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Research Article

Estimation of durability properties of self-healing concrete influenced by different bacillus species

Chereddy Sonali Sri Durga[1,a], Chava Venkatesh*[1,b], T. Muralidhararao[1,c] , Ramamohana Reddy Bellum[2,d], B. Naga Malleswara Rao[1,e]

[1]Dept. of Civil Eng., CVR College of Engineering, Hyderabad, India
[2]Dept. of Civil Eng., Aditya Engineering College (Autonomous), India

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Abstract

The study was carried out with the influence of four Bacillus species, namely Bacillus subtilis (BS), Bacillus licheniformis (BL), Bacillus halodurans (BH), and Bacillus cereus (BC), in order to improve the impermeable nature of concrete. The selected bacterial agents were cultured according to the nutrient broth medium method and prepared with two variable cell concentrations of 10^8 and 10^9. In this study, bacteria were used in two different modes, i.e., as an additive to the concrete and as a curing agent. However, all the concrete specimens were cracked with a 65% stress level concentration and then cured in calcium lactate (only for bacteria-induced concrete specimens) and bacterial solution (only for normal concrete specimens) for crack healing. The durability behavior of these concrete specimens was monitored before crack, after crack, and after healing. In this regard, the following durability tests were performed: Rapid Chloride Permeability Test (RCPT), Water absorption test, Open porosity test, and Acid attack test. From the experimental observations, a decline in the passage of coulombs by 1352 C, water absorption by 3.47%, open porosity by 4.61%, and increased resistance against acid attack was found in normal concrete specimens cured under bacterial solution (especially in Bacillus halodurans with cell concentrations of 10^9) compared to other ones. Based on the analysis of the results, bacterial cultures have enriched the durability of the concrete by filling the voids and cracks with calcite crystals. However, Bacillus halodurans has shown better durability performance in both types of concrete, i.e., bacterial concrete cured in calcium lactate and normal concrete cured in bacterial solutions.

1. Introduction

Concrete is one of the most extensively utilized building material and also cracks are unavoidable because it is brittle in tension yet robust in compression [1-4]. When fractures appear in concrete, it can shorten the life of the structure. So, it is mandatory to attend the cracks and rebuilt them before failure of structure happens. A lot of cracks repairing and maintenance techniques are available, but they all are time consuming and extremely expensive [5]. Microbially induced carbonate precipitation (MICP) has been investigated as a solution for stone crack restoration because calcium carbonate minerals are a homogeneous substance that is compatible with concrete & stone which are environment friendly [6]. MICP is an old natural phenomenon that has altered the earth, as calcium carbonate minerals are generated from calcium and carbonate ions in this process [7-8]. This technology has been marketed for healing broken surfaces with induced $CaCO_3$ production named as self-healing concrete can be broadly classified into autogeneous self-healing and autonomous self-healing. In most of the traditional concrete mixtures, 20-30%

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*Corresponding author: chava.venkatesh@cvr.ac.in
[a] orcid.org/0000-0003-0942-9252; [b] orcid.org/0000-0003-0028-7702; [c] orcid.org/0000-0002-7768-3298; [d] orcid.org/0000-0002-0040-5812; [e] orcid.org/0000-0002-5543-168X

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of cement particles are left unhydrated. If the concrete's cracking occurs, the unreacted cement grains may become exposed to moisture and fill the cracks by precipitating calcite crystals termed as autogenous self-healing. If external agents, viz any chemical or biological agents are used for self-healing process, this process termed as autonomous healing [9].

The studies from various researchers stated that the healing process was carried out by introducing the bacteria into the concrete. The bacterial spores activate the healing process with the presence of moisture; thereby the cracks in concrete are healed by microbial-induced calcite precipitation [10]. The bacteria in the former are engineered to produce urease, an enzyme that catalyses the conversion of urea to ammonium and carbonate. As ammonia dissolves, the pH shifts from neutral to alkaline, resulting in the formation of carbonate ions [11]. Bacterial cell walls are negatively charged, allowing them to capture positively charged calcium ions and deposit them on their surface. For the latter, the metabolic conversion of an organic calcium source, which mixes with the leached $Ca(OH)_2$ to make additional $CaCO_3$ crystals, may result in the emission of carbon dioxide during the respiration process of some spore-forming alkali-resistant bacteria [12-13]. The investigations were continued in this area majorly focus on the mechanism of bacteria based self-healing concrete, characterization methodologies and crack self-healing ability. It was stated from the test results that there is an improvement in bacteria based self-healing concrete corelate to conventional ones in terms of healing percentage and reduction of crack width [14-15].

Along with the self-healing behavior of concrete study, the mechanical properties of concrete were also investigated [16-19]. Some researchers have also a study on engineered cementitious composites [20-22] consisting of cement, aggregate, water, fibers and chemical additives. As this fiber helps to improve the tensile strength of the concrete and acts as a crack resistor. The observation was done for both the specimen's engineered cementitious composites (ECC) and fiber-reinforced (FR) mortar. The specimen's load-bearing capacity characterized the strain hardening even after cracking was seen in ECC specimens as no changes were observed in FR mortar. However, the carbon dioxide present in atmosphere combines with moisture and reacts with calcium ions present in the concrete results in calcium carbonate formation [23]. This is why crack reduction happens and leads to repair of the whole crack, but the crack was healed to 100μm only due to natural process. To extend the reduction of crack width, both the biological and chemical agents need to be incorporated into concrete. Some of the bacterial studies focused on durability, as mentioned here. Liu et al. [24] used Denitrifying bacteria in concrete to improve concrete performance. This study concluded that compressive strength increased by around 30.3%, split tensile strength by 19.2%, and water absorption decreased by 33%. Naveet et al. [25] studied chloride ion permeability, water absorption, and compressive strength of Sporosarcina pasteurii bacteria-added concrete. Their study concluded that a 22% strength increment was observed. Compared to reference concrete, bacterial concrete exhibited 4 times more water absorption reduction and eight times less chloride ion penetration. Another study conducted by Priya et al. [26] utilized Bacillus sphaericus as a microbial agent in concrete to investigate durability and strength properties. The study concluded that compressive strength increased by 9.15%, while water absorption and sorptivity values decreased by 15.46% and 27.78%, respectively, compared to non-bacterial concrete. There is a need to focus on potential Bacillus species, particularly "Bacillus subtilis (BS), Bacillus licheniformis (BL), Bacillus halodurans (BH), and Bacillus cereus (BC)", due to the lack of sufficient literature. In the current research work, the methodology of incorporating both Bacillus species and calcium lactate into concrete to fill voids and cracks and decrease porosity and permeability was followed. The durability tests were performed to assess the fresh and hardened properties of the concrete in the present investigation.

2. Materials and Methods

2.1. Materials

The materials used in the current study are ordinary Portland cement of grade 53 confirming the specifications of ASTM C 150-19 [27][42], naturally available river sand and crushed stone aggregate are with the limitations of IS 383-2016 [28][43], potable water satisfying the limits of IS 456-2000 [29] for mixing of concrete. Along with these, four types of bacillus species were selected and purchased from National Collection of Industrial Microorganisms (NCIM)-Pune.

2.2. Experimental Methods

In the present study, all the concrete specimens were prepared with M40 grade concrete and mix proportions (as shown in Table 1) are determined as per the IS 10262-2009 [30]. The number of test specimens are used and their dimension are mentioned in the Table 2. All the samples cured in normal water at 28 days before its cracking.

Table 1. Mix calculations (Kg/m^3)

Mix	Cement	Fine aggregates	Coarse aggregates	Water	Bacteria (cell/ml of water)
Normal concrete	450	624	1220	186	-
Bacterial agents induced concrete	450	624	1220	186	5.79×108 5.79×109

Table 2. Details of specimens used in the entire research work

S.No.	Name of the test	Dimensions of specimen	Specimens for each Set	Total
1.	RCPT	100mm Φ and 50mm thick	Normal concrete - 30 Bacteria induced concrete - 24	54
2.	Open Porosity	150 mm × 150mm × 150mm	Normal concrete - 30 Bacteria induced concrete - 24	54
3.	Water absorption	150 mm × 150mm × 150mm	Normal concrete - 30 Bacteria induced concrete - 24	54
4.	Acid attack (HCl)	150 mm × 150mm × 150mm	Normal concrete - 30 Bacteria induced concrete - 24	54
			Total	216

2.3 Bacterial Culture Process

The four bacillus species (*Bacillus subtilis, Bacillus licheniformis, Bacillus halodurans and Bacillus cereus*) adopted in the present study are due to their calcium carbonate mineralization efficiency and capacity to survive in high alkaline environments. The bacterial cultures are grown using nutrient broth medium contains peptone 5gms, beef extract 3gms, sodium chloride 5gms diluted in 1000ml of distilled water that was sterilized at 121°C for 20 minutes. Allow time for the medium to cool before inoculating it with culture and after inoculation, the solution was incubated for 24hrs at 37°C at a speed of 85rpm in an incubator shaker. In order to prepare the bacterial solution in terms of cell concentration, a serial dilution technique was utilized and again the cells were counted with hemocytometer method.

2.4 Direct Usage of Bacteria

In this process, the bacteria were cultured and two different cell concentrations of 10^8 and 10^9 are prepared and are incorporated into the concrete directly during its mixing process. After mixing, the specimens were cast in order to evaluate the performance of concrete by conducting various tests. Along with this, the specimens are placed in curing tank for a specified time of curing after 24hrs of casting.

2.5 Indirect Usage of Bacteria

This procedure entails to utilize the bacteria solution as one of the curing agents (indirectly to concrete) for healing purposes. The normal concrete specimens are prepared, cured for 28 days and are cracked artificially using 65% stress level concentration. The bacterial solution of two varied cells 10^8 and 10^9 are developed and the cracked normal concrete specimens are placed in this solution for sealing cracks.

2.6 Durability Studies on Concrete

Durability can be defined as the capability of concrete offering resistance against chemical attack, abrasion, attrition, and weathering actions by maintaining its desired properties. The durability of concrete was estimated by conducting water absorption, open porosity, acid attack, and rapid chloride permeability test in the present study. The test procedures are explained detailly in this section and there are many factors influencing the durability i.e., cement content, temperature, moisture, permeability, aggregate and water quality.

2.6.1 Rapid Chloride Permeability Test

The rapid chloride permeability test (RCPT) was used to calculate the resistance offered by concrete samples through chloride ion permeability. As per ASTM C 1202(2012) [31] specifications, the performance of concrete was evaluated using a specimen with size of 100mm diameter and 50mm height. Figure 1 shows the RCPT setup, which consists of two reservoirs as one is filled with 0.3M of NaOH and the other is filled with 3% NaCl solutions. A constant voltage of 60V direct current was maintained and passed through the concrete specimens for chloride ions movement and readings were noticed. The total charge passed in 6hrs was estimated using Equation 1. The RCPT ratings as per ASTM C 1202(2012) were listed in Table 3.

$$Q = 900 \times (I_0 + 2\times I_{30} + 2\times I_{60} + 2\times I_{120} \ \ldots\ldots + I_{360}) \tag{1}$$

Table 3. RCPT ratings

Charge passed	> 4000	2000-4000	1000-2000	100-1000	< 100
Chloride ion permeability	High	Medium	Low	Very low	Negligible

Fig. 1 RCPT setup

2.6.2 Water Absorption Test

A water absorption test was conducted to measure the amount of water absorbed by the concrete specimens when submerged in water. This test was performed for the concrete specimens with size 150mm×150mm×150mm as per BS 1881-122:2011 [32] limitations. The water absorption was measured in percentage (%); the lower the value indicates better the result. The percentage of water absorption can be calculated by using the formulae in Equation 2. The experimental setup for the water absorption test was shown in Figure 2.

$$w(\%) = \frac{Ww - Wd}{Wd} \times 100 \tag{2}$$

Where W(%) = Percentage of water absorption, Ww = Wet weight of the samples, Wd = Dry weight of the samples

Fig. 2 Experimental setup for water absorption test

2.6.3 Open Porosity Test

The porosity can also be known as pore space, a measure of air and void space between the concrete particles. As per guidelines of ASTM C642-06 [33], the test was performed for the concrete specimens of size 150mm×150mm×150mm. The porosity of concrete is the ratio of the volume of pore space in a unit of material to the total volume of material. The porosity of concrete is calculated using the following Eq 3.

$$p = \frac{(W_{ssd} - W_d)}{(W_{ssd} - W_w)} \times 100\% \tag{3}$$

Where p is the porosity, W_{ssd} is the specimen weight in saturated surface dry (SSD) condition, W_d is the specimen dry weight, W_w is saturated submerged specimen's weight.

2.6.4 Acid Attack Test

A highly soluble calcium salt byproduct was formed from the reaction between the acid and calcium hydroxide portion of the cement paste in the acid attack test. The specimens of size 150mm×150mm×150mm were prepared to execute the acid attack test as per ASTM C1898-20 [34] standards. The specimens were placed in the acid solution for 28 days which was diluted with 5% HCl. In the acid attack test, the acid durability loss factor (ADLF) is calculated by the product of acid mass loss factor (AMLF), acid attack factor (AAF), and acid strength loss factor (ASLF). The formula for ADLF is in Eq 4, and the test setup for the acid attack test is in Figure 3.

$$\text{Acid durability loss factor (ADLF)} = \text{AMLF} \times \text{AAF} \times \text{ASLF} \tag{4}$$

Where Acid mass loss factor (AMLF) = (Change in a mass of specimen after immersion in acid/Initial mass of specimen before immersion in acid) ×100

Acid attack factor (AAF) = (Change in a dimension of diagonals after immersion in acid/Original diagonal dimension before immersion in acid) ×100

Acid strength loss factor (ASLF) = (Change in strength after immersion in acid/Original strength before immersion in acid) ×100

Fig. 3 Experimental setup for acid attack test

3. Results and Discussion

3.1 Rapid Chloride Permeability Test

Figure 4 and Figure 5 illustrates chloride ions passage (measured in terms of coulombs) of normal concrete specimens cured in various bacterial solutions (such as *Bacillus subtilis, Bacillus cereus, Bacillus halodurans,* and *Bacillus licheniformis*) with two different cell concentration (10^8 and 10^9). However, the chloride ions passage was measured for the concrete specimens before cracking, after cracking and after healing with a stress level concentration of 65%. The passage of coulombs was measured in uncracked concrete samples were around 1900 C to 2001 C. Similarly, after cracking, the passage of coulombs has been increased up to 2828 C in *Bacillus subtilis*, 2886 C in *Bacillus cereus*, 2837 C in *Bacillus halodurans,* and 2802 C in *Bacillus licheniformis*. Afterward, the cracked concrete samples were cured in bacterial solutions (10^8 cell concentration) for 28days and once again, chloride ions permeability has been measured. Thus, the results have shown that the passage of coulombs was reduced up to 1941 C in *Bacillus subtilis*, 2033 C in *Bacillus cereus*, 1566 C in *Bacillus halodurans,* and 1729 C in *Bacillus licheniformis*. These results confirm the calcite precipitate formation by the bacterial solution that has filled the concrete cracks and voids. Similarly, the cracked concrete samples cured in bacterial solution with a cell concentration of 10^9 has been reduced from 2802 C to 1717 C in *Bacillus subtilis*, 2841 C to 1988 C in *Bacillus cereus*, 2820 C to 1452 C in *Bacillus halodurans,* and 2855 C to 1595 C in *Bacillus licheniformis*.

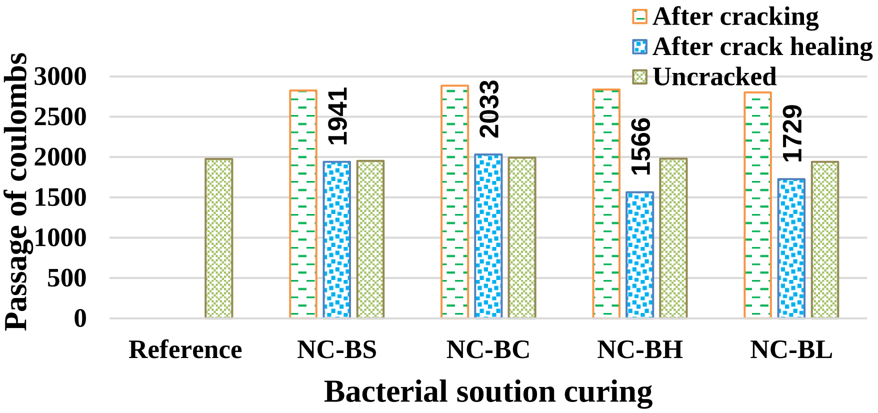

Fig. 4 Passage of coulombs Vs bacterial solution curing with cell concentration of 10^8

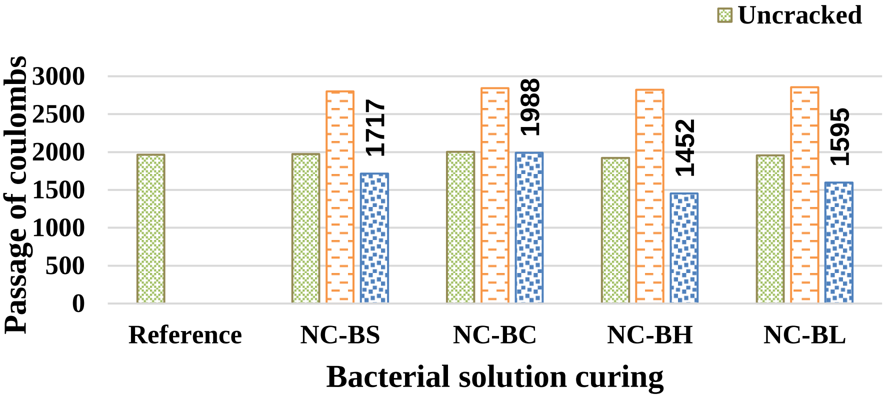

Fig. 5 Passage of coulombs Vs bacterial solution curing with cell concentration of 10^9

In the present research work, various bacterial concretes have been prepared with cell concentrations 10^8 and 10^9. Moreover, all the concrete samples were cured in calcium lactate after cracking, and the chloride ions permeability has measured before cracking, after cracking, and after crack healing as represented in Figure 6 and Figure 7. No significant variations were observed in bacterial concrete than reference concrete from the obtained results in uncracked samples. Afterward, the crack was created for the concrete specimens with a stress level concentration of 65% in both cell concentrated samples via 10^8 and 10^9, as all the cracked concrete samples have cured in calcium lactate solution, and it was observed that bacterial concrete permeability was reduced due to the filling of cracks by calcite formation. However, the passage of coulombs has reduced from 2868 C to 2467 C in reference concrete, 2914 C to 1984 C in *Bacillus subtilis*, 2866 C to 1954 C in *Bacillus cereus*, 2805 C to 1632 C in *Bacillus halodurans*, and 2857 C to 1787 C in *Bacillus licheniformis* for the bacterial concrete specimens with a cell concentration of 10^8. Similarly, bacterial concrete with cell concentration 10^9 also reduced the passage of coulombs from 2852 C to 1962 C in *Bacillus subtilis*, 2852 C in 1866 C in *Bacillus cereus*, 2769 C in 1571 C in *Bacillus halodurans*, and 2798 C to 1659 C in *Bacillus licheniformis*. Similar results were found by this research work [35] that the bacterial concrete had reduced the electrical current passage through the concrete. The bacteria have formed calcite sediments into the voids/pores. Hence the permeability of the concrete has been reduced.

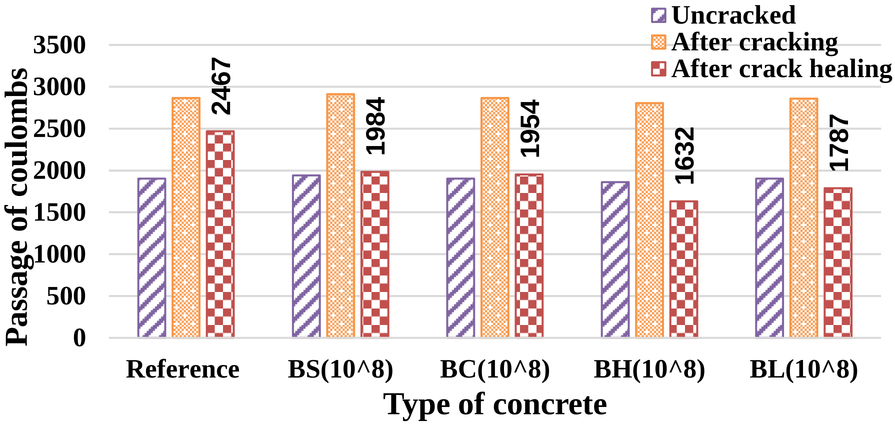

Fig. 6 Passage of coulombs Vs bacterial concrete cured in calcium lactate (10^8)

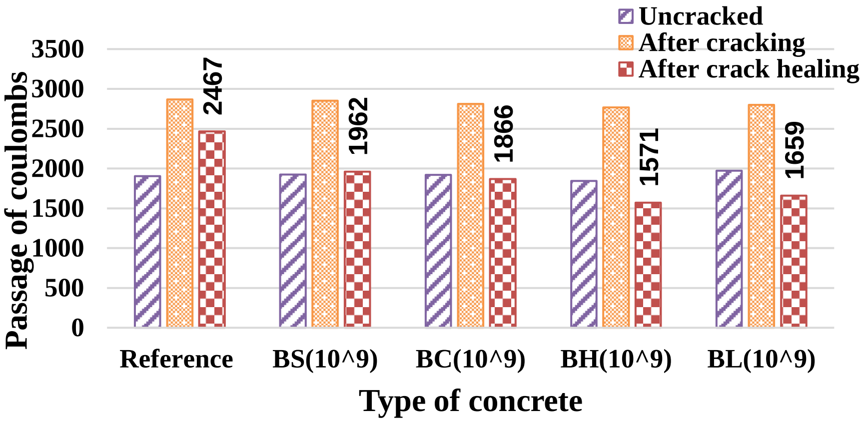

Fig. 7 Passage of coulombs Vs bacterial concrete cured in calcium lactate (10^9)

4.3 Water Absorption Test

Figure 8 and Figure 9 show the water absorption of normal concrete samples cured in various bacterial solutions (such as *Bacillus subtilis, Bacillus cereus, Bacillus halodurans,* and *Bacillus licheniformis*) when concrete samples were cracked (with 65% of stress level concentration). The water absorption for the cracked concrete samples has increased from 4.81 % to 8.62%, 4.54 % to 8.56%, 4.75 % to 8.81%, and 4.87% to 8.59%. However, all the cracked concrete samples were cured in various bacterial solutions (cell concentration of 10^8) for 28days. Afterward, once again, the water absorption has measured. The obtained results showed that water absorption of the cracked samples reduced to 5.58% in *Bacillus subtilis*, 5.63% in *Bacillus cereus*, 4.29% in *Bacillus halodurans,* and 4.56% in *Bacillus licheniformis*. This effect could be explained by the bacterial solution calcite precipitation, which has filled/sealed the cracks. Similarly, cracked samples cured in 10^9 cells concentrated bacterial solution reduced the water absorption values from 8.69% to 5.08% in *Bacillus subtilis*, 8.76% to 5.36% in *Bacillus cereus*, 8.85% to 4.17% in *Bacillus halodurans*, and 8.49% to 4.48% in *Bacillus licheniformis*.

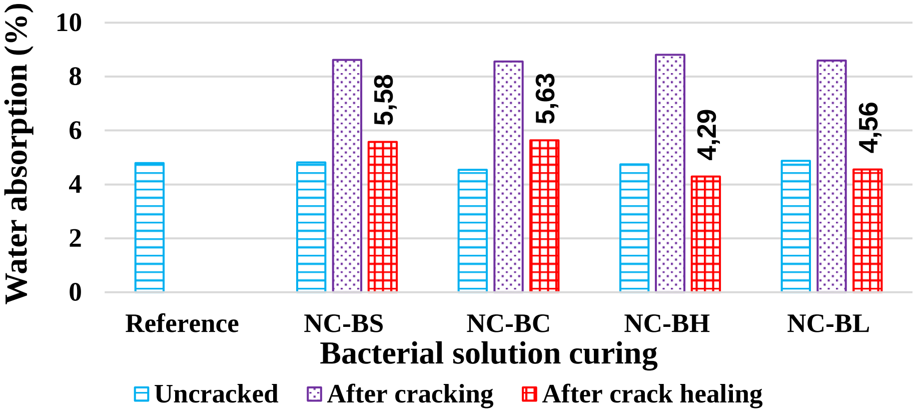

Fig. 8 Water absorption vs bacterial solution (10^8)

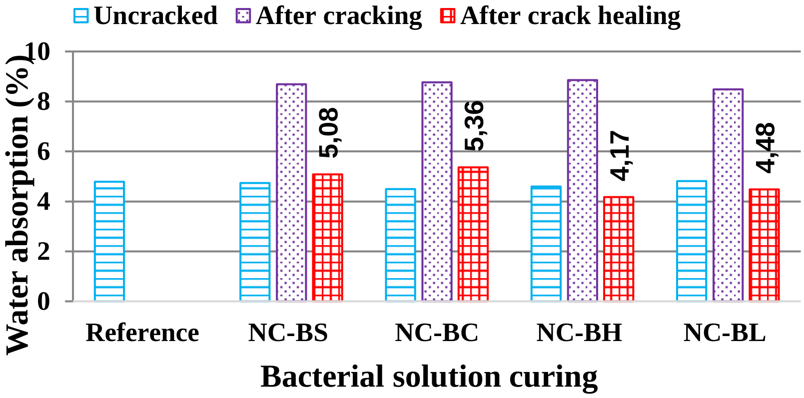

Fig. 9 Water absorption Vs bacterial solution (10^9)

In the present research work, four selected bacteria cultures were incorporated into the concrete, and water absorption has measured before and after cracking. All the cracked concrete samples were cured in calcium lactate solution and once again, the water absorption for the cracked samples was measured. From the obtained results, the uncracked concrete samples have shown 4.91% in reference, 4.87% in *Bacillus subtilis,* 4.61% in *Bacillus cereus,* 4.74% in *Bacillus halodurans,* and 4.79% in *Bacillus licheniformis* of water absorption values. The water absorption for the cracked bacterial concrete samples with a cell concentration of 10^8 has reduced from 8.86% to 7.15% in reference, 8.54% to 5.69% in *Bacillus subtilis,* 8.79% to 5.31% in *Bacillus cereus,* 8.5% to 4.23% in *Bacillus halodurans* and 8.82% to 4.48% in *Bacillus licheniformis* after cured into the calcium lactate as shown in Figure 10. Similarly, Figure 11 depicts the water absorption of cracked bacterial concrete specimens with a cell concentration of 10^9 reduced from 8.6% to 5.26% in *Bacillus subtilis,* 8.42% to 5.45% in *Bacillus cereus,* 8.72% to 4.09% in *Bacillus halodurans,* and 8.44% to 4.39% in *Bacillus licheniformis* after cured into the calcium lactate. A study [36] reported that water and gas permeability of concrete decreased after activation of bacterial spores in the concrete, as the calcite crystals are formed, and the cracks are filled. Similarly, in the study [37] it was reported that bacterial cultures would precipitate the calcite on the surface of the concrete which seals pores or voids. Moreover, there was a reduction of 65% to 95% water absorption, the permeability and sorpitivity of concrete also will be reduced.

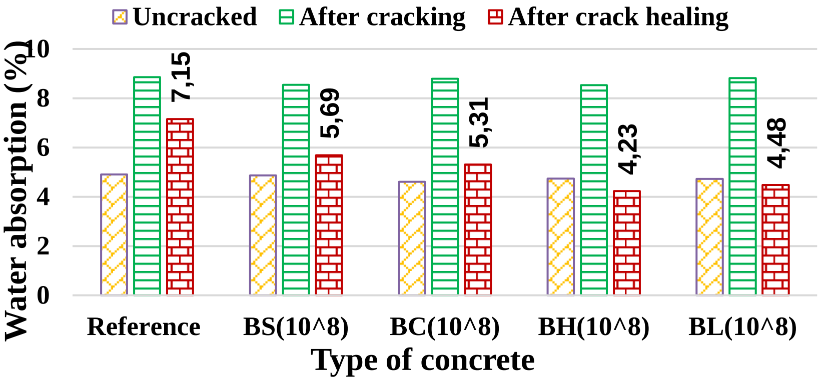

Fig. 10 Water absorption Vs bacterial concretes cured in calcium lactate (10^8)

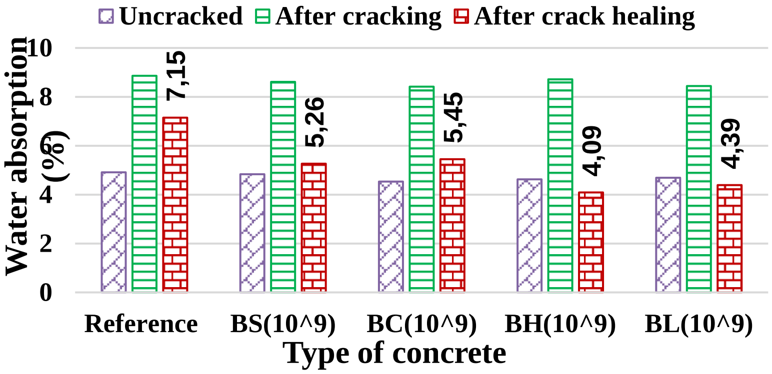

Fig. 11 Water absorption Vs bacterial concretes cured in calcium lactate (10^9)

4.4 Open Porosity Test

In the present research work, open porosity test has been performed to know the bacterial cultures filling effect in the concrete pores and cracks. This test was conducted when concrete samples were uncracked, cracked (with 65% of stress level concentration), and after crack healing (cracked samples were immersed in various bacterial solutions such as *Bacillus subtilis, Bacillus cereus, Bacillus halodurans,* and *Bacillus licheniformis*. Thus, the obtained results in Figure 12 and Table 4 demonstrate that concrete's porosity has reduced, possibly due to calcite filling in the cracks and voids.

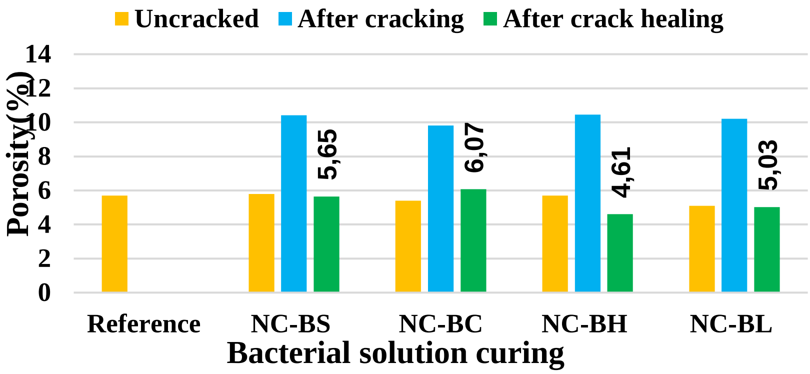

Fig. 12 Porosity Vs bacterial solution curing (10^9)

However, the cracked samples (with cell concentration of 10^9) porosity reduced from 10.42% to 5.65% in *Bacillus subtilis*, 6.82% to 6.07% in *Bacillus cereus*, 10.45% to 4.61% in *Bacillus halodurans,* and 10.21% to 5.03% in *Bacillus licheniformis*. Similarly, the results were observed in the concrete samples with 10^8 cell concentrations curing, and there is a reduction of 9.73% to 5.65% in *Bacillus subtilis*, 9.66% to 6.28% in *Bacillus cereus*, 10.05% to 4.82% in *Bacillus halodurans,* and 10.01% to 5.24% in *Bacillus licheniformis*.

Table 4. Porosity Vs bacterial solution curing (10^8)

			Porosity (%)		
	Reference	NC-BS(10^8)	NC-BC(10^8)	NC-BH(10^8)	NC-BL(10^8)
Uncracked	5.7	5.1	5.6	5.9	5.3
After cracking	-	9.73	9.66	10.05	10.01
After crack healing	-	5.65	6.28	4.82	5.24

Figure 13. and Table 5 shows the porosity of the bacterial concretes cured in calcium lactate. The results showed that cracked samples (with cell concentration of 10^9) porosity reduced from 10.67% to 8.69% in the reference sample, 10.19% to 5.83% in *Bacillus subtilis*, 9.52% to 6.24% in *Bacillus cereus*, 10.25% to 5.14% in *Bacillus halodurans* and 10.31% to 5.35% in *Bacillus licheniformis* as shown in Figure 14. In this research work, [38] it was concluded that concrete porosity and water absorption had approximated and decreased from 48% to 55% and 50% to 55%. It might be due to calcite formation in the concrete microcracks and pores. A similar justification given by a researcher, [39] that bacillus species could precipitate $CaCO_3$ and act as a filler material and reduce the concrete's porosity implies strength and durability improvement.

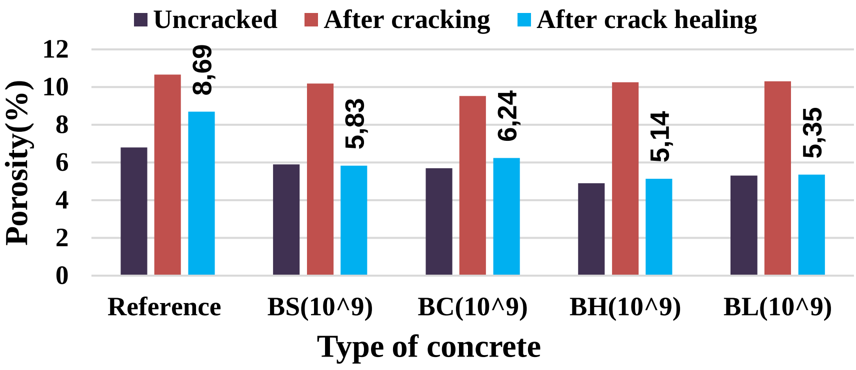

Fig. 13 Porosity Vs bacterial concretes cured in calcium lactate (10^9)

Similarly, concrete samples incorporated with various bacterial cultures of cell concentration 10^8 cured using calcium lactate has reduced the porosity values from 10.67% to 8.69% in the reference sample, 10.25% to 5.97% in *Bacillus subtilis*, 10.66% to 6.49% in *Bacillus cereus*, 10.35% to 5.43% in *Bacillus halodurans* and 10.41% to 5.51% in *Bacillus licheniformis* as illustrates in Table 5.

Table 5. Porosity Vs bacterial concretes cured in calcium lactate (10^8)

	Porosity (%)				
	Reference-CL	BS(10^8)-CL	BC(10^8)-CL	BH(10^8)-CL	BL(10^8)-CL
Uncracked	6.8	6.2	5.8	5.7	5.68
After cracking	10.67	10.25	10.66	10.35	10.41
After crack healing	8.69	5.97	6.49	5.43	5.51

4.5 Acid Attack Test

In the present research work, an acid attack test has been conducted to determine the durability behavior of bacterial concretes cured in calcium lactate, and normal concretes cured in bacterial solution. However, normal concrete samples cured in bacterial solutions have better durability than calcium lactate cured bacterial concrete samples as represented in Table 6. Similarly, bacterial cultures incorporated concrete having more durability than reference concrete. Moreover, *Bacillus halodurans* have shown better performance than other bacterial cultures as enlisted in Table 7. The bacterial cultures give protection to crack through calcite formation which enriches the structure's lifespan.

Moreover, it acts as a curing agent and helps to develop secondary pozzolanic properties and high strength [40 & 41]. However, low acid durability loss factors have been observed (Viz., 12.96 and 10.73) in normal cracked concretes cured in the *Bacillus halodurans* solution as represented in Table 8. Similarly, the *Bacillus halodurans* incorporated in concrete cracked samples cured in calcium lactate have shown low acid durability loss factors such as 17.54 and 10.56 as shown in Table 9.

Table 6. mass loss (%), strength loss (%) and dimension loss (%) of normal concrete samples cured in various bacterial solutions

CC	Mix	Mass Loss (%)			Dimension Loss (%)			Strength loss (%)		
		UC	AC	AH	UC	AC	AH	UC	AC	AH
	Ref	2.95	4.2	2.15	2.15	4.86	3.16	5.95	6.76	6.51
10^8	NC-BS	2.43	4.32	2.12	2.15	4.52	2.29	4.5	6.21	4.6
	NC-BC	2.29	4.83	2.89	1.85	4.67	2.97	4.12	6.92	4.22
	NC-BH	2.6	4.36	2.97	1.34	4.92	1.39	4.14	6.64	3.14
	NC-BL	2.59	4.84	2.39	1.58	4.52	1.62	4.76	6.1	4.34
10^9	NC-BS	2.41	4.45	2.98	2.09	4.37	2.16	4.38	5.94	4.49
	NC-BC	2.13	4.37	2.78	1.79	4.61	2.85	3.97	6.02	4.11
	NC-BH	2.48	4.84	2.74	1.27	4.21	1.31	3.95	6.35	2.99
	NC-BL	2.91	4.83	2.51	1.51	4.33	1.54	3.29	6.64	3.17

CC-Cell concentration, Ref-reference, UC- uncracked concrete, AC- after cracking, AH- after healing

Table 7. Mass loss (%), strength loss (%), and dimension loss (%) of bacterial concrete cured in calcium lactate

CC	Mix	Mass Loss (%)			Dimension Loss (%)			Strength loss (%)		
		UC	AC	AH	UC	AC	AH	UC	AC	AH
	Ref	2.75	5.86	4.22	2.5	3.12	3.56	6.03	7.12	6.15
10^8	BS	2.37	5.06	3.99	2.29	3.1	2.36	4.61	7.02	5.81
	BC	2.12	5.94	3.73	1.96	3.96	2.11	4.21	7.36	5.42
	BH	2.54	5.26	2.79	1.43	3.23	1.5	4.21	7.97	4.19
	BL	2.87	5.57	2.27	1.67	3.5	1.75	4.45	7.02	4.89
10^9	BS	2.33	5.84	3.86	2.17	3.42	2.29	4.47	7.56	4.67
	BC	2.08	5.05	3.54	1.85	3.61	2.98	4.09	7.37	4.29
	BH	2.42	5.36	3.54	1.34	3.57	1.42	4.12	7.01	2.1
	BL	2.83	5.22	3.04	1.58	3.46	1.67	4.37	7.1	2.78

CC-Cell concentration, Ref-reference, UC- uncracked concrete, AC- after crack, AH- after healing

Table 8. Acid durability loss factor for normal concretes cured in a bacterial solution

CC	Mix	Acid Durability Loss Factor		
		UC	AC	AH
	Ref	37.74	137.99	85.37
10^8	NC-BS	23.51	121.26	32.87
	NC-BC	17.45	156.09	36.22
	NC-BH	14.42	142.44	12.96
	NC-BL	19.48	133.45	16.8
10^9	NC-BS	22.06	115.51	28.9
	NC-BC	15.14	121.28	32.56
	NC-BH	12.44	129.39	10.73
	NC-BL	14.46	138.87	12.25

Table 9. Acid durability loss factor for bacterial concretes cured in calcium lactate

CC	Mix	Acid Durability Loss Factor		
		UC	AC	AH
	Ref	41.46	130.18	92.39
10^8	BS	25.02	110.12	54.71
	BC	17.49	173.12	42.66
	BH	15.29	135.41	17.54
	BL	21.33	136.85	19.43
10^9	BS	22.6	150.99	41.28
	BC	15.74	134.36	45.26
	BH	13.36	134.14	10.56
	BL	19.54	128.23	14.11

5. Conclusions

In the present research work, four different bacterial cultures "(i.e., *Bacillus subtilis (BS)*, *Bacillus cereus (BC)*, *Bacillus halodurans (BH)*, and *Bacillus licheniformis(BL)*)" are used in the concrete as additive and also as a curing agent. To assess the durability behaviour of bacterial concrete; "chloride ions permeability, open porosity, water absorption, and acid resistance tests" were conducted.

- The selected Bacillus species, namely Bacillus subtilis, Bacillus cereus, Bacillus halodurans, and Bacillus licheniformis, have the potential to heal surface cracks and enhance the durability of concrete when compared to non-bacterial concrete.
- The passage of coulombs (1352 C) has decreased significantly in normal concrete specimens cured under bacterial solution (Bacillus halodurans with a cell concentration of 10^9) compared to other bacterial solutions and calcium lactate curing, attributed to reduced void discontinuity.
- There is a decrease in water absorption (3.47%) and open porosity (4.61%) observed in normal concrete specimens cured under bacterial solution (Bacillus halodurans with a cell concentration of 10^9) in comparison to others.
- Normal concrete specimens cured with bacterial solution (Bacillus halodurans at a cell concentration of 10^9) exhibit higher resistivity of 9.94 against acid attack.
- Bacillus licheniformis has demonstrated optimal performance following Bacillus halodurans in all conditions.
- Although no significant difference in results was found between bacterial cell concentrations of 10^9 and 10^8, samples with a concentration of 10^9 cells have exhibited greater durability.
- The analysis of results reveals that bacterial cultures have improved concrete durability by filling voids and cracks with calcite, leading to reduced coulombs, water absorption, porosity values, and enhanced resistance against chemical attacks.
- However, Bacillus halodurans has exhibited superior durability performance in both types of concrete (i.e., bacterial concrete cured with calcium lactate and normal concrete cured with bacterial solutions). Thus, this Bacillus species is recommended for use in real-time repair and rehabilitation projects.

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