the microstructure of mortar, the following techniques were used in the present study: spectroscopy, X-ray powder diffraction (XRD), and field emission scanning electron microscope (FESEM). XRD analysis gives information of various compounds present in the mortar sample including calcium carbonate. Calcium carbonate can exist in three polymorphic forms: calcite, aragonite, and vaterite. Calcite is the most stable and the least soluble one among the three [21]. XRD spectra in the present study was obtained by using the spectrometer model PW 3040/00 with a Cu anode (30 kV and 20 mA) and semi-angle range of 10°–80°. X-ray diffraction is based on the fact that the intensity of a diffraction peak is directly proportional to the compound present in the sample [8]. A small amount of mortar powder sample was collected and sieved through 100 µm IS sieve and was tested in XRD.

Morphological, qualitative, and semi-quantitative analyses of the deposited CaCO$_3$ crystals were carried out with scanning electron microscopy coupled with energy dispersive X-ray. A small amount of sample was collected from the core of the cube used for compressive strength testing. The samples were gold coated with a fine coater prior to examination for 5 min. Using a Nova NanoSEM/FEI machine, FESEM images for control
and bacterial specimens were obtained with the same magnification for comparison of their microstructure.

3. Results and discussion

3.1. Tests on fresh cement mortar

Standard tests on cement were conducted for the consistency of the cement as per Indian standard IS 4031 Part 4 [22]. The consistency of the cement paste was found to be 32%. In order to check the effect of bacteria in the initial and final setting time of the cement, standard tests for setting time were conducted on the cement mortar as per Indian standard IS 455 [18].

Previous literature on the study of the influence of bacteria on the setting time is very limited. However, no study has been reported on the initial and final setting time of cement by using *B. sphaericus*. Initial and final setting times of cement paste with and without bacteria were recorded and are presented in Table 3. The initial setting time of mortar with inclusion of bacteria is 50 minutes, which is marginally lower than normal mortar (52 minutes). Similarly, final setting time of mortar with bacteria is 6 hours 7 minutes while for normal mortar it is 6 hours. It can be seen from the table that the effect of bacteria on the setting time is very negligible.

3.2. Variation of compressive strength for different cell concentrations

The cubes were tested in a load-controlled universal testing machine to obtain the compressive strength at 7 days and 28 days. The compressive strengths of all the specimens are presented in Table 4. It can be observed from the table that as the cell concentration increases, the compressive strengths at both 7 days and 28 days also increase initially and then decrease. It can be seen that the maximum percentage increase in compressive strength of about 58% is observed (at 7 days) in the mortar cube having a cell concentration of $10^7$ cells/ml. The variation of the compressive

| Table 3. Comparison of setting time of cement. |
|-----------------|-----------------|-----------------|
| Specimen                  | Initial setting time (minutes) | Final setting time |
|-----------------|-----------------|-----------------|
| Cement paste (Normal) | 52               | 6 hours 00 minutes |
| Cement paste (with bacteria) | 50               | 6 hours 07 minutes |

| Table 4. Compressive strength of mortar cubes with different bacteria concentrations. |
|-----------------|-----------------|-----------------|-----------------|
| Mortar cube ID | Cell concentration (cells/ml) | Mean compressive strength (% increase) at 7 days (MPa) | Mean compressive strength (% increase) at 28 days (MPa) | Remark |
|-----------------|-----------------|-----------------|-----------------|-----------------|
| CT               | 0 (Control)     | 3.44             | 5.90             |               |
| CUC              | 0 (Control)     | 3.01 (~14%)      | 5.67 (~4%)       |               |
| B1               | $10^6$          | 4.46 (29.70%)    | 6.98 (18.30%)    |               |
| B2               | $10^6$          | 5.34 (55.23%)    | 7.02 (18.98%)    |               |
| B3               | $10^7$          | 5.44 (58.23%)    | 7.28 (23.38%)    | Maximum compressive strength |
| B4               | $10^8$          | 4.91 (42.73%)    | 6.19 (4.90%)     |               |
| B5               | $10^9$          | 4.71 (36.90%)    | 6.10 (3.38%)     |               |
strength at 7 day and 28 day is also expressed graphically in Figure 3. The maximum strength at 28 days is about 23% over the control specimen and this occurs at a cell concentration of about $10^7$ cells/ml and hence this cell concentration can be treated as optimum dosage to obtain maximum compressive strength.

Maximum percentage increase in compressive strength in a previous study shows similar results. Ghosha et al. [23] reported that the maximum improvement in compressive strength of mortar at 28 day is about 25% with $10^5$ cells/ml by using *Bacillus pasteurii*. Achal et al. [24] reported increase in compressive strength of mortar cube as about 23% and 36% at 7 days and 28 days, respectively, by adding *Bacillus sphaericus*. An increase of about 18% in strength of mortar at 28 days is reported by Ramachandran et al. [25] by using *B. pasteurii*. Ramachandran et al. [25] found that in initial days of curing, bacteria shows good nourishment due to porosity of mortar cubes, and as the mortar hardens, pores are blocked, which reduces the flow of nutrient, leading to death of bacteria cells.

The compressive strength at 28 days of the specimen CUC, cured using 2% urea and 25 mM CaCl$_2$ per ml (curing solution) of curing water, is found to be about 5.67 MPa. The compressive strength of CT specimen, cured with normal tap water at 28 days, is about 5.9 MPa. The change in compressive strength due to curing solution is found to be negligible. Negligible difference in compressive strength implies that curing solution alone has no significant effect on the specimen. The increase in compressive strength in other bacterial specimens (B1, B2, B3, B4, and B5) is due to the bacterial activity in the presence of curing solution (2% urea and 25 mM CaCl$_2$ per ml of curing water).

### 3.3. Increase in compressive strength for different cell concentrations reported in previous studies

Table 5 displays a comparison of percentage increase in compressive strength of mortar in the present study with respect to previous studies in terms of the mortar mix, type of bacteria, and cell concentrations. Ramachandran et al. [25] and Ghosha et al. [23] used
Bacillus pasteurii while the present study used Bacillus sphaericus. At different values of cell concentration (cells/ml) such as $10^5$, $10^6$, $10^7$, $10^8$, and $10^9$, the percentage increases in compressive strength at 7 days and 28 days are presented in Table 5. From this limited number of test results, it can be seen that Bacillus sphaericus could improve the compressive strength at 7 days by about 58.2%, against the 17.0% reported by Ramachandran et al. [25]. The maximum improvement in the compressive strength at 28 days due to Bacillus sphaericus is about 23.4%, which is marginally lower than the maximum strength, 25.3%, achieved by Ghosh et al. [23].

Studies on the effect of bacteria in the presence of supplementary cementious materials such as fly ash or silica fume show that the maximum improvement in compressive strength of concrete cubes with addition of 10% silica fumes and 10% fly ash is about 23% at a concentration of $10^5$ cells/ml of Sporosarcina pasteurii bacteria [8]. Maximum improvement in the compressive strength of concrete cube is about 22% using S. pasteurii [26]. With the addition of fly ash content of 10%, 20%, and 30%, improvements in compressive strength of 20%, 15%, and 11%, respectively, is observed at a concentration of $10^5$ cells/ml. However, to draw general conclusions on this aspect requires further studies in this direction.

### Table 5. Comparison of percentage increase in compressive strength on mortar cubes w.r.t. previous studies.

| Parameter                  | Percentage increase at 7 days compressive strength | Percentage increase at 20 days compressive strength |
|----------------------------|---------------------------------|----------------------------------|
| Cement to Sand ratio       | 4:11               | 1:3                              | 1.6               | 4:11               | 1:3                | 1:6               |
| Type of bacteria           | Bacillus pasteurii   | Bacillus pasteurii              | Bacillus sphaericus   | Bacillus pasteurii | Bacillus pasteurii   |
| Cell concentration (cells/ml)$10^5$ | --                     | 16.7                             | 29.70              | --                 | 25.3               | 18.3               |
| Cell concentration (cells/ml)$10^6$ | --                     | 9.5                              | 55.23              | --                 | 14.7               | 19.0               |
| Cell concentration (cells/ml)$7.6 \times 10^8$ | 6.4                       | --                              | No change          | --                 | --                 |
| Cell concentration (cells/ml)$7.6 \times 10^9$ | 17.0                     | --                              | No change          | --                 | --                 |

3.4. Sorptivity of bacteria mortar cubes

Sorptivity test is conducted for both control and all bacterial specimens (B1, B2, B3, B4, and B5). The cumulative water absorption versus square root of time in hours ($\sqrt{t}$) for all bacterial specimens were computed and are presented as a plot in Figure 4. From the durability point of view, sorptivity coefficient should be minimum. Sorptivity coefficient, $S$ (mm/h $0.5$), the slope [27] of the trend lines, for all the specimens can be seen in Figure 4 and have been listed in Table 6. It can be seen that sorptivity coefficient is minimum (0.79) for B3, where bacteria concentration is about $10^7$ cells/ml. In order to understand the correlation between the compressive strength and the sorptivity coefficient, a graph is plotted between the two
parameters in Figure 5. It can be seen that, as the sorptivity coefficient decreases, the compressive strength increases in a linear fashion. The lesser values of sorptivity coefficient imply that concrete is denser. This may be due to sealing of the pores by carbonation, which in turn increase durability. Similar observations were also reported elsewhere [27].

3.5. X-ray diffraction (XRD) spectrometry

The addition of bacteria into the mortar cubes resulted in the formation of calcite crystals. The intensities of various compounds have been plotted for both bacterial and control specimens in Figure 5. It can be seen in Figure 5 that the number of calcite peaks (nine numbers of ‘C’) is higher in bacterial mortar cube sample and lower in control sample (four numbers of ‘C’). The increase in number of peaks signifies that the presence of calcite is greater in bacterial cubes than in control cubes. The crystallinity of the calcium carbonate is also found out. Similar conclusions were also reported elsewhere [26]. This increase in calcite content is perhaps responsible for the increase in compressive strength of bacterial mortar cubes.

3.6. XRD of thin layer deposited in curing solution

Bacterial cube specimens were kept in curing solution (2% urea and 25 mM CaCl₂ per ml). A white precipitation was observed to be formed as a layer over the surface of curing solution, which can be seen in Figure 6. This layer was collected and dried to obtain a white powder. The XRD analysis of this layer was carried out to understand its mineralogy, and the intensities of various compounds were obtained from XRD analysis,
which are presented in Figure 6. It can be seen that all the peaks obtained from the graph are of calcite. Thus it can be concluded that the calcite layer is produced by the bacteria. This layer is not observed in the curing solution with control specimen as there are no bacteria in the curing solution.

### 3.7. FESEM on mortar cubes

Figure 7 shows rod-shaped impression, which is consistent with the shape of *B. sphaericus* [8]. While Figure 8 shows the FESEM images of samples from control and bacterial
specimen, B4 for 7 days of curing, Figure 9 shows the images of the same samples for 28-day curing. White patches of calcite crystals can be observed in these images and it can be seen that the amount of calcite crystals is more in the bacterial samples than in...
the control samples. The presence of the crystalline calcite is perhaps the reason for the improvement of compressive strength.

To check the continuous variation in the microstructure of the cement mortar due to the presence of bacterial calcite precipitation, analysis of FESEM for 7, 14, and 28 days were carried out. Figures 10(a–c) show the FESEM images of mortar cubes (B4) after 7, 14, and 28 days curing, respectively. It can be seen that needle-shaped structures are more in concentration and growing as the number of days of curing increases and are less when it reaches a curing period of 28 days. The concentration of lamellar rhombohedral structures increases as the curing period increases.

4. Conclusions

The important conclusions drawn from the above study are as follows:

(i) It is observed that *Bacillus sphaericus* can survive in alkaline concrete-like environment and can produce calcium carbonate better in comparison to *Bacillus cereus*. Addition of bacteria alone cannot improve the properties of concrete/cement mortar. Ureolytic bacteria requires urea and a source of calcium to produce CaCO$_3$.

(ii) Compressive strength (at 7 days and at 28 days) of mortar cube is found to be increasing with the increase of bacteria cell concentration up to $10^7$ cells/ml.
However, it is found that further increase of bacteria concentration reduces the compressive strength of cement mortar.

(iii) The optimum doses of bacteria is found to be $10^7$ cells/ml and the corresponding increments of average compressive strength are observed to be 58% and 23% at 7 days and 28 days, respectively.

(iv) Sorpitivity coefficient decreases as the concentration of bacteria increases, which increases the durability of the specimen. The minimum sorpitivity coefficient is obtained for a cell concentration of $10^7$ cells/ml.

(v) *Bacillus sphaericus* bacteria is found to be not altering the setting times of the cement paste.

(vi) The XRD study shows the presence of more calcite peaks in bacterial mortar sample at 28 days than the control specimen.

(vii) A layer is observed over curing water of bacterial specimen after a few days, which is not observed on the curing solution with control specimen. The XRD analysis confirmed that it is of calcite produced by bacteria, which is responsible for the improved compressive strength.

(viii) The morphology of the bacterial calcite is studied by FESEM. The direct involvement of bacteria in calcite production can be inferred by rod-shaped impressions, which is consistent with the dimensions of the bacteria on the calcite crystals.

**Disclosure statement**

No potential conflict of interest was reported by the authors.

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