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ORIGINAL PAPER

Study on properties of bacteria-embedded fly ash concrete

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Abstract

This paper focuses on adopting a new biotechnology tool for enhancing the property of fly ash concrete. Much of the coal fly ash generated from power plants is being dumped regularly. However, environmental situations do not permit dumping of fly ash in huge quantity. To cope with this problem, biotechnology tool is being implemented in the current research work for the utilization of fly ash in concrete. Strength and durability properties of bacteria-embedded fly ash concrete are examined for various ratios of bacterial solution to water. Experimental results show that incorporating alkaliphilic endospore-forming bacteria into fly ash concrete exhibited higher strength and durability than conventional concrete for bacterial solution to water ratios of 0.3 and 0.6. Interestingly, the ratio of compressive strength to split tensile strength of bacteria-embedded fly ash concrete was lower than that of ordinary concrete. It was observed that fly ash replacement up to 30% exhibited better strength and durability properties than normal concrete. Scanning electron microscopic analysis revealed direct involvement of the strain in precipitating calcite and was confirmed by XRD.

Keywords Bacteria-embedded fly ash concrete · Strength · Durability · SEM · Bacterial solution to water ratio

Introduction

Ever since power generation started in the year 1920, many millions of tonnes of fly ash have been generated (Ahmaruzzaman 2010). Production of coal ash in coal-burning power plants is always on the higher side in recent decades (Yoshitake et al. 2011). Worldwide annual production of fly ash is around 500 million tonnes which constitute around 70–80% of total ash produced. (Ahmaruzzaman 2010). Disposal of large quantity of fly ash absorbs large amount of water, energy and in addition to this it requires large cultivable land (Knoben 2011). In view of this, disposing such a large quantity of fly ash causes serious environmental problem. Fly ash particles are known to be highly contaminating. Considerable research is going on worldwide to utilize fly ash and minimize disposal of fly ash into landfill which can pose great threat to the environment (Kosior-Kazberuk and Lelusz 2007). To mitigate the environmental impact, fly ash has to be utilized in various applications (Yoshitake et al. 2011). Fly ash is being currently employed as partial replacement for cement in the construction sector which brings ample economical, technical as well as environmental benefits leading to cleaner environment, high-durability service and cost-effective structures (Kosior-Kazberuk and Lelusz 2007). Fly ash a pozzolanic material affects the properties of mortar and concrete by its pozzolanic characteristics. Pozzolanic activity of fly ash is due to the presence of surplus quantity of silica, alumina and iron oxide (Yazici and Arel 2012). At early age, strength gain in fly ash concrete is significantly low, while at later age, strength gain is observed. The key factor for low strength at early age for fly ash concrete is unavailability of required quantity of $Ca(OH)_2$ (Barbhuiya et al. 2009).

Cement, a basic raw material in construction industry, industry is one of the most energy-intensive industries consuming about 4Gj/tonne of cement. It contributes to

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about 11–14% of the total energy utilized by the industry (Najjar and Waite 2016). Excessive use of cement has as a negative effect on the environment. Hence, concrete cannot be termed as a sustainable material (Jonkers et al. 2010). FA when used in concrete could substantially contribute to reduce the overall CO_2 footprint of the final concrete product. Fly ash used in concrete acts as a supplementary material (Yoshitake et al. 2011). Due to fineness of fly ash, movement of harmful substances into the matrix of concrete sustainably decreases thereby enhancing durability performance. The strength gain of fly ash concrete is inferior in comparison with normal concrete at young age. With longer duration of age, fly ash concrete strength tends to increase due to pozzolanic reaction (Yazici and Arel 2012). The present study aims to investigate the effect of biological technology on enhancing the property of fly ash concrete by varying the bacterial solution to water ratio. The concept is based on the application of specific microorganisms to produce biominerals for the sake of environmental engineering (Vahabi et al. 2015). This biological technique is termed as microbiologically induced calcite precipitation. Addition of few microbes to the concrete improves the performance by deposition of calcite in the pores of concrete by biological process. Bacteria addition mediates the precipitation of minerals by microbiologically induced calcite precipitation technique (MICP) (Wang et al. 2015). Several studies pertaining to repair of cracks in concrete have been reported. From various literature studies reported on crack healing, it is seen that the genus Bacillus is being used as bio-agent for biomineralization process (Sahoo et al. 2016). Bacilli are Gram-positive, rod-shaped, endospore-forming bacteria commonly found in soil. The precise role of the bacteria in calcite precipitation process is yet not clear, but various bacteria are capable of precipitation of calcium carbonate. Precipitation occurs as a byproduct of common metabolic processes such as photosynthesis, sulphate reduction and urea hydrolysis. Upon hydrolysis of urea, carbonate ions are produced in a calcium-rich environment and thereupon produce calcite.

Several research teams till date have reported remediation of cracks in concrete or mortar. Luo et al. (2015) developed bacteria-based self-healing concrete. The effect of bacteria was studied with varying conditions such as depth and curing conditions. Authors concluded that microbial self-healing agent would be applied to meet the goal of concrete crack self-healing. Kim Van Tittelboom in his work used ureolytic bacteria to precipitate calcite by conversion of urea into ammonium and carbonate. Precipitation of calcite fills cracks in concrete which creates a bridge in the cracks (Van Tittelboom et al. 2010). Biotechnology tool was adopted by Lucas et al. (2018) for remediation of cracks in concrete. In their experiments, bacteria were blended with cement–sand mixture and were used as bio-agent for crack remediation in concrete. They concluded that efficient remediation of cracks in concrete was obtained.

In addition to this, application of biological method in enhancing the property of concrete has been studied. Andalib et al. (2014) conducted experimental studies on biotechnological method to achieve high-strength bio-concrete durability. Authors specified that Bacillus species can withstand boiling water. Bacteria (Bacillus) of 30×10^5 cells/ml concentration were introduced into different structural concrete grades (40, 45 and 50 MPa). The authors observed that maximum gain in strength and weight loss occurred in H_2SO_4 immersion compared to HCl. The investigation showed better results in the presence of bacteria compared to conventional concrete. The micro-structural study was undertaken to confirm calcite precipitation by biochemical process. It was concluded that SEM images showed good evidence of calcite precipitation in the concrete matrix. Vahabi et al. (2015) showed that the optimum pH for calcite precipitation for *B. licheniformis* AK01 strain is between 7 and 11. Results show around 15% improvement in strength property. Results by V Achal show that inclusion of bacteria enhanced the transport property. Ghosh et al. (2005) in their study showed that thermophilic anaerobic microorganism of different concentrations when mixed with concrete improved strength by 25%. De Muynck et al. (2008) used *B. sphaericus* for enhancing the transport property of mortar specimens. Around 40% decrease in water absorption and permeability for bacterial specimens was observed. The results obtained with biotechnological treatment were similar to that of conventional treatments. Ramachandran et al. (2001) reported that *Sporosarcina pasteurii* have the potential to enhance the properties of cementitious composites. Siddique et al. (2016) have developed a relationship between compressive strength and chloride permeability and sorptivity of bacterial concrete. A high value of coefficient of determination shows better correlation between strength and durability property. Sahoo et al. (2016) carried out experimental investigation to study the potential of *B. subtilis* on improving the property of recycled coarse aggregate concrete (RCAC). The growth kinetics was developed to select the desired concentration of *B. subtilis* required to be added to concrete. Significant improvement in (18.62%) strength and durability property was reported in *B. subtilis*-blended RCAC. SEM study showed more crystalline calcium carbonate in recycled coarse aggregate concrete embedded with bacteria compared with normal concrete. It was also mentioned that XRD images show high calcite formation with higher intensity in bacterial concrete. The current paper aims to apply the promising bio-deposition technique to fly ash concrete to strengthen the concept of sustainability in concrete industry by combining the use of FA with *S. pasteurii* strains. The performance is assessed by evaluating compressive strength, split tensile strength, water absorption and sorptivity properties at various ratios of bacterial solution to water. The performance of bacteria-embedded fly ash concrete was assessed by comparing with conventional specimens. Conventional specimen is prepared without the addition of bacteria. However, normal concrete is prepared without the addition of fly ash and bacteria. The knowledge obtained through this study can be instrumental in developing sustainable fly ash concrete thereby substantially reducing the disposal of fly ash.

Experimental program

Material selection

Cement and aggregates

Commercial Ordinary Portland cement of grade 43 was used throughout this study. Natural river sand conforming to zone II of IS 383:1970 was used as fine aggregate. Natural gravel with maximum size 20 mm and specific gravity 2.66 was used as coarse aggregate. The fly ash used for this study was procured from Raichur thermal power plant. The properties of cement and fly ash are shown in Table 1.

Bacterial strains

The bacterial strain considered for this research is *S. pasteurii* (Ramachandran et al. 2001). This strain (Sierra-Beltran et al. 2014) was procured from National Collection of Industrial Microorganisms (NCIM), Pune. *S. pasteurii* bacteria are alkaliphilic (alkali resistant) spore-forming bacteria (Sierra-Beltran et al. 2014). Liquid medium solution for *S. pasteurii* was prepared using nutrient broth solution supplemented with urea and $CaCl_2$ (15). *S. pasteurii* spores have the ability to withstand high mechanical stress and have long-term viability up to 200 years under dry conditions (Sierra-Beltran et al. 2014). The cells of *S. pasteurii* do not aggregate and thus it ensures a high cell surface to volume ratio that is essential for efficient cementation initiation (Abo-El-Enein et al. 2013).

Table 1 Properties of Portland cement and fly ash used in this study

Constituents	Percentage (%)	
	Portland cement	Fly ash (%)
SiO_2	22.0	61.9
Al_2O_3	4.71	27.0
Fe_2O_3	2.76	7.00
CaO	64.3	0.70
MgO	1.00	0.60
Na_2O	0.14	0.93
K_2O	0.61	0.90
LOI	0.90	0.80
Specific gravity	3.15	2.30

Experimental methods

Concrete specimen preparation and curing regimes

The concrete mix proportion was done as per IS10262-2007. Cement, fine aggregate and coarse aggregate were weighed and mixed according to mix proportion for M25 grade of concrete and the details are provided in Table 2. Cement was partially replaced by fly ash in different propositions such as 0%, 10%, 20% and 30%. For this dry mix, *S. pasteurii* of bacterial dosage 10^5 cells/ml of mixing water was added. Dosage of 10^5 cells/ml was found to be optimum from our previous study (Santosh et al. 2017). Water to cement ratio maintained was 0.5. Experiments were performed for 0.3, 0.4 and 0.6 ratios of bacterial solution to water. Control concrete was prepared with plain water (without the addition of bacterial solution). Mixing was done in a mixer for duration of 8 min. No additional supplements were added during mixing and preparation of concrete cubes. After 24 h, the specimens were demoulded and transferred to curing tank and allowed to cure in tap water for 7, 28, 60 and 90 days in the absence of urea.

Compressive strength development

Samples of dimensions 150 × 150 × 150 mm were cast for M25 grade of concrete. Cubes were compacted in a table vibrating machine. Bacterial and conventional specimens were cured for 7, 28, 56, 90 and 180 days and compressive strength test was performed in compliance with IS 516:1959. Tests for compressive strength were performed in triplicate. Compressive strength was determined using digital testing machine of 2000 kN.

Tensile strength

Cylinder of diameter 150 mm and length 300 mm was cast for the determination of split tensile strength. The concrete was compacted in a table vibrating machine. The tests were carried out in triplicate and average values were reported. Split tensile strength was determined in compliance with IS 5816:1999 at 14, 56 and 180 days. Tensile strength was determined using digital compression machine of 2000 kN capacity.

Table 2 Mixture proportions of concretes with and without fly ash

Cement, kg/m^3	Fine aggregates, kg/m^3	Coarse aggregates, kg/m^3	W/C ratio	Water, kg/m^3	Fly ash replacement (%)	Bacterial solution to water ratio	Bacterial 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

Water absorption

Bacterial and control specimens for water absorption tests were prepared in the same way as mentioned for compressive strength. All the specimens were dried in an oven at 105° C. Weights of specimens were noted and allowed to cool to room temperature. The specimens were immersed in water for 14, 56 and 180 days and weight was noted regularly. This procedure was continued until it became saturated. Saturated water absorption (IS 3495:1992) was evaluated by the following formula:

$$[W2 - W1/W1] \times 100,$$

where $W2$ is the saturated weight, kg; $W1$ is the oven-dried weight, kg.

Sorptivity

Sorptivity is a measure of the capillary forces exerted by the pore openings which draw fluids into the matrix of the concrete. The bacteria-blended fly ash concrete specimens were kept on small supports and immersed in water to a depth of 10 mm. At regular intervals of time, rise in water level which was observed by dark colour was noted. This procedure was followed until the rise of water level was stopped. The sorptivity of (IS 4031–1988) bacteria-blended specimens was calculated by the following formula:

$$S = i/\sqrt{t},$$

where S is sorptivity in mm/min$^{1/2}$, i is rise of water level in mm and t is elapsed time in s

Results and discussion

All the test results are obtained for bacterial solution to water sample ratios of 0.3, 0.4 and 0.6. As per ASTM or IS standard testing method, no fixed proportion is available for bacteria solution. Therefore, in this study, experiments were performed to find the effect of variation of bacterial solution to water sample ratio on the strength and durability properties of various concrete samples.

Compressive strength

This research investigates the potential of *S. pasteurii* on improving the strength properties of fly ash concrete. Various researchers have performed work pertaining to fly ash concrete. Until now, research was focussed on the addition or replacement of cement with fly ash. Strength gain has not been on the higher side for fly ash concrete in comparison with conventional concrete. To overcome this drawback, a novel idea of using urease-producing bacteria to improve the mechanical properties of fly ash concrete is implemented. Bacterial cell concentration was kept constant throughout the research (10^5 cells/ml). The results of compressive strength are as shown in Table 3 for bacterial solution to water ratios of 0.3, 0.4 and 0.6. Strength gain recorded for

Table 3 Effect of *S. pasteurii* on strength properties of fly ash concrete

Fly ash replacement (%)	Average compressive strength (MPa)							
	14 days		28 days		56 days		120 days	
	Conventional concrete	Bacterial concrete	Conventional concrete	Bacterial concrete	Conventional concrete	Bacterial concrete	Conventional concrete	Bacterial concrete
Bacterial sample solution/water = 0.6								
0	28.39	35.46	33.33	41.33	37.62	44.62	41.30	46.20
10	26.40	31.60	31.84	38.65	36.40	42.40	40.80	44.60
20	25.40	29.80	30.59	36.47	35.20	40.20	39.90	43.90
30	23.80	27.21	29.18	33.93	33.20	38.90	37.80	43.10
Bacterial sample solution/water = 0.4								
0	28.39	34.12	33.33	42.21	37.62	43.74	41.30	45.90
10	25.70	29.90	31.84	37.45	36.4	40.78	40.80	44.10
20	24.80	28.71	30.59	35.86	35.2	39.81	39.90	42.60
30	23.40	25.83	29.18	32.83	33.2	37.87	37.80	41.40
Bacterial sample solution/water = 0.3								
0	28.39	31.4	33.33	39.40	37.62	41.30	41.30	45.20
10	25.70	28.1	31.84	36.10	36.40	40.20	40.80	43.60
20	24.80	26.30	30.59	33.44	35.20	37.70	39.90	42.40
30	23.40	24.60	29.18	32.10	33.20	35.90	37.80	40.90

bacteria-embedded fly ash concrete was higher than conventional concrete at all test ages. Maximum increment in terms of percentage was observed to be 24.89% for bacterial concrete at 14 days for bacterial solution to water ratios of 0.6 compared to conventional concrete. A higher bacterial solution to water ratio has influenced higher compressive strength. A decreasing trend was observed with decrease in ratio. At a bacterial solution to water ratio of 0.3, lower gain in strength was observed but higher compared to conventional or normal concrete. This may be due to the presence of lower concentration of bacterial cells.

Results demonstrate that with curing time compressive strength was found to increase with high gain of strength at early age with gradual decrease at longer duration for all types of bacteria-embedded concrete as shown in Table 3. At young age, concrete specimens are porous which may lead to movement of oxygen and water into the matrix of specimens (Farmani et al. 2015). This may have resulted in high rate of biomineralization process during the initial 14 days in comparison with 56 or 120 days. However, after certain age, porosity may decrease which minimizes the movement of water and oxygen into the matrix of concrete. At this stage, bacteria will go into dormant state and activity of bacteria may come to halt. The spores of the bacteria can survive for decades without food or water. With age, micro-cracks may start to develop which may allow movement of water into the pores of concrete (Zhu et al. 2015). This awakens the bacteria and biomineralization process may start to take place. It was observed that for 120 days of curing, the bacteria-embedded fly ash concrete with up to 30% cogeneration plant fly ash gave a compressive strength up to 8% higher than the conventional concrete at all ratios of bacterial solution to water. The enhancement in compressive strength may be attributed to the bio-deposition process where bacteria influence the precipitation of calcite by the production of urease enzyme (Siddique and Chahal 2011). As a result, these pores may get filled with calcite near the bacterial cells which leads to increase in strength property. The urea enzyme secreted by the above-mentioned bacteria acts as a biocatalyst (Farmani et al. 2015). Use of fly ash up to 30% as cement replacement will have dual advantage. Primarily, fly ash utilization minimizes the consequences faced by the fly ash disposal. Use of fly ash up to 30% reduces the amount of cement requirement which minimizes CO_2 gas emissions from cement industry (Van Tittelboom et al. 2010). Moreover, at an age of 56 days, bacteria-embedded fly ash concrete can gain strength, which can be achieved by conventional concrete only in 120 days.

A simple equation to predict compressive strength with bacterial solution to water ratio was developed by multiple regression. The software Minitab 17 was used for developing regression equation. The general multiple regression equation is given below (Devore and Berk 2012):

$$Y = \alpha + \beta_1 X_1 + \ldots + \beta_k X_k \pm e,$$

where Y is the independent variable; α is the Y-intercept; β_1 and β_k are the slopes associated with X_1 and X_b; X_1 and X_b represent the values of independent variables; e represents the error. The model developed to predict compressive strength at any bacterial solution to water ratio for concrete (with and without fly ash) at various ages is presented as given below:

$$f_c = 30.3 + 3.71 f_r - 0.233 f_f \quad R^2 = 0.81 (14\,\text{days})$$

$$f_c = 36.7 + 6.91 f_r - 0.241 f_f \quad R^2 = 0.82 (14\,\text{days})$$

$$f_c = 38.5 + 10.2 f_r - 0.178 f_f \quad R^2 = 0.93 (14\,\text{days})$$

$$f_c = 42.2 + 7.61 f_r - 0.126 f_f \quad R^2 = 0.95 (14\,\text{days})$$

where f_c, f_r and f_f are compressive strength, bacterial solution to water ratio and fly ash percentage, respectively. The empirical correlation equation developed is reliable since the value of correlation coefficient R^2 is greater than 0.75.

Ratio of compressive strength to split tensile strength

The ratio of compressive strength to split tensile strength is presented in Table 4 for 14 and 56 days. The ratio value for bacteria-embedded fly ash concrete was lower than the conventional concrete for 14 and 56 days. The ratio values were found to be varying between 9.10 and 9.58 for 14 days and similarly, for 56 days, it varies between 10.28 and 11 for bacteria-embedded fly ash concrete. The ratio for ordinary concrete is in the range between 10.5 and 15.2 (Shetty 2005). This shows that *S. pasteurii* and fly ash have pronounced effect on split tensile strength. This may be due to the deposition of layer of calcite on the surface of specimens (Siddique and Chahal 2011). Moreover, the increase in the split tensile strength is slightly slower at 56 days compared to 14 days of curing. At longer duration of time, porosity of the specimens may reduce which resists movement of oxygen or water into the matrix of concrete. In view of this, rate of biomineralization process may be retarded.

Water absorption

Water absorption test results are displayed in Table 5. It can be seen that incorporating *S. pasteurii* substantially decreases water absorption with respect to control concrete at ratios of 0.3 and 0.6. However, 10% replacement with fly ash proved to be more effective. Minimum water absorption for conventional concrete was observed to be 5.36% for 10% replacement with fly ash at 180 days. For bacteria-embedded fly ash concrete, this was achieved in 56 days for 10% replacement with fly ash for both the ratios. It can be seen in Table 5 that for 180 days, up to 30% replacement of

Table 4 Compressive and split tensile strength

Mixtures	Compressive strength (MPa)		Split tensile strength (MPa)		Compressive/split tensile strength	
	14 days	56 days	14 days	56 days	14 days	56 days
Bacterial sample solution/water = 0.6						
Conventional concrete	28.39	37.62	2.79	3.06	10.18	12.29
0	35.46	44.62	3.75	4.17	9.46	10.70
10%	28.39	42.4	3.47	4.05	9.10	10.46
20%	29.80	40.2	3.13	3.91	9.58	10.28
30%	27.21	38.90	2.94	3.53	9.30	11.00

Table 5 Effects of *S. pasteurii* on water absorption property of fly ash concrete

Fly ash replacement (%)	14 days		56 days		180 days	
	Conventional concrete	Bacterial concrete	Conventional concrete	Bacterial concrete	Conventional concrete	Bacterial concrete
Bacterial sample solution/water = 0.6						
0	6.80	5.91	6.62	5.78	6.30	5.63
10	5.80	4.99	5.54	4.90	5.36	4.72
20	6.01	5.34	5.90	5.16	5.68	4.96
30	6.34	5.67	6.28	5.58	5.90	5.18
Bacterial sample solution/water = 0.3						
0	6.80	6.14	6.62	5.98	6.30	5.87
10	5.80	5.12	5.60	5.08	5.36	4.94
20	6.01	5.60	5.90	5.57	5.68	5.37
30	6.34	5.92	6.28	5.88	5.90	5.49

bacteria-embedded fly ash concrete showed water absorption much lesser than 5.36 which was achieved by conventional concrete for both the ratios. The deposition of a layer of calcite crystals near the cells and the surface of concrete resulted in less water absorption compared to conventional concrete (Achal et al. 2011). Formation of calcite layer avoids movement or ingress of water or any harmful liquids into the matrix of concrete. Promising test results clearly confirm that combination of fly ash and *S. pasteurii* has a great potential in resisting water absorption into the matrix of concrete.

Sorptivity

Figures 1 and 2 present the influence of *S. pasteurii* on the sorptivity properties of control and bacteria-embedded fly ash concrete. The sorptivity property follows the same trend as water absorption does. Over a period of 90 days, the bacteria-embedded fly ash concrete absorbed up to 30% less water than conventional concrete for both the ratios. This may be due to the presence of *S. pasteurii* cells (Achal et al. 2011). *S. pasteurii* cells influence the biomineralization process in the concrete and result in filling of pores in the matrix of concrete. This leads to significant decrease in capillary water absorption of bacterial specimens. However, 0.6 ratio of bacteria-embedded fly ash concrete was superior to ratio of 0.3. It can be inferred from the current investigation that the fly ash and bacteria cells have a profound effect on the sorptivity property of concrete specimens.

Scanning electron microscopy (SEM)

Scanning electron microscopy was used to check the $CaCO_3$ crystals in fly ash concrete. Fourteen-day sample of normal and bacteria-embedded concrete was crushed into small pieces and scanning electron microscopic analysis was carried out at Shivaji University, Kolhapur. Figure 3 shows SEM image of control concrete. Several micro-pores and pores can be seen clearly. On the contrary, Fig. 4 shows an image of bacteria-embedded fly ash concrete where several openings are filled and covered with calcite crystals. As a result of this, a major improvement in the property of fly ash concrete is observed due to the incorporation of bacteria.

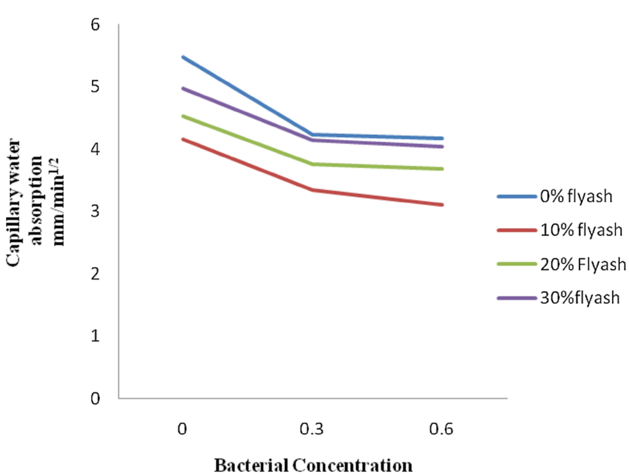

Fig. 1 Capillary absorption at 28 days of curing for varying proportions of bacterial solution to water ratio. All the tests were performed in triplicate and presented results are the average values

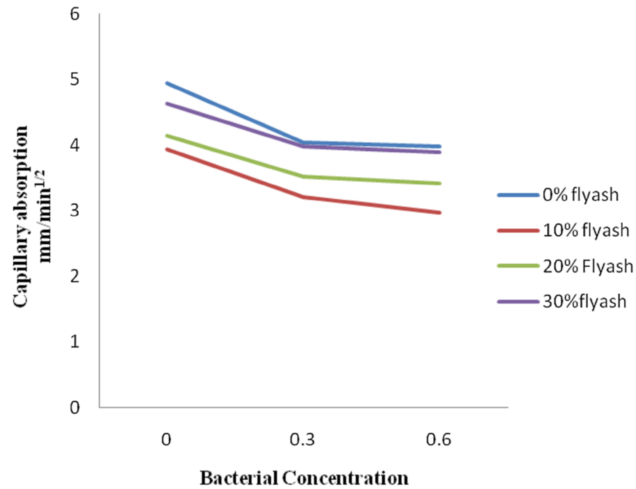

Fig. 2 Capillary absorption at 90 days of curing for varying proportions of bacterial solution to water ratio. All the tests were performed in triplicate and presented results are the average values

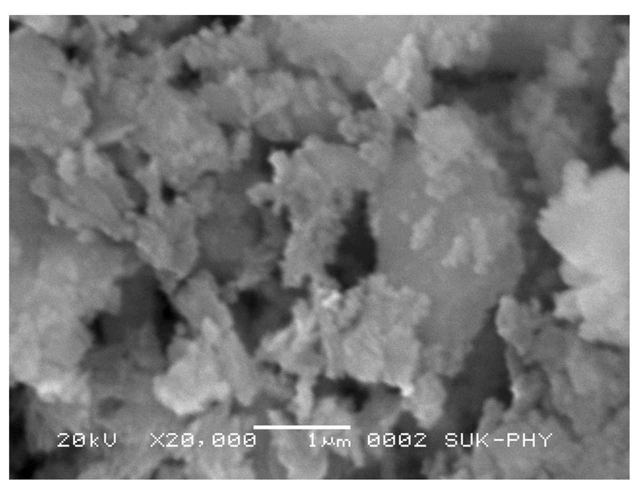

Fig. 3 Scanning electron micrograph of control concrete at 28 days of curing

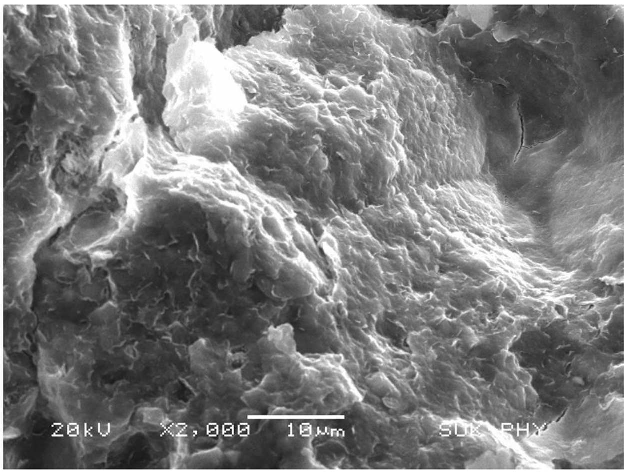

Fig. 4 Scanning electron micrograph of bacteria-embedded fly ash concrete at 28 days of curing

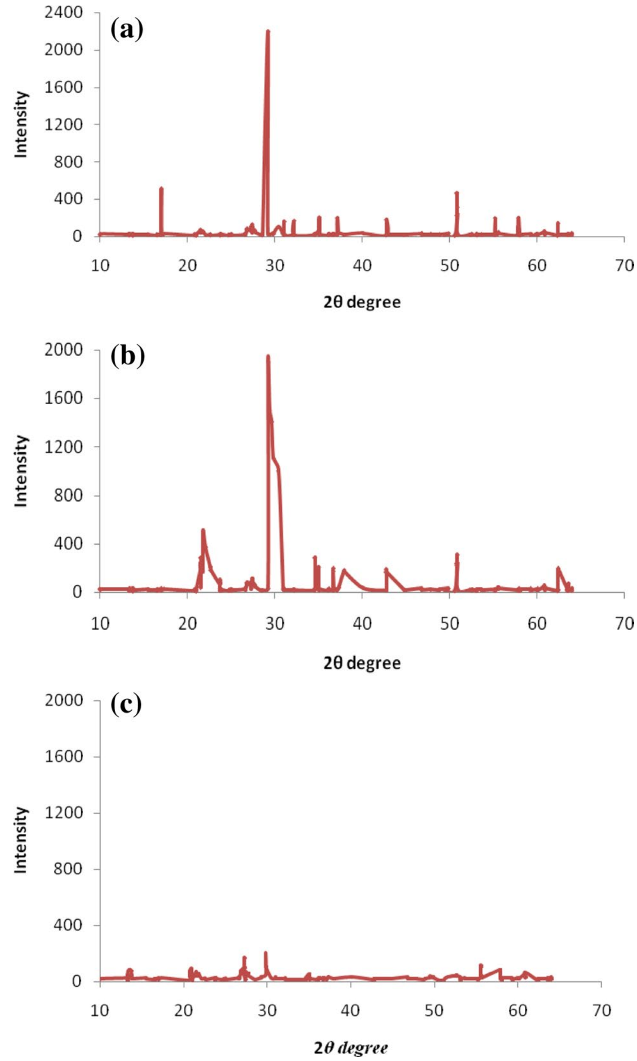

Fig. 5 XRD pattern of concrete samples at age of 28 days of curing: **a** bacterial concrete (bacterial solution to water ratio of 0.6), **b** bacterial concrete (bacterial solution to water ratio of 0.5) and **c** control concrete

X-ray diffraction (XRD) spectroscopy of concrete samples

In view of verifying calcite formation, the concrete samples were subjected to XRD analysis. The XRD analyses of bacterial and control samples are shown in Fig. 5, respectively. It is observed that the analysis result from PDF card shows more calcite is precipitated with higher intensity in concrete due to bacterial activity. The highest peak was observed at 2 theta (2 h) value of 29.27 for bacterial solution to water ratio of 0.6. Similar trends were observed for bacterial concrete for bacterial solution to water ratio of 0.4. This analysis confirms that the material produced is calcite. The above results confirm that *S. pasteurii* have the potential to precipitate calcite. XRD spectra of control samples showed no extra peaks. The results obtained were similar to that obtained by Chahal and Siddique (2013).

Conclusion

Based on the test results, the following major conclusions can be drawn:

1. Current study successfully demonstrated that incorporating alkaliphilic endospore-forming bacteria and fly ash increased strength properties up to 25% for concrete specimens for bacterial solution to water ratios of 0.6 and 0.4. However, it was less effective for a ratio of 0.3.
2. At early days of curing, i.e., 14- and 28-day bacterial-blended fly ash concrete showed improvement of around 25% in compressive strength compared to control concrete. Biomineralization technique overcomes the limitation of early strength gain in fly ash concrete. However, at longer duration, water may enter through small pores developed which activates the cells and process of biomineralization may restart leading to deposition of calcite.
3. Alkaliphilic endospore-forming bacteria and fly ash decreased capillary water absorption up to 30% for bacterial solution to water ratios of 0.6 and 0.3 and have the potential to enhance the service life of concrete exposed to any kind of fluid mixture.
4. The results show that the improvement in strength property of concrete specimens was dependent on the bacterial solution to water ratio. However, transport property performance was comparable for all ratios of bacterial solution to water.

5. Observation from test results reveals that the presence of fly ash does not hinder the rate of biomineralization process.
6. A modern biotechnology concept seems to be a cost-effective tool and also serves as an eco-friendly tool to prevent environmental pollution.
7. Use of alkaliphilic endospore-forming bacteria and fly ash (up to 30% replacement of cement) acts as a promising combination in the construction sector which greatly increases the service life of concrete structures. Fly ash utilization for up to 30% replacement of cement greatly reduces the burden of high cost of cement. Moreover, it leads to dual environmental benefit. First, fly ash disposal from coal power plant into landfill is greatly retarded. Second, cement replacement by fly ash lowers cement production thereby greatly reducing green house gas emission of cement plant.
8. Thus, this study proves that sustainable bacteria-embedded fly ash concrete can be produced by a novel biotechnology method which provides greener and economic options.

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Compliance with ethical standards

Conflict of interest We wish to inform that there is no conflict of interest associated with this publication.

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