Suez Canal Engineering Energy and Environmental Science Journal
Faculty of Engineering – Suez Canal University
2025, Volume 3, NO. 2 , pages 51– 64

The Mechanical Properties of Bacteria Based Self-Healing Concrete

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DOI: 10.21608/sceee.2025.359992.1069

Article Info:

Article History:
Received: 11\02\2025
Accepted: 17\03\2025
Published: 30\04\2025

DOI: 10.21608/sceee.2025.359992.1069

Abstract

Two decades of research on concrete self-healing using bacteria, with Sporosarcina pasteurii being one of the best choice due to its fast urease activity and biochemical properties. The optimal dosage is $3kg bacteria\ beads/m^3 concrete$. *Bacterial self-healing mortar samples show lower compressive strength, particularly when bacteria are added to concrete water before mixing. Compressive strength decreases with increasing dosage, with a13%,17.14% and 41.6% reduction observed for 3,5and 9kg/*m^3 *respectively. All of that at an early age and it was found that compressive strength equals control samples at age of 28 days. The bacteria used have proven their efficiency in self-healing concrete to treat cracks up to 2 mm, if they are immersed in water, which makes them of utmost importance in water structures, which is reflected in the calculations of the non-crack section reinforcement. The results indicate that the addition of bacteria in the mixing water before mixing has a great impact on self-healing, its productivity and the effectiveness of calcium carbonate produced by bacteria from its counterpart that uses bacteria beads on its gradually dry condition. Porosity studies showed a reduction in the porosity percentage after self-healing to 0.2% compared to 5.50% in control concrete sample.*

Keywords: *BIO Concrete, Self-Healing Concrete, MICP (Microbial induced calcium carbonate precipitation), Bacteria Based.*

Suez Canal Engineering, Energy and Environmental Science Journal (2025), ***Vol.3***, No. 2.

1. Introduction

1.1. Background

Concrete, a widely used construction material, is vulnerable to deterioration due to its strength, durability, and commercial availability. Concrete cracks, which are often caused by a combination of factors, can negatively impact the structure's performance and appearance. Non-structural cracks are caused by low-quality building materials and exposure to changing ambient conditions. Concrete cracks, categorized into active and dormant types, can be costly to repair due to differences between repairing agents and concrete's strength and elastic modulus. Strategies include appropriate mix design, materials selection, curing regimes.Self-healing, a crack recovery process, has been studied over the past 20 years, with approaches divided into autogenous healing and autonomous healing. Autogenous healing occurs when calcium hydroxide in cement continues to hydrate or carbonate, but is only effective for microcracks and is water-dependent (VanTittelboom et al., 2011). Active and passive vascular systems can introduce external substances during autonomous healing processes. One method is bacterium-based self-healing, where bacteria, deposition agents, and essential nutrients are added to the concrete matrix. Studies show effectiveness in promoting self-healing behavior since the late 2000s (Jonkers and Schlangen, 2009; Wang, De Belie, and Verstraete, 2012). In a nutshell, the theory postulated that when concrete cracks and water seeps in, bacteria already existing at the crack surface will be stimulated and should precipitate $CaCO_3$ to seal the gaps, blocking the entry of water. Concrete must have both the carbonate-precipitating bacteria and their necessary nutrients introduced to it during the mixing process in order for calcium carbonate $CaCO_3$ precipitation to take place. Bacteria release carbonate when a calcium solution is present, and different bacterial species create distinct $CaCO_3$ minerals. In practically every natural ecosystem on Earth, active microbes and the mineral precipitation they produce coexist with biological processes (López-Garca et al., 2005; Shen, Buick, and Canfield, 2001). The interaction between ions Ca_2+ from the environment and one or more metabolic products CO_3 generated by organisms provides the basis for mineral precipitation during MICP. Several mechanisms, including urea hydrolysis (Dhami, Reddy, and Mukherjee, 2013; De Muynck et al., 2010; Stocks-Fischer, Galinat, and Bang, 1999). Recently, bacterially induced carbonate precipitation has been employed to improve cementitious materials' self-healing capabilities, increase their strength, and repair cracks in building materials. This technology has also been investigated as a potential way to increase the resilience of cementitious materials (Ramakrishnan et al., 2005).

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1.1. Problem statement

Innovative techniques to prevent cracks in concrete structures are needed to prolong their lifespan. One solution is bio-mineralizing calcium carbonate using specific bacterial species. This self-healing bacteria can repair fissures in concrete, increasing durability and strength, and reducing the environmental impact of periodic repairs.

1.2. Objectives and methodology

The goal of the ongoing project is to examine the long-term performance of cement mortar that has been infused with various carbonate-producing bacteria for use in construction applications. Based on their urease activity, PH tolerance, and spore formation, five different species of bacteria have been chosen. The effect of these bacterial species on the properties of cement mortar has been studied. Calcium urea, yeast extract, and bacteria are added to cement mortar as nutrients, and their effectiveness in promoting self-healing properties is tested. In-depth experimental research is conducted to comprehend how these biological agents affect cement mortar when used as a self-healing material. The only selected bacterial species have been used to examine the performance of bacterial cement mortar during normal weather exposure degree. Bacterial mortars are exposed to outdoor weathering environments. As a result, it is important to examine the long-term performance of bacteria-based self-healing mortar under various exposure conditions in order to better understand these processes. The following major goals are suggested to accomplish the project's goals:

1- Select best bacterial species by assessing their urease activity and spore formation for calcite precipitation in concrete.
2- Check out how encapsulated bacterial spores and nutrients, which are self-healing agents, affect mortars' mechanical characteristics, including as their compressive strength at different ages using different dosage with different ways to add to concrete.
3- examine concrete Using scanning electron microscope (SEM) to examine the microstructural changes resulting from self-healing.
4- evaluate concrete composition using energy dispersive x-ray spectroscopy (EDX)
5- Study the effect of bacteria based self-healing on concrete permeability.

2. Materials and methods

2.1. Overview of experimental program

The experimental program was organized into three main sections. In the first phase, choose one of five bacterial strains with the best ability to precipitate calcium carbonate and prepare beads for that strain. In the second phase, the objective was to investigate the behavior of bacterial concrete, A variety of techniques will be used to incorporate varying dosage of bacteria into the concrete cubes by analyzing their behavior. A strategy has been devised to investigate the impact of the quantity of bacteria introduced to the concrete cubes, employing multiple dosages, ranging from 3 kg to 5 kg and 9 kg, per cubic meter of concrete. In order to determine the best way to use these dosages, it is introduced to add before mentioned bacterial dosages to the water used to mix the concrete, to activating the bacteria for an hour before mixing those techniques named as MICP using concrete water before the concrete mix (MW). Second way to add bacteria by Adding the bacteria straight to the concrete mixture while they are still in their granular form that techniques named as MICP added on its dry conditions after concrete mixing (MD). In order to evaluate the initial compressive stress after seven days, 6 test cubes are created by using the method (MW), then 6 more cubes are created using the same settings to test them after 28 days. All of 12 test cubes tested the best dosage and the preferable way to add selected bacterial beads to the concrete. In addition, previous bacterial test cubes compared its results to control test cube of ordinary concrete cubes contain the same composition and mixing conditions to create 6 controls cubes of 18 total test cubes. 18 total test cubes divided into three trials describes dosage used. Hence, 18 cubes in all are cast, 9 of which are examined after 7 days and the remaining 9 after 28 days. In the meanwhile, the cubes that are tested at 28 days not for test compressive strength but for loading the cubes to make artificial cracks to evaluate the ability of bio-concrete self-healing by external observations and that test cubes undergo (SEM & EDX) to track the behavior of bacteria in fixing the cracks, gather data, and subsequently compare the outcomes to the test cubes free of bacteria(control cubes) by (SEM & EDX). Following data collection, the maximum compressive strength is examined. Both bacterial and non-bacterial concrete cubes may withstand it the advantages of incorporating the investigated microorganisms into concrete, as well as the best way to do so and the boundaries of their operation, are established. In the third phase, after investigation of the best practice to the way to add bacteria and the proper dosage, four tests cubes are pored and tested for concrete porosity using cone to create water head two of that cubes are bacterial concrete using the result of the investigation of best bacterial beads dosage one cube mixing bacterial beads using (MD) and another using (MW) and that two tested cubes have artificial cracks. The other two test cubes for control but one of them has artificial crack to test the ability of self-hydration for concrete during curing time. Figure (1).

2.2. Characterization of used Materials

2.2.1. Cement

Ordinary Portland cement (OPC) grade 42. Characteristics of cement Egypt's ES 4756/1-2022 (CEM IV/A) criteria are followed throughout its production. The physical and chemical properties of the cement used are shown in Table (1) and (2), respectively, as obtained from the producer.

Table (1): Chemical properties (from product data).

Item	--	Spec. Limit	Test Result
SiO_2	%	----	23.77
Al_2O_3	%	----	5.41
Fe_2O_3	%	----	6.20
CaO	%	----	57.14
MgO	%	----	2.28
SO_3	%	3.5 max.	2.25
Na_2O	%	----	0.64
K_2O	%	----	0.25
Cl	%	0.10 max.	0.07
Cr (Hexavalent)	ppm	2.0 max.	1.10

Table (2): Physical and Mechanical properties (from product data)

Fineness, Blaine	Cm^2/gm	----	4238
Heat of Hydration (7 d)	KJ/kg	270 max.	225
Setting time (Vicat): Initial	minutes	60.0 min	140
Final	minutes	----	195
Le chatelier expansion	mm	10.0 max	1.0
Compressive Strength	$Mp_a(N/mm^2)$		
2 days		10 min	23.78
7 days			34.13
28 days		42.5 min., 62.5 max	49.50

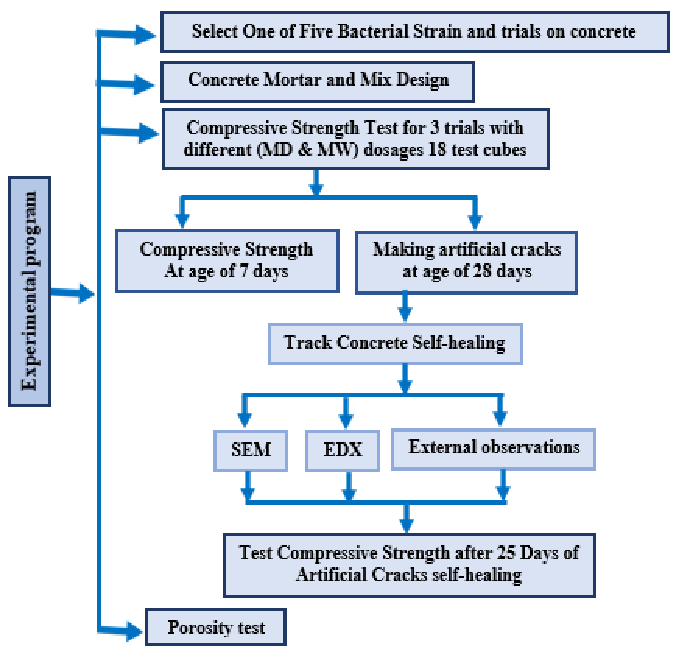

Figure (1): illustrated the experimental program, (source by Researcher).

2.2.2. Aggregate

Crushed dolomite aggregates, and natural sand are used as coarse and fine aggregate, respectively. The aggregates were mixed by percentages of 65% for coarse aggregate, and 35% for fine aggregate by weight. Figure (2) shows the sieve for fine, and coarse aggregate, as well as the mixed aggregates analysis. Tables (3) show the grain size distribution, while Tables (4) and (5) show the physical properties of coarse and fine aggregates.

Table (3): Aggregate Grain Size Distribution (source by researcher).

sieve size (mm)	% passing			
	natural sand	limits	crushed dolomite	limits
19	100	_	100	100
12.5	100	_	100	100-995
9.5	100	100	98.95	100-85
4.75	100	100-95	87.86	80-45
2.36	94.1	100-80	0.86	15-0
1.18	78.7	85-50	0.45	10-_
0.6	43.18	60-25	0	_
0.3	14.79	30-5	0	_
0.15	2.14	10-_	0	_
0.075	0.51		0	

Table (4): Physical Properties of Coarse Aggregate (source by researcher).

Property	Test Results	Specifications (ES 1109-2021)
Specific Gravity	2.75	_
Bulk density (t/m3)	1.83	_
Water absorption %	1.84	_
Crushing value %	14.25	not more than 45%
Coefficient of abrasion %	5.35	not more than 16%
Clay and fine dust content %	0.545	not more than 1%

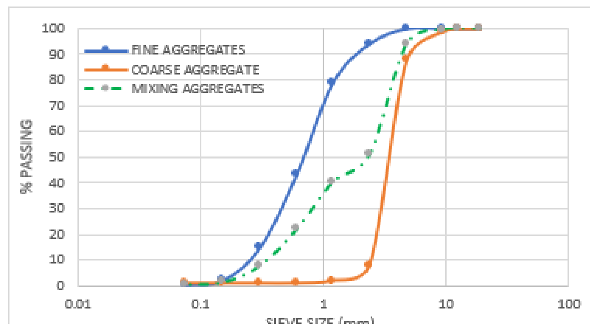

Figure (2): Sieve Analysis for Fine, Coarse, and Mixed Aggregate, (source by Researcher).

2.2.3. Water

The water used in the mix design was potable water from the water- supply network system. It was free from suspended solids and organic materials, which may affect concrete properties.

2.2.4. Bacteria

Plain concrete mixed with Sporosarcina pasteurii Figure (3)-1. Two samples examined under light microscope the first sample mixed with Bacillus Rosetted Figure (3)-2, the second one for plain concrete without Bacillus Rosetted Figure (3)-3 and those samples examined using light microscope with magnifications 1000X as shown in Figure (4 & 5).

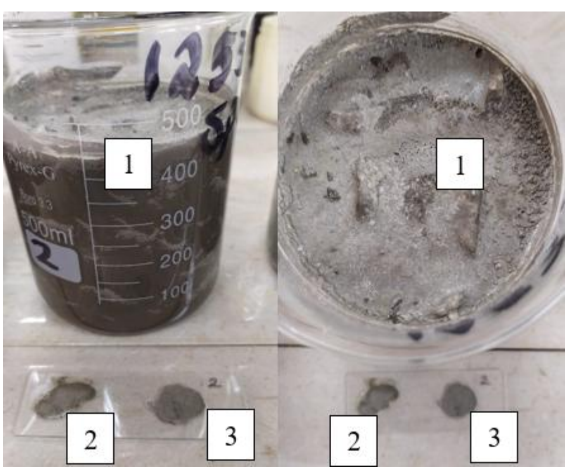

Figure (3): Sporosarcina pasteurii mixed with plain concrete, (source by Researcher).

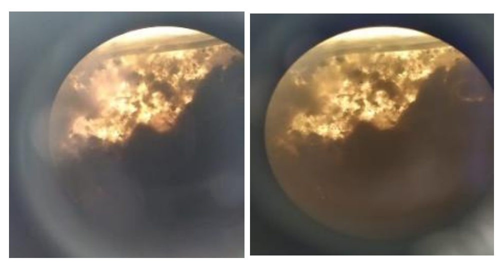

Figure (4): Sample with Sporosarsina, (source by Researcher).

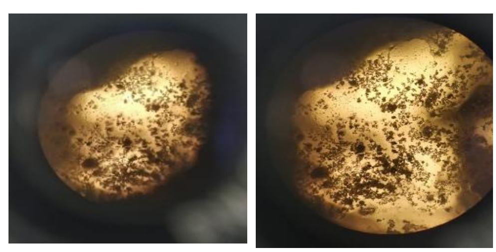

Figure (5): Sample without Sporosarsina, (source by Researcher).

2.3. Concrete mix design

It is a concrete mixture to which bacteria are added in different quantities. There are several methods for adding them to the concrete mixture, as shown in the table (6).

Table (6): test cubes mix design, (source by researcher).

TEST CUBES MIX DESIGN / CUBIC METER CONCRETE																		
MATERIALS	TEST CUBES NAME																	
	TRIAL NO.(1)						TRIAL NO.(2)						TRIAL NO.(3)					
	01-07-O	01-28-O	01-07-MD	01-28-MD	01-07-MW	01-28-MW	02-07-O	02-28-O	02-07-MD	02-28-MD	02-07-MW	02-28-MW	03-07-O	03-28-O	03-07-MD	03-28-MD	03-07-MW	03-28-MW
Cement (kg)	350						350						350					
Fine aggregete (kg)	700						700						700					
Coarce aggregate (kg)	1085						1085						1085					
Water (litre)	215						215						215					
Bacteria dosage (kg)	—	—	3	3	3	3	—	—	5	5	5	5	—	—	9	9	9	9
Adding method	—	—	MD	MD	MW	MW	—	—	MD	MD	MW	MW	—	—	MD	MD	MW	MW

Adding method abbreviations:

*MD: add bacteria dosage on its granular shape after concrete mix

*MW: add bacteria dosage into concrete mixing water before mixing with an 1 hour

2.4. **Mixing**

The mixing procedures were as follows:

The first step is Weighting all constituent materials, as shown in the Tables (6). The second step is for MD cubes: add bacteria dosage on its granular shape after concrete mix. The third step is for MW cubes: add bacteria dosage into concrete mixing water before mixing with a 1 hour to active bacteria before mixing. The fourth step is fine aggregate was added to the mix and mixed for 3 minutes. The fives step is coarse aggregate was added to the mix and mixed for another 4 minutes until a homogenous mix was obtained.

2.5. **Casting**

150mm Cubes moulds were filled in three equal well compacted lifts. After the moulds were filled the specimens were placed on a bench in the casting room at ambient conditions for one day until curing began.

2.6. **Curing**

The specimens were demolded and cured (water tank) at a temperature of 20^{o} C until the day of testing.

2.7. **Concrete tests**

2.7.1. **Compressive test**

Cubes of 150mm were cast for compressive strength test. Then they were well compacted. After that, the specimens were de-molded after 24 hours and cured at room temperature in normal pure water until the day of testing. The compressive strength was determined by testing the samples at 7 and 28 days of water curing as per ASTM C109. ELE testing machine (for mortar cubes only), shown in Figure (6), was used to test the mortar samples.

2.7.2. **External observations**

External observations of self-healing bio-concrete cracks reveal their functionality. Key indicators include visible crack closure, white, chalky calcium carbonate deposits, and slight differences in surface texture or color compared to the original concrete.

Figure (6): ELE Compressive Testing Machine for Cement Mortars, (source by Researcher).

2.7.3. **(SEM & EDX) tests**

SEM is a high-resolution imaging technique that provides detailed observations of material surface morphology and composition, offering nanometer scale resolutions and 3D insights when combined with EDS. EDX is a crucial analytical tool in self-healing concrete research, enabling researchers to analyze material composition, characterize healing agents, examine microstructure, assess healing efficiency, and serve as a quality control tool, enhancing performance and longevity.

A. Test program and abbreviations:

A sample of bacteria is put in a dish under standard conditions of containment pollution and suitable temperature and photographed using SEM technology. In chapter 4 we review SEM images for partial self-healing cracks for only 4 samples with different dosage The dose used is controlled to be 3 kg bacteria/cubic meter of concrete and 9 kg bacteria/cubic meter of concrete to form 2 samples and these doses are added in two different ways, The first is to add the previously mentioned dose to the concrete mixture water and leave it for about an hour to enable the bacteria to grow and ensure the complete spread of the bacteria throughout the sample. The second is to add the bacteria in their initial state after mixing the concrete directly and mixing well again to ensure the distribution of the bacteria throughout the sample. The four samples are left until they harden, and the samples are treated with the same conditions mentioned above for treating the concrete. When the samples arrive at the age of 17 days and with the creation of artificial cracks in the samples, the four samples are placed in clean water at a temperature of 20 Celsius and within the other 15 days the self-healing process is observed, and the first SEM was taken for the four samples where the Olympus optical microscope (OM) (BX41M-LED, Tokyo, Japan and SEM Quanta FEG250-FEI-made in USA as shown in figure (7). Parts of cracks up to 2:3 mm were discovered and photographed, treated, and confirmed that the bacteria had formed into calcium carbonate. Sample abbreviations to be: S1:sample1 contains 3kg bacteria/cubic meter added after concrete mixing. S2:sample2 contains 3kg bacteria/cubic meter added in mixing concrete water within 1 hour before mixing. S3:sample3 contains 9kg bacteria/cubic meter added after concrete mixing. S4:sample4 contains 9kg bacteria/cubic meter added in mixing concrete water within 1 hour before mixing.

Figure (7): SEM & EDX machine, (source by Researcher).

2.7.4. **Porosity test**

Concrete porosity tests are essential for assessing the permeability and durability of concrete. Porosity affects the resistance of the material to water penetration, chemical attacks and freeze-thaw cycles. In this test, the porosity of two self-healing concrete cubes will be tested after creating artificial cracks by adding bacteria to these cubes 3Kg (Bacteria)/ m^3(Concrete) using the method of (MD) add bacteria dosage on its granular shape after concrete mix and (MW) add bacteria dosage into concrete mixing water before mixing with a 1 hour. The results are compared with two cubes of concrete, one of which has an artificial crack.

A. Material required:

Concrete 100mm cubes Sample (1): concrete with wet dosage (MW) and artificial cracks Sample (2): concrete with dry dosage (MD) and artificial cracks Sample (3): ordinary cube with artificial cracks Sample (4): ordinary cube without artificial cracks, Water reservoir cone (elevated to maintain a constant head), Stopwatch and Sealing material (e.g., rubber gaskets or epoxy) to prevent leakage around the sample edges as shown in figure (8).

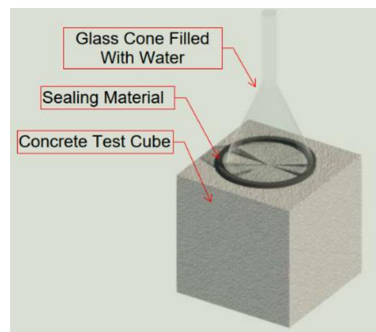

Figure (8): porosity test setup, (source by Researcher).

B. Procedure and porosity calculations**:**

Apply a Constant Water Head to maintain a fixed water head (10–30 cm) above the concrete sample to create pressure, Record the initial water level, Collect the water that seeps through the sample in the container, Record the amount of water infiltrated at regular intervals (every 10 minutes), Stop the test after a set duration (120 minutes), Measure the Infiltration, Collect the water that seeps through the sample in the tray or container, Record the amount of water infiltrated at regular intervals (every 10 minutes), and Stop the test after a set duration (120 minutes).
Calculate Porosity Calculate the total volume of water infiltrated (Vinfiltrated) Determine the total volume of the concrete sample (Vsample).

3. Results and discussion

3.1. **Compressive strength at age of 7 days and after 28 days of self-healing of artificial cracks**

After pouring concrete according to the criteria mentioned above in the design mix, the eighteen cubes were placed in a water basin as a stage for curing the concrete, where each type of cubes of the trials (O, MW, and MD) were placed separately from each other, as the initial experiments noted that bacteria can move through the water and form on the outer surface of the adjacent elements. At the age of seven days, the compressive stress test was performed on nine cubes, three of each type, and the results were as mentioned in Trial (1, 2, and 3) Cubes Compressive Strength at Age 7 Days and after self-healing of artificial cracks as shown in figure (9, 10 and 11)

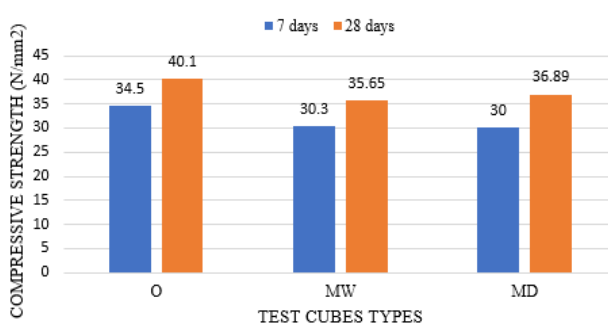

Figure (9): trial 1 cubes compressive strength, (source by Researcher).

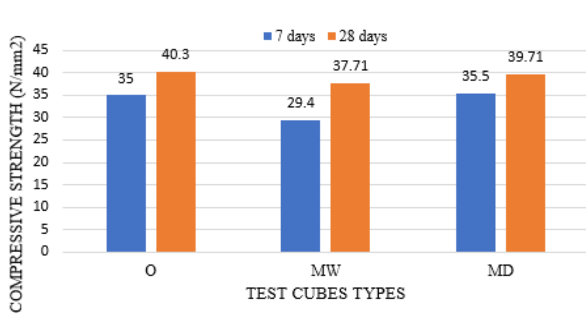

Figure (10): trial 2 cubes compressive strength, (source by Researcher).

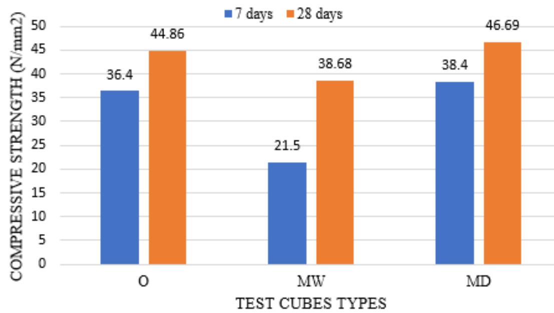

Figure (11): trial 3 cubes compressive strength, (source by Researcher).

It was confirmed that more than 75% of the concrete compressive strength was achieved in the first seven days of concrete life. However, it was noted that the higher the dose using the MW method, the lower the initial compressive strength in the seven days, and the opposite is true in the MD method. The higher the dose, the higher the stress, but at rates reaching 5.49%.

3.2. External observations Results for cracks self-healing.

After 28 days of concrete cube age, using a concrete compressive strength machine, the concrete cubes are loaded with a load of 75% of the design load of the concrete mix. The aim of this step is to create artificial cracks in the remaining nine cubes at this age of the concrete, through which the self-healing of the cubes that work with MICP (microbial induced calcium carbonate precipitation) technology can be monitored. In the preceding circumstances, after 17 days, cracks have been seen to mend For concrete cubes of type (MW) in which bacteria were added to the concrete mixing water approximately one hour before mixing the concrete mixture and with different amounts of added bacteria according to the concrete mixture design as mentioned in table (6), it was shown from external observation that these cubes had self-healed up to 2 mm, and the more the amount of added bacteria increased, the more efficient the self-healing was, as shown in Figure (12, 13 and 14).

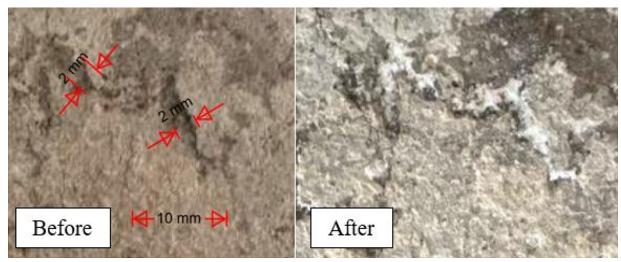

Figure (12): Cube 01-MW before and after self-healing of artificial cracks, (source by Researcher).

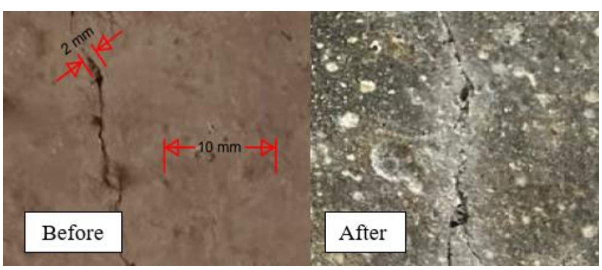

Figure (13): Cube 02-MW before and after self-healing of artificial cracks, (source by Researcher).

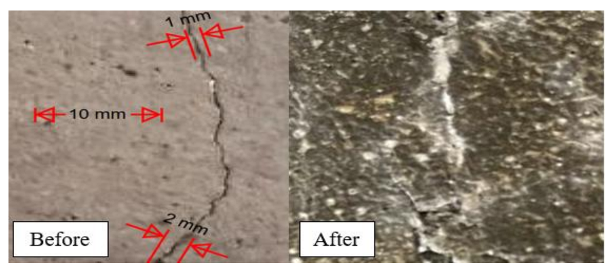

Figure (14): Cube 03-MW before and after self-healing of artificial cracks, (source by Researcher).

In the preceding circumstances, after 17 days, cracks have been seen to mend For concrete cubes of type (MD) in which bacteria were added to the concrete after concrete mixture on its generally form and with different amounts of added bacteria according to the concrete mixture table in Chapter Three, Table (6), it was shown from external observation that these cubes had self-healed up to 2 mm, and the more the amount of added bacteria increased, the more efficient the self-healing was, as shown in Figure (15, 16 and 17).

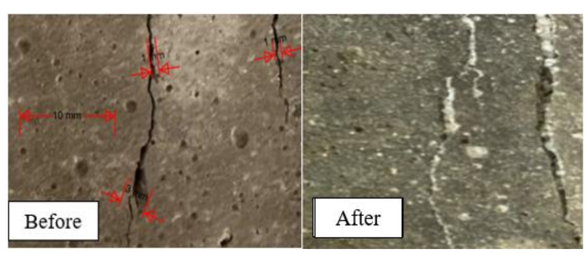

Figure (15): Cube 01-MD before and after self-healing of artificial cracks, (source by Researcher).

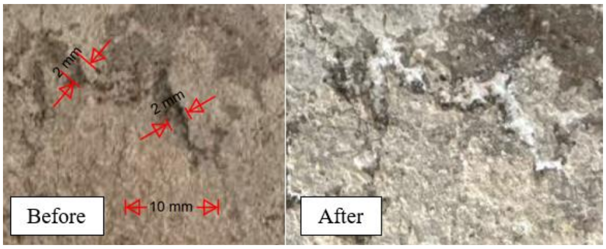

Figure (16): Cube 02-MD before and after self-healing of artificial cracks, (source by Researcher).

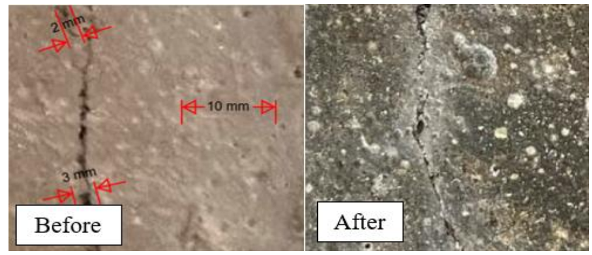

Figure (17): Cube 03-MD before and after self-healing of artificial cracks, (source by Researcher).

3.3. Scanning electron microscopy (SEM)

To define the polymorphic form of precipitated calcium carbonate, a small amount of used bacteria was placed in a test dish. After the precipitation process occurred, a SEM test was done to determine the specific form of the transformed bacteria as it is in Figure (18 and 19) using Olympus optical microscope (OM) (BX41M-LED, Tokyo, Japan). SEM has been taken at different magnification levels such as 10 micro and 20 micron as shown in Figure (20 and 21) This is to facilitate the distinction between concrete, its components, and calcium carbonate deposited by bacteria during self-healing.

According to the test program for Scanning Electron Microscopy (SEM) that mentioned in chapter three and for four samples of S1 that contain 3kg bacteria/cubic meter added after concrete mixing, S2 that contain 3kg bacteria/cubic meter added in mixing concrete water within 1 hour before mixing, S3 that contain 9kg bacteria/cubic meter added after concrete mixing and S4

that contain 9kg bacteria/cubic meter added in mixing concrete water within 1 hour before mixing. SEM has been done for each sample after creating 2mm artificial cracks to clarify the self-healing of those cracks as shown in Figure (22 to 25).

Figure (18)

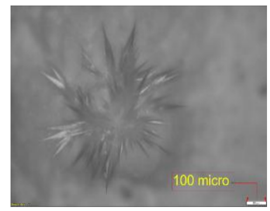

Figure (19)

Figure (18) Test dish contains transformed bacteria into calcium carbonate, Figure (19) Polymorph of single created calcium carbonate 100 micron scale, (source by Researcher).

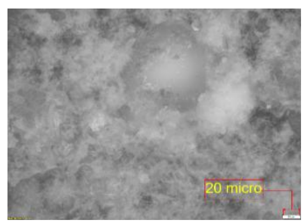

Figure (20)

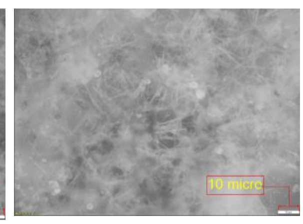

Figure (21)

Figure (20) Polymorph of created calcium carbonate 20 micron scale, Figure (21) Polymorph of created calcium carbonate 10 micron scale, (source by Researcher).

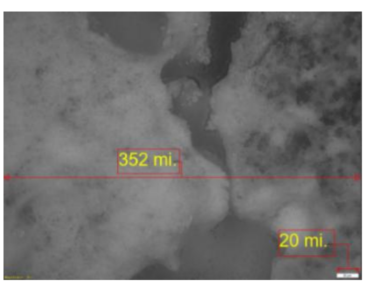

Figure (22): S1-part of crack concrete self-healing 20micron, (source by Researcher).

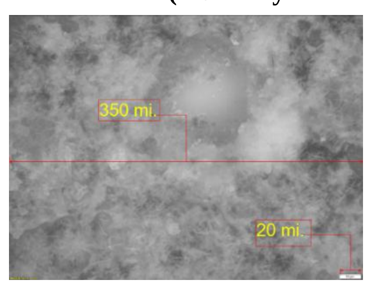

Figure (23): S2-part of crack concrete self-healing 20micron, (source by Researcher).

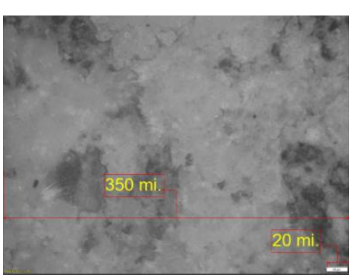

Figure (24): S3-part of crack concrete self-healing 20micron, (source by Researcher).

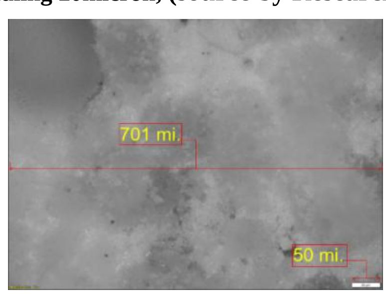

Figure (25): S4-part of crack concrete self-healing 50micron, (source by Researcher).

From the previously mentioned Figures and the external observation as mentioned in figure (12 to 17) of the cracks formed in the samples, it is clear that the bacteria work efficiently and it was found that when the bacteria are added to the mixing water before mix the concrete, they become more effective, noting their negative effect on the concrete's resistance to pressure (compressive strength). On the contrary, adding bacteria in their solid state after mixing the concrete has a noticeable effect on equalizing the concrete stress to the pressure for the concrete (O Type) cubes. Note that with the increase in bacteria in this way, the concrete's compressive strength may increase, as shown in previous figures (9, 10 and 11). From all the previous results, it is clear that when bacteria are added to the concrete line water one hour before the mixing process, this method is considered very effective. Therefore, a sample of S2 and S4 was chosen to conduct a SEM test using a device SEM Quanta FEG250-FEI - made in USA. The metadata of SEM of S2 are HV (High Voltage): 30.00 kV, HFW (Horizontal Field Width): 829 µm Magnification: 500x Detector (det): ETD (Everhart-Thornley Detector) Working Distance (WD): 10.1 mm Scale Bar: 300 µm Label: EDRC according to all those specs clarify concrete artificial crack self-heling as shown in figure (26).

Figure (26): S2-SEM clarify crack self -healing, (source by Researcher).

By zooming in indicated crack mentioned in previous figure and The metadata of SEM of S2 are HV (High Voltage): 30.00 kV, HFW (Horizontal Field Width): 207 µm Magnification: 2000x Detector (det): ETD (Everhart-Thornley Detector) Working Distance (WD): 10.5 mm Scale Bar: 50 µm Label: EDRC according to all those specs clarify concrete artificial crack self-heling As shown in figure (27).

The metadata of SEM of S4 are HV (High Voltage): 30.00 kV, HFW (Horizontal Field Width):414µm Magnification: 1000x Detector (det): ETD (Everhart-Thornley Detector) Working Distance (WD): 11.3mm Scale Bar: 100 µm Label: EDRC according to all those specs clarify concrete artificial crack bacterial based self-heling by create calcium carbonate As shown in figure(28).

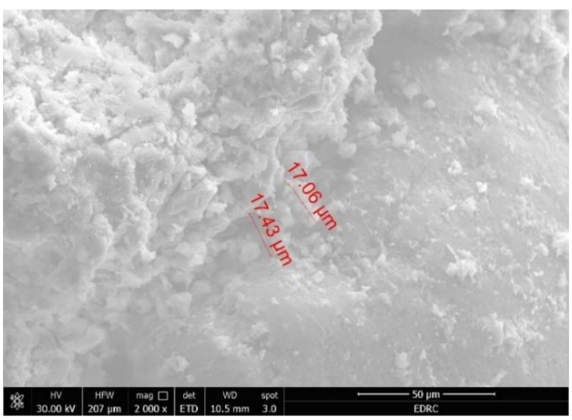

Figure (27): S2-SEM clarify crack self -healing with dimensions, (source by Researcher).

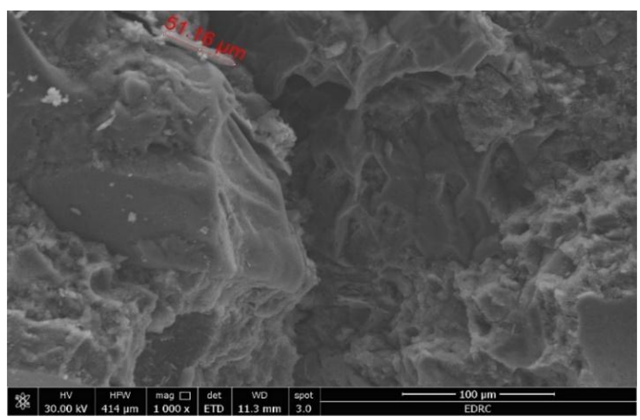

Figure (28): S4-SEM clarify MICP with dimensions, (source by Researcher).

By zooming in indicated crack mentioned in previous figure and The metadata of SEM of S2 are HV (High Voltage): 30.00 kV, HFW (Horizontal Field Width): 82.9 µm Magnification: 5000x Detector (det): ETD (Everhart-Thornley Detector) Working Distance (WD):11.2mm Scale Bar: 30 µm Label: EDRC the crack self-heling As shown in figure (29). For another position of S4 clarified 3.365 µm crack It was closed by calcium carbonate precipitation resulting from bacterial transformation The metadata of SEM of S4 are HV (High Voltage): 30.00 kV, HFW (Horizontal Field Width): 82.9 µm Magnification: 5000x Detector (det): ETD (Everhart-Thornley Detector) Working Distance (WD): 12.9 mm Scale Bar: 30 µm Label: EDRC according to all those specs clarify concrete artificial crack self-heling part (1) As shown in figure (30). The metadata of SEM of S4 part (2) are HV (High Voltage): 30.00 kV, HFW (Horizontal Field Width):414µm Magnification:10000x Detector (det): ETD (Everhart-Thornley Detector) Working Distance (WD): 12.7 mm Scale Bar: 10 µm Label: EDRC according to all those specs clarify crack self-heling as shown in figure (31).

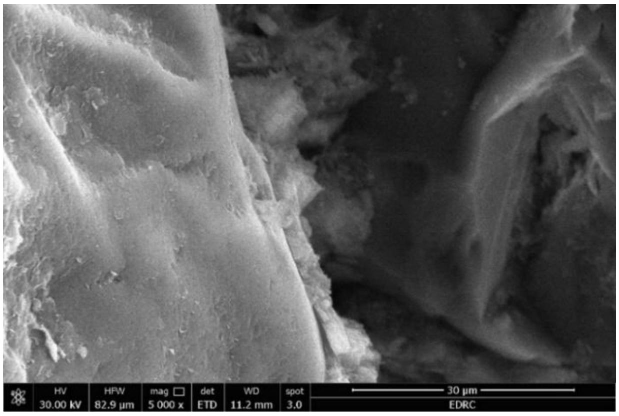

Figure (29): S4-SEM clarify crack full of calcium carbonate precipitation, (source by Researcher).

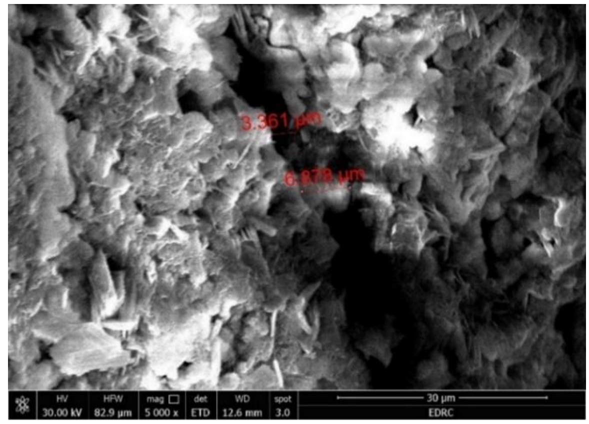

Figure (30): S4-SEM clarify calcium carbonate filling hair cracks part (1), (source by Researcher).

3.4. **Energy dispersive X-ray spectroscopy (EDX).**

All of the above SEM test results was found to address the cracks, but there was an important question that must be answered that is this self -treatment result of calcium carbonate resulting from the transformation of bacteria, to answer this question it has been done EDX analysis for the polymorphs found at gaps shown in figure (27) for sample S2 and figure (31) for sample S4 . At next will clarify EDX for sample S2 and S4. for sample S2 shown in figure (32) clarifying the EDX analysis for indicated point of crack self-healing to prove that it consists of calcium carbonate from bacterial transformation the specs of the EDX analysis are Image Resolution: 512 by 442, Image Pixel Size:0.09 µm, Acc. Voltage: 30.0 kV, Magnification: 7992.

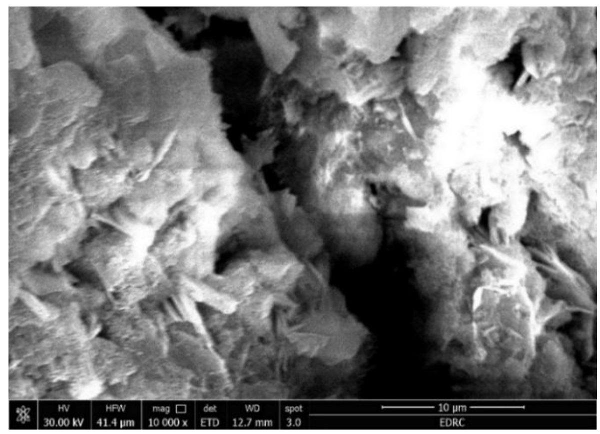

Figure (31): S4-SEM clarify calcium carbonate filling hir cracks part (2), (source by Researcher).

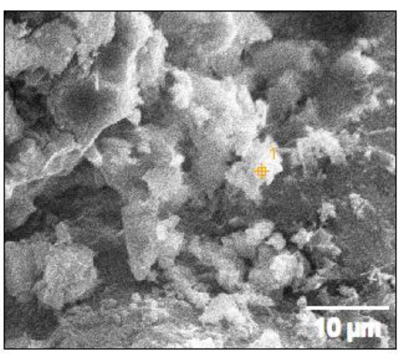

Figure (32): S2-EDX analysis point, (source by Researcher).

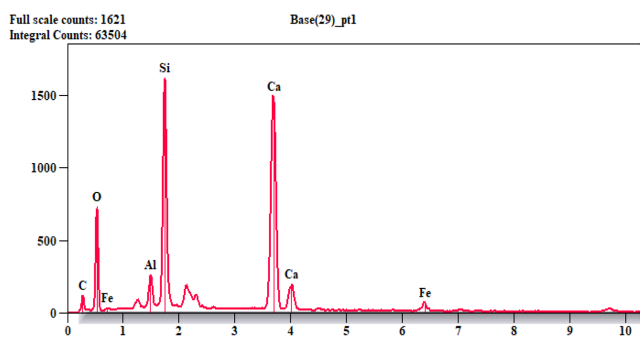

Figure (33): S2 Element Composition Analysist, (source by Researcher).

Table (7): S2 Element Composition Analysist- Weight %, (source by researcher).

	C	O	Al	Si	Ca	Fe
Base(S2)	5.9	46.9	2.2	17.4	25.7	1.9

Table (8): S2 Element Composition Analysist- Atom %, (source by researcher).

	C	O	Al	Si	Ca	Fe
Base(S2)	10.2	61.1	1.7	12.9	13.4	0.7

It's now clearly clarified that the indicated point (1) mentioned in in figure (32) and according to figure (33) tables (7 and 8) composite of silica rounded by calcium produced from bacteria transformed by MICP techniques.
for sample S4 shown in figure (34) clarifying the EDX analysis for indicated point of crack self-healing to prove that it consists of calcium carbonate from bacterial transformation the specs of the EDX analysis are Image Resolution: 512 by 442, Image Pixel Size:0.15 µm, Acc. Voltage: 30.0 kV, Magnification: 4995.

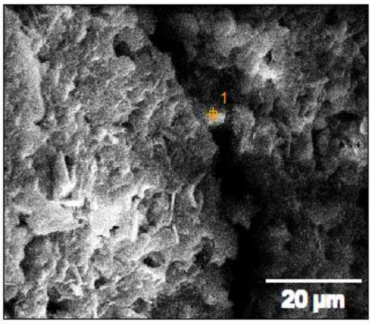

Figure (34): S4-EDX analysis point, (source by Researcher).

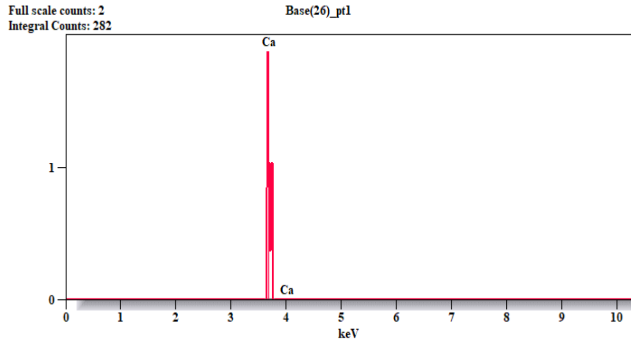

Figure (35): S4 Element Composition Analysist, (source by Researcher).

Table (9): S4 Element Composition Analysist- Weight%, (source by researcher).

	Ca
Base(S4)	100.0

Table (10): S4 Element Composition Analysist- Atom %, (source by researcher).

	Ca
Base(S4)	100.0

It's now clearly clarified that the indicated point (1) mentioned in figure (34) and according to figure (35) tables (9 and 10) composite from pure calcium produced from bacteria transformed by MICP techniques.

And to determine the best in terms of the most benefiting from the possibility of increasing the processed self -Healing by using bacteria, is it by adding bacteria to the concrete mixture water before mixing to nearly the hour better or adding it in its solid condition after mixing the concrete mixture. Therefore, start thinking about the work of EDX for two full eyes, and S3 and S4 samples were chosen and these two samples have the same concrete mixture and the same dose of bacteria, but each of them took a way to add bacteria in the concrete mixture.

for sample S3 shown in figure (36) clarifying the EDX analysis for the full sample according to the specs of the EDX analysis are Image Resolution: 512 by 442, Image Pixel Size:14.2 µm, Acc. Voltage: 30.0 kV, Magnification: 53.

Table (11): S3 Element Composition Analysist- Weight%, (source by researcher).

	C	O	Al	Si	S	Ca	Fe
Base(S3)	4.5	48.3	1.9	12.5	1.2	28.6	2.8

Table (12): S3 Element Composition Analysist- Atom %, (source by researcher).

	C	O	Al	Si	S	Ca	Fe
Base(S3)	8.0	64.0	1.5	9.5	0.8	15.2	1.1

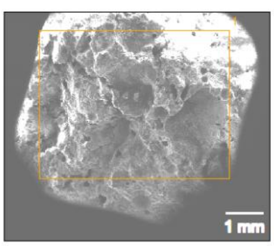

Figure (36): FULL S3-EDX, (source by Researcher).

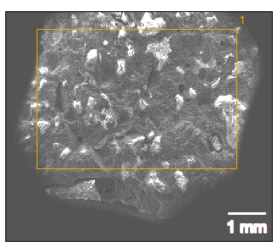

Figure (38): FULL S4-EDX, (source by Researcher).

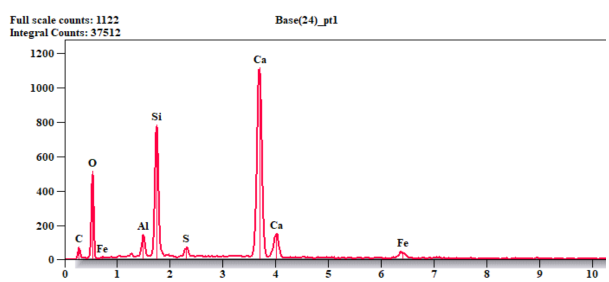

Figure (37): FULL S3 Element Composition Analysist, (source by Researcher).

for sample S4 shown in figure (38) clarifying the EDX analysis for the full sample according to the specs of the EDX analysis are Image Resolution: 512 by 442, Image Pixel Size:14.2 µm, Acc. Voltage: 30.0 kV, Magnification: 53.

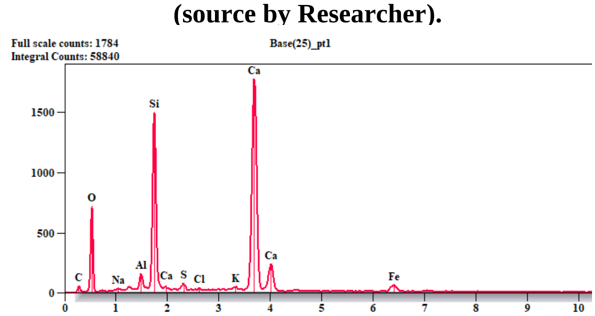

Figure (39): FULL S4 Element Composition Analysist, (source by Researcher).

Table (13): S4 Element Composition Analysist- Weight%, (source by researcher).

	C	O	Na	Al	Si	S	Cl	K	Ca	Fe
Base(S4)	2.7	46.9	0.3	1.4	15.5	0.8	0.1	0.3	29.7	2.3

Table (14): S4 Element Composition Analysist- Atom %, (source by researcher).

	C	O	Na	Al	Si	S	Cl	K	Ca	Fe
Base(S4)	4.8	63.9	0.3	1.1	12.0	0.5	0.1	0.2	16.1	0.9

The previous results indicate that the addition of bacteria in the mixing water before it at about an hour has a great impact, its productivity and the effectiveness of calcium carbonate produced by bacteria from its counterpart in the other sample, as the percentage of calcium is higher in the same.

3.5. **Porosity test**

Water Collected Over Time One of the porosity test results that clarify the cumulative volume of water passing through the concrete sample as time progresses in figure (40) shows that the concrete with wet dosage can heal the artificial cracks and have low water collect over time followed by the concrete cube with dry dosage. The aim of measuring the porosity of normal concrete with artificial cracks was to measure the effectiveness of autogenous healing where, in comparison, the curing period that preceded the porosity measurement process and the surrounding conditions were not useful for the previously mentioned cube.

The second results from porosity test Flow Rate Over Time that rate at which water flows through the sample, is calculated as the change in water volume per unit time. The cube of concrete with wet dosage has lowest flow rate over time after self-healing of artificial cracks as shown in figure (41).

Here is a bar chart showing the porosity percentages for different concrete samples. The chart highlights the variations in porosity, calculated as the ratio of pore volume to total volume for each sample. Each bar is annotated with the corresponding porosity percentage for clarity as shown in figure (42).

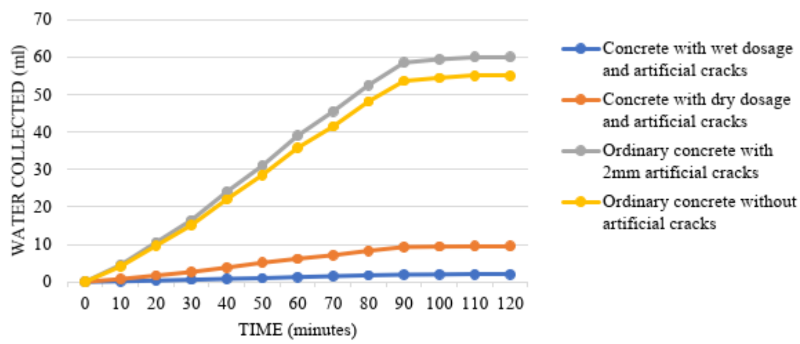

Figure (40): water collected over time, (source by Researcher).

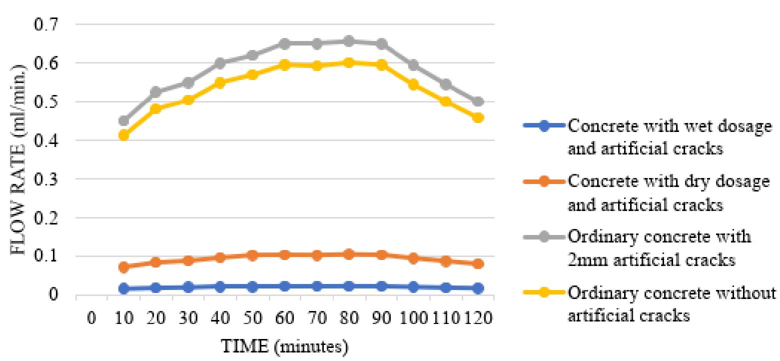

Figure (41): flow rate over time, ((source by Researcher).

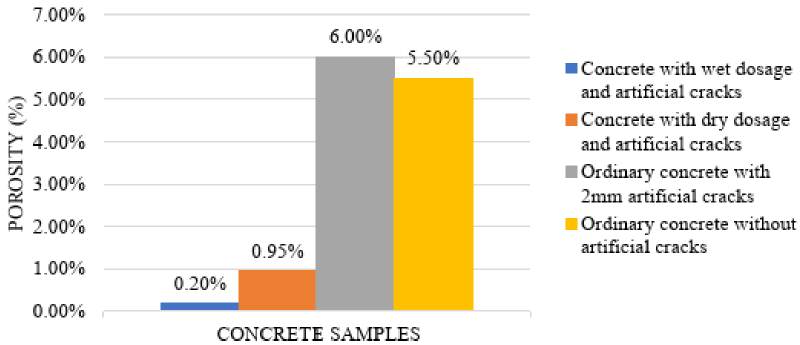

Figure (42): concrete porosity results, (source by Researcher).

4. Conclusions.

1) Based on the phenotypic characteristics, such as its cell morphology and biochemical properties, the isolate were found to be Bacilli as they all gram positive and had short to long rod shape. They were all able to grow under aerobic conditions. The strains were able to sporulate when exposed to adverse environmental conditions (starvation).

2) Regarding the urease activity of the urease positive bacterial species such as sporosarcina pasteurii , that specie displayed very fast urease activity. The difference in this activity could be attributed to different capabilities of these species providing varied types of urease enzymes and the difference in the level of urease enzyme among these tested strains.

3) Considerable work was undertaken to prepare calcium alginate beads to encapsulate. sporosarcina pasteurii spores and evaluate beads ability to preserve. sporosarcina pasteurii viability. The beads were prepared by mixing sodium alginate and Pyrophyllite (Pyrax) clay solution and then cross-linked them with $CaCl_2$ solution. The characterization of calcium alginate-clay beads encapsulated with. sporosarcina pasteurii was thoroughly examined. analysis results showed that the functional group of pyrophyllite and alginate has strong interaction with each other. The surface and cross section characteristics of Ca-alginate-Pyrophyllite beads shown by the SEM micrographs have supported this conclusion. It appears from thermogravimetric analysis that pyrophyllite was well dispersed in alginate matrix; therefore, the Ca-alginate-pyrophyllite beads structure was stable, and firm compared with alginate beads. Furthermore, the matrix was suitable for. sporosarcina pasteurii encapsulation and preservation of spore's viability over time.

4) It has been found that the optimum quantity of beads that must be added to cement mortar mixture was found to be 3kg(bacteria)/m3 (concrete) without adversely affecting the strength properties of cement mortar. It has also been indicated that 3kg(bacteria)/m3 (concrete) is the optimum amount of reagent can be used not only to avoid the deterioration effect of nutrient.

5) Bacterial self-healing mortar samples exhibited lower compressive strength than the control After cycles of wetting and drying especially in case added bacteria with concrete water before mortar mixing the compressive strength decreases by increasing bacterial dosage It has been detected that a reduction of 13% was found in compressive strength for wet dosage 3kg (bacteria)/m3(concrete), whereas for 5 and 9kg (bacteria)/m3(concrete) strength dropped by 17.14% and 41.6%, respectively. This behaviour could be attributed primarily to the bacterial Acquisition of concrete water causes transformation in water behaviour to be protein cells about that pure water needed for cement hydration All of that at early age and it was found that compressive strength equal to the control samples at age of 28 days This behaviour could be attributed primarily to the increase in the bacterial metabolic activities which responsible for precipitation of calcium carbonate as the temperature increased to +15 °C . Previously, it was not possible to rely on improving the compressive strength of concrete using self-healing concrete using bacteria in practical life because it requires laboratory precautions to achieve the desired results that are difficult to achieve on a large scale.

6) The bacteria used, regardless of the method of adding them to the concrete mixture or the dose used, have proven their efficiency in self-healing concrete to treat cracks up to 2 mm, provided that they are immersed in water, which makes them of utmost importance in water structures, which is reflected in the calculations of the reinforcing steel used when designing concrete non crack section to control crack width.

7) The previous results indicate that the addition of bacteria in the mixing water before it at about an hour has a great impact, its productivity and the effectiveness of calcium carbonate produced by bacteria from its counterpart in the other sample, as the percentage of calcium is higher in the same.

8) Concrete samples in which bacteria were added to the concrete water before mixing for an hour at a rate of 3 kg (bacteria)/m3 (concrete) Porosity studies showed a reduction in the porosity percentage after self-healing to 0.2% compared to its counterpart in control concrete sample, which reached 6% and less by adding bacteria after the concrete was finished in the solid state of bacteria and the result achieved a porosity percentage of 0.95%.

5. References.

All the tables and figures previously mentioned are from the researcher.

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