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Bio-healing Cement mortar by use of hydrogel encapsulated bacterial spores

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Abstract

In this study, hydrogel capsules were used as bacterial carriers for crack healing improvement. The hydrogel capsules were resistant to the high pH of concrete and humidity sensitive. Upon cracking, capsules in crack zone will break. Spores in the broken capsules can germinate and precipitate $CaCO_3$ in the presence of water in the cracks, causing crack healing by calcite. The purpose of this study was to demonstrate the viability of using hydrogel capsulated spores to self-heal cracks in a cementitious matrix. The enhanced self-healing efficiency in cracked specimens contributed by encapsulated bacterial spores was demonstrated based on the experimental results from light microscopy. The specimens with bacteria had much higher crack healing ratio compared to control. From our results, the bacterial encapsulation was recommended to be used as bacterial carriers for crack healing and strength improvements.

Keywords: *encapsulation, bacterial concrete, bio-healing of cracks*

Introduction

Concrete is the most commonly used construction material worldwide. However, concrete structures suffer from cracking that leads to deterioration and shorter service life (Virginie Wiktor and Henk M. Jonkers, 2011). The main cause of deterioration in concrete is the

appearance of cracks, which threaten the safety, durability, and the functionality of concrete structures (Xianfeng Wang *et al.*, 2019)

Self-healing concrete is a damage management concept, where a damage or crack is healed without any external help. Autogenously healing can be defined as a natural process of filling and sealing cracks that does not require any external operations or works (Guadalupe Sierra-Beltran *et al., 2014).* The process of bacterial conversion takes place either in the interior or exterior of the microbial cell or even some distance away within the concrete. The application of bio-mineralogy concepts in concrete resulted in the development of a new material known as bacterial concrete. It refers to a new generation of concrete that incorporates selective cementation via microbiologically-induced $CaCO_3$ precipitation for the remediation of micro-cracks (Gautam 2018).

Bacteria must meet certain criteria in order to be used as a curing agent in concrete, such as 1-Their ability to withstand an alkaline atmosphere in concrete for the generation of calcium carbonate. 2-Without being impacted by calcium ion concentration, it should contain a significant amount of calcium carbonate and be able to withstand high pressure. 3-Using a lot of oxygen and reducing steel corrosion should be oxygen-brilliant. The main mechanism of bacterial crack healing is that the bacteria act as a catalyst, converting the components of the precursor into an efficient filler material (Jonkers 2011). It should be noted that there is a possibility that the bacterial cells or spores will be damaged during the mixing and cement hydration stages. This is due to the cement-based matrix gradually becoming a dense structure during the hydration process. Mechanical forces during mixing may also harm the bacteria. Therefore, encapsulation of bacteria before addition is preferable (Wang *et al.*, 2014).

Wang *et al*., 2014 showed that the addition of microencapsulated methyl methacrylate-based healing agent into carbon microfiber-reinforced mortar can greatly improve the crack resistance and toughness under fatigue loading. This demonstrated that the concept of using microcapsules as carriers for self-healing agents in concrete was feasible and very promising.

Materials and Methods

Bacterial isolation

Endospore forming bacterium in Fayoum Governorate, Egypt was isolated from active sludge. Procedure for Fulton endospore staining was done to detect bacterial endospores. Alkaline nutrient agar plates were prepared by the addition of NaOH solution droplets till reaching pH 14

then, bacterial isolates were spread on plates and incubated for 48 hrs. The most alkali tolerant bacterium was preserved in slants for further tests.

Encapsulation of bacteria

In this study, hydrogel Capsules were used as bacterial spores' carriers. Extrusion-ionic gelation was the encapsulation technique applied, according to the methodology described by Sun and Griffiths, 2000, with some modifications. Before the encapsulation assay, the lyophilization of bacterial suspension was prepared by their growth in alkaline nutrient liquid media for 24 hrs, then the lyophilized bacteria were mixed with sucrose as an adsorbent. Capsules are resistant to the high pH of concrete and humidity sensitive. This means that the alkaline capsules can withstand the mixing process and are easily broken when cracks appear. Upon cracking, capsules in crack zone will break as shown in **Figure (1).** The food of bacterial spores (yeast extract) and the deposition agents including urea and Ca source (Ca-chloride) were incorporated together with the capsules during the mixing process.

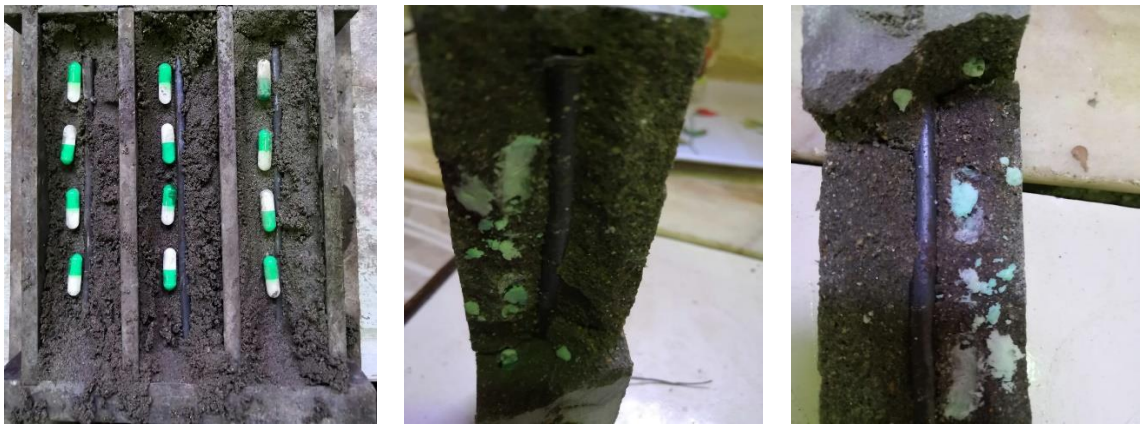


Figure (1) Shows Capsulation of Bacterial Spores.

Molecular Identification of the bacterial isolate

The genomic DNA was isolated for molecular identification using conventional bacterial techniques outlined in Molecular Cloning (Sambrook & Russell, 2000). The PCR mix was made as follows: 1L (20pmole/L) of each primer, 10L (10x) PCR buffer, 3L (50mM) $MgCl_2$, 10L (10x) PCR buffer (10mM) dNTPs, 0.5L (2.5U) Taq polymerase, 2L gross DNA extract, and sterilized distilled H2O to a final volume of 100L. PCR was carried out for 35 cycles under the following conditions: Stage of denaturation at 90-94°C for 40 seconds, The annealing phase was set to 55°C for 1 minute, the extension step to 72°C for 2 minutes, and the final expansion to 72°C for 10 minutes. The gel containing 0.5g/mL ethidium bromide in the Tris-Borate-EDTA (TBE) buffer was then observed using a UV transilluminator by the buffer loading and electrophoresis analysis on 0.7 percent horizontal agarose (60 min at 15V/cm). GATC Biotech in

Constance, Germany, completed the sequencing of the amplified fragments. The NCBI Data Base was used to align DNA sequences (www.ncbi.nlm.nlh.gov). The phylogenetic trees were created with TREEVIEW software (1.6.6) using a neighbor-joining strategy based on gene sequences of 16S rDNA from some phylogenetic near strains to the isolated strain.

Self-healing agent preparation

Bacterial isolate was cultured in alkali nutrient broth for 24 hrs, Bacterial concentration using the measured term in solution $Y= 8.59\times10^{7} X ^{1.3627}$ (Khaliq & Ehsan, 2016). Where the bacterial concentration per mL is Y, and X is the reading at OD_{600}. With a spectrophotometer, the bacterial concentration was found to be 1.29 X 10^{7} cells/ml. Based on these data, the concentration of spore was preserved in samples equal to 1.3 X 10^{7} cells/cm^{3} of concrete mixture. The nutrients, containing forty g/L calcium chloride anhydrous, sixty-five g/L urea and two g/L yeast extract were dissolved in sterilized tap water to prepare the bio-based concrete.

Experimental design

The mixture design and testing program was conducted in accordance with ECP and ASTM standards. This program included two types of bacteria, different carrier compounds with two different introduction techniques in concrete mixture as follow the bacteria were introduced in concrete thorough various carrier compounds namely, direct incorporation, and capsules to evaluate the self-healing performance. Fifteen mixtures containing different type, and carries of bacterial spores was designed as shown in **Table (1)**. The compression and self-healing were carried out for hardened concretes at different curing times of 7, 14, and 28 days. 40x40x160 mm beam molds were used. In Group (1), the mixture doesn't contain any type of bacterial spores called control. In Group (2), the mixtures contain type (1) of bacterial spores called *Bacillus wiedmannii*. While, in Group (3) the mixtures contain type (2) of bacterial called *Bacillus paramycoides.*

Table (1) Mortar Mixtures Quantities.

Type of mortar	Water (kg/m^3)	Cement (kg/m^3)	Sand (kg/m^3)	Type of Bacterial Spores	Type of carrier
M1-1	140	350	1050	No bacterial spores	
M2-1	140	350	1050	*Bacillus wiedmannii*	Direct
M2-3	140	350	1050		Capsules
M3-1	140	350	1050	*Bacillus paramycoides*	Direct
M3-3	140	350	1050		Capsules

Test specimens

After 24 h of casting, specimens were removed from the molds and were cured with water. Beam molds for mortar specimens (40×40×160 mm) were used. Compression and self-healing were conducted at various curing times of 7, 14, and 28 days for hardened concrete. To track microstructural changes due to mineral formation. Stereomicroscope was used for self-healing measurements.

Flexural strength test

The static flexural test took place 28 days after the casting of the specimens. This test was carried out in conjunction with the ASTM C78 standard for simple beams undergoing third-point loading. The rate of loading for the static flexural test was maintained at 900 kPa per minute. three beams were tested at 28 days and their average value is reported by using the following Equation (3) below for the calculation of the modulus of rupture which is determined by dividing the product of peak load (P) and specimen clear span (L) by the product of specimen width (b) and depth (d) squared.

$$R = \frac{PL}{bd^2} \qquad \textbf{Equation (1)}$$

Results

Identification by 16S rRNA Gene Sequencing

The nucleotide sequence in molecular identification was compared with known sequences using the Blastx software (BLAST), The National Knowledge Center for Biotechnology. The bacterial isolate was identified as *Bacillus wiedmannii* having 92% similarity with *Bacillus wiedmannii* strain FSL W8-0169 as shown in **Figure (2)** and *Bacillus paramycoides* having 98% similarity with *Bacillus paramycoides strain MCCC 1A04098* as shown in **Figure (3)**.

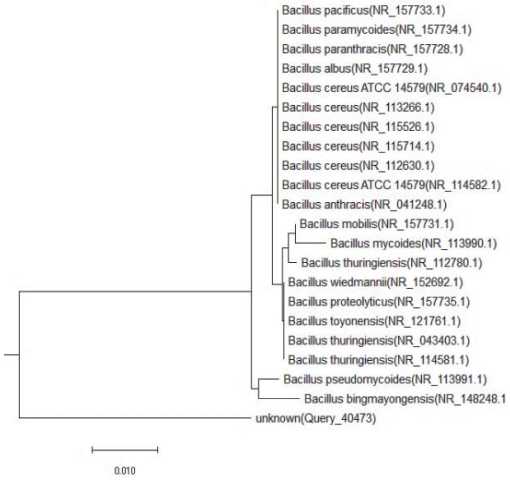


Figure (2) Neighbor – joining phylogenetic tree based on bacterial 16S rRNA sequence data

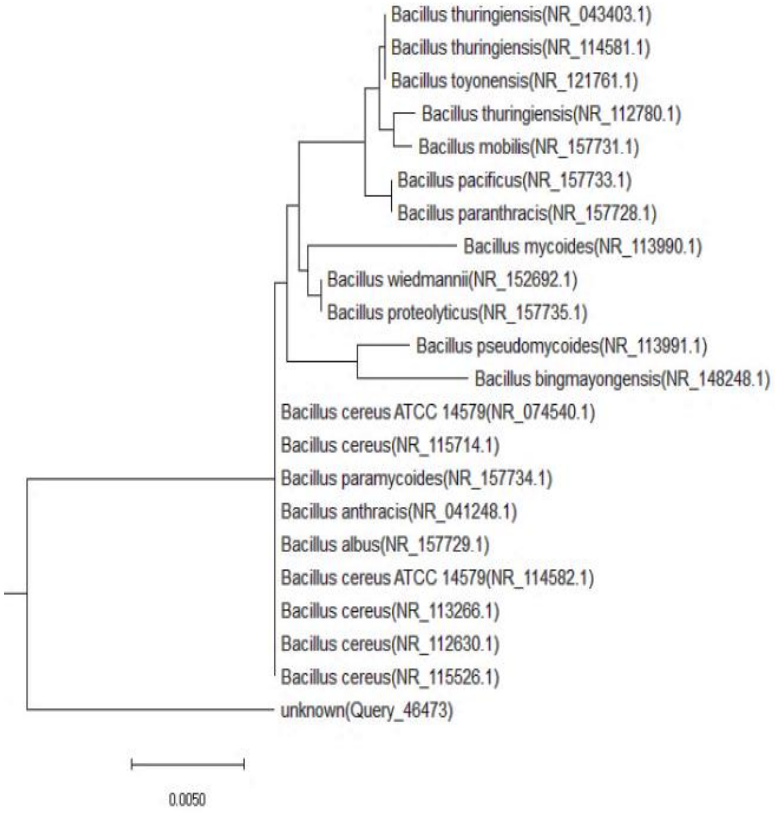
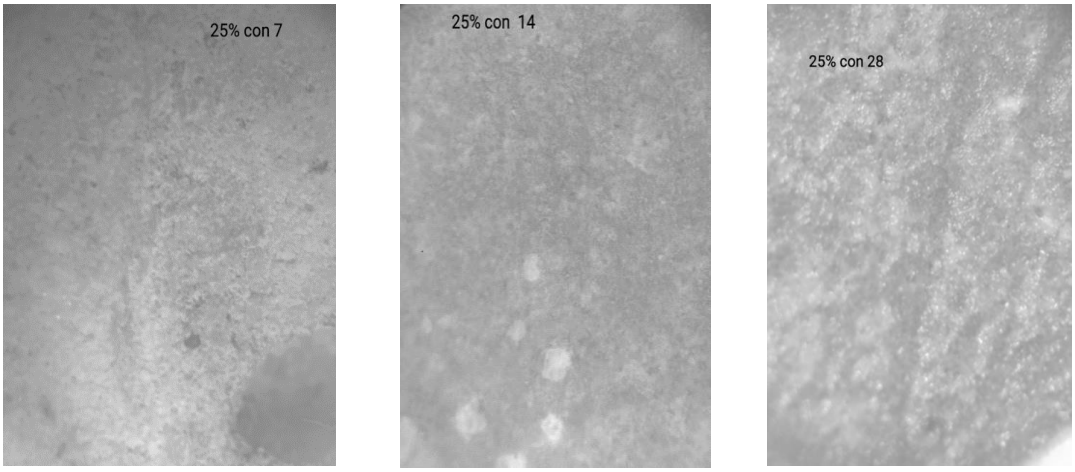


Figure (3) Neighbor – joining phylogenetic tree based on bacterial 16S rRNA sequence data

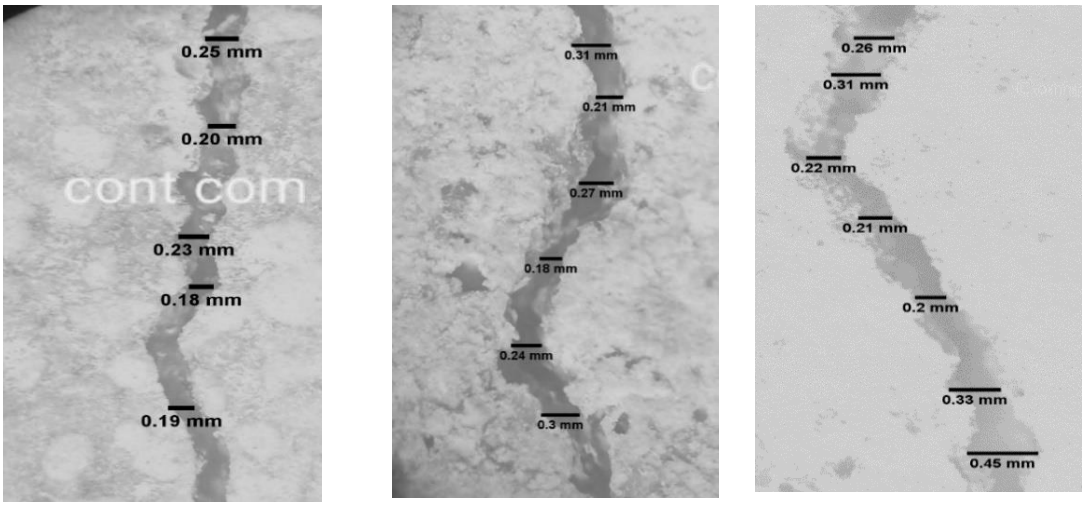
Quantification of crack healing by bacteria

In Group (1), the mixture doesn't contain bacteria. It is noted that the cracks completely healed at 25% of maximum load after two months of curing as shown in **Figure (4)**. At maximum load and 50%, 75% of maximum load, unhealed cracks were observed as shown in **Figures (5), (6),** and **(7)**.



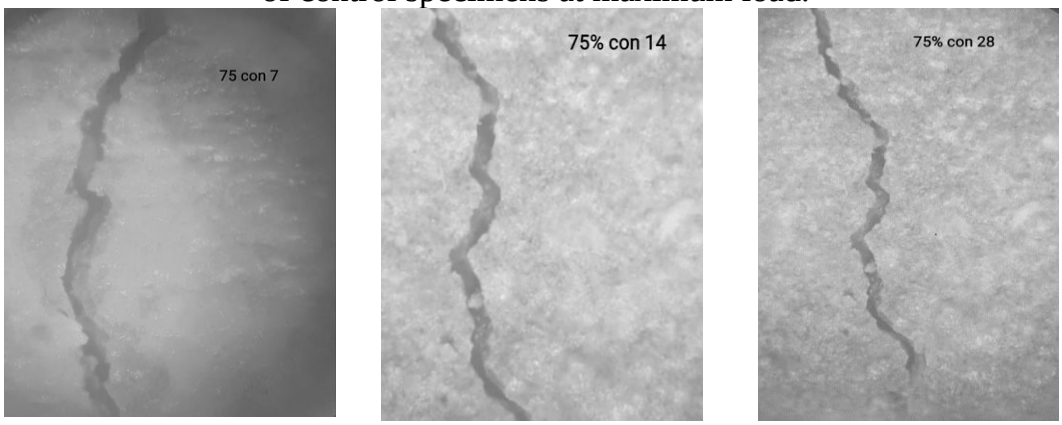
after 7 days after 14 days after 28 days

Figure (4) Crack healing in the pre-cracked specimens at 7, 14, and 28 days of control specimens at 25% of maximum load.



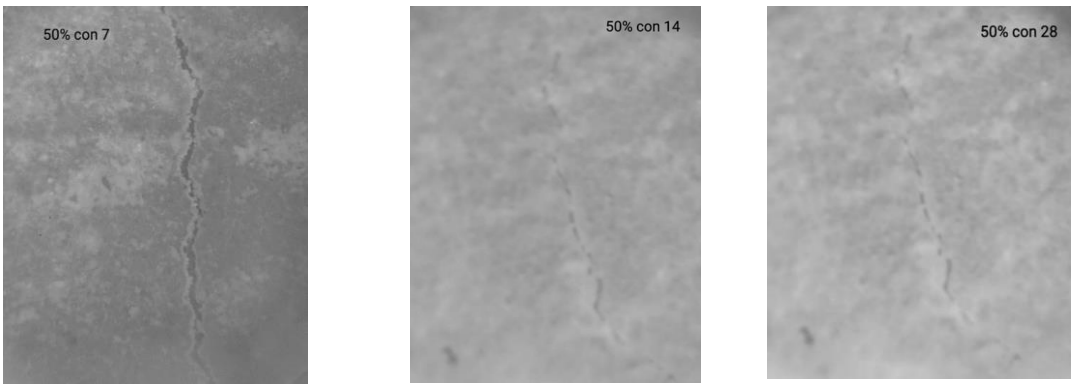
after 7 days after 14 days after 28 days

Figure (5) Crack healing in the pre-cracked specimens at 7, 14, and 28 days of control specimens at maximum load.



after 7 days after 14 days after 28 days

Figure (6) Crack healing in the pre-cracked specimens at 7, 14, and 28 days of control specimens at 75 % of maximum load.

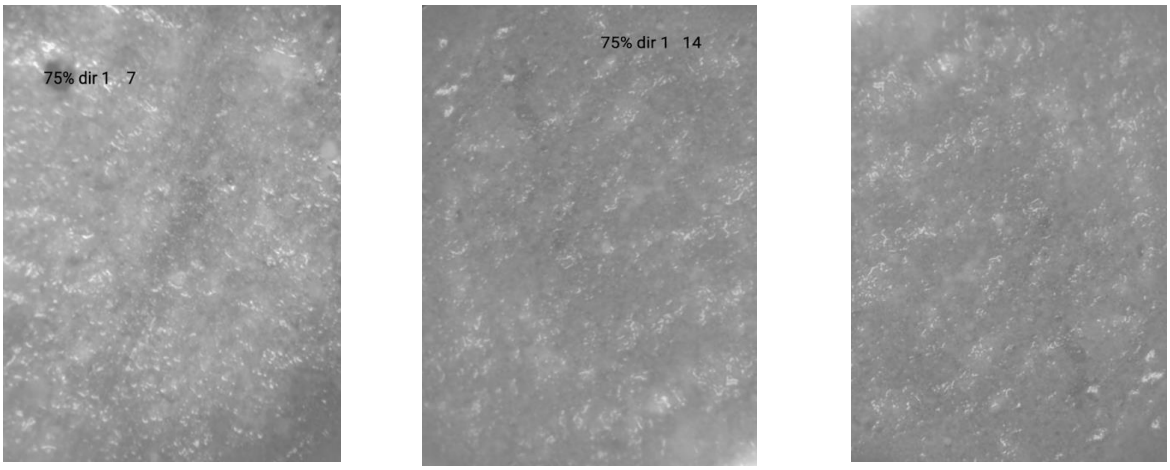


after 7 days after 14 days after 28 days

Figure (7) Crack healing in the pre-cracked specimens at 7, 14, and 28 days of control specimens at 50% of maximum load.

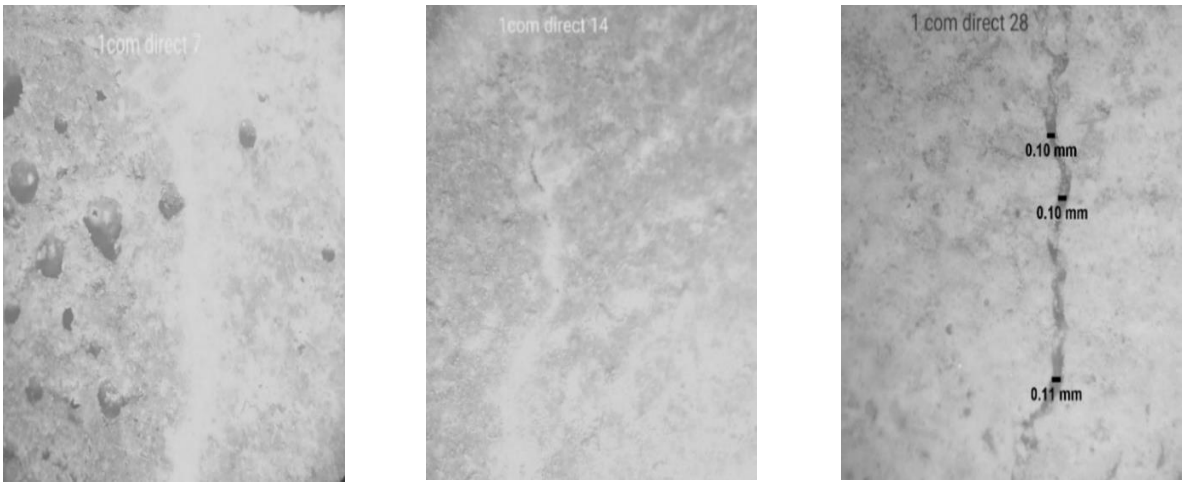
In Group (2), the mixtures contain type (1) of bacterial called *Bacillus Wiedmannii*. For specimens which have bacterial spores that were incorporated directly it is noted that the cracks

completely healed at 25%, 50%, and 75% of maximum load after two months of water curing in the pre-cracked specimens at 7, 14, and 28 days as shown in **Figure (8)**. At maximum load, it is noted that cracks completely healed after two months of water curing only in the pre-cracked specimens at 7, 14days as shown in **Figure (9)**. For specimens which bacterial spores have been encapsulated, it is noted that the cracks completely healed at 25%, 50%, and75% of maximum load after two months of water curing in the pre-cracked specimens at 7, 14, and 28 days as shown in **Figure (10)**. At maximum load, it is noted that cracks completely healed after two months of water curing in the pre-cracked specimens at 14, 28 days as shown in **Figure (11)**.



after 7 days after 14 days after 28 days

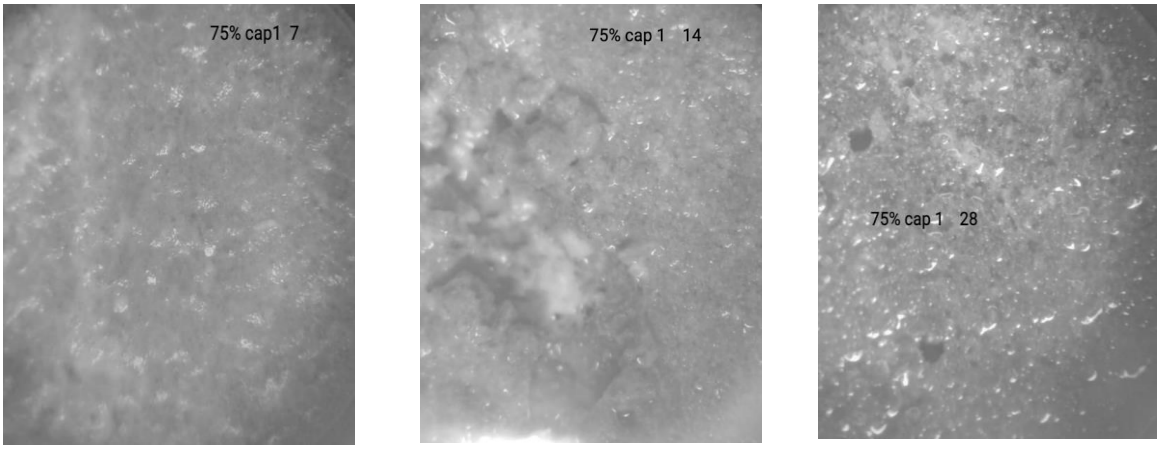
Figure (8) Crack healing in the pre-cracked specimens at 7, 14, and 28 days for group (2) bacterial spores were incorporated directly at 75 % of Maximum Load.



after 7 days after 14 days after 28 days

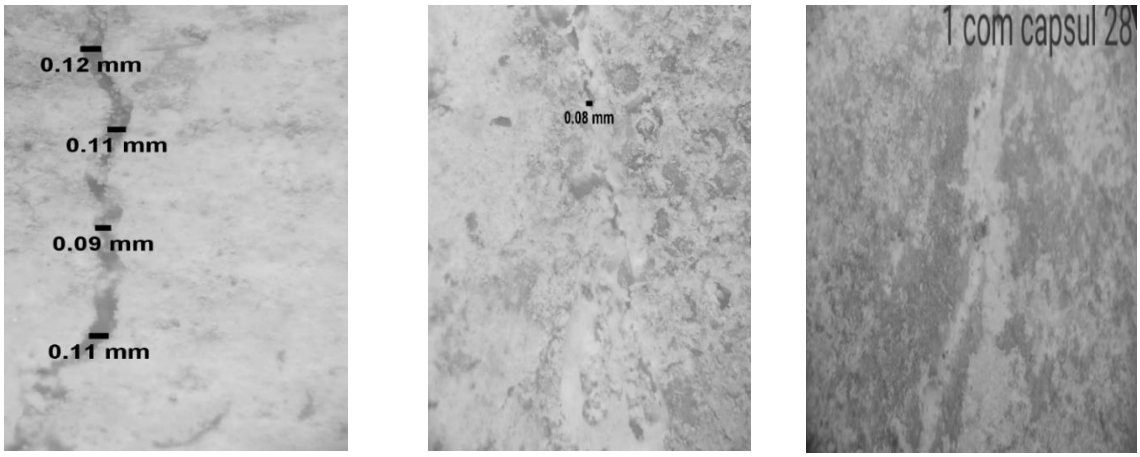
Figure (9) Crack healing in the pre-cracked specimens at 7, 14, and 28 days for group (2) bacterial spores were incorporated directly at Maximum Load.

Volume: 7; Issue: 10; October 2021; pp 2258-2271; ISSN: 2454-5422



after 7 days after 14 days after 28 days

Figure (10) Crack healing in the pre-cracked specimens at 7, 14, and 28 days for group (2) specimens which bacterial spores have been encapsulated at 75% of maximum load.

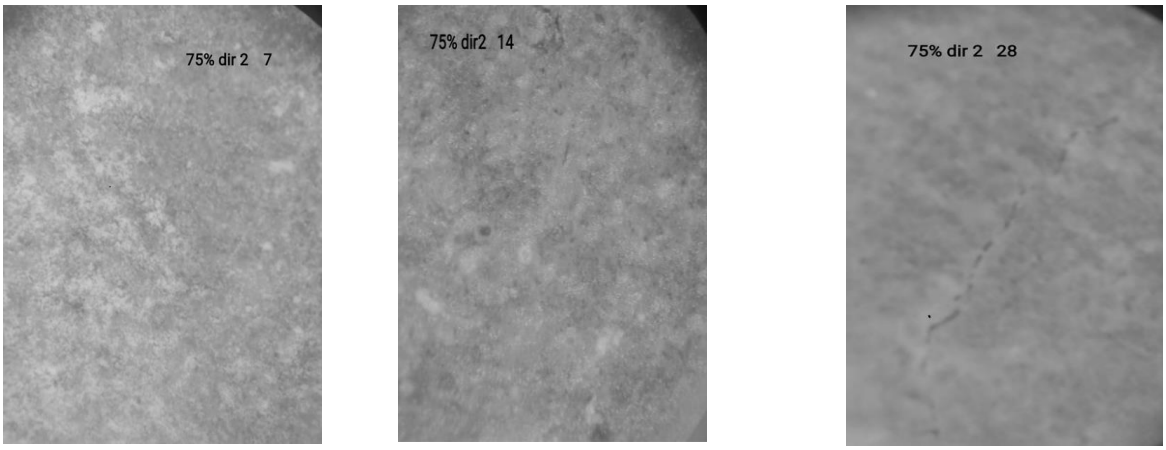


after 7 days after 14 days after 28 days

Figure (11) Crack healing in the pre-cracked specimens at 7, 14, and 28 days for group (2) specimens which bacterial spores have been encapsulated at maximum load.

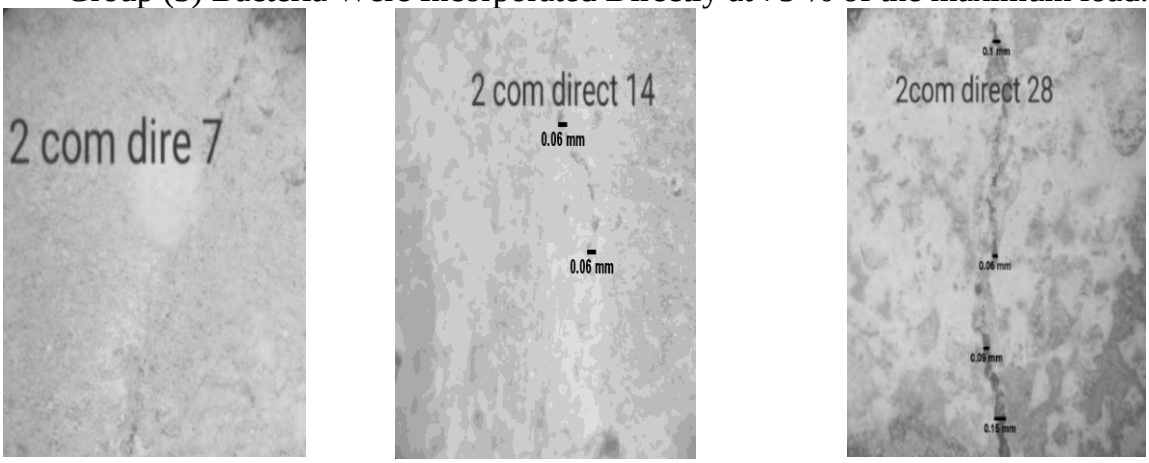
In Group (3), the mixtures contain type (2) of bacterial called *Bacillus Paramycoides*. For specimens which have bacterial spores that were incorporated directly it is noted that the cracks completely healed at 25%, 50%, and 75% of maximum load after two months of water curing in the pre-cracked specimens at 7, 14, and 28 days as shown in **Figure (12)**. At maximum load it is noted that cracks completely healed after two months of water curing in the pre-cracked specimens at 7, 14days as shown in **Figure (13)**.

Volume: 7; Issue: 10; October 2021; pp 2258-2271; ISSN: 2454-5422



after 7 days after 14 days after 28 days

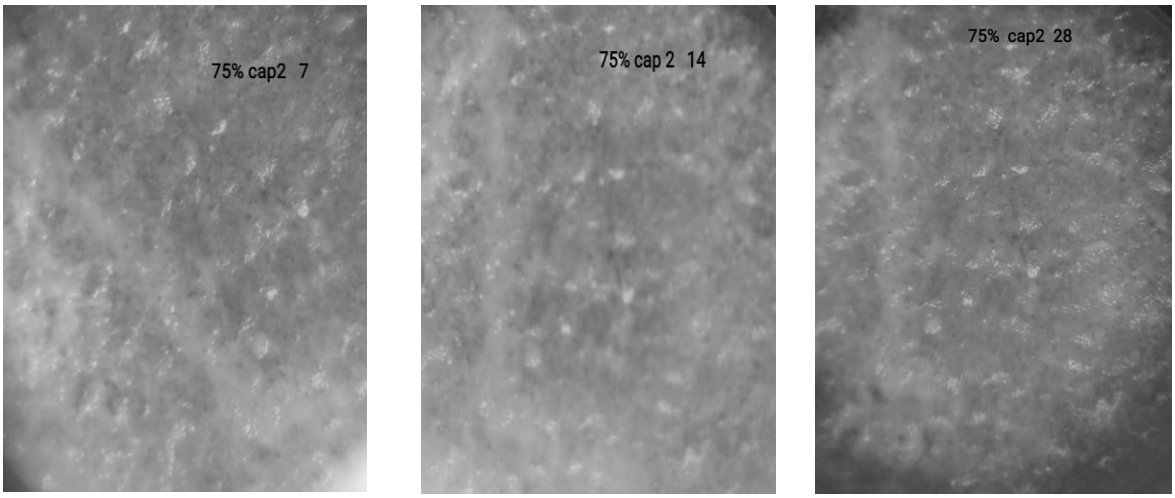
Figure (12) Crack Healing in The Pre-Cracked Specimens at 7, 14, And 28 Days for Group (3) Bacteria Were Incorporated Directly at 75 % of the maximum load.



after 7 days after 14 days after 28 days

Figure (13) Crack Healing in The Pre-Cracked Specimens at 7, 14, And 28 Days for Group (3) Bacteria Were Incorporated Directly at maximum load.

For specimens which bacterial spores have been encapsulated it is noted that the cracks completely healed at maximum load and 25%, 50%, and 75% of maximum load after two months of water curing in the pre-cracked specimens at 7, 14, and 28 days as shown in **Figure (14)**. At maximum load it is noted that cracks completely healed after two months of water curing in the pre-cracked specimens at 14, 28 days as shown in **Figure (15).**



after 7 days after 14 days after 28 days

Figure (14) Crack Healing in The Pre-Cracked Specimens at 7, 14, And 28 Days for Group (3) Specimens which bacterial spores have been encapsulated at 75% of maximum load.

after 7 days after 14 days after 28 days

Figure (15) Crack Healing in The Pre-Cracked Specimens at 7, 14, And 28 Days for Group (3) Specimens which bacterial spores have been encapsulated at maximum load.

Flexural Strength Test

Measured flexural strength of self-healing specimens is presented in **Table (2)**, and **Figure (16)**. It can be seen that all bacterial spore incorporation techniques result in increased flexural strength of the mixture.

Volume: 7; Issue: 10; October 2021; pp 2258-2271; ISSN: 2454-5422

Table (2) Flexural Strength of Mortar Specimens (kg/cm^2).

Type of concrete	Flexural strength (days)			Type of Bacterial	Type of carrier
	7	14	28		
M1-1	10.5	14.25	18	Control	No bacterial
M2-1	10.5	15	19.5	*Bacillus wiedmannii*	Direct
M2-3	9.75	13.5	16.5		Capsules
M3-1	9.45	14.25	18.45	*Bacillus paramycoides*	Direct
M3-3	9.3	12.75	15		Capsules

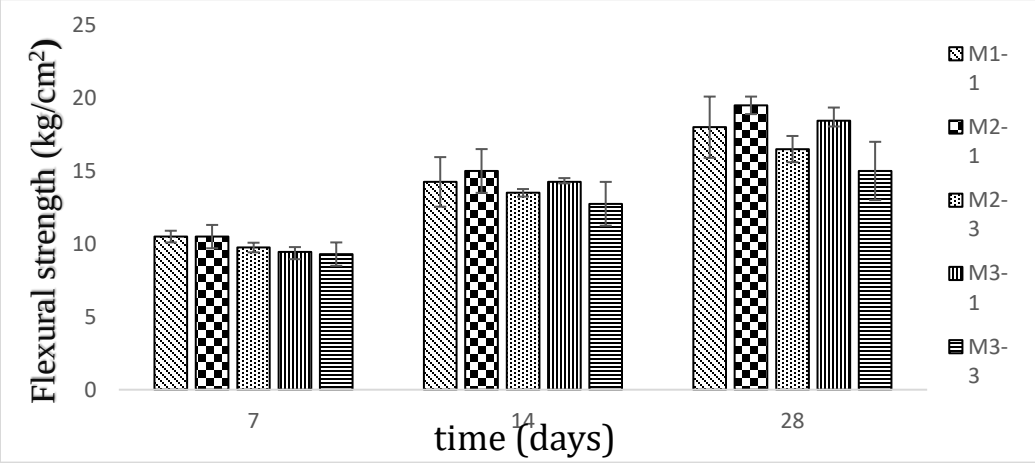


Figure (16) Comparison Between Flexural Strength Values for Group (1), Group (2) and Group (3).

Discussion

Bacteria's capacity to form endospores allows them to endure intense mechanical and chemical stresses during concrete mixing (Stanaszek-Tomal, 2020), and these endospores can live for up to 200 years (Seifan *et al., 2016*). Water entry into fissures activates the embedded bacterial spores, and CaCO3 is generated by germination of spores into vegetative cells as a result of microbial nutrition metabolism (Schreiberová *et al.,* 2019*).* Both bacterial isolates may precipitate CaCO3 due to their ability to hydrolyze urea into ammonia and carbonate. SEM micrographs showed the presence of $CaCO_3$ crystals in bacterial specimens and these results are in agreement with Chahal *et al.,* 2011; Fujita *et al.,* 2000 Who also illustrated that specific calcite crystals were screened in the bio-based concrete and the matrix of untreated specimens seemed to be amorphous, exhibiting no apparent growth of crystals.

Compared to samples, particularly after 28 days, the decrease in crack healing of direct inoculation is due to a decline in the feasibility of bacteria survival in the concrete under the

pressure applied during the mixing stage and that formed because of the formation of a dense microstructure (Schreiberová *et al.*, 2010; Wang *et al.*, 2014). The loss of bacteria by removal due to the formation of dense microstructures formed after 28 days of hydration in the concrete can be attributed to variations in the attitude of self-healing in direct bacterial inoculation. It can be shown that all methods of bacterial integration contribute to increased flexural strength of the mixtures, due to this deposition of $CaCO_3$ on the microorganism cell surfaces and inside the mortar pores (Kathirvel Parthiban & Kaliyaperumal Saravana Raja Mohan *et al.*, 2017).

Direct incorporation of bacteria also showed an increase in flexural mortar strength due to the presence of bacteria producing calcite in the mixture. This calcium carbonate continuously manufactured by the bacteria, urea, and calcium chloride provided as organic precursor makes the internal structure of concrete more compact.

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