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EFFECT OF TYPE OF NUTRIENT ON CRACK HEALING PERFORMANCE OF BACTERIAL CONCRETE

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Abstract: Any concrete structure that develops cracks represents a strength failure, and it is an unremarkable observation leading the material attain high permeability and thereby escalating the risk of deterioration. Durability of concrete can be increased by adopting bio-chemical self-healing agents into the concrete. In the current study, an attempt is made to examine the healing tendency of the micro-cracks developed in the concrete by incorporating bacteria of *Bacillus* species, through the process of biomineralization. Survival of the bacteria is of great concern and it needs to be supplemented by certain nutrients or precursors. Hence this research acquisition, emphasizes the significance of the effect on the addition of nutrients on self-healing performance of bacterial concrete. This paper investigated the effect on healing of the micro-cracks developed in the bacteria based concrete along with the nutrients. In the present work, two types of nutrients viz calcium lactate (1% by weight of cement) and combination of calcium lactate (0.6% by weight of cement) and calcium chloride (0.4% by weight of cement) were intended to analyse their influence on the bacterial concrete specimens. The cultured form of *Bacillus subtilis* at a required concentration of $1x10^5$ cells/mL was added into the concrete matrix, post addition of the nutrients at various dosage in mixing water. The experimentation ensues that there was enhancement on the performance of the bacterial concrete along with increasing curing age period (3,7,14,21,28,56 days) along with conventional concrete. Water absorption and the porosity were diminishing for the bacterial concrete compared to conventional concrete. The maximum healing of the crack width of about 0.8mm at 56 days was observed for bacterial concrete administered with combined nutrient effect.

1 INTRODUCTION

Cracks adversely affect the concrete durability as they provide pathways for aggressive agents such as ions, gas, liquids, and humidity to penetrate leading to reduction in the material's mechanical strength, structure's safe [1-2]. A novel technique is introduced in which bacteria undergoes biomineralization process thereby producing the calcite precipitation that seal the cracks and pores in concrete.

Many researchers have proposed this Microbially Induced Calcium Carbon Precipitate (MICP) as an substitute and environmentally-safe crack repair technique [3]. For crack repair and durability improvement, a variety of bacteria have been incorporated into the concrete formulation [2]. Certain isolated spores from the bacteria were discovered during the search has focalized on those microbes that thrive in alkaline environments, such as alkali lakes in Russia, carbonate rich soils in desert areas of Spain, and soda lakes in Egypt [4-5]. Because the pH

of the water-cement mixture is high, a bacterium that can adapt to such a high alkaline environment should be added [3]. *Bacillus subtilis, Bacillus sphaericus Bacillus pasteurii, Bacillus cohnii,* and *Bacillus megaterium* are among the *Bacillus* genus species bacteria that can withstand extremely alkaline condition. Prior to the preparation of the concrete mixture, nutrients namely calcium lactate ($C_6H_{10}CaO_6$, L), calcium nitrate ($(CaNO_3)_2$, N), calcium acetate ($C_4H_6CaO_4$, A), calcium chloride ($CaCl_2$, CL), and calcium formate ($Ca(HCOO)_2$, F) are being adopted. Spore-forming bacteria with the gene *B. subtilis* are commonly used as crack repair agents, because they produce spores with specialized cells that can withstand high mechanical forces and harsh environments. Some strains are known to produce extremely long-lived spores with low metabolic activity that can live for up to 200 years [6-7].

2 BIOCALCIFICATION: AUTONOMOUS CRACK HEALING MECHANISM

This mechanism involves incorporating a unique self-healing agents into the concrete in which bacteria, aids in the mineralization and eventually heads in closure of newly developed cracks, reducing concrete permeability, improving durability, and preserving embedded steel reinforcement from corrosion and hence it was discovered to be the most promising mechanism. The presence of four key variables such as calcium content, Dissolved Inorganic Carbon (DIC) concentration, pH, and the availability of nucleation sites, drives the fairly straightforward chemical process of calcium carbonate precipitation [8]. Calcium carbonate provides a promising and long-lasting restorative alternative due to the fact that it works with concrete. A chemical molecule known as a nutrient provides the reagents needed to produce calcium carbonate, particularly the calcium ion (Ca^{2+}) and carbonate ion (CO_3^{2-}) [9]. The process of calcite precipitation depends on bacteria's ability to alter the environment around them in a way that encourages the precipitation of carbonates. A rise in pH and dissolved organic carbon around the microorganisms as a result of bacterial metabolic processes including urea hydrolysis and organic source oxidation leads to MICP in the presence of calcium [10]. Some bacteria alter the chemistry of their microenvironments to promote carbonate precipitation by metabolic processes like ureolysis, photosynthesis, ammonification, denitrification, sulphate reduction, and methane oxidation [11]. MICP can help to strengthen and extend the life of concrete that has flaws. In MICP technology, most researchers employ Chloride "CL" as the calcium source [9]. Self-healing concrete is defined as concrete that produces limestone through a biological process to repair surface cracks in concrete structures as seen in **Figure 1**. This limestone hardens over time, filling up the fractures in the damaged surface. When the bacteria exist in the concrete, calcium based precursors are proactively metabolized and results in efficient bacteria-based self-healing concrete as seen in Equation 1 [12]. Concrete's strength was seen to enhance as a result of Lactate "L" ability to create additional calcium ions.

$$C_6H_{10}O_4 + 7O_4 \rightarrow CaCO_3 + 5CO_2 + 5H_2O \quad (2)$$

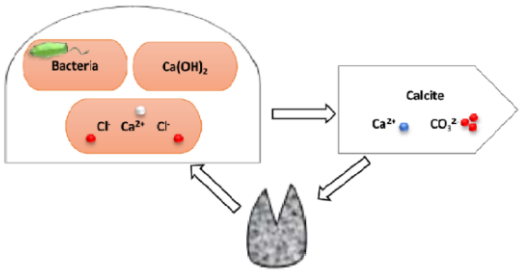


Figure 1: Schematic representation of $CaCO_3$ production in existence of microbe and "CL" crystal (Stanaszek-Tomal 2020).

2.1 Effect of various nutrients and bacteria in concrete

Dosage of "L" 0.25, 0.5, and 1% were administered with bacteria at concentrations of 10^4, 10^5, 10^6 cfu/mL. The maximum strength was attained with 10^5 cfu/mL and 0.5 % "L".

Addition of "L" at higher amounts and more dosage of the bacterial medium had generated a complex dense structure. However, on the flip side it diminishes the concrete strength at increasing concentrations [13]. The bacterial spores were counted using a haemocytometer with three bacterium concentrations employed in the batches: $4x10^7$, $7x10^7$, and $10x10^7$ spores/mL at all ages, the maximal augmentation in strength was obtained at cell concentrations of 10^5 cells/mL, or cell concentrations of 10^3 cells/mL, 10^5 cells/mL, and 10^7 cells/mL respectively. **Table 1** explains the impact of increasing the amount of nutrients and dosage of microbe [14]. This study's objective is to determine which nutrient among "L" and combination of "L" & "CL" for the production of best performing self-healing concrete.

Table 1: Comparison of bacteria and nutrients with their performances

Ingredients	Concent -ration	Strength Properties	Crack Healing (Precipit ation)
"Bacteria**"**	⬆	⬇	⬆
"L"	⬆	⬇	⬆
"CL"	⬆	⬇	⬆

3 EXPERIMENTAL PROGRAM

3.1 Materials

The cement used in this study was Ordinary Portland Cement (OPC) of 53 grade complying with IS 12269: 2013. The properties of cement were found to be Fineness 2.33%, standard consistency 33%, initial and final setting time 86 min and 380 min respectively and specific gravity of 3.15. The fine aggregate was collected from the locally available natural river sand. Fine and coarse aggregate's physical characteristics were specific gravity 2.6 (Zone II) and 2.7, water absorption 0.5% and 0.55% respectively. Potable water conforming with IS 456: 2000 was used.

3.2 Microbe strain characterization and culture media

The genus species *B. subtilis* in lyophilized form are procured from Proprenz Biotech Pvt Ltd, Hyderabad. The microorganism was cultured in the Microbiology and Food Science & Technology laboratory, GITAM University, Visakhapatnam. Schaeffer-Fulton endospore staining was used to determine whether the *Bacillus* spores could produce endospores. The cultured bacterial solution maintaining samples pH of 11, were then processed for further characteristically investigations.

3.2.1 Microbial cell quantification

The microbial cell concentration in a suspension, contains thousands/ millions of cells, hence dilution is necessary as, reasonably can't be counted. After 24 h of incubation, a sterile pipette was used to place 0.1 µL of cell solution in a test tube containing 1000 µL of a dilutant. This solution furtherly diluted 10 times more than initial sample, and the right dilution factor (DF) was determined to be 10^{-1}. The solution is now inscribed into cleaned and washed Hemocytometer (counting chamber) consisting of grid approximately in 5 square blocks, volume of each gird being 0.1 mm^3. The hemocytometer was then observed under microscope with 40X focusing lenses and then spores count was derived to be 63 cells and the cell concentration of the solution computed to be 1.26×10^6 cells/mL

3.3 Precursors

The role of the precursors in the bacterial concrete has become an essential supplementary ingredient as it develops the growth and survival period of the bacteria and also in functionalizing the process of biomineralization. In this study calcium based sources adopted were "L" procured from Triveni Interchem Pvt Ltd., Gujarat, India and "CL" purchased from United scientific & chemicals, Visakhapatnam, India. The properties of the "L" and "CL" are presented in **Table 2**.

Table 2: Properties of Calcium Lactate and Calcium Chloride

Properties	Calcium Lactate	Calcium Chloride
Form	White powder	White crystalline salt
Chemical Formula	$C_6H_{10}CaO_6$	$CaCl_2$
Appearance	White or off-white powder	white powder, granules or flakes
Solubility in water	yes	yes
Odour	Efflorescent	Odourless

3.4 Mix proportioning

In this investigation, concrete mix design was executed using IS 10262:2019. This study employs three sorts of mixtures and compared the effect of nutrients as single agent and in combinations on the strength and healing properties of concrete. “L” and “CL” were the two nutrients confronted. The water-cement ratio arrived was 0.48. Mix 1 (CC) is the conventional concrete mix containing no amount of microbe and nutrient. Mix 2 (BC-L) is composed of bacterial solution of required concentration (10^5 cells/mL) and “L” at concentration of 1% by weight of cement. Mix 3 (BC-LCL) contains bacterial broth with combination of “L” (0.6%) and “CL” (0.4%) by weight of cement. **Table 3** elaborates the detail of designed mix and their quantities necessitated for three mixes.

Table 3: Materials and their quantities

Type of mix → / Materials ↓	CC	BC-L	BC-LCL
Cement (Kg/m³)	350	350	350
Fine aggregate (Kg/m³)	715.97	715.97	715.97
Coarse aggregate (Kg/m³)	1207.84	1207.84	1207.84
Water (Kg/m³)	168	168	168
Bacterial solution (cells/mL)	-	10^5	10^5
“L” dosage	-	1%	0.6%
“CL” dosage	-	-	0.4%

4 EXPERIMENTAL METHODS

4.1 Concrete specimens and testing

Concrete mix was prepared as per mix proportions and casting of cubes, beams and cylinders of size 100x100x100 mm, 100x100x500 mm and 150x300 mm were fabricated respectively, for the aforementioned age periods. For the bacterial mixes, required calcium source is then weighed, mixed /diluted in the pure water in such a way that the total measured quantity was added. Later, into the concrete mixture, the quantified bacterial solution of required concentration (10^5 cells/mL) was added. The concrete specimens were demoulded after 24 hours and submerged into the curing tank and then tested at various age periods.

4.2 Tests on fresh concrete

4.2.1 pH of concrete

pH is tested using pH papers to check the alkalinity of concrete with and without bacteria.

4.2.2 Slump Test

The concrete's workability is demonstrated by the slump type. By adding three equal layers of concrete to the slump cone and tamping it down 25 times for each layer before raising the cone upright, a volume of concrete was slump tested. The slump is represented by the subsidence in height between the cone and the concrete. The concrete's workability is demonstrated by the slump type. The examination was conducted in accordance with IS: 1199-1959.

4.3 Tests on hardened concrete

4.3.1 Compressive strength test

According to IS 516: 2021, the compressive strength was determined at 3, 7, 14, 21, 28, and 56 days by performing compressive strength test on both conventional and bacterial specimens using compression testing machine. A specimen measuring 100mm x100mm x 100mm is put through the test on

the compression testing equipment. A consistent load of 140 kg/cm^2 has been applied until the test specimen arrives to fatigue condition.

4.3.2 Split tensile strength test

As per IS 516: 2021, the split tensile strength test was carried out to estimate the tensile strength on two types of concrete specimens using compression testing machine. For each blend, cylinder specimens of size 150mm x 300 mm were subjected to the increasing load within the range of 1.2 N/mm^2/min to 2.4 N/mm^2/min for aforementioned age periods.

4.3.3 Flexural strength test

In accordance with IS 516: 2021, the modulus of rupture, was determined for the concrete specimens of size 500mm x 100mm x 100mm as mentioned above at all age periods. The rate of loading was maintained at 1.8 kN/min till a further load cannot be substantiate.

4.3.4 Water absorption

The water absorption of concrete was determined in accordance with IS 2386 Part III:1963 by oven drying the cubes 100mm x 100mm x 100 mm for 24 hours and immersing them in water for next 24 hours. Dry and wet weights (M_{dry} and M_{sat}) were taken to compute the water absorption for each mix using the equation 2.

$$W_a = \frac{M_{sat} - M_{dry}}{M_{dry}} X100 \qquad (2)$$

4.3.5 Porosity

The porosity on the cubical specimens of size 100mm x 100mm x 100 mm was measured as per code DIN 1048 [15], so as to comprehend the process of water transport within pore structure and to estimate the linked pore space as, at all age periods.

$$P = \frac{Msat - Mdry}{\rho_w V} \qquad (3)$$

V_v = volume of voids = M_{sat} - M_{dry};
V = total volume of specimen;
ϱ_w = the unit mass of water;
M_{sat} = weight of saturated specimen;
M_{dry} = weight of oven dried specimen (105 °C)

4.3.6 Crack healing analysis

Healing of cracks in bacterial concrete was studied to assess self-healing effect at various nutrient dosage and age periods. The microscope crack detector was used to measure the surface crack widths after 3 and 28 days of curing for crack healing quantification**.** The bacterial specimens were subjected to loading until the fissures emerged on the surface. The induced crack widths ranged from 0.1 mm to 1.2 mm. The samples were then immersed in water.

5 RESULTS AND DISCUSSIONS

5.1 Strain Characterization

The bacterial spores were cultivated in petri plates by using nutrient agar media at pH-11 and the bacterial growth was observed in 10^{-3} and 10^{-4} serial dilution plates after 24 h of incubation at 37^{o}C in an incubator. The urease test discloses that bacteria doesn't release any urease enzyme and remains light orange colour of the broth after 24h, which was in agreement with [16]. The prepared bacteria smears were washed, blotted and then examined under a research compound microscope (100X) as shown in **Figure 2**. **Table 4** illustrates the remarks obtained by performing various tests on the microorganism and its culture medium.

Table 4: Tests on bacteria

Test conducted	Indication	Result
Gram staining reaction	Violet colour	Gram positive
Morphological Characterization	Aerobic condition, rod shape in structure	Bacterium identified as *Bacillus subtilis*.
Urease Test	Light orange colour	Urease negative; Non-ureolytic
Endospore staining	Colour appearance	Pink-Vegetative cell Green-Endospore

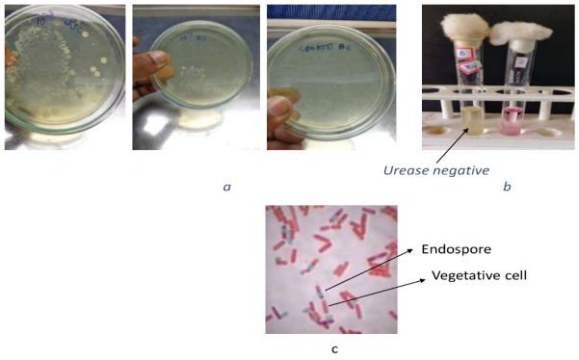


Figure 2: a) Bacterial growth on plates with control b) Urease test c) Endospore staining.

5.2 pH Values

The pH of cement and water is more than 13. When bacteria are combined together, it creates an unfavourable environment for them to live in. The majority of organisms perish in environments with a pH of 10 or above (for review see [17-19]. Bacterial viability was maintained as shown in **Figure 3** in a cement extract solution containing nutrients (both "CL" and "L") as these nutrients do not impose any effect as evinced in the study [9] on the pH of the concrete shown in **Table 5,** also the non-ureolytic bacteria is capable of living and precipitating $CaCO_3$ in high pH environments.

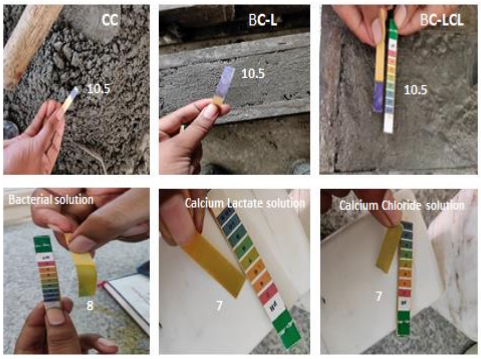


Figure 3: pH detection.

Table 5: pH determination

Component	pH value	Pertinent reference
CC	12	(Jonkers et al. 2010) [6]
BC-L	10.5	(Tobler et al. 2011) [31]
BC-LCL	10.5	
Bacterial solution	8	(Phillips et al. 2013) [32]
Lactate solution	7	
Chloride solution	7	

5.3 Mechanical Properties

It can be distinctly observed from **Figure 4** that both the mixes BC-L and BC-LCL with bacterial cell concentration of 10^5 cells/mL exhibited compressive strength at the early age i.e., 7days with an increase in percentage of 30.9% and 74.2% compared to CC. The similitude potency has been depicted by CL, CCL in the previous study of [20,14,13,21,15]. From **Figure 5** it is evident that the **i**ncrease in flexural strength was obtained for BC-L and BC-LC at 7days with the increase being 24.65% & 14.13% respectively which is equivalent to 21 days' strength of CC. Also from **Figure 6** increase in tensile strength obtained for BC-L and BC-LCL specimen at 7days was 75.7% & 60.6% respectively which is equivalent to 28 days' strength of CC, the similar effect was established in the study of "L" and "CL" respectively [15,9]. This is because voids are being enclosed due to constant hydration process and also the influence of mineral precipitation has shown

the development undoubtedly which improved the strength qualities of concrete.

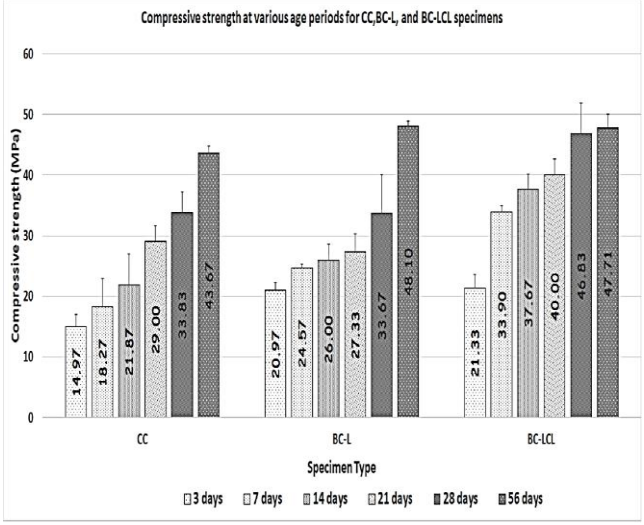


Figure 4: Compressive strength of conventional and bacterial specimens.

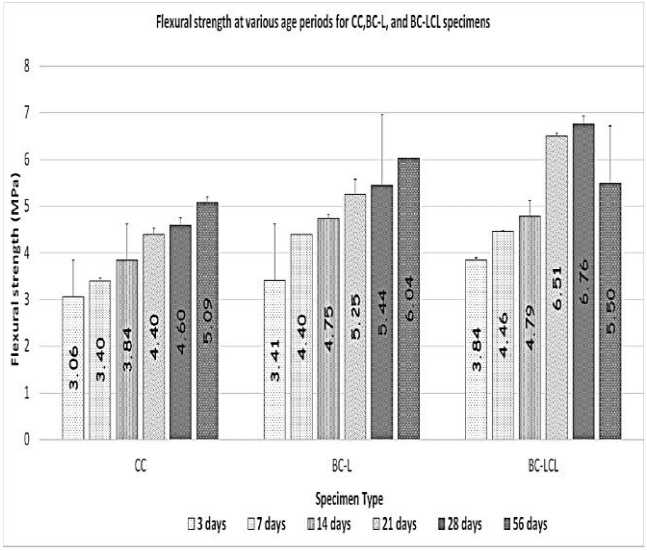


Figure 5: Flexural strength of conventional and bacterial specimens.

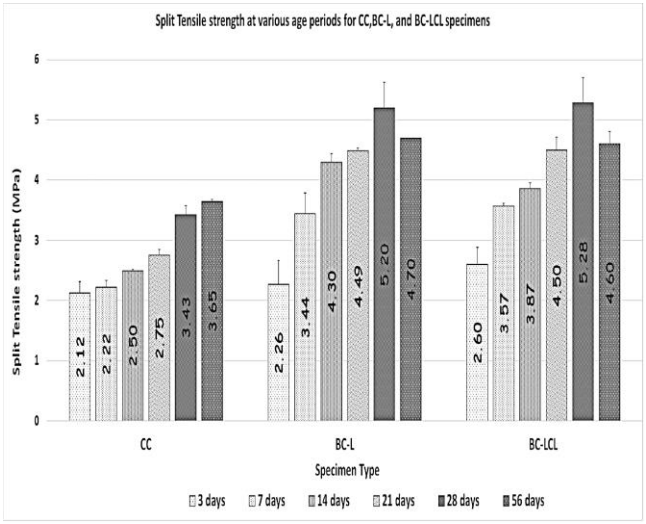


Figure 6: Split tensile strength of conventional and bacterial specimens.

5.4 Water absorption and Porosity

Reduction in the water absorption was observed to be about 14% and 11% for bacterial cubes BC-L, BC-LCL respectively compared to CC at 28 days. The same trend of increment or decrement was affirmed in a study of "L" [20,22, 23,15]. This is due to the deposition of calcium carbonate precipitate on the surface of concrete resulted in a decrease of water absorption. The porosity for BC-L specimens have shown porosity of 6.3% which was analogous in the research made by [15,24,25] where as that of CC is 7% at 28 days. However, BC-LCL it arrived to be 6% as the "CL" aids in precipitating in high amounts. Calcite mineral precipitation in the pores have also lowered the average pore radius of concrete. **Figure 7**, bestows the water absorption and porosity of three different mixes CC, BC-L and BC-LCL at 28 days with aid of a graph plotted using the experimental data. The predicted equation and their related regression coefficient R^2 values depicted a linear relation between two parameters.

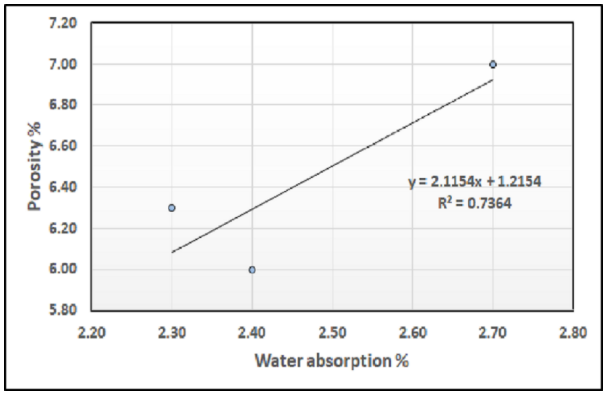
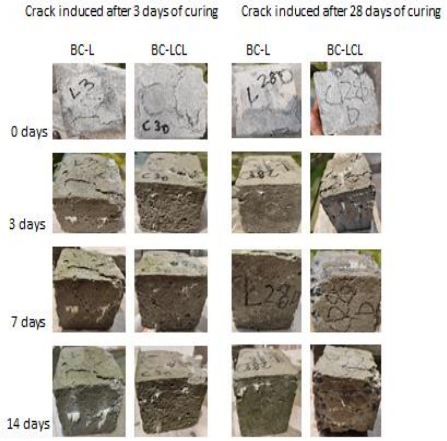


Figure 7: Relation between water absorption and porosity of the plain and bacterial specimens.

5.5 Crack healing performance

Cracks were artificially induced on both 3 days of curing and 28 days of curing by applying minimal load through Compression Testing Machine (CTM) until the cracks are formed. For the mix of M20 grade bacterial concrete crack widths were varying approximately from 0.05mm to 1mm. Cracks up to 0.6mm were reported to be healed in the BC-L and BC-LCL for 3-day cured specimens as shown in **Figure 8**. It was observed that on 56th day cured BC-L specimen's maximum cracks up to 0.7mm, this significant effect on healing was also remarked for "L", "CL" specimens in the earlier studies [25-30, 1,15] and in case of BC-LCL specimens up to 0.8mm crack width was healed. The calcite precipitation as shown in **Figure 9** was observed with aid of Microscope crack width detector with optical magnification of X40 (C & D Microsystem Limited, UK). This shows that the bacteria are active due to the availability of nutrients for a longer period.



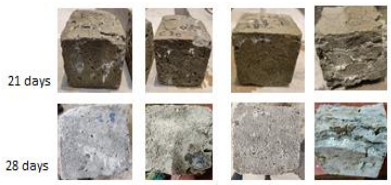


Figure 8: Self-Healing through $CaCO_3$ precipitation observed on the surface cracks of the bacterial cubes.

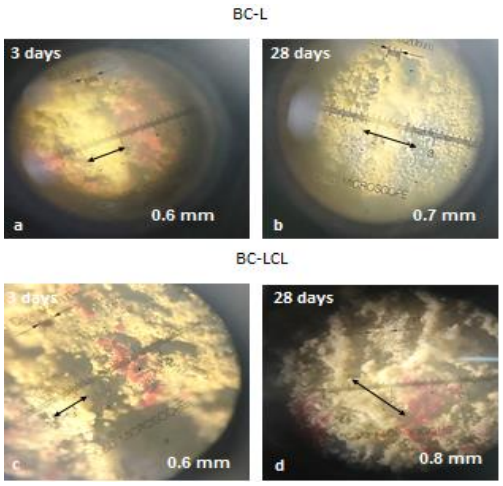


Figure 9: Observing the formation of $CaCO_3$ and healing of the surface cracks on the specimens BC-L, and BC-LCL on the 28th day post application of loading after 3 days (a, b) and 28 days (c, d) of curing respectively.

6 CONCLUSIONS

By incorporating the precursors along with the bacterial medium in the concrete matrix, the compressive, flexural and tensile strengths of both BC-L and BC-LCL increased with age similar to that of CC. Both BC-L and BC-LCL exhibited higher strengths than CC at all ages. But the percentage growth of increase in strength reduced with the increase of age. BC-LCL achieved high early strength than BC-L. BC-LCL achieved 28days strength of CC at an age of 7days itself. Both the BC-L and BC-LCL showed lesser absorption of water than CC. Porosity was less for both BC-L and BC-LCL than CC. BC-LCL showed lowest porosity value i.e., 6%. Maximum healing of crack i.e., 0.8mm was observed in BC-LCL at 56 days than BC-L and CC as "CL" showed high formation of carbon precipitation. It can be concluded that the combination of "L" and "CL" as nutrient is the best fit for producing high performance bacterial concrete as BC-LCL performed exceedingly well than BC-L in

terms of crack healing.

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