Investigating the effect of biological material on the property of concrete

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Abstract. Biomineralization is an innovative technology adopted in the area of construction field. Biomineralization method involves use of biological material to improve the property of the concrete structure. The present study investigated the effect of prepared biological material of Bacillus sparcisus strain introduced into fly ash concrete as a new approach on its durability and mechanical property. Mechanical and durability property of bacterial blended fly ash concrete were studied at cell concentration of $10^5$ cells/ml with varying concentration of bacterial solution to water. Test results revealed that, in comparison with conventional samples, those containing alkaliphilic endospore-forming bacteria material cured in tap water, recorded increase in compressive strength up to 30 % and decrease of durability property to 30 %. The ratio of bacteria solution to water played important role in improving the properties of concrete. At higher ratio, calcite precipitation by bacteria resulted in improvement of properties of concrete. Scanning electron microscope and XRD tests were performed to confirm the formation of calcite in the opening spaces of concrete.

Keywords: Bio-material, Bacillus sphericus, Compressive strength, Water absorption, SEM observations

1. Introduction

Owing to higher mechanical strength, good durability property and ease handling of concrete material, it has become an important and commonly used construction material globally [1]. Worldwide cement is the first choice as concrete material since it is strong and easily available [2]. In coming future, devolvement in the area of civil engineering is going to increase. Concrete is the back bone of concrete material. The life of concrete depends on mechanical and durability of concrete. Every year 60 billion tons of constructional materials is being utilized globally. The key material in preparation of concrete is cement which is manufactured in large scale [3]. 5000 million tons of cement was manufactured in the year 2014. Cement industry requires large of energy compared to other industry. Cement unit also is known to contribute of around 7% CO$_2$ discharge into the surroundings. One of the ways to reduce CO$_2$ discharge is use of supplementary cementing materials. Fly-ash, ground granulated blast furnace slag (GGBFS), cement kiln dust, rice husk ash (RHA) are commonly being successfully used in cement manufacturing process [4]. Use of these materials contributes to reducing CO$_2$ discharge from cement industry. Due to drawbacks of cement manufacturing cement cannot be termed as a sustainable material [2]. Several researches is going on to cut down on the use of cement being used. To bring sustainability in concrete SCM is used as a partial replacement for cement. [5]. Another
major limitation encountered in concrete is cracking due to multiple reasons such as autogenous shrinkage, freeze-thaw reactions, mechanical compressive and tensile forces [6]. Very fine cracks may not directly affect the strength of concrete [7] but will lead to movement of fluid particles (water and other chemical solutions) resulting in concrete cement matrix degradation and consequently weakens the reinforcement in concrete [8]. To mitigate this problem and to bring sustainability in construction materials, biological concept was adopted. Biological concept (biological concrete) is defined as biomineralization process which involves use of metabolic pathways of bacteria for the formation of calcite [9]. Biomineralization tool is being adopted as a biological method in producing bio-concrete. This is proving to be sustainable method which overall improves property of concrete [10]. Biomineralization process is employed as a bio-cementation technology which is emerging as a sustainable method to heal cracks in concrete, improve concrete property [11, 12]. The calcite materials get deposited in the pores of concrete to heal cracks which reduces porosity of concrete [13].

In literature different variety of bacteria has been reported for incorporation in the concrete formulation for crack reparation and property enhancement. Most scientists focus on selection of ureolytic bacteria to improve CaCO$_3$ precipitated capacity. Ureolytic bacteria secretes urease enzyme (biological catalyst) which helps in precipitation of calcium carbonate precipitation [14]. The application of bacteria in cement based materials can be cost-effective and environment-friendly alternative method to improve the service life of buildings. Various studies have explored biological tool as a research domain to repair cracks in concrete, surface treatment and enhancement in the properties of concrete. Ramakhrisnan [15] and his team conducted experimental investigation on bioconcrete. The potential of $B$. pasteurii in improving the strength was assessed. The presence of bacteria promoted strength by around 25% to 30% at the curing of 28 days. Ramachandran et al [16] and his colleagues carried out the work on biomineralization work using spore-forming bacteria, Sporosarcina pasteurii which produces calcite through various biochemical reactions. Luo [17] assessed the ability of biological method in self-healing concrete. Study was conducted at various depth and at age different of concrete. Authors in their conclusion mentioned that that microbial self-healing agent can meet the goal of concrete crack self-healing. Sahoo [18] worked on recycled coarse aggregate concrete (RCAC) to improve upon the property of concrete by biological method. Strain of $B$. Subtilis was used for their work. The kinetics study was done to select the desired dosage. Bacterial RCAC concrete gave around 19% improved strength compared to normal concrete. SEM showed more crystalline calcium carbonate in RCAC bacterial embedded concrete. XRD images showed more crystals of calcite with higher intensity in bacterial concrete. Balam [19] and his team carried our research work to reduce concrete porosity and water absorption of lightweight aggregate concrete. The authors reported that biological techniques are environmental friendly which reduces opening spaces in concrete there by gaining strength and decrease in fluid movement in concrete. Qiu et al [20] investigated the potential of Sporosarcina pasteurii, for the treatment of recycled concrete aggregates. $E$. coli strain were used by ghosh et al [21] in improving the strength of mortar specimens. The authors mentioned greater strength improvement was around 25% for 28 days of curing blended with bacteria at cell dosage of $10^5$ cells ml$^{-1}$. However no major effect was reported by the use of $E$. coli bacteria. In conclusion it was reported that key parameter for successes of bio-mineralization is appropriate selection of bacteria.

The current study focuses on the optimization of the fly-ash (FA) content and biological material in concrete to achieve superior mechanical and durability properties of concrete. The experimental work is designed to evaluate the properties of conventional and bacteria (biological material) blended fly-ash concrete containing varying propositions of FA. The effect of bacteria solution (biological material) to water ratio on the properties of concrete is also assessed. In the final part, SEM and XRD analyses was done to confirm formation of calcite particles in the concrete structure.
2. Experimental programme

2.1 Materials
2.1.1 Cement and aggregates
The common materials employed in concrete are cement, fine and coarse aggregates. Portland cement of grade 43 was used for preparing concrete cubes. Natural river sand confirming to IS 383:1970 was used as fine aggregates. Coarse aggregates of size 20mm and specific gravity of 2.66 was used. For this research work FA was obtained from Raichur thermal power plant. Table 1 highlights the properties of cement and fly ash.

Table 1. Characteristics of materials employed for research work.

| Constituents | Portland cement (%) | Fly ash (%) |
|--------------|---------------------|-------------|
| SiO₂         | 22.0                | 61.9%       |
| Al₂O₃        | 4.71                | 27.0%       |
| Fe₂O₃        | 2.76                | 7.00%       |
| CaO          | 64.3                | 0.70%       |
| MgO          | 1.00                | 0.60%       |
| Na₂O         | 0.14                | 0.93%       |
| K₂O          | 0.61                | 0.90%       |
| LOI          | 0.90                | 0.80%       |
| Specific gravity | 3.15            | 2.30%       |

2.1.2 Bacterial strains.
The thrust of research for biological concrete is selection of appropriate bacteria. The key factor for selection of bacteria is survivable of bacteria inside concrete and the compatibility of bacteria with various types of materials as replacement for cement such as rice husk, cement kiln dust etc. Based on these criteria Bacillus sphaericus strain was selected for our research work. This is an alkaliphilic (alkali-resistant) spore-forming bacteria which can survive under high pH and have the ability to undergo dormant stage. Bacillus sphaericus can withstand high temperature up to 53 °C [Ganesh 22]. Oxygen is essential during bacteria growth and germination, but it is not mandatory essential factor for bacterial urea decomposition (Wang 2017). Bacteria for experimental work were obtained from National Collection of Industrial Microorganisms (NCIM), Pune.

2.2 Methods
2.2.1 Concrete specimens preparation and curing regimes.
The mix design for concrete was done in line with IS10262-2007. All the three materials (cement, sand and coarse aggregate) were taken in a right proportion for M25 grade of concrete (table 2). The fly ash percentage was varied from 10 to 30% as a replacement for cement. Bacillus sphaericus of cell concentration 10⁵ cells/ml was introduced with water at different ratio (0.2, 0.4 and 0.6) in dry mixture. Cell concentration of 10⁵ cells/ml was decided from my previous work. [23]. Experiments were conducted for ratio of bacterial solution to water ratio 0.2, 0.4 and 0.6. Plain concrete was prepared without addition of bacteria. All mixture was done in a mixer for duration of 8 min. During preparation of concrete cubes no additional supplements were added. The cubes were demoulded after one day and curing was done in tap water without addition of supplements for 7, 24, 56 and 90 days. The designations used for conventional specimens are normal concrete which refers to concrete without fly-ash and bacteria and control concrete refers to concrete with fly-ash and without bacteria. The tests outlined below shows the methods adopted to compare the biological fly-ash blended concrete specimens with the control ones (without bacteria) in terms of their mechanical properties and durability properties.
### Table 2. Mix proportions of concrete material

| Cement kg/m³ | Fine aggregates kg/m³ | Coarse aggregates kg/m³ | W/C ratio | Water kg/m³ | Fly ash replacement (%) | Bacteria solution to water ratio | Bacteria solution |
|--------------|-----------------------|-------------------------|-----------|-------------|--------------------------|-----------------------------|------------------|
| 440          | 796.4                 | 1315.6                  | 0.5       | 218         | 0                        | -                           | -                |
| 396          | 796.4                 | 1315.6                  | 0.5       | 218         | 10                       | -                           | -                |
| 352          | 796.4                 | 1315.6                  | 0.5       | 218         | 20                       | -                           | -                |
| 308          | 796.4                 | 1315.6                  | 0.5       | 218         | 30                       | -                           | -                |
| 440          | 796.4                 | 1315.6                  | 0.5       | 154         | 0                        | 0.3                         | 64               |
| 396          | 796.4                 | 1315.6                  | 0.5       | 154         | 10                       | 0.3                         | 64               |
| 352          | 796.4                 | 1315.6                  | 0.5       | 154         | 20                       | 0.3                         | 64               |
| 308          | 796.4                 | 1315.6                  | 0.5       | 154         | 30                       | 0.3                         | 64               |
| 440          | 796.4                 | 1315.6                  | 0.5       | 130         | 0                        | 0.4                         | 88               |
| 396          | 796.4                 | 1315.6                  | 0.5       | 130         | 10                       | 0.4                         | 88               |
| 352          | 796.4                 | 1315.6                  | 0.5       | 130         | 20                       | 0.4                         | 88               |
| 308          | 796.4                 | 1315.6                  | 0.5       | 130         | 30                       | 0.4                         | 88               |
| 440          | 796.4                 | 1315.6                  | 0.5       | 90          | 0                        | 0.6                         | 128              |
| 396          | 796.4                 | 1315.6                  | 0.5       | 90          | 10                       | 0.6                         | 128              |
| 352          | 796.4                 | 1315.6                  | 0.5       | 90          | 20                       | 0.6                         | 128              |
| 308          | 796.4                 | 1315.6                  | 0.5       | 90          | 30                       | 0.6                         | 128              |

#### 2.2.2 Compressive strength test.
Specimens of dimensions 150x150x150 mm were cast for M25 grade of concrete. Biological and conventional samples were cured for 14, 28, 56, 120 days. Strength of all the samples were done in accordance with IS 516:1959. Strength was determined using digital testing machine of 2000kN. The experiments were performed in triplicates at various curing periods and average value of the results is reported as representative compressive strength. The relative load rate was applied to the concrete cubes and the total maximum load was noted to evaluate compressive strength as follows [24]:

\[
\text{CS} = \frac{P}{A}
\]

where CS is compressive strength (MPa); \( P \) is total maximum load (N) and \( A \) is area of loaded surface mm².

#### 2.2.3 Water absorption test.
Cubes were prepared as done for compressive strength. Drying of cubes was done in oven (105°C) Weight of cubes were noted and allowed to cool to room temperature. Curing of the samples was done in tap water for 14, 56 and 180 days and regularly weight were noted till the weight was constant. This procedure was continued till it became saturate. Water absorption (IS 3495: 1992) was determined using the equation [19]
\[
\frac{(S_2 - S_1)}{S_1} \times 100
\]

S2 = Saturated weight, kg  ;  
S1 = Oven dried weight, kg

3. Results and discussion

There is no standard procedure reported for using biological solution in biomineralization process as per ASTM or IS standard. In this research work, the quantity of bacteria solution was decided on the ratio of bacteria solution to water. The effect of bacteria solution to water ratio on strength and durability property is discussed below.

3.1 Compressive strength

The compressive strength of 14, 28, 56 and 90 days was determined. Table 3 depicts the mean value of compressive strength of specimens at 14, 28, 56 and 90 days.

| Fly ash replacement (%) | 14 days | 28 days | 56 days | 90 days |
|-------------------------|---------|---------|---------|---------|
|                        | Conventional Concrete | Bacterial Concrete | Conventional Concrete | Bacterial Concrete | Conventional Concrete | Bacterial Concrete | Conventional Concrete | Bacterial Concrete |
| 0                      | 28.39    | 35.21   | 33.33   | 43.11   | 37.62   | 44.22   | 41.30   | 43.10   |
| 10                     | 26.40    | 32.12   | 31.84   | 39.14   | 36.40   | 43.24   | 40.80   | 43.22   |
| 20                     | 25.40    | 30.61   | 30.59   | 38.01   | 35.20   | 39.11   | 39.90   | 43.18   |
| 30                     | 23.80    | 27.11   | 29.18   | 34.12   | 33.20   | 37.21   | 37.80   | 41.26   |

(Bacterial solution/ water = 0.4)

| Fly ash replacement (%) | 14 days | 28 days | 56 days | 90 days |
|-------------------------|---------|---------|---------|---------|
|                        | Conventional Concrete | Bacterial Concrete | Conventional Concrete | Bacterial Concrete | Conventional Concrete | Bacterial Concrete | Conventional Concrete | Bacterial Concrete |
| 0                      | 28.39    | 35.21   | 33.33   | 43.11   | 37.62   | 44.22   | 41.30   | 43.10   |
| 10                     | 26.40    | 30.90   | 31.84   | 38.61   | 36.40   | 41.16   | 40.80   | 43.20   |
| 20                     | 25.40    | 28.61   | 30.59   | 36.22   | 35.20   | 40.11   | 39.90   | 41.80   |
| 30                     | 23.80    | 25.21   | 29.18   | 33.11   | 33.20   | 37.91   | 37.80   | 40.23   |

(Bacterial solution/ water = 0.2)

| Fly ash replacement (%) | 14 days | 28 days | 56 days | 90 days |
|-------------------------|---------|---------|---------|---------|
|                        | Conventional Concrete | Bacterial Concrete | Conventional Concrete | Bacterial Concrete | Conventional Concrete | Bacterial Concrete | Conventional Concrete | Bacterial Concrete |
| 0                      | 28.39    | 29.2    | 33.33   | 37.23   | 37.62   | 40.19   | 41.30   | 42.10   |
| 10                     | 26.40    | 26.4    | 31.84   | 34.90   | 36.40   | 29.10   | 40.80   | 43.10   |
| 20                     | 25.40    | 25.11   | 30.59   | 31.57   | 35.20   | 35.90   | 39.90   | 42.28   |
| 30                     | 23.80    | 24.12   | 29.18   | 30.78   | 33.20   | 34.49   | 37.80   | 40.14   |

As can be seen in table 3, with increase in fly-ash percentage as a replacement for cement, strength tends to decrease for control concrete. Fly-ash concrete gaver lower strength compared to normal concrete. To mitigate with this problem a novel concept of biomineralization was applied to increase the strength of fly-ash concrete. Bacterial cell dosage was maintained constant in this work (10^5 cells/ml). The optimum bacteria concentration was obtained from our previous study [25]. The lowest strength was recorded for FA concrete made up of 30 % FA as a substitute for cement. Table 2 displays that adding bacteria of cell dosage 10^5 cells/ml increased strength for 30 % replacement of cement with fly-ash for all types of ratios. Highest increment was observed to be around 25% in comparison with normal concrete at bacteria solution to water ratio as 0.6. Higher bacterial solution to water ratio contains gave better strength results. A decline trend in compressive strength was noted with decrease in ratio. However at all ratio, biological concrete gave better results in comparison with normal and control concrete. Curing age of biological concrete plays an important role. As can be observed in table, as age of curing increases, strength gain does not increase much. When bacteria are blended with concrete, the nano space in concrete gets filled with bacteria and starts to germinate.
calcite which will be harder [22]. During initial period, pores are slightly open which allows movement of oxygen and water in to the matrix of concrete. This may have resulted in higher rate of biochemical reaction and more amount of calcite formation for 14 to 28 days in comparison with 56 and 90 days [11]. After certain age of curing, opening pores gets reduced which retards movement of fluid particles into concrete. During this period bacteria may will be in dormant state where the activity of bacteria will be reduced. The bacterial spores are known to survive for decades in the absence of food or water. After certain age minute openings formation start to take place which permits flow of fluid particles into the concrete [17]. As the bacteria receive water or air, biomineralization process may start. The urease enzyme acts as biocatalyst in this biomineralization process [26]. The compressive strength of plain concrete is 33.33 MPa after 28 days. For 30% FA biological concrete, strength is 34.12 MPa. The strength obtained for this biological concrete is greater than normal concrete at 28 days. Use of fly-ash in concrete does not give higher strength. But with bacteria this limitation is overcome. BBFC of 30% FA can be used which will have two major benefits. Primarily, fly-ash usage lowers the environmental damage faced by fly-ash disposal. The FA utilization minimize amount of cement used. CO$_2$ gas emissions from the cement industry will be reduced due to use of fly-ash and helps in greener environment formation.

### 3.2 Water absorption

Table 4 reports the mean value of three trails in each curing period of water absorption measurements for biological and conventional concrete. The concrete age considered for absorption studies were 7, 56, and 120 days. Water absorption of control concrete at 7 days was 6.34%, 5.38%, 5.74 % and 6.10 % for 0% , 10 %,20% and 30% fly-ash concrete. At 7 days biological concrete for 10 % fly-ash showed water absorption of 5.84 %, 4.86 %, 5.27% and 5.53 %. Inclusion of bacteria *bacillus sphaercius* decreased water absorption by around 30% compared to normal and control concrete. Clearly, the water absorption of all biological concrete reduced drastically compared to non biological concrete at ratio of 0.3 and 0.6. Fly ash blended biological concrete (10% replacement of fly ash with cement) gave much better results among all biological concrete. Lower water absorption for non biological concrete conventional was noticed (5.38 %) for 10 % FA concrete at 120 days. For biological blended fly ash concrete this was achieved in 7 days for 10% replacement with fly ash for 0.6 ratio of bacteria solution to water. It can be seen in table 4 that the water absorption for normal concrete was 6.6 %. For Biological blended fly ash concrete of 30% fly-ash showed water absorption of 5.27 % which is lower than that of normal concrete for ratio of 0.6. The biomineralization process resulted in formation of calcite crystals near the cell which acted as resistance for the movement of water particles into the concrete [27]. At ratio of 0.6, least water absorption was observed. For biological concrete, water absorption was found to be less for ratio of 0.6 compared to ratio of 0.3. Higher quantity of bacteria at ratio might have led to precipitation of more calcite compared to 0.3 ratio. To favour increase in rate of biomineralization process, some researchers have used urea as supplements during mixing and curing period. This will lower the utilization of cement and reduce green house effect due to discharge of CO$_2$ in to the atmosphere and gain improvement in durability property.
Table 4. Water absorption rates (%)

| Fly-ash replacement (%) | Bacterial sample solution/ Water = 0.6 | 7 days | 56 days | 120 days |
|-------------------------|----------------------------------------|--------|---------|----------|
|                         | Normal Concrete                        | Bacterial Concrete | Normal Concrete | Bacterial Concrete | Normal Concrete | Bacterial Concrete |
| 0                       | 6.60                                   | 5.64    | 6.62    | 5.81      | 6.34    | 5.48    |
| 10                      | 5.70                                   | 4.58    | 5.54    | 4.86      | 5.38    | 4.99    |
| 20                      | 5.92                                   | 5.17    | 5.90    | 5.11      | 5.74    | 5.14    |
| 30                      | 6.22                                   | 5.43    | 6.28    | 5.57      | 6.10    | 5.27    |
|                         | (Bacterial sample solution/ Water = 0.3)|        |         |           |         |        |
| 0                       | 6.60                                   | 6.22    | 6.62    | 5.82      | 6.34    | 5.81    |
| 10                      | 5.70                                   | 5.18    | 5.60    | 5.11      | 5.38    | 5.08    |
| 20                      | 5.92                                   | 5.72    | 5.90    | 5.42      | 5.74    | 5.37    |
| 30                      | 6.22                                   | 5.97    | 6.28    | 5.59      | 6.10    | 5.48    |

3.3 Scanning electron microscopy (SEM)

Figure 1 to 3 shows scanning electron microscopy analysis of conventional and bacterial concrete. Fourteen days sample of normal and bacterial blended concrete samples was subjected to scanning electron microscopy analysis. The image of (figure 2 and 3) biological concrete shows calcite precipitation in the openings of the concrete matrix. This might be due to bacterial activity which caused specimens to be denser. This biological activity enhanced the property of concrete. On the contrary figure 1 shows SEM image of normal concrete where several openings were observed. Similar observations were made by Siddique [18] and [28].

Figure 1. Scanning electron micrographs of normal concrete
Figure 2. Scanning electron micrographs showing presence of CaCO$_3$ in biological concrete at bacteria solution to water ratio of 0.6

Figure 3. Scanning electron micrographs showing presence of CaCO$_3$ in biological concrete at bacteria solution to water ratio 0.4.

3.4 X-ray diffraction (XRD) spectroscopy of concrete samples

Figure 4 to 5 shows x-ray analyses of the normal and bacterial concrete. In bacterial specimens some extra peak were observed as shown in figure 5. No extra peaks were noticed in plain concrete. For bacterial concrete highest peak was obtained at 2 theta (2h) value of 29.38$^\circ$ (Figure 5). Similar observation was also shown by Herrington [29], Khaliq [30] and Kirti [31]. This confirms the precipitation of calcite crystals in the concrete. The calcite peak was confirmed by comparing with international centre for diffraction data [32]. The normal concrete showed no extra peaks.
4. Conclusion and future prospects

Based on experiment conducted following are the conclusions drawn.

1. Incorporating alkaliphilic endospore biological material in to concrete successfully demonstrated that strength properties enhanced by 25 % for a bacterial solution to water ratio of 0.6 and 0.4.
2. Curing age is a key parameter in the enhancement of mechanical and durability property. At young age of 14 and 28 days it showed up to 28 % improvement in strength property for biological concrete compared to normal and control concrete. Biomineralization technique over comes the limitation of gaining strength at early age.
3. Bacteria and fly-ash combination proves to be an excellent combination to protect concrete against penetration of any fluid particles.
4. The ratio of bacteria solution to water is deciding factor for biological concrete.
5. The rate of bio chemical reaction is hindered due to presence of FA.
6. Thus the biominearlization process will help to bring sustainability in the construction field.
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