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Quantification of Self-Healing Effect of Bacillus Subtilis on Cementitious Material

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Abstract: The creation of smart self-healing materials and preventative repair techniques is required due to the growing concern for the safety and sustainability of buildings. Although the development of microcracks rarely affects a building's structural qualities, the increased permeability caused by microcrack networking poses a significant threat to the durability of concrete structures by increasing the likelihood that aggressive materials will enter the structure. This study evaluates the self-healing capacity of bacterial concrete with that of traditional concrete. Bacterial spores and calcium lactate, a two-component biochemical agent, are released from the particle by crack ingress water upon fracture development. The self-healing agent is embedded into the cement mortar and its behavior is studied. Control and bacterial specimens are cast and the tests like compressive strength, porosity, UPV test, ESEM, EDAX are done. The results were studied for 15,30 and 45 days of healing. After 45 days submerged in water, experimental results revealed crack-healing of up to 7.6% of cracks in bacterial concrete but only up to 2.46% of cracks in control specimens.

Keywords: Self-healing agent**,** compressive strength, porosity, calcium precipitates, microscope, UPV test, ESEM, EDAX

1. Introduction

Crack formation in concrete is a phenomenon that cannot be completely avoided due to shrinkage reactions of concrete and tensile stresses occurring in the structures. Large cracks in concrete structures largely affect the structural integrity therefore repair actions are required whereas small cracks with a crack width of about 0.05 to 1mm are generally considered unproblematic. Even though these micro-cracks do not affect the structure, they contribute to the porosity and permeability of concrete. Ingress of aggressive chemicals such as chlorides, sulphates and acids lead to the degradation of concrete and corrosion in embedded steel reinforcement which affects the durability of concrete structures. Chloride ions penetrating the matrix through cracks destabilize the passivation film of the steel reinforcement and accelerate corrosion. Sulphate ions penetrating the matrix results in ettringite formation, a reaction in which a high-density phase is transformed into a low-density phase, causing expansion and further cracking of the material. Similarly, CO_2 diffusing through the crack (a process called carbonation) reacts with $Ca(OH)_2$ in the cement which results in lowering of pH of the concrete medium and again de-passivating the protective film on the steel reinforcement. Hence, to increase the durability of the structure the crack healing technique should be adopted. In several studies, it is found that concrete has the capacity for autonomous healing of such micro cracks. It is due to the presence of unhydrated cement in the concrete after the hydration process. When moisture present in the atmosphere comes in contact with the concrete matrix through the crack,

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unhydrated cement present in the concrete matrix reacts with moisture and seals the crack to some extent which is not effective enough. Even though, this can be avoided by demolishing and reconstructing the structure, it is not widely preferred as it is not cost-effective. Other techniques such as injecting chemicals into the concrete, which seals the crack can be considerable but still unhydrated cement will be present and also using chemicals will affect the environment. Instead of using chemicals, in the view of biological origin bacteria can be used as a self-healing agent which is also eco-friendly. An active self-healing mechanism in concrete does not require manual checking and repair since it is not labour-intensive. This saves a huge amount of money.

Extensive studies have been performed to assess the performances of bacterial concrete. H. M. Jonkers found that bacterial spore viability increased from 2 to more than 6 months when added immobilized (protected) inside porous expanded clay particles compared to direct (unprotected) addition to the concrete mixture. Virginie Wiktor, Henk M. Jonkers studied the Oxygen consuming bacteria, the healing agent that acts as an O2 diffusion barrier protecting the steel reinforcement from corrosion. Dr. K. V. Ramana Reddy and M. Padmaja conducted an experimental investigation on bacillus subtilis in healing micro-cracks in concrete. The compressive strength of concrete at the age of 28 days increased by 31.2% due to the action of self-healing bacteria. Water absorption decreased by 27.2% and permeability of concrete decreased by 69.7%. E. Schlangen, H. Jonkers, S. Qian and A. Garcia study found that bacteria are used to precipitate calcite in cracks in concrete. This method did not lead to strength improvements of the structure but by filling the cracks the path to the reinforcement is blocked. Herewith the ingress of liquids and the ions which caused corrosion in the reinforcement was stopped and thus durability of the structure was enhanced. Hans-Wolf Reinhardt, Martin Jooss studied the permeability and self-healing of cracked concrete as a function of temperature and crack width. Regarding self-healing of cracks in (High-Performance Concrete) HPC, the conclusion was drawn that at a hydraulic gradient of approximately 1 MPa/m and under the assumption of non-moving crack edges, cracks of width less than or equal to 0.10 mm was regarded as smooth and closed by self-healing processes. Mostafa Seifan, Ali Khajeh Samani, Aydin Berenjian analysed the topic called “Bio-concrete: next generation of self-healing concrete”. The study revealed the ability of bio self-healing concrete in filling deep micro cracks as well as restricting crack development. Reduction in porosity of structure, made the concrete watertight. Vijeth N Kashyap and Radhakrishna published a paper on the study of the effect of Bacteria on Cement composites. In this paper two different types of bacteria called Bacillus sphaericus and Sporosarcina Pasteurii were obtained from Microbial type culture collection and gene bank, Chandigarh in a freeze-dried condition. The SEM and XRD analysis showed the presence of calcite inside cement composite specimens which are produced microbial processes. Microbes enhance the strength and durability of cement composites.

This paper involves culturing Bacillus Subtilis bacteria for mixing with concrete to get self-healing bacterial concrete. The control and bacterial specimens were cast in cubes and prisms. The casted specimens are subjected to compressive strength test, pull-out test, UPV test, Porosity measurement and ESEM and EDAX analysis to quantify the self-healing percentage of the bacterial specimens and the control specimens by the results obtained from the tests.

2. Selection of Bacteria

The bacteria we used in our project is Bacillus subtilis. This is gram-positive bacteria producing colonies of dry, flat, and irregular with lobate margins. They also produce Circular, pinhead colonies which are convex with entire margins. The colonies of this gram-positive appear as the colour of the agar or whitish. Since these bacteria produce calcium carbonate and due to ease of availability, it is used for future investigation. It can survive in a temperature range of about 23-25^{o}C and needs a pH range of 8- 9. This type of bacteria can be dormant and alive for about 200 years under unfavorable conditions. These bacteria act as a catalyst in the crack healing process. It is economical and easily available.

3. Bacteria Culture

In this project, Bacillus subtilis is cultured by using broth and it is mixed in the mortar. The materials required are bacterial LB Broth, conical flask, boiling tubes, cotton plugs, autoclave, laminar air medium.

Media preparation:

Luria – bertani (LB) broth: PH -7
Nutrient broth for 150 ml
Beef extract - 0.45g
Peptone - 0.75g
NaCl - 0.75g

After weighing, these reagents are added to 150 ml of distilled water in a conical flask to get the nutrient broth medium. For our project, three nutrient broth media of 150ml each are prepared. The conical flasks are plugged with cotton to prevent the entry of external agents from the atmosphere.

Fig. 1 - Broth medium

Fig. 2 - Bacterial solution

4. Materials Used for Concrete Mix

Ordinary Portland cement of 53 grade available in the local market is used for the investigation. Locally available clean, well-graded, natural river sand conforming to IS 383-1970 is used as fine aggregate. Locally available potable water conforming to IS 456-2000 is used for casting the specimen. The prepared bacterial culture is used for casting the bacterial specimen. The calcium lactate is used as a nutrient for bacterial growth in the bacterial specimen.

5. Specimen Details

Cube and prism specimens are used for testing purposes. The size of the cubes used is (7x7x7)cm^3. Ten cube specimens are used for control specimens and another ten specimens are used for Bacterial specimens. The control specimens are named C1, C2, C3, C4, C5, C6, C7, C8, C9. Similarly, the bacterial specimens are named B1, B2, B3, B4, B5, B6, B7, B8, B9. The prism specimens used are of size (4x4x16) cm^3. 8mm steel rod is inserted to half the length. Six prisms are used for control and bacterial specimens. The Control specimens are named C1, C2, C3, C4, C5, C6 and bacterial specimens are named B1, B2, B3, B4, B5, B6.

6. Experimental Investigation

6.1 Compressive Strength Test

The cube specimens of C1, C2, C3, B1, B2, B3 are used for 7-day compressive strength and C4, C5, C6, B4, B5, B6 are used for 28-day compressive strength.

Fig. 3 - Compressive strength testing

6.2 Environmental Scanning Electron Microscopy (ESEM) And Energy-Dispersive X-Ray Analysis (EDAX)

To examine the chemical compositions of the specimens near the cracked region ESEM and EDAX tests are done to check the formation of calcium carbonate. These tests are done for a bacterial specimen named B8.

6.3 Pull Out Test

This test is done to determine the ultimate strength of concrete by pulling the rod inside it. The clamp at the top is not tightened so that it does not hold the rod whereas the clamp at the bottom is tightened to hold the rod. The pressure applied to the jack pulls the bottom clampdown which pulls the rod. The breaking load is determined and for inducing internal cracks 70% of the breaking load is applied. The prism specimens named B4, C5 and C6 are used for this test.

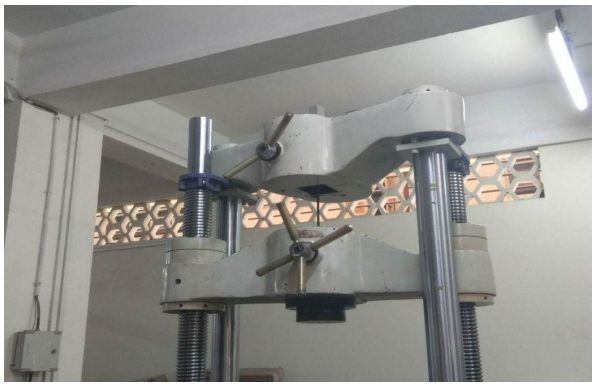

Fig. 4 - Universal testing machine

6.4 Ultrasonic Pulse Velocity Test

In the UPV test healing of micro-cracks are measured. For this test, 4 no of control specimens and 4 numbers of bacterial specimens of size 4cmx4cmx16cm (prism) are taken. For inducing micro-cracks, pull out test is carried out. Pull out test requires a rod of diameter 8mm and it is inserted in the prism from one end to half of its length. Then the velocity is measured for the uncracked specimen. Then by carrying out the pull-out test breaking load is identified which is 20kN. By applying 75% of breaking load micro-cracks are induced. Now the velocity is again measured. The measured velocity is observed to be lesser than the velocity of the uncracked specimen. This is because of the presence of micro-cracks which increased the path length of the pulse. It is expected that as the healing takes place, the path length of the pulse decreases, and the velocity gets increased and at one point it will be merely equal to the velocity of the uncracked specimen. The test is carried out at a regular interval of 15 days (15th day, 30th day, 45th day). The prism specimens used for the tests are C1, C2, C3, C4, B1, B2, B3, B5, B6.

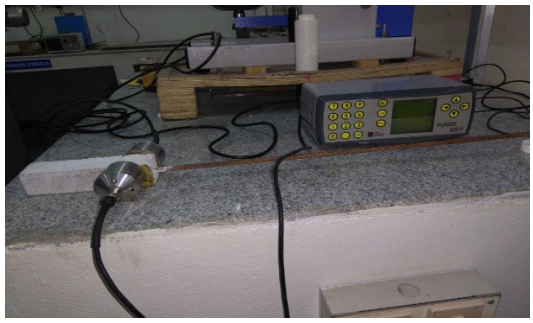

Fig. 5 - UPV testing

6.5 Porosity

The saturated water absorption of concrete is a measure of the pore volume or porosity in hardened concrete which is occupied by water in a saturated condition. The cube specimens used for the porosity test are C7, C8, C9, B8, B9. The porosity obtained from absorption tests is designated as effective porosity. It is determined using the following formula:

Effective porosity = (Ws-Wd) *100 / (Ws-Wsub)

Where,
Ws = weight of specimen at fully saturated condition.
Wd = weight of the oven-dried specimen
Wsub = submerged weight of the specimen in water.

7. Results and Discussions

The results of the above-mentioned tests are discussed here. The discussion is based on two categories: the effect of bacteria over strength and healing. Compressive strength results deal with the strength aspect. ESEM, EDAX, UPV, porosity measurements deal with the healing aspect.

7.1 Effect of Bacteria Over Compressive Strength

The test is conducted on 7th and 28th days of the curing period. The results are shown in Table 1. The average 7^{th}-day compressive strength of the control specimen is 38.37MPa and the bacterial specimen is 28.36 MPa.The average 28^{th}-day compressive strength of the control specimen is 53.17MPa and the bacterial specimen is 42.03MPa. The percentage reduction in strength of bacterial specimens on the 7^{th} day is 26.10%. The percentage reduction in strength of bacterial specimens on the 28^{th} day is 20.87%. This reduction in the compressive strength is due to the retardation effect of calcium lactate which is used as the nutrient for the bacteria.

Table 1 - Compressive strength at 7th and 28th day

Specimen	Compressive strength at 7 days (MPa)	Average (MPa)	Specimen	Compressive strength at 28 days (MPa)	Average (MPa)
C1	41.04		C4	54.58	
C2	37.04	38.37	C5	52.46	53.17
C3	37.04		C6	52.46	
B1	29.03		B4	42.37	
B2	26.03	28.36	B5	40.35	42.03
B3	30.03		B6	43.38	

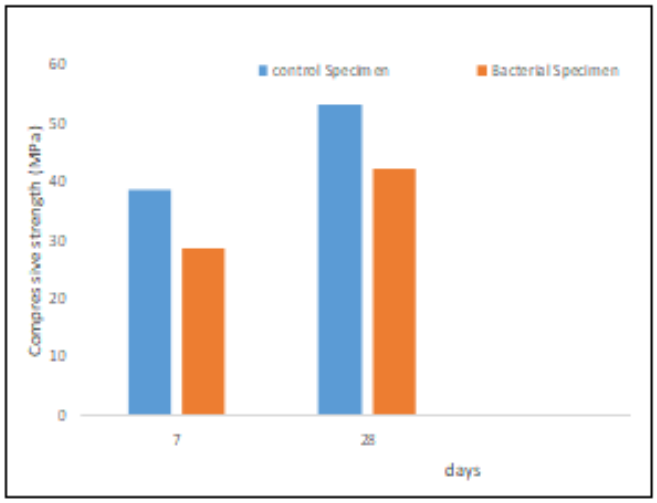

Fig. 6 - Compressive strength at 7th and 28th day

7.2 Effect of Bacteria Over Healing

7.2.1 Environmental Scanning Electron Microscope (ESEM)

ESEM is used to observe the various crystal morphologies. For ESEM analysis specimen B8 is taken. The crack is induced using the compression test and ESEM requires the specimen of size 1cmx1cm which is prepared by cutting near the crack region. Then the samples are scanned using ESEM and the images are shown in fig 7:

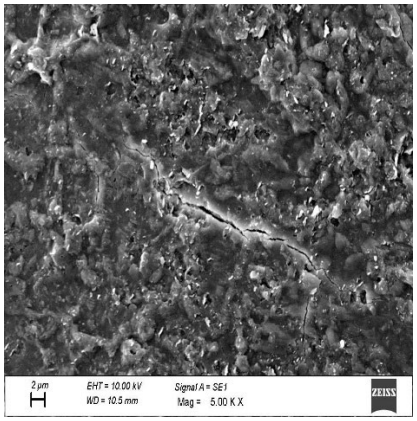

Fig. 7 (a)

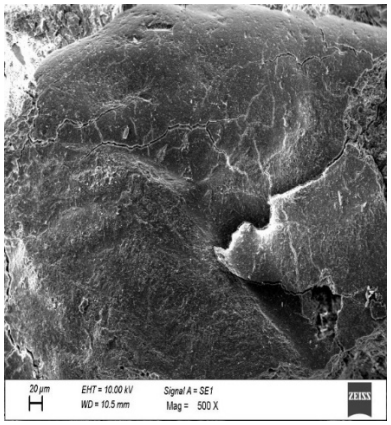

Fig. 7 (b)

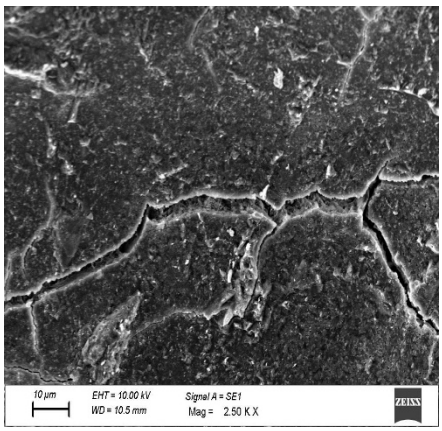

Fig. 7 (c)

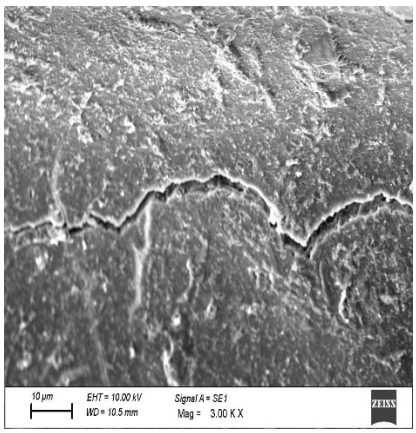

Fig. 7 (d)

Fig 7 - (a); (b); (c); (d) Microscopic images of crack filling precipitates after crack healing of a bacteria-based specimen

7.2.2 EDAX

Element composition analysis using energy dispersive spectroscopy (EDAX) Shows the precipitates formed on the crack surfaces of bacterial specimens are essentially an association of calcium, oxygen and carbon atoms which is nothing but the calcium carbonate. The Y-axis shows the counts (number of X-rays received and processed by the detector) and the X-axis shows the energy level of those counts(keV). On the surface of the specimen, 8 points are located, and the elemental composition of each point is analysed using EDAX. From table 2 the presence of calcium, carbon and oxygen is confirmed in the bacterial specimen. Therefore, it was concluded that this calcium carbonate present in the bacterial specimen is responsible for self-healing.

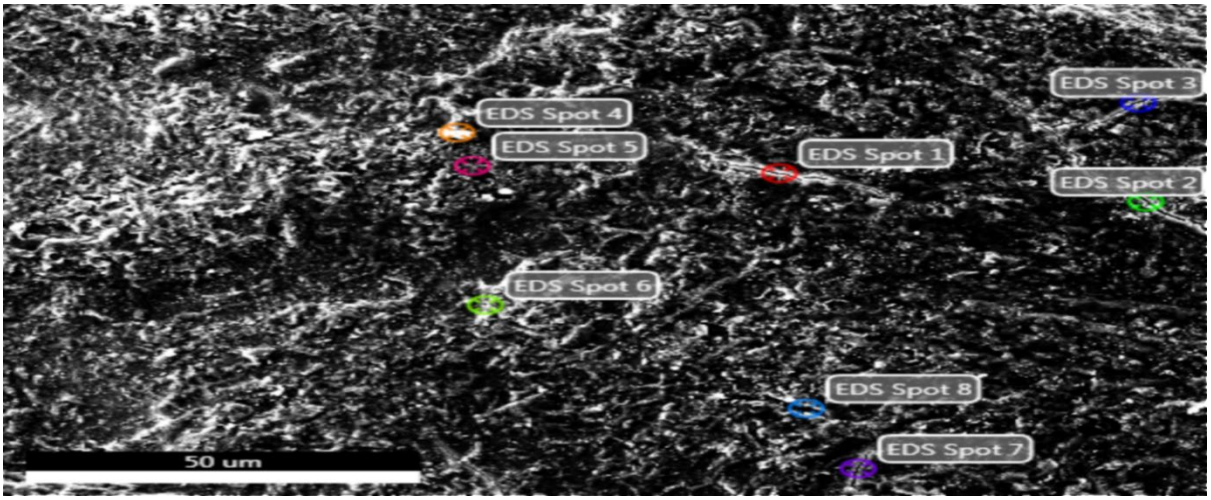

Fig 8 - Image showing the bacterial specimen with 8 different locations for EDAX analysis

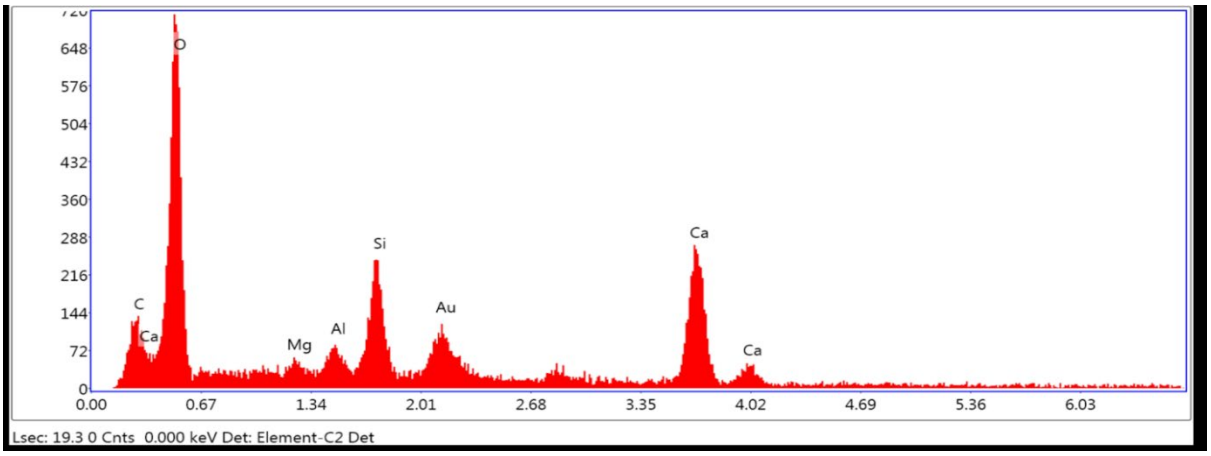

Fig 9 - Represents the EDAX analysis results of the prepared bacterial specimen at spot 8 of the loaded sample

Table 2 - Weight per cent of various elements present in the bacterial specimen at 8 locations as per EDAX results

Element	Weight (%)								Average
	spot 1	spot 2	spot 3	spot 4	spot 5	spot 6	spot 7	spot 8	
Ca	37.05	41.02	21.33	17.21	23.25	30	45.30	43.32	32.31
C	1.84	2.18	2.78	3.93	2.90	0	2.23	0.44	2.04

O	35.52	31.33	40.22	46.38	37.11	47.83	32.40	43.20	39.24

7.2.3 Ultrasonic Pulse Velocity

The values obtained in the UPV test in every path before loading, after loading, after 15 days, after 30 days and after 45 days are shown in table 3 and table 4. The positive values in tables 3 and 4 show that the velocity values have been increased which in turn indicate that the cracks had healed in those paths. It was found that not much healing occurred after 15 days. But a considerable amount of healing occurred in 45 days. The average percentage healing of the control specimen at 15, 30, 45 days are 0.8825%,2.092%,2.467% respectively. For the bacterial specimen, the average percentage of healing at 15,30 and 45 days are 5.68%, 6.475% and 7.607% respectively. From figure 10, the linear correlation has an r^2 value of more than 0.9 which implies a good linear fit against test data. The slope of this line indicates the rate of healing. the slope of the control specimen is 0.792 whereas for the bacterial specimen is 0.963 which clearly indicates a faster rate of healing than the control specimen.

Table 3 - UPV values before and after crack, after 15, 30 and 45 days of crack

Specimen number	Velocity before crack(m/s)	Velocity after crack(m/s)	Velocity after 15 days of crack(m/s)	Velocity after 30 days of crack(m/s)	Velocity after 45 days of crack(m/s)
C1	2951.5	2531	2515.5	2533	2530.5
C2	3212.5	3112.5	3116	3115	3113
C3	2633	2523	2527	2524.5	2528.5
C4	2867	2711.5	2711.5	2712.5	2712.5
B1	3137	3064.5	3073	3073.5	3075
B2	3238.5	2806.5	2867	2867	2868
B3	2888.5	2622.5	2622.5	2628	2630.5
B5	3135	3018.5	3015	3015.5	3017

Table 4 - Average healing percentage after 15, 30, 45 days for control and bacterial specimen

Specimen	% Healing					
	15 days		30 days		45 days	
	% Healing of each specimen	Average % healing	% Healing of each specimen	Average % healing	% Healing of each specimen	Average % healing
C1	-3.6	0.8825	0.47	2.092	0.63	2.467
C2	3.5		3.5		3.6	
C3	3.63		3.76		5	
C4	0		0.64		0.64	
B1	11.72	5.68	12.41	6.475	14.48	7.607
B2	14		14		14.23	
B3	0		2.06		3	
B5	-3		-2.57		-1.28	

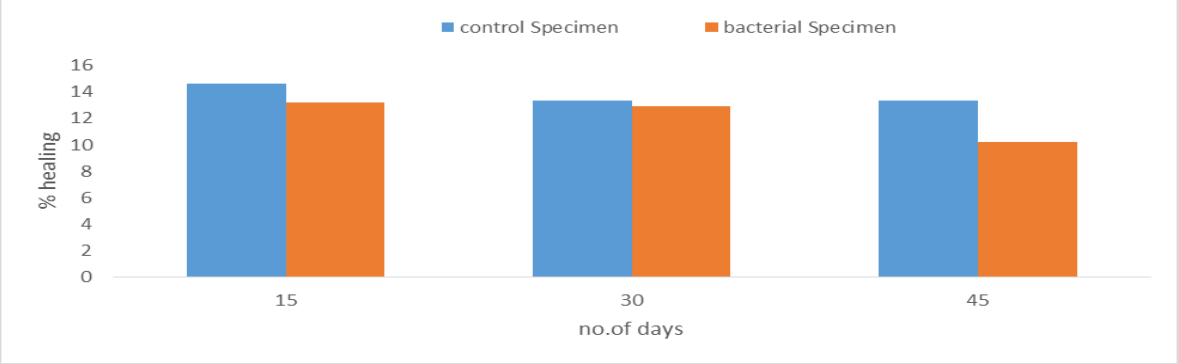

Fig. 10 - Average healing percentage after 15, 30, 45 days for control and bacterial specimen

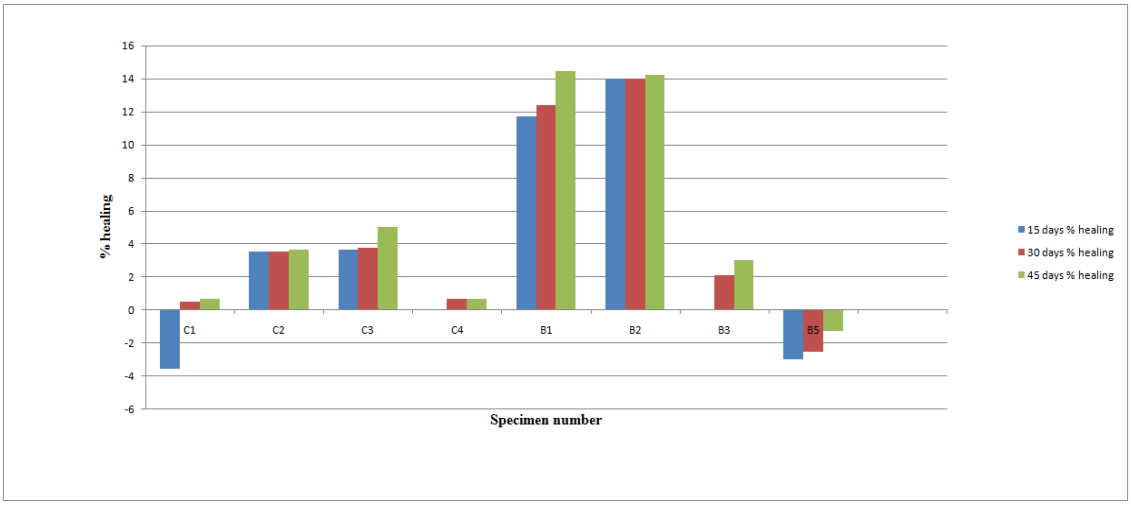

Fig. 11 - Healing percentage of the various specimens after 15, 30 and 45 days

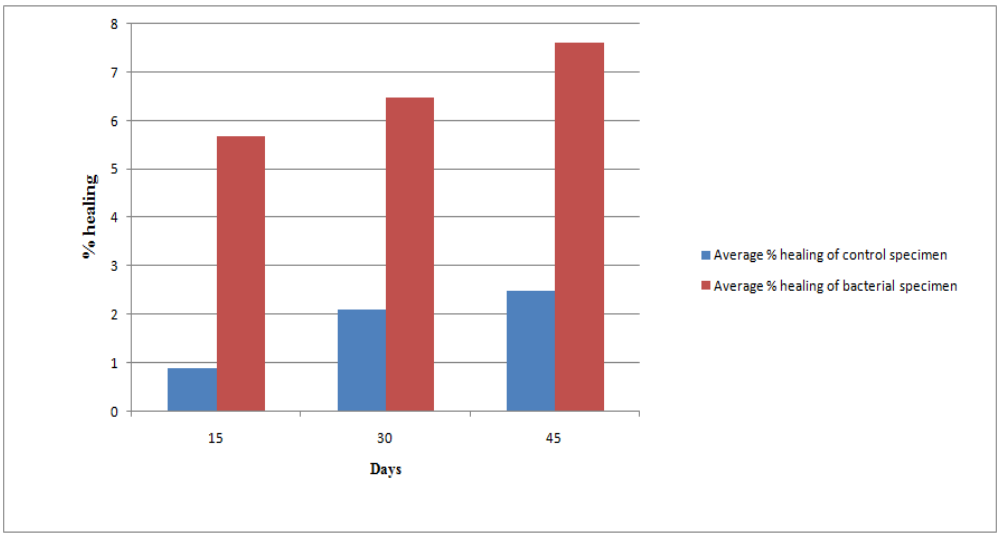

Fig. 12 - Average healing percentage after 15, 30, 45 days for control and bacterial specimen

7.2.4 Porosity

The porosity test results are shown in Table 5. In table 5, the porosity of each specimen is calculated using the formula which is discussed in the theory and the average is taken. At 15 days the average porosity of the control specimen is 14.617% and that of the bacterial specimen 13.187%. Similarly, at 30 days the average porosity of the control specimen is 13.371% and that of the bacterial specimen 12.911%. At 45 days the average porosity is observed to be 13.307% for the control specimen and 10.185% for the bacterial specimen. These results show that the porosity decreases in both control and bacterial specimen. From graph 13. we see that the porosity decreases for both bacterial and control specimens. The rate of decrease in porosity of bacterial specimen is higher than that of control specimen. The decrease in porosity of the control specimen is due to the autogenic healing process whereas the bacterial specimen is due to bacterial reaction.

Table 5 - Saturated, submerged, and dry mass of various specimens after 15, 30, 45 days

Specimen	15 days			30 days			45 days		
	m_{sat}	m_{sub}	m_{dry}	m_{sat}	m_{sub}	m_{dry}	m_{sat}	m_{sub}	m_{dry}
C7	0.816	0.454	0.764	0.814	0.45	0.766	0.814	0.462	0.77
C8	0.826	0.454	0.774	0.822	0.456	0.774	0.819	0.430	0.778
C9	0.824	0.452	0.776	0.820	0.458	0.77	0.822	0.514	0.77
B7	0.181	0.454	0.772	0.818	0.452	0.77	0.810	0.410	0.764
B9	0.810	0.446	0.776	0.808	0.446	0.762	0.808	0.312	0.764

Table 6 - Average porosity of various specimen after 15, 30 and 45 days

Specimen	15 days		30 days		45 days	
	Porosity of each specimen (%)	Average porosity (%)	Porosity of each specimen (%)	Average porosity (%)	Porosity of each specimen (%)	Average porosity (%)
C7	14.36	14.617	13.186	13.371	12.5	13.307
C8	13.90		13.114		10.539	
C9	15.59		13.812		16.883	
B7	12.637	13.187	13.115	12.911	11.5	10.185
B9	13.736		12.707		8.871	

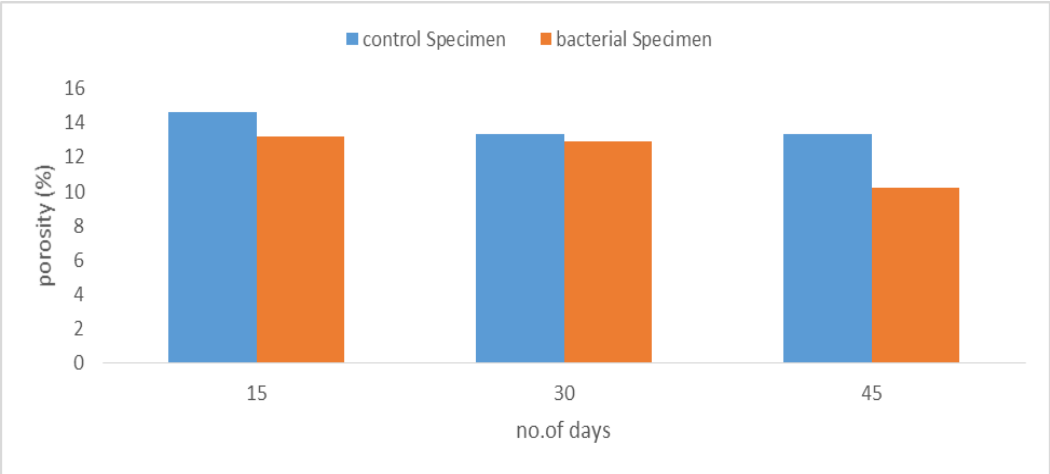

Fig. 13 - Average porosity of control and bacterial specimen after 15, 30 and 45 days

8. Conclusion

From the Experimental investigation, the following conclusions are inferred

- The percentage reduction in strength of bacterial specimen at 7th day and 28th day is 26.10% and 20.87 respectively. There is not much increase in compression strength of mortar, but the reduction in the strength is caused due to retardation of calcium lactate.
- ESEM images show the traces of the accumulation of calcium carbonate precipitates near the crack region which is visual evidence.
- EDAX graphs reveal that the precipitates formed on the crack surfaces of bacterial specimens are essentially an association of calcium, oxygen and carbon atoms which is nothing but the calcium carbonate.
- Ultrasonic Pulse velocity test and porosity test gives good insight into the healing of micro-cracks inside the specimen. The rate of healing of bacterial specimens is higher in UPV where the porosity is more in bacterial specimens than the control specimen, which could directly correlate to the healing of bacteria.

9. Future Scope

The efficiency of healing process can be studied by adding bacteria in concrete. The healing nature of cement mortar specimens can be studied by varying the volume of bacteria added to the cement mortar mix.

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