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RESEARCH ARTICLE - CIVIL ENGINEERING

A Laboratory Investigation on the Production of Sustainable Bacteria-Blended Fly Ash Concrete

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Abstract This paper outlines the possibility of producing a novel, green and economical concrete based on biomineralization. Cement an important ingredient of concrete is expensive, and production of cement leads to around 8–10 % of CO_2 emissions. Significant efforts have been put by researchers to find alternative that will reduce burden of high cost, high energy consumed and environment impacts. Fly ash (FA) has been used as an alternative to reduce impact of cement. However, its replacement to cement limits to low percentage. The present work aims to develop a sustainable FA concrete by blending with varying dosages of bacteria. The concrete specimens were tested for compressive strength, split tensile strength, shear strength and sorptivity test at 7 and 28 days of curing. Bacterial concrete with 0 % FA obtained best results for strength property, but with increasing the percentage of FA the strength property was found to decrease. Results showed that bacterial concrete was more effective in minimizing sorptivity property in the presence of FA. The improvement in property of bacterial concrete was confirmed due to the calcium carbonate precipitation as evidenced in SEM analysis. The bacterium incorporated into the concrete mixture is safe and eco-friendly. Thus, findings suggest that the combination of bacteria and FA minimizes the demand of cement and reduces emission of greenhouse gases and adds sustainability to the concrete.

Keywords Biomineralization · Bacteria-blended fly ash concrete · Novel and green concrete · Calcite precipitation · Properties of concrete

1 Introduction

Concrete is the major component in the construction industry and is the second most consumed

substance after water [1]. It can be cast into any desirable shape. Plain concrete, however, possesses very low tensile strength [2], limited ductility and little resistance to cracking [3]. Apart from this, cracking of concrete is a significant problem in the construction industry. However, these cracks in concrete are expected to expand further if proper treatments are not done. At present, modern technology involves use of organic coatings consisting of volatile organic compounds [4]. Traditional inorganic coatings consist of calcium–silicate compounds, which exhibit a composition similar to cement. Surface treatments with water repellents such as epoxy injections with pore blockers and various synthetic agents such as silanes or siloxanes [5] are being presently used today, but with a certain drawbacks such as degradation with time, high constant maintenance, and these materials are environmentally unfriendly.

The massive production of cement contributes to significant amount of CO_2 into the atmosphere, making the carbon footprint associated with the construction quite high [4]. Cement industry is major contributor for global CO_2 emissions after electricity generation Company. From envi-

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ronment point of concern, concrete does not seems to be a sustainable material [1]. Hence, there is urgency in the development of sustainable concrete for environment reasons. The best way of controlling and reducing the emission of these gases is by minimizing the use of conventional building materials and using alternate technology. Another way to enhance the properties of concrete or protect concrete and mortar surfaces is to use a new research domain of biological treatment, which has been studied by several researchers in healing of cracks in concrete [4].

Smart materials react to changes in stimuli (temperature, moisture or pH) and can simulate biological, human-like behaviour. Humans have the ability to precipitate minerals in the form of bones and teeth continuously. This ability is not only confined to human beings; even some bacteria can continuously precipitate calcite. This phenomenon is called microbiologically induced calcite precipitation (MICP). Microbiologically induced calcite precipitation is a technique that comes under a broader category of science called biomineralization. It is a process by which living organism forms inorganic solids [1]. In this method, a new membrane-like layer of calcite near bacterial cells formation takes place over the surface of the already existing cement mortar layer. Calcite has a coarse crystalline structure that readily adheres to surfaces in the form of scales. It has the ability to continuously grow itself and is insoluble in water and is environment friendly and natural [2]. Due to its inherent ability to precipitate calcite continuously, bacterial concrete can be called “Smart Bio Material”.

1.1 Fly ash

Fly ash is available in large quantities as a by-product from thermal power stations [6]. Worldwide annual production of fly ash constitutes to about 500 million tonnes [7]. Disposal of unused fly ash is posing serious ecological problem in addition to occupying large area of cultivable land. Fly ash disposal makes major negative environment impact such as air pollution and ground water quality problem due to leaching of metals from the ashes. Utilization of fly ash as a partial replacement material for Portland cement offers a holistic approach to meet the rising demand of concrete. Fly ash use in the concrete improves many of its properties with little or no extra cost and is better alternate option of disposing fly ash. Its use reduces heat of hydration, permeability, alkali aggregate reaction, improves workability, increases resistance to sulphate attack and corrosion thus making concrete mass more strong and durable. Several works have been carried out with replacement level of fly ash up to 30 % [8]. Major drawback for FA as a replacement is lower gain in strength at early age due to slow pozzolanic reaction in comparison with normal concrete [9–11]. This acts as a barrier for the application of fly ash in concrete field. To overcome this shortcoming, a novel biotechnology tool is applied to fly ash concrete to enhance the properties of concrete at early age. Application of fly ash in construction sector would save national revenue and solves huge dumping problem which would cause severe environmental nuisance.

1.2 Biomineralization

Biomineralization has attracted significant attention to the several researchers and presently is a research hot spot. Biomineralization is a metabolic process of formation of hard structures, surfaces or scales by combining minerals with organic compounds of some specific microorganisms. Biomineralization involves various microorganisms, pathways and environments. It is a process which involves precipitation of calcium carbonate (calcite) by microorganisms. This technique is also called microbiological induced calcite precipitation. Microbiological induced calcite precipitation technique has various applications such as consolidation of sand columns, for repair of limestone monuments and to a smaller extent for purification of water [3]. The precipitation of calcite by bacteria forms a layer of calcite on the surface of the specimens. This results in decrease in capillary water uptake and porosity [3]. This biological treatment helps to improve the overall behaviour of concrete. MICP technique is a novel, eco-friendly, self-healing and energy efficient technology for remediation of building materials and enhancement in the durability characteristics of concrete. This technology may bring new approaches in the construction industry.

The possible biochemical reaction involved in the generation of calcium carbonate is given below [2].

$$NH_2CONH_2 + H_2O \rightarrow NH_2COOH + NH_3 \tag{1}$$

$$NH_2COOH + H_2O \rightarrow NH_3 + H_2CO_3 \tag{2}$$

$$H_2CO_3 \rightarrow HCO_3 + H^+ \tag{3}$$

$$2NH_3 + 2H_2O \rightarrow 2NH^{4+} + 2OH^- \tag{4}$$

$$HCO_{3-} + H^+ + 2OH^- \rightarrow CO_3^{2-} + 2H_2O \tag{5}$$

$$CO_3^{2-} + Ca^{2+} \rightarrow CaCO_3 \tag{6}$$

$$NH_2CONH_2 + 2H_2O + Ca^{2+} \rightarrow 2NH^{4+} + CaCO_3 \tag{7}$$

The urease enzyme is common in many microorganisms, and ureolysis can be induced in a laboratory by adding urea. One mol of urea is hydrolysed intracellularly to 1 mol of ammonia and 1 mol of carbamate (Eq. 1), which spontaneously hydrolyses to form an additional 1 mol of ammonia and carbonic acid (Eq. 2). These products subsequently equilibrate in water to form bicarbonate, 2 mol ammonium and 2 mol of hydroxide ions (Eqs. 3 and 4). The latter give rise to a pH increase, which in turn can shift the bicarbonate equilibrium, resulting in the formation of carbonate ions (Eq. 5), which in the presence of soluble calcium ions precipitate as $CaCO_3$ Eq. (6). Equation (7) is an overall reaction for the system, showing that urea and calcium are added to the system and ammonium and calcium carbonate are products [1].

Various bacteria in the past few years have been used for repairing cracks as well as in improving the mechanical property of concrete specimens [12]. Past research demonstrated that the addition of *Bacillus subtilis* into recycled concrete aggregate resulted in 20 % increase in compressive strength at cell concentration of 10^6cells/ml. Deposition of calcium carbonate enhanced the property of RCA concrete [12]. Achal et al. [11] indicated that porosity of mortar specimens decreased by 50 % due to calcium carbonate precipitation. Biomineralization technique was successful in healing the cracks of depth 27.2 m in mortar specimens. In another study, application of *B. sphaericus* for consolidation of limestone at various temperatures (10, 20, 28, 37 °C) showed up to 46 % decreased sorptivity in limestone specimens compared to control. They concluded that MICP occurred in larger quantities at greater depths in macroporous stones than in microporous stones [13]. In another study, application of *B. sphaericus* for consolidation of limestone at various temperatures (10, 20, 28, 37 °C) showed up to 46 % decreased sorptivity in limestone specimens compared to control. They concluded that MICP occurred in larger quantities at greater depths in macroporous stones than in microporous stones [14].

Jonkers et al [1] reported that bacterial spores added directly into the cement paste mixture remained viable for up to 4-month duration. Bacteria act a catalyst in the material matrix and provide a truly autonomous repair mechanism. Wang et al. [15] developed silica gel-immobilized bacteria for self-healing of cracks in concrete. They reported that silica gel-immobilized bacteria showed greater activity, and hence, more $CaCO_3$ precipitated. Bang et al. [16] studied potential of *B. sphaericus* to heal cracks in concrete. *B. sphaericus* showed high urease activity and continuous formation of calcite. Pacheco-Torgal et al. [17] indicated that genus Bacillus bacteria have potential of naturally precipitating calcium carbonates. This ability of bacteria is due to several activities such as photosynthesis, ammonification, denitrification, sulphate reduction and anaerobic sulphide oxidation. The literature on study of bacteria in concrete containing fly ash as partial replacement is not extensive. The principal aim of this paper is to demonstrate the ability of bacterial strain *Bacillus* sp. in improving the mechanical and durability properties of fly ash concrete and to work on proper combination of cement and fly ash by blending bacteria to get the desired properties of concrete specimens.

2 Materials

2.1 Cement and Sand

The present study was carried out using Portland cement of 43 grade and fine aggregates confirming to zone II of IS 383:1970 having specific gravity of 2.59 and fineness modulus of 2.81. Locally available coarse aggregate having the maximum size of 20 mm and down as per IS 383:1970 was used in the current work. The cement was analysed for various properties as per IS 8112-1989 shown in Table 1. The specific gravity of cement was found to be 3.15. The fly ash C type used in this investigation was obtained from Raichur Thermal Power Plant (Karnataka). Physical properties of fly ash are reported in Table 2

Table 1 Physical analysis of cement

Particulars	Experimental result
1. Fineness	248 m^2/kg
2. Soundness	
a. By Le Chatelier mould	1.00 mm
b. By autoclave	0.14
3. Setting time (min)	
a. Initial set	196 min
b. Final set	270 min
4. Comp strength (MPa)	
a. 3 days	31
b. 7 days	43
c. 28 days	58
Temperature during testing	28 °C

Table 2 Properties of fly ash

Particulars	Fly ash properties
Sp. surface (m^2/kg)	483.7
Lime reactivity (MPa)	8.5 N/mm^2
Cement reactivity (%)	88.00 %
SiO_2	61.90 %
SiO_2+ Al_2O_3+ Fe_2O_3	94.50 %
CaO	0.70 %
MgO	0.60 %
IR	93.50 %
LOI	0.80 %
Total alkalis as Na_2O	0.93 %
Chloride	0.06 %

2.2 Selection of Bacteria

The fresh concrete is highly alkaline in the range 11–13 [1]. The selected bacteria have to survive under this high alkaline condition and resist mechanical stress during mixing [10]. In the current investigation,*Bacillus sphaericus* NCIM NO 2478 was procured from National Collection of Industrial Microorganisms, Pune. *B. sphaericus* which facilitates the precipitation of calcium carbonate is safe and non-pathogenic. High dosage of *bacillus Sphaericus* was injected to laboratory animals, and no measurable health effects were observed (29). This microbe was selected based on the experiment conducted at various pH. The *B. sphaericus* was able to grow at pH of 9, 10, 11 and 12. Hence, it is called alkaliphilic (alkali-resistant) spore-forming bacteria since it is able to grow above 9 pH [18]. The selected strain has high urease activity, longer survival time and withstands high alkalinity. The liquid medium composition required for growth of culture includes nutrient broth solution supplemented with urea [19] which was initially sterilized using autoclave for 20 min at 120 °C. The final pH of the broth solution was 7.2.

2.3 Growth Curve

Growth curve for *Bacillus sphaericus* NCIM NO 2478 was obtained by inoculating spores of *B. sphaericus* in liquid broth solution. Liquid broth solution is prepared by adding nutrient broth supplemented with 2 % urea. Optical density was noted every hour by using spectrophotometer. Growth curve was developed as shown in Fig. 1

2.4 Mixture Proportions and Casting of Specimens

Concrete mixture design was done according to IS 10262-2007. Cement, fine aggregate and coarse aggregate were weighed and mixed according to mix proportion for M25 grade of concrete (W/C = 0.5). The mix proportion of concrete used in the current study was cement (1): fine aggregate (1.81): coarse aggregate (2.99). Fly ash was incorporated with replacement levels of 0, 10, 20 and 30 % by weight of cement. All the ingredients were mixed in a mixer for 10 min in dry conditions. For this dry mix, required amount of water and bacterial solution in the ratio 0.5 was added where upon concrete mixture was mixed for 6 more minutes. Workability of mixtures was conducted using slump test according to ASTM C143. The specimens were kept in a sheet under moistened for 24 h. After 24 h, the specimens were demoulded and transferred to curing tank where they were allowed to cure for 7, 28, 60 and 90 days in tap water only depending up on the type of test. No additional supplements were provided during curing of concretes in tap water. Concrete specimens without addition of fly ash and bacteria serve as conventional concrete.

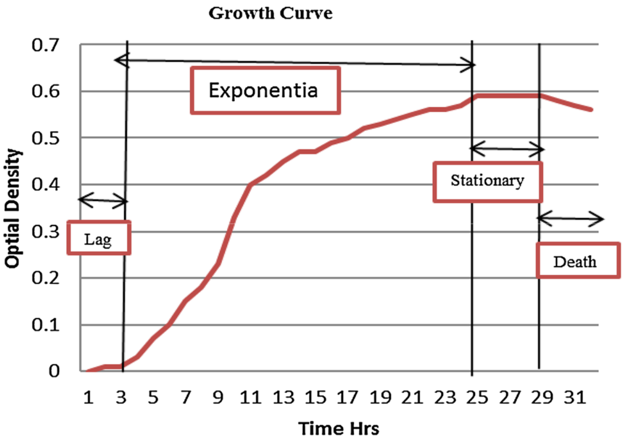

Fig. 1 Growth curve of *Bacillus sphaericus* bacteria

3 Testing Methods

3.1 Compressive and Split Tensile Strength

Concrete cubes of dimensions 150×150×150 mm for testing compressive strength and cylindrical specimens of diameter 150 mm and length 300 mm for tensile strength were prepared. All the tests were performed in triplicates. The compressive testing was determined in compliance with IS 516:1959 at 7, 28 60 and 90 days and tensile property in compliance with IS 5816:1999 at 28 days. Compressive and tensile strength was determined using 2000-kN capacity testing machine.

3.2 Flexural Strength

All the specimens for flexural strength test having dimensions of 100×100×500 mm were cured for 28 days in tap water only (in the absence of nutrients). The two-point loading was placed at a distance of 133 mm, and bottom was placed at an effective span of 400 mm as per IS 516-1959. The load was applied without shock and increasing continuously at a rate of 1800 N/min.

3.3 Shear Strength

Bio-based and control concrete of L-shaped specimens was prepared to evaluate shear strength. These specimens were tested on 2000-kN capacity compression testing machine. A loading arrangement was made such that a direct shearing force was applied on the shorter arm of the "L"-shaped specimen (i.e. over an area of 150 mm × 60 mm). The maximum applied load (P) was noted down. The failure load (F) due to the applied shear force is obtained by using the relation

Failure load $(F) = Px/(x+y)$,

where P failure load in KN, $x = 25$ mm, $y = 25$ mm

The shear strength is given by the relation

Shear strength $= F/A$,

where F failure load, A area on which shear force is applied $= 150\text{ mm} \times 60$.

3.4 Sorptivity Test

Sorptivity is a measure of the capillary forces excreted by the pore structure causing fluids to be drawn into the specimens [18]. To measure sorptivity, specimens (bacterial and control concrete) were kept in a water tray on small supports in a manner such that only a lowest 10 mm of prism was submerged. The rise in water level in concrete which manifests itself by dark colour was noted down at every regular interval of 15 min. This was continued until the further rise in water level was noted. The sorptivity of the cubes specimens (bacterial and control concrete) was calculated by the equation of $S = i/\sqrt{t}$ [20].

S sorptivity in mm/min$^{1/2}$, i cumulative water absorption per unit area of the surface (m^3/m^2), t time elapsed for this rise in minute.

3.5 Scanning Electron Microscope Analysis

Bacterial blended and conventional concrete was crushed into small pieces after 28 days of curing. Samples were dried at room temperature and then examined for calcite precipitation using SCM analysis.

3.6 Results

3.7 Observations Made from growth Curve of *Bacillus sphaericus*

Figure 1 shows the growth curve of *B. sphaericus*. Figure 1 shows that lag phase exists up to 4 h. At this duration, bacteria try to adjust to the high-nutrient environment and prepare for fast growth [3]. Exponential growth was observed up to 25 h. At this stage, growth of cells is faster and consumes nutrients at a faster rate [3]. Highest optical density was observed to be 0.58. The stationary phase is observed between 25 and 29 h. Due to depletion of nutrients, growth rate gets hindered.

3.8 Mechanical Properties and Sorptivity

The mechanical and durability property of various concrete mixes is graphically presented in Figs. 2,3, 4, 5, 6, 7 and 8.

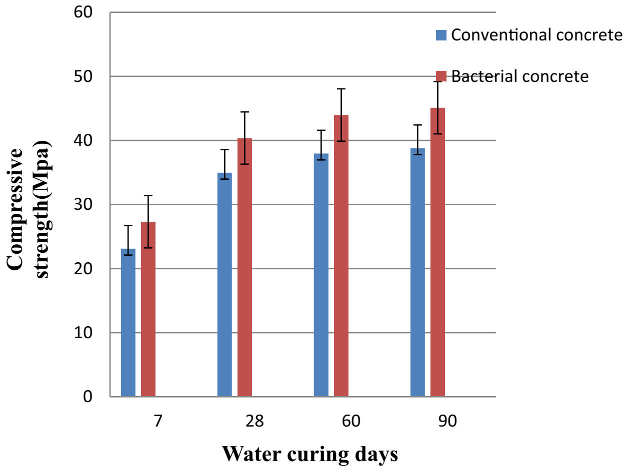

Fig. 2 Effect of curing conditions on compressive strength of conventional and bacterial concrete

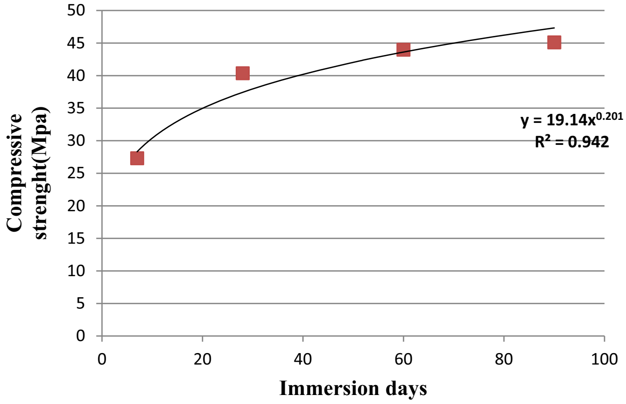

Fig. 3 Relationship between compressive strength and immersion of bacterial concrete specimens in tap water

Addition of various dosages of *B. sphaericus* promoted MICP biochemical process which resulted in enhancement of strength properties. The effect of *B. sphaericus* on the strength properties of concrete specimens is summarized in the form of Tables 3 and 4. Each presented value is the average of three measurements. The maximum increment in mechanical property was found to be at a cell concentration 10^5 cell/ml for bacterial blended concrete (with and without fly ash). Compared to conventional concrete, there was maximum of 15.47 % improvement in compressive strength for bacterial blended concrete with the addition of 10^5cells/ml at 28 days of curing as observed in Fig. 4 and Table 3. For 7 days, highest gain in percentage in compressive strength was found to be 18.17 % compared to conventional specimens (Table 3). Maximum increment in flexural strength was found to be 16.43 % at a concentration of 10^5cells/ml for 10 % replacement with fly ash. Similar trends were observed for other mechanical properties of concrete specimens. From experiment trials, optimum dosage was found to be 10^5cells/ml. Bacterial blended fly ash concrete at 30 % replacement level

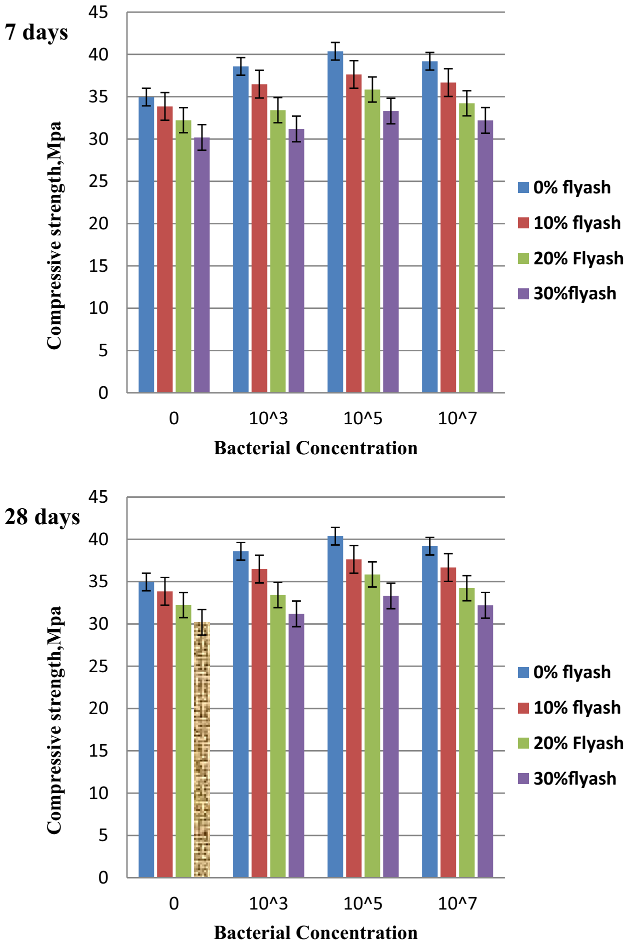

Fig. 4 Compressive strength at age of 7 and 28 days of curing

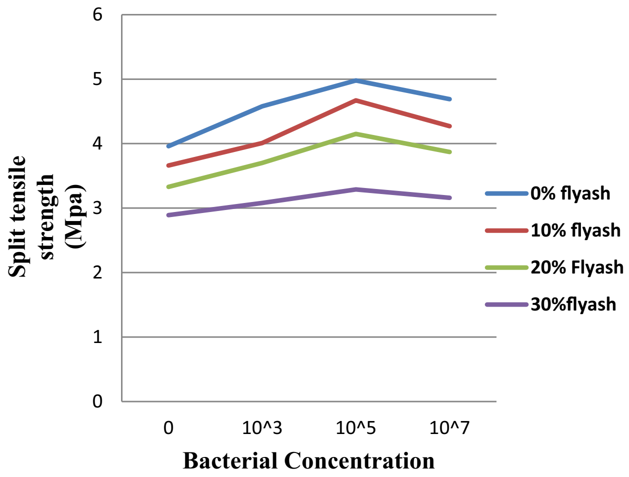

Fig. 5 Effect of various dosages of *B. sphaericus* on split tensile strength of fly ash concrete at 28 days of curing

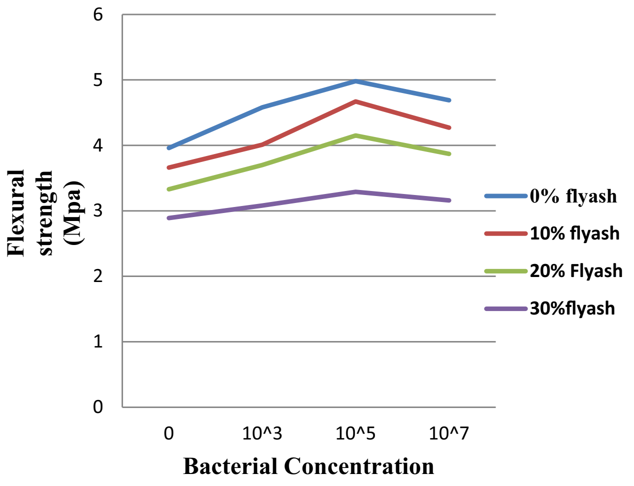

Fig. 6 Effect of various dosages of *B. sphaericus* on flexural strength of fly ash concrete at 28 days of curing

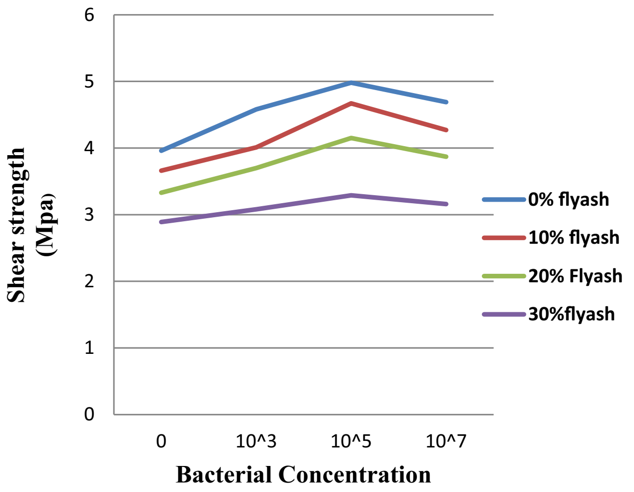

Fig. 7 Effect of various dosages of *B. sphaericus* on shear strength of fly ash concrete at 28 days of curing

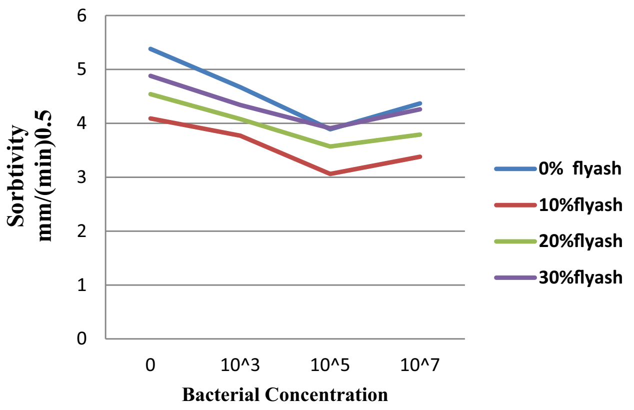

Fig. 8 Effect of various dosages of *B. sphaericus* on sorptivity of fly ash concrete at 28 days of curing

showed same trend as that of conventional concrete. The compressive strength to split tensile strength

Table 3 Compressive strength results for 7 and 28 days of normal and fly ash bacterial concrete with variation in bacterial cell concentration

Description of concrete	Bacteria concentration (cells/ml)	Compressive strength for 7 days (MPa)	Percentage increase	Compressive strength for 28 days (MPa)	Percentage increase
Concrete with 0 % fly ash as replacement of cement	0	23.11	Conventional	34.96	Conventional
	10^3	25.64	10.94 %	38.59	10.38 %
	10^5	27.31	18.17 %	40.37	15 %
	10^7	26.4	14.23 %	39.19	12.09 %
Concrete with 10 % fly ash as replacement of cement	0	22.1	Control	33.85	Control
	10^3	24.19	9.4 %	36.48	7.77 %
	10^5	25.6	15.83 %	37.63	11.17 %
	10^7	24.82	12.3 %	36.67	8.33 %
Concrete with 20 % fly ash as replacement of cement	0	21.8	Control	32.22	Control
	10^3	23.4	7.3 %	33.41	3.69 %
	10^5	24.43	12.06 %	35.85	11.26 %
	10^7	24.1	10.5 %	34.22	6.21 %
Concrete with 30 % fly ash as replacement of cement	0	20.4	Control	30.18	Control
	10^3	21.2	3.92 %	31.19	1.83 %
	10^5	23.2	13.72 %	33.31	10.37 %
	10^7	22.43	9.96 %	32.20	6.69 %

Table 4 Split tensile strength results for 28 days of normal and bacteria-blended fly ash concrete with various dosages of bacteria

Description of concrete	Bacteria concentration (Cells/ml)	Split tensile strength (MPa)	Percentage increase	Ratio of compressive strength to tensile strength
Concrete with 0 % fly ash as replacement of cement	0	3.96	Conventional	8.8
	10^3	4.58	15.65 %	8.42
	10^5	4.98	20.75 %	8.10
	10^7	4.69	18.43 %	8.35
Concrete with 10 % fly ash as replacement of cement	0	3.66	Control	9.24
	10^3	4.01	9.56 %	9.09
	10^5	4.67	18.59 %	8.05
	10^7	4.27	16.66 %	8.58
Concrete with 20 % fly ash as replacement of cement	0	3.33	Control	9.7
	10^3	3.7	11.11 %	9.029
	10^5	4.15	17.62 %	8.64
	10^7	3.87	16.21 %	8.84
Concrete with 30 % fly ash as replacement of cement	0	2.89	Control	10.44
	10^3	3.08	6.5 %	10.12
	10^5	3.29	13.84 %	10.13
	10^7	3.16	9.3 %	10.18

ratio was observed to be in the ratio 8.05–10.18. For conventional concrete, it was observed in the range 8.8–10.44. This shows that *B. sphaericus* and fly ash have pronounced effect on split tensile strength. The equation to predict tensile strength of bacteria-blended fly ash concrete at 28 days of curing at optimum bacterial concentration of 10^5cells/ml is presented in the form of multiple regression equation. The software tool Minitab 17 was used for developing multiple regression equation.

$$f_t = 9.3 - -0.080 f_f - -0.103 f_c \quad (R^2 = 0.954)$$

Where f_t, f_f and f_c are tensile strength (Mpa), fly ash percentage and compressive strength (Mpa), respectively. The higher values of correlation coefficient indicate strong relationship between the properties split tensile strength and compressive strength with variation in fly ash proposition for optimum value of bacteria concentration.

The compressive strength of bacterial and conventional concrete improved with age as shown in Fig. 2. However, bacterial concrete showed higher compressive strength than conventional concrete at all ages (Fig. 2). At early age, compressive strength of bacterial concrete was found to increase with high gain, and at latter age gain in strength tends to slow down for 60 and 90 days (Figs. 2, 3). Figure 3 depicts the relationship between compressive strength of bacterial concrete and age of curing. The high value of R^2 expresses good correlation between compressive strength of bacterial concrete and age of curing.

Filling of pores by crystals of calcite near the cells may be an important reason for gain in strength. It is due to this reason fraction of opening spaces in the matrix of concrete reduces. At early age, concrete specimen is porous which may lead to movement of oxygen or water into the matrix of specimens [21]. This may have resulted in high rate of biomineralization process during initially 7 or 28 days in comparison with 60 and 90 days.

The influence of Bacillus*sphaericus* on sorptivity is represented in the form of Fig. 7. It can be observed from the figure that the penetration of water decreases with increase in bacteria concentration up to 10^5 cells/ml. The lowest sorptivity of the specimens was found to be 3.06 mm/min$^{1/2}$ at a bacterial concentration of 10^5cells/ml at 10 % replacement with fly ash. The sorptivity of 30 % replacement with fly ash bacterial blended concrete showed 30 % less sorptivity than conventional concrete.

4 Discussions

Calcite most common mineral on earth constitutes 4 wt% of the earth crust. Bacteria by the phenomenon of MICP deposit calcite [2]. This technique has been an area of research for construction materials. The current investigation aims to study the effect of eco-friendly microbial process on the mechanical and durability property of the concrete (with and without fly ash). The strain used in the present work is alkaliphilic (alkali-resistant) spore-forming bacteria. These cells are known to resist high mechanically and chemically induced stresses [16]. The curing of various concrete mixes was done in tap water for 7 and 28 days, respectively. No additional nutrients were added to the water during curing process. Different researchers have proposed their work where curing was done in water in the presence of nutrients [3].

At all level, addition of bacteria resulted in better performance in comparison with conventional concrete. With increase in bacteria dosage up to 10^5cells/ml, strength and durability property improved significantly. However, there onwards a reverse trend was observed. The improvement in strength properties may be attributed to deposition of layer of calcium carbonate on the bacterial cells which plugs the pores inside the matrix of concrete [16]. This led to reduction in total pore volume which in turn enhanced the sorptivity and mechanical properties of concrete specimens. A comparable increase in strength was reported by other researchers where curing was done in the presence of nutrients [22]. At early age, more gain in strength is observed in bacterial blended concrete. However, bacteria remain viable for prolonged duration once penetrated into the matrix of concrete [1]. Bacterial blended fly ash concrete had significantly less sorptivity than conventional concrete. The deposition of layer of calcium carbonate could have hindered water movement into the matrix of concrete [23]. As concentration increases, the microbial calcite precipitation increases which fills some of the open pores resulting in less water penetration [1]. From the experiment results, it can be concluded that 10^5 cells/ml was optimum for enhancing mechanical and durability properties of concrete (with or without fly ash). At concentration of 10^3 and 10^7cells/ml, increase in strength was more or less the same with respect to control. As per growth curve in Fig. 1, 10^3cells/ml concentrations was achieved during O.D of 0.1. At this duration, cells are adapting to the high-nutrient environment and preparing for fast growth [2]. Hence, due to which the biomineralization process might be slow which resulted in less calcite formation. Concentration 10^7 cells/ml was obtained in the range 26–28 h. At this duration, the microbes are at the end of stationary phase. During this phase, there is depletion of nutrients. The cells reduce their metabolic activity and consume non-essential cellular proteins [2]. Due to shortage of nutrients, biomineralization process may not be proper and results in less calcite precipitation. Hence, findings from the experiment suggest that biomineralization is a promising technique [24] which has dual benefits. Primarily, it can be used effectively in enhancing the mechanical and durability properties [25] of concrete (with and without fly ash). Also, microbial addition into fly ash concrete matrix would save money and environment.

5 Scanning Electron Microscope Investigations

Microbial calcite precipitation in concrete specimens was visualized using SEM.

Figure 9 shows the SEM picture of control concrete where pores can be easily seen. Figure 10 shows the SEM analysis of bacterial concrete which was found in intimate contact with the calcite crystals found on the surface. Rod-shaped impressions consistent with the dimensions of *B. sphaericus* were found in the calcite crystal, which formed on the sur-

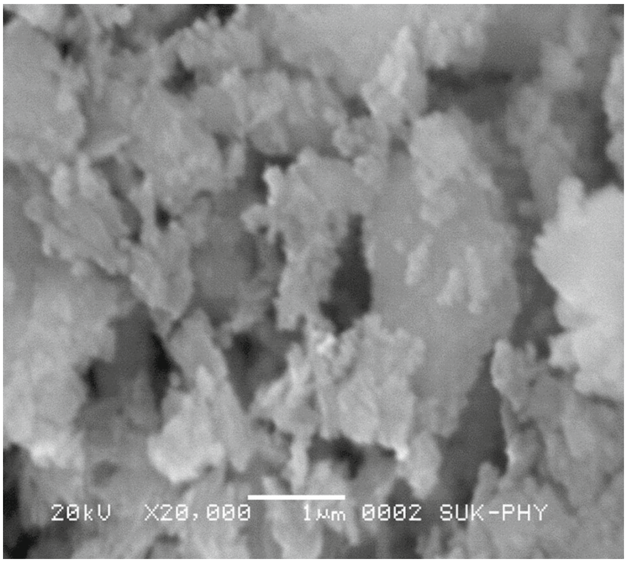

Fig. 9 SEM image of normal concrete at 28 days of curing

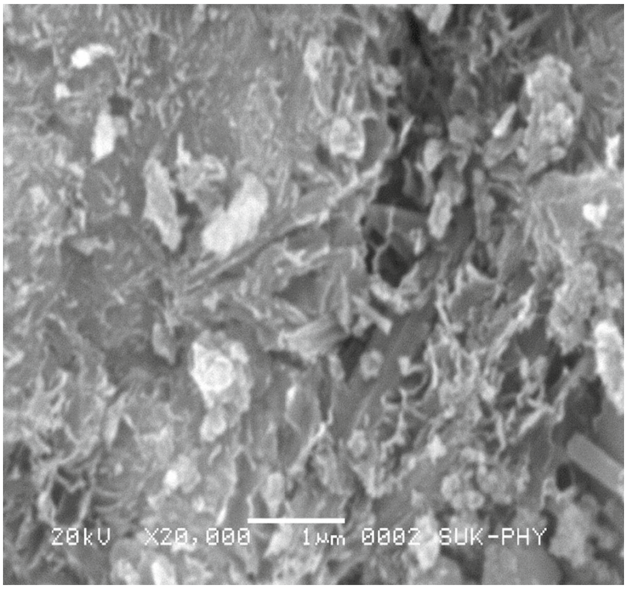

Fig. 10 SEM image of bacterial concrete at 28 days of curing

face of the specimens. Similar observations were also noticed by [12]. These microscopic observations confirm the mechanism of microbial calcite precipitation in concrete [26,27]. The deposition of calcite results in filling of pores which results in decrease in porosity [28]. Hence due to filling of pores, improvement in mechanical and sorptivity properties is observed [15].

6 Conclusions

The major conclusions that emerge from the experimental work are listed below:

1. The use of *B. sphaericus* in concrete improves the mechanical and durability properties of concrete (with or without fly ash) at all ages. However, higher gain in percentage with respect to control concrete was observed at 7 days of curing. The increase in strength properties is mainly due to filling of the pores inside the concrete by precipitation of calcite by bacteria. It can be concluded from experiment results that with 30 % replacement of fly ash for bacterial concrete the strength property is comparable with normal concrete.
2. The improvement in the mechanical and durability properties of concrete was superior while using a bacterial dosages of 10^5 cells/ml.
3. It has been observed from the present study, maximum amount of calcite precipitation takes place during log phase, i.e around 20–24 h from the time of inoculum.
4. Test results reveal that the presence of fly ash does not hinder the rate of biomineralization process. These findings suggested that biomineralization process can be implemented in improving the mechanical and durability property of fly ash concrete. Test results suggest that the combination of fly ash and microbes leads to further enhancement of durability properties and it acts as a better combination.
5. Implementation of this technique leads to production of novel, green and economical concrete since it is pollution-free and natural. This technology may bring new approaches in the construction industry.
6. Mechanical property at 30 % replacement with fly ash for bacterial blended fly ash concrete was observed to be similar with that of normal concrete at 28 days of curing.
7. Based on the above studies, the combination of 30 % fly ash and 10^5cells/ml of *B. sphaericus* would prove to be a cost-effective approach to get more or less the same mechanical properties as that of normal concrete. Moreover, with the above combination a reduction of 30 % in sorptivity property is observed with respect to normal concrete.

The above conclusion suggests that use of MICP technique to fly ash concrete leads to production of eco-friendly, highly durable and sustainable construction material. However, it is recommended to study the effect of bacteria on high volume of fly ash concrete.

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