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Influence of bacteria on the compressive strength, water absorption and rapid chloride permeability of fly ash concrete

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ABSTRACT

This paper presents the results of an experimental investigation carried out to evaluate the influence of *Sporoscarcina pasteurii* bacteria on the compressive strength and rapid chloride permeability of concrete made without and with fly ash. Cement was replaced with three percentages (10, 20 and 30) with fly ash by weight. Three different cell concentration (0, 10^3,10^5,10^7 cells/ml) of bacteria were used in making the concrete mixes. Tests were performed for compressive strength, water absorption and rapid chloride permeability at the age of 28 days. Test results indicated that inclusion of *S. pasteurii* in fly ash concrete enhanced the compressive strength, reduced the porosity and permeability of fly ash concrete. Maximum increase (22%) in compressive strength and four-times reduction in water absorption was observed with 10^5 cells/ml of bacteria. This improvement in compressive strength was due to deposition on the bacteria cell surfaces within the pores.

Calcite deposition in concrete observed nearly eight times reduction in chloride permeability of fly ash concrete. The present work highlights the influence of bacteria on the properties of concrete made with supplementing cementing material such as like fly ash. Usage of bacteria like *S. pasteurii* improves strength and durability and strength of fly ash concrete through self-healing effect.

1. Introduction

Concrete is the most widely used man made construction material in civil engineering world. It has specialty of being cast in any desirable shape but plain concrete however possesses very low tensile strength, limited ductility and little resistance to cracking. As a matter of fact, advancement in concrete technology has been generally on the strength of concrete. It is now recognized that strength of concrete alone is not sufficient, the degree of harshness of the environmental condition to which concrete is exposed over its entire life is very important. Therefore, both strength and durability have to be considered explicitly at the design stage. To do this, a durable structure needs to be produced. For concrete buildings, one of the major forms of environmental attack is chloride ingress, which leads to corrosion of the reinforcing steel and a subsequent reduction in the strength, serviceability and aesthetics of the structure. This may lead to early repair or premature replacement of the structure. A common method of preventing such deterioration is to prevent chlorides from penetrating the structure by using relatively impenetrable concrete. The ability of chloride ions to penetrate the concrete must then be known for design as well as quality control purposes. The penetrability of concrete is obviously related to the pore structure of the cement paste matrix. This will be influenced by the water–cement ratio of the concrete, the inclusion of supplementary cementing materials which serve to refine the pore structure and the degree of hydration of the concrete. The highly developed pore structure occurs due to greater amount of heat of hydration which inturn depends on the age of concrete. This is especially true for concrete containing slower reacting supplementary cementing materials such as fly ash require a longer time to hydrate.

Fly ash is generally used as replacement of cement, as an admixture in concrete, and in manufacturing of cement. This study explores the possibility of replacing part of cement with fly ash as a means of incorporating significant amounts of fly ash. All building materials are porous. This porosity of building material along with ingress of moisture and other harmful chemicals such as acids, chlorides and sulfates affect the material and seriously reduce their strength and life. An additive that seals the pores and cracks and thus reduces the permeability of the structure would immensely improve its life. Conventionally, a variety of sealing agents such as latex emulsions and epoxies and surface treatments with water repellents such as silanes or siloxanes are used to enhance the durability of the concrete structures. However, they suffer from serious limitations of incompatible interfaces, susceptibility to ultraviolet radiations, unstable molecular structure and high cost. They also emanate toxic gases.

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In order to overcome the shortcomings of conventional sealing agents, materials with self-healing capability can be used effectively. Use of urease producing bacteria such as *Sporoscarcina pasteurii* addresses these problems effectively, as these continue to survive and grow within the concrete structure after the initial use. Urease helps in mineralization of calcium carbonate, by hydrolyzing urea present in the environment. It releases carbon dioxide from urea that combines with calcium ions resulting in deposition of calcium carbonate in the form of calcite.

2. Literature review

The compressive strength development and corrosion-resisting characteristics of concrete mixes in which fly ash was used as partial replacement (equal quantity of sand replacement). Concrete mixtures were made with fly ash additions of 0%, 20%, and 30%, and water–cement ratios of 0.35, 0.40, 0.45, and 0.50. Based on the test results, it was concluded that addition of fly ash as an admixture increases the compressive strength and long-term corrosion-resisting characteristics of concrete [1]. The superior performance of these mixes compared to plain concrete mixes was attributed to the densification of the paste structure due to pozzolanic action between the fly ash and the calcium hydroxide liberated as a result of hydration of cement.

Berg and Neal [2] indicated that municipal solid waste bottom ash (MSWBA) could be used as an aggregate in concrete to produce CMU that meets ASTM C 90 standards when it is processed for maximum size and gradation and ferrous removal by magnetic separation.

Hwang et al. [3] studied the effects of fine aggregate replacement on the rheology, compressive strength, and carbonation properties of fly ash and mortar. Water-to-cement ratio were 0.3, 0.4, and 0.5. Cement was replaced with 25% and 50% levels. Test results showed that rheological constants increased with higher replacement level of fly ash and that, when water to Portland cement ratio was maintained, the strength development and carbonation properties were improved.

Bakoshi et al. [4] used coal bottom (10–40%) as replacement for fine aggregate, and observed that the compressive strength and tensile strength of bottom ash concrete generally increases with the increase in replacement ratio of fine aggregate and curing age. The freezing–thawing resistance of concrete using bottom ash is lower than that of ordinary concrete and abrasion resistance of bottom ash concrete is higher than that of ordinary concrete.

Biomineralisation of calcium carbonate is one of the strategies to remediate cracks in building materials because cracks not only influence the service durability on concrete structure, but also harmful for the structure safety [5]. Bacterial deposition of a layer of calcite on the surface of the specimens resulted in a decrease of capillary water uptake and gas permeability [6].

Muynck et al. [6] indicated that durability of mortar specimens with different porosity was affected by bacterial carbonate precipitation (biodeposition). The surface deposition of calcium carbonate crystals decreased the water absorption with 85% depending on the porosity of the specimens.

Bang et al. [7] developed immobilization technique for remediation of cracks in concrete, where microbial cells are encapsulated in polymers has been adapted to enclose calcium carbonate precipitation in the gap to enhance the strength for selective concentration. Microbial calcite precipitation (MCP) occurs as a by-product of common microbial metabolic process, such as urea hydrolysis, photosynthesis, sulfate reduction. These different metabolic processes increase the alkalinity (pH and dissolved inorganic carbon) and thereby favouring the calcium carbonate precipitation. Calcium carbonate precipitation is a general process in the bacterial world under appropriate conditions.

Castainer et al. [8] and Riding [9] reported that bacteria and fungi can induce precipitation of calcium carbonate extracellularly through a number of processes that include photosynthesis, ammonification, denitrification, sulfate reduction and anaerobic sulfide oxidation.

Braissant et al. [10] studied that *Bacillus pasteurii* a common soil bacterium can induce the precipitation of calcite. As a microbial sealant, $CaCO_3$ exhibited its positive potential in selectively consolidating simulated fractures and surface fissures in granites and in the consolidation of sand. Besides this, a durability study on concrete beams treated with bacteria, exposed to alkaline, sulfate and freeze–thaw environments were also studied. The durability performance increased with increase in the concentration of bacteria [11].

3. Experimental program

3.1. Isolation and morphology of bacteria

Calcium carbonate precipitating bacteria were isolated from Rhizopheric soil (tulsi plant) and Alkaline soil. The samples were suspended in a sterile saline solution (0.85% NaCl), diluted properly and plated on precipitaion agar containing urea (20 g/l), $NaHCO_3$ (2.12 g/l), NH_4Cl (10 g/l), Nutrient broth (3 g/l), $CaCl_2 \cdot 2H_2O$ (25 g/l). Incubation was done at 28 °C. Colonies were assessed every 5 days with a stereo microscope (Zeiss) and selected as positive based on visual crystal formation within 10 days. Positive isolates were purified through repetitive dilution and plating (as described above). The isolated bacteria were identified as *Sporosarcina pasteurii* also known as *B. pasteurii* from older taxonomies. This bacteria has the ability to solidify organic nitrogen source through the process of biological cementation. *S. pasteurii* has been proposed to be used as an ecologically sound biological construction material [12]. On the basis of Calcinate formation one dose of bacteria was selected for final preparation of concrete mixture by partial replacement of cement with fly ash. Different concentrations of cells (10^3, 10^5, 10^7 cells/ml) were obtained by growing culture for different time followed by centrifugation at 8000 rpm for 10 min at 4 °C.

3.2. Properties of ordinary Portland cement

Ordinary Portland cement was used. It was tested as per Indian Specifications IS: 8112-1989 [13]. Its physical and chemical properties are shown in Tables 1 and 2.

3.3. Properties of fly ash

Physical and chemical properties of fly ash from Bathinda thermal power plant (Punjab, India) was analyzed as per ASTMC 618 [14]. Fly ash has a very high content of amorphous silicon dioxide and consists of fine spherical particles along with small amounts of iron, magnesium, and alkali oxides were found. Test results are shown in Tables 3 and 4 respectively.

3.4. Properties of fine and coarse aggregate

Natural sand with a 4.75-mm maximum size as fine aggregates and coarse aggregate with 12.5 mm nominal size was used. They were tested as per Indian Standard Specifications IS: 383-1970 [15]. Their physical properties are given in Table 5.

3.5. Concrete mixture proportions

Control concrete mixture was designed as per IS 10262-1982 [16] to have 28-day compressive strength of 28 MPa. Then cement was partially replaced with 0%, 10%, 20%, and 30% fly ash by weight of cement with varying amount of bacterial culture (*S. pasteurii*). Various amounts of bacterial culture of *S. pasteurii* was mixed containing (0%, 10%, 20%, and 30%) fly ash.

Table 1
Physical properties of ordinary Portland cement (OPC).

Physical property	Value
Consistency of standard cement paste (%)	36
Initial setting time (min)	123
Final setting time (min)	174
Compressive strength (MPa)	
3 days	16
7 days	35
28 days	46
Specific gravity	2.9
Standard consistency (%)	34

Table 2
Chemical properties of ordinary Portland cement (OPC).

Chemical	Constituent%
SiO_2	21.04
Al_2O_3	5.02
Fe_2O_3	3.12
CaO	62.11
MgO	2.44
$K_2O + Na_2O$	1.04
SO_3	3.12

Table 3
Physical properties of fly ash (ASTM C 618).

Color	Dark gray
Specific gravity	2.4
Bulk density (kg/m^3)	700
Surface area (kg/m^2)	19,000

Table 4
Chemical properties of fly ash (ASTM C618).

Compound	% By mass
SiO_2	58.11
Al_2O_3	27.21
Fe_2O_3	5.23
CaO	2.14
MgO	0.72
$K_2O + Na_2O$	1.0
Loss on ignition	1.52

Table 5
Physical properties of fine and coarse aggregate.

Property	Value	
	Fine aggregate	Coarse aggregate
Specific gravity	2.58	2.15
Fineness modulus	2.18	5.57
Unit weight (kg/m^3)	1672	1598

Table 6
Concrete mix proportions with and without fly ash (FA).

Mixture no.	M-1	M-2	M-3	M-4
Cement (kg/m^3)	390	390	390	390
Natural sand (kg/m^3)	568.7	568.7	568.7	568.7
Fly ash (%)	0	10	20	30
Coarse aggregate (kg/m^3)	1164.12	1164.12	1164.12	1164.12
W/C ratio	0.5	0.5	0.5	0.5
Water (kg/m^3)	185	185	185	185
Slump (mm)	90	85	80	80

3.6. Preparation of test specimens

Concrete cubes were prepared with different concentrations of *S. pasteurii.* The cell concentration was determined from the bacterial growth curve made by observing optical density at 600 nm. Control concrete cubes were cast without the addition of microbes. All the experiments were performed in triplicates. Further following properties were studied at the age of 28 days: Compressive strength (IS: 516-1959) – 150 mm cubes [17], Rapid chloride permeability test (ASTM C1202) – 100 × 50 mm cylindrical specimens [18] and Water porosity – 70 mm specimens ASTM C 642 [19]. Mixture proportions are given in Table 6.

3.7. Compression strength

Fly ash was added by replacing the amount of cement at the concentrations of 0%, 10%, 20% and 30%. To study the compressive strength test of cement mortar, *S. pasteurii* was grown in medium (described above). Concrete as per specifications of Compressive strength cubes were cast [17]. Sand and cement were thoroughly mixed, adding along with grown culture of *S. pasteurii*. Cubes were cast and compacted in a vibration machine. After de-molding, all specimens were cured compression testing at 28 days. Control specimens were also prepared in similar way where water and medium (described above) replaced bacterial culture. Compression testing was performed using automatic compression testing machine.

3.8. Water absorption

The water absorption test was conducted as per ASTM C 642 [19] in order to determine the increase in resistance towards water penetration in concrete. The cube molds of 70 mm were prepared both with and without bacteria and fly ash. The concrete specimens were cured for 28 days. After curing, the specimens were oven dried at 110 °C in oven, establishing a mass equilibrium of less than 0.5% between two measurements at 24 h intervals. Then the specimens were immersed in water at approximately 21 °C for 48 h and saturated mass after immersion was calculated. Then the specimens were placed in suitable receptacle, covered with tap water and were boiled for 5 h, further the saturated mass after boiling was calculated. The specimens were suspended by a wire and the apparent mass in water was calculated as per the formula:

$$\text{Volume of permeable voids } \% = (C - A)/(C - D) \times 100$$

where A is the mass of the oven dried sample in air, grams, C is the mass of sample after immersion and boiling, grams, and D is the apparent mass of sample in water after immersion and boiling, grams.

3.9. Rapid chloride permeability

Corrosion is mainly caused by the ingress of chloride ions into concrete annulling the original passivity present. Rapid chloride permeability test (RCPT) has been developed as a quick test able to measure the rate of transport of chloride ions in concrete. This test was conducted as per ASTM method [18]. Details of experimental set up are shown in Fig. 1. Concrete disc of size 100 mm diameter and 50 mm thickness with and without bacterial culture were cast and allowed to cure. After curing the concrete specimens were subjected to RCPT by applying 60 V. Two halves of the specimens are sealed with PVC container of diameter 90 mm. One side of the container is filled with 3% sodium chloride solution (that side of the cell will be connected to the cathode terminal of the power supply) and other side sodium hydroxide solution (0.3 N) was poured and connected to anode terminal. The interpretation is that the larger the Coulomb number or the charge transferred during the test, the greater the permeability of the sample. The concrete which is more permeable will show higher charge transfer vice versa. The method has shown good correlation with chloride tests. The following formula, based on the trapezoidal rule can be used to calculate the average current flowing through one cell.

$$Q = 900(I_0 + 2I_{30} + 2I_{60} + 2I_{90} + 2I_{120} + \cdots + 2I_{300} + 2I_{330} + I_{360})$$

where Q is the current flowing through one cell (coulombs), I_0 is the current reading in amperes immediately after voltage is applied, and I_t is the current reading in amperes at t minutes after voltage is applied.

Table 7 shows the rating of chloride permeability according to ASTM method [18].

3.10. Scanning electron microscopy (SEM)

The deposition of calcite inside the microcracks of concrete by bacteria (*Sporoscarcina pateurii)* were analyzed under SEM. The samples for SEM were taken after the compressive strength test; the broken specimens were collected and dried at

Fig. 1. Rapid chloride permeability apparatus.

100 °C in oven for 3 days and then examined at accelerating voltages ranging from 30 to 35 kV by a SEM (Zeiss EVO50). Samples were gold coated with a sputter coating Emitech K575 prior to examination.

4. Result and discussion

4.1. Compression strength

Effect of *S. pasteurii* bacteria on the 28-day compressive strength of all concrete is given in Table 8 and shown in Fig. 2. It is evident that compressive strength of fly ash concrete increased with increase in bacteria cell concentration up to 10^5 cells/ml, and then there was reduction in the strength at 10^7 cells/ml. Maximum increase in compressive strengths was achieved at 10^5 cells/ml for all fly ash concretes.

For control concrete (0% fly ash) with 10^5 cells/ml bacterial cells, there was 22% improvement in the compressive strength (28 MPa) with respect to compressive strength (24 MPa) of control concrete with out bacteria cells.

In fly ash concretes, there was 20% improvement in compressive strength of concrete (10% fly ash) with the inclusion of 10^5 cells/ml bacterial cells. Similarly, there was 15% and 11% improvement in compressive strengths of concretes with 20% and 30% fly ash contents with the addition of 10^5 cells/ml bacterial cells. The improvement in compressive strength by *S. pasteurii* is probably due to deposition of $CaCO_3$ on the microorganism cell surfaces and within the pores of, which plug the pores within the binder matrix. Similar results were reported by other researchers [20–22]. The results from the study showed that due to inclusion of bacteria in fly ash concrete, compressive strength was improved which would in-turn increase the overall durability performance of the concrete. The increase in compressive strengths is mainly due to filling of the pores inside the cement mortar cubes with microbiologically induced calcium carbonate precipitation.

4.2. Water absorption

The influence of bacteria on the water absorption of fly ash concrete is given in Table 9 and shown in Fig. 3. Water absorption test at 7-days was conducted as per ASTM C 642 [19]. It can be seen from this figure that with the inclusion of bacteria, water absorption capacity of fly ash concretes decreased with the increase in bacteria concentration. Maximum reduction in water absorption was observed with 10^5 cells/ml for all fly ash concretes; however, concrete with 10% fly ash concrete gave 3.25% water absorption (minimum).

The presence of bacteria resulted in a significant decrease in the water uptake compared to control specimens. The deposition of a layer of calcium carbonate on the surface and inside pores of the concrete specimens resulted in a decrease of water absorption and permeability. Once the pores are sealed, reduction in water ingress is observed. This bacterial action deposition can seal the pores, voids and microcracks, where other sealants are unable to work through. Nemati and Voordouw [23] noticed a decrease in the permeability of sandstones cores after injecting calcium carbonate forming reactants. Hence, from this experiment, it is clear that the presence of a layer of carbonate crystals on the surface by bacterial cells has the ability to improve the resistance of cementitious materials towards degradation.

Table 7
RCPT ratings (as per ASTM C1202-97).

Charge passed (C)	Chloride ion penetrability
>4000	High
2000–4000	Moderate
1000–2000	Low
100–1000	Very low
<100	Negligible

Table 8
Effect of Bacteria (*Sporoscarcina pasteurii)* on compressive strength of fly ash concrete.

Mixture no.	Bacteria concentration (cells/ml)			
	0	10^3	10^5	10^7
M-1 (0% fly ash)	24	25	28	26
M-2 (10% fly ash)	23	24	27.6	25
M-1 (20% fly ash)	22	23	26	24
M-1 (30% fly ash)	21	22	25	23

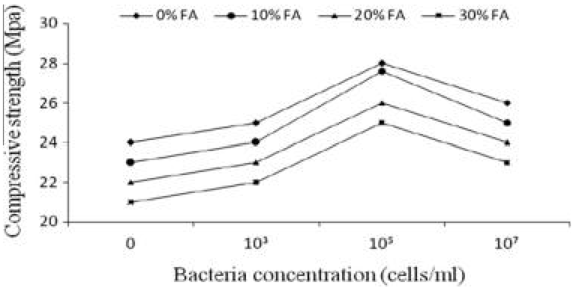

Fig. 2. Effect of bacteria (*Sporoscarcina pasteurii)* on compressive strength of fly ash concrete at 28 days.

Table 9
Effect of bacteria (*Sporoscarcina pasteurii*) on water absorption of fly ash concrete.

Mixture no.	Bacteria concentration (cells/ml)			
	0	10^3	10^5	10^7
M-1 (0% fly ash)	17.7	14	13	13.7
M-2 (10% fly ash)	14	4	3.25	3.7
M-1 (20% fly ash)	16	6.9	5.2	7
M-1 (30% fly ash)	17.4	7.8	6.9	8

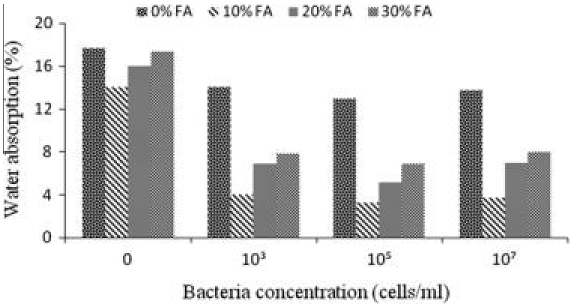

Fig. 3. Effect of bacteria (*Sporoscarcina pasteurii*) on water absorption of fly ash concrete at 7 days.

Table 10
Effect of bacteria (*Sporoscarcina pasteurii*) on the rapid chloride permeability of fly ash concrete.

Mixture no.	Bacteria concentration (cells/ml)			
	0	10^3	10^5	10^7
M-1 (0% fly ash)	1988	1210	989	1382
M-2 (10% fly ash)	1943	1189	915	1268
M-1 (20% fly ash)	1604	1062	789	1293
M-1 (30% fly ash)	1266	853	762	1120

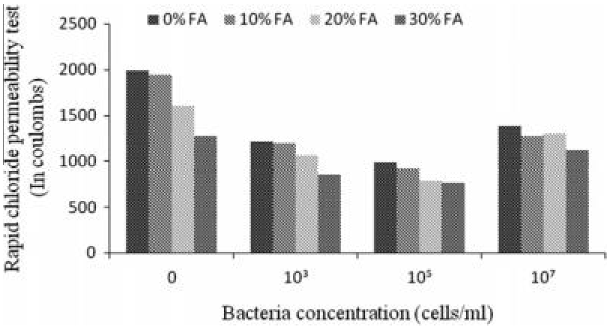

Fig. 4. Effect of bacteria (*Sporoscarcina pasteurii*) on the rapid chloride permeability of fly ash concrete at 28 days.

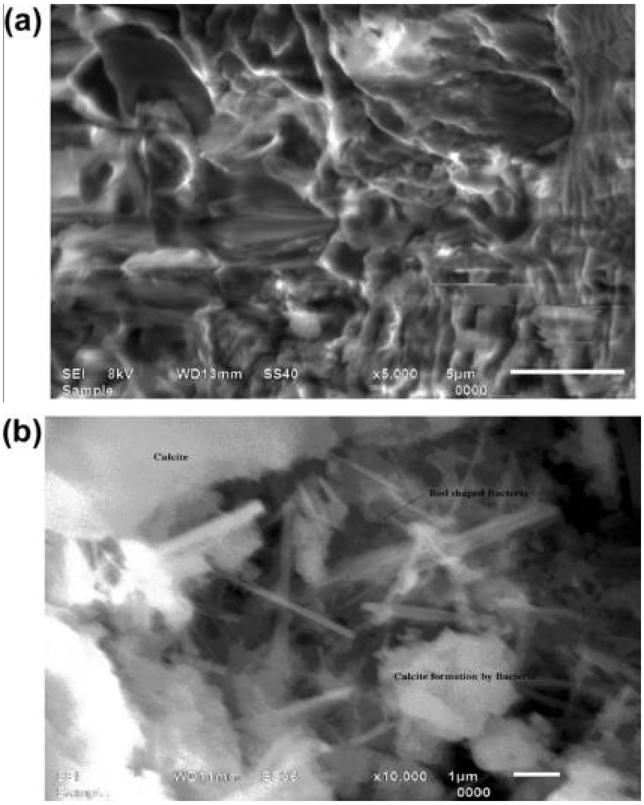

Fig. 5. SEM images of (a) control concrete and (b) fly ash concrete (containing 10% fly ash + 10^5 cells/ml of bacteria) showing bacterial induced calcite deposition in micro cracks.

4.3. Rapid chloride permeability

Results of the effect of bacteria on the rapid chloride permeability of fly ash concrete at the age of 28 days, is given in Table 10 and shown in Fig. 4. It is clear from this figure that with the inclusion of bacteria, chloride ingress capacity of fly ash concretes decreased with the increase in bacteria concentration. Maximum reduction in chloride ions was observed with 10^5 cells/ml for all fly ash concretes; however, concrete with 30% fly ash concrete gave 762 coulombs penetration which is considered to be very low. The ability of concrete to resist the penetration of chloride ions is a critical parameter in determining the service life of concrete structures exposed to deicing salts or marine environments. The concrete containing fly ash along with optimized dose of bacteria (*S. pasteuri*) showed good resistance towards rapid chloride penetration.

4.4. Scanning electron microscopy

Calcite precipitation in fly ash concrete was carried out by SEM analysis. Fig. 5a shows the SEM picture of control concrete, wherein, pores can be easily seen inside it. The SEM analysis of fly ash concrete with *S. pasteurii* has revealed distinct calcite crystals embedded in concrete. High calcium amounts in it confirmed that calcite was present in the form of calcium carbonate due to bacteria. Fig. 5b shows the presence of crystalline calcium carbonate associated with bacteria. The deposition of calcite serves as barrier to harmful substances and thus improves impermeability.

5. Conclusions

- Bacteria *S. pasteurii* plays a significant role in increasing the compressive strength of fly ash concrete by up to 22% at the age of 28 days.
- The increase in compressive strength is mainly due to consolidation of the pores inside the fly ash concrete cubes with bacterial induced calcium carbonate precipitation.
- *S. pasteurii* causes four times reduction in water absorption which could inturn increase durability of concrete structures.
- Bacterial calcite deposition observed nearly eight times reduction in chloride permeability, hence the shelf life of the concrete structures can be increased.

6. Suggestions for future research

- This study could also be conducted for other types of cement.
- Long term investigation of the properties could also be carried out. For which, investigations are already in progress and would be communicated in future publications.

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