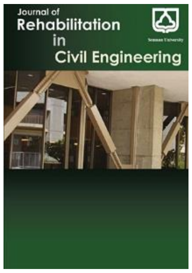

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Microstructure Analysis of Bio Engineered Concrete under the Concept of Self-Healing

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ABSTRACT

The aspects of the durability of concrete that need significant attention in the current world demand the intense focus towards inventions in self-repairable materials. Bioengineered concrete using Bacillus sphaericus is one such novel trial. One needs to arrive at such an optimum combination of materials that involve the contribution of bacteria to resolve the issues of repair and rehabilitation effectively. The inherent properties of Bacillus sphaericus paved the way to arrive at the compatible combination of bacteria with nutrients in concrete to achieve self-repairable bioengineered concrete. Since the repairing agent used here performs at a micro level to precipitate stable calcite there is an indeed study carried out through SEM, XRD, EDAX, and Digital microscopic images to come out with an ideal solution for concrete repair work at the micro-level along with standard tests such as compressive strength. The results of these different tests on the bioengineered concrete using four Bacillus species of bacteria show that the best performance both in strength and durability is attained by bacteria Bacillus sphaericus with calcium lactate as a nutrient medium.

1. Introduction

The material concrete as a constructional material can perform utmost at compression state in a structure. In continuation with the demand, the performance of concrete as a flexural member is expected with the use of reinforcement to enhance the tensile property. Since material concrete which is

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been the 2nd highest used manmade material on earth which is not only expected for effective strength properties but also for durability properties 0. The challenging part of adopting concrete as a basic building material is assuring its quality and maintenance for a longer period. The performance of concrete for its lifetime with minimum repair and maintenance demands dense microstructure and also fewer cracks. Since concrete is a heterogeneous material it is difficult to assess the role of individual components of concrete in attaining better strength and durability properties. The study on traditional methods of testing concrete possesses its limitations many a time in giving thin line bifurcations of the performance reasoning, especially the changes in the microstructure of concrete. Hence there is an in-depth study requirement at the micro level for deciding the cause and case distinctly.

If the material concrete is articulated to arrest even the micro-level cracks at the initial stage itself there will be no need for a stage of repair to happen. The basic combination of ingredients of concrete inherently possesses the autogenous healing capacity as stated by Carola Edvardson in 1999 0 where the reduced water-cement ratio into the mix retains some cement particles unreacted by hydration process by lack of water supply. This results in those unreacted cement particles to wait for a crack or an air gap to occur to supply the required moisture and thereby concentrating the production of C-S-H gel at the cracked point and clog the crack hence called autogenous healing. About the above concept, there is a chance to add a supplementary ingredient that will perform the same task in a similar mechanism that can address the cracked locations at the micro-level.

Numerous research works have been carried out and still going on in various eminent universities in this view to arriving at the compatible combination of the healing agent with concrete components giving rise to a renowned concept called Self-Healing Concrete. This combination has inspired many kinds of research to work on the magic of healing cracks automatically without human interference and the practical applications also been studied as in the article 0. Unlike other researches this concept also holds its limitations towards practical adaptability such as material availability, influencing medium, cost-effectiveness, eco-friendly, thermophilic, alkaliphilic, reusable, multitasking properties etc. The bacillus species being such one suitable healing agent. There were many experimentations adopted on these species such as Bacillus subtilis, Bacillus Megaterium and many more 0.

In the present study, an innovative trial has been made to incorporate the bacteria in solution form as a healing agent which can fit into the utmost limitations of the self-healing concrete. As a bacterium so chosen is alkaliphilic, aerobic, thermophilic, endospore-forming in nature, it can compete with a maximum of the other mechanisms of self-healing. Various bacteria have been researched under this platform as stated in articles 0000.

2. Materials

The study on bioengineered concrete has been initiated on the mortar mix as the performance of bacteria is mainly dependent on the alkalinity of the matrix which in turn depends on the cement as studied in the article 0. The role of the bacteria Lysinibacillus sphaericus with different calcium sources to achieve self-healing was explored in 2018 by Christine et al., 0. The role of the selection of bacteria majorly depends on its tendency to be alkaliphilic to bear the concrete high alkaline environment and also their potential in crack repair work being spore-forming has been explored by T.K Sharma in 2017 0. The standard mortar ingredients such as OPC cement and fine aggregates conforming to zone II are used for the present study. The special ingredients as healing agents are chosen as bacteria *Bacillus subtilis* (MTCC No.2413), *Bacillus megaterium* (MTCC No.1684), *Bacillus sphaericus* (MTCC No.9523)and consortia [combination of all three bacteria] along with nutrient supply as calcium lactate for its efficiency in bacterial metabolism and calcite precipitation as studied in the article 0. These bacteria are induced into clay pellets for immobilization of microorganisms for compressive strength properties. The bacteria are procured from the Microbial Type Culture Collection and Gene Bank (MTCC), Chandigarh. The previous studies even focus on endospore-forming bacterial species to assure the effectiveness of the bacteria for a longer period and also the significance of encapsulation of these to check its viability against the harsh environment in concrete 0.

3. Methodology

3.1. Mix proportioning

A standard mortar mix of proportion 1: 3[Cement: sand] is applied. Optimum bacterial cell count is chosen as 106cells/ml based on the influence of cell concentration on the performance of bacterial concrete. Calcium lactate of 69grams/ml of water is optimized based on fundamental bacterial testing.

3.2. Casting of cubes and curing

The work is planned in two stages, formerly the bacteria-induced clay pellets are induced into the mortar specimens with four different species of bacteria to examine the compressive strength enhancing property of bacteria through cube specimens. Later the bacteria were tested for their efficiency in crack healing capacity by Ultrasonic Pulse velocity test, digital microscopic observations through plain mortar disks. These mortar disks were artificially cracked and experimented with. The UPV test and microscopic observations are carried out simultaneously to ensure the significance of calcite deposition in enhancing the UPV values. Cement to sand ratio 1:3 is used. Cubes of 70.6*70.6*70.6 mm size and mortar disks of 100mm diameter and 15mm thick are prepared. Weighed cement and sand are dry mixed in the laboratory batch mixer until uniformity. Water is added simultaneously for the uniform wet mix approximately for 3 minutes. The homogenous wet mix of mortar is filled into the cube moulds in 3 layers with 25 blows as per standards. The cubes after 24 hours are demoulded and cured for 28 days in a water bath.

3.3. Culturing of bacteria

The nutrient Broth was weighed as required. The nutrient broth in dissolving in distilled water. The conical flasks with the nutrient broth were Plugged with non-absorbent cotton. Then the Conical flasks were placed in a Sterilization cooker. Autoclaving is done to sterilize in cooker. After cooling the nutrient broth, to inoculate the bacteria into the broth Horizontal Airflow Chamber is setup. Bacteria in the lyophilized form are placed inside the Airflow chamber. The inoculation loop is heated with a spirit lamp and the bacteria are placed inside the conical flask. A conical flask containing bacterial solution was plugged in non-absorbent cotton. Bacterial solution flasks are kept in a rotary shaker for 24hrs, rotated at the rate of 140 rpm for uniform growth of bacteria. In the 24 hours of germination period, the bacteria in the conical flask looks turbid indicating the germination. The required cell concentration is obtained by an equation specified by Santhosh K in his article 0 that is $Y=8.59x10^7X^{1.3627}$ where Y denotes the cell concentration and X denotes the Optical density reading in 600nm wavelength.

3.4. Initiation of cracks

The concept of crack healing is a significant role in a self-healing bacterial agent and has been explored through previous researches such as through bacterial microcapsules by K.Paine. 0, Expanded Perlite encapsulated bacteria by Mohamed alazhari 0, Tubular Macro capsules encapsulated Primer plastigel by Giovanni Anglani 0. This concept was attempted in this study by initiating the crack and injecting the bacterial solution. This method of examining is so chosen only to focus on the significance of immobilization of bacteria into the localized cracks. The mortar cubes and disks were cast and cured for 28 days. These are subjected to compression under the rate of loading 0.233MPa/sec until the primary initiation of cracks.

3.5. Injection of the bacterial solution into the cracks

The four different bacterial solutions with 10^6cells per ml was injected into the micro cracks of disks separately to check the comparative recovery of distress by calcite precipitation.

4. Testing and observations

The bioengineered mortar specimens are tested in comparison with the control specimen under-strength property test such as the compressive strength test. For assessing the healing capacity and densification of microstructure, UPV test, Scanning Electron Micrographic observations, XRD plots, EDAX testing, and also the digital microscopic study at different intervals are performed and analysed.

4.1. Compressive strength test

This test is conducted to examine the survival and performance of these set of bacteria in a high alkaline mortar medium and enhance the compressive strength by calcite deposition. The compressive strength of the mortar cubes is tested at 7, 14 and 28 days from the day of being cast for each set. The compressive strength is tested by loading the specimens with a Compressive Testing Machine at the rate of 35MPa/min until failure. The average compressive strength of the mortar cubes induced with different bacterial solutions is as given in table 1 and Figure 1.

Table. 1. Compressive strength of bioengineered mortar specimens in contrast with control specimens.

Mortar cube specimens	7th-day strength (MPa)	14th-day strength (MPa)	28th-day strength (MPa)
Control sample	**35.47**	**45.39**	**51.4**
Bacillus subtilis	33.68	37.52	41.03
Bacillus megaterium	29.213	37.763	45.69
Lysinibacilluss phaericus	**36.123**	**39.083**	**46.523**
Consortia	31.50	38.91	45.73

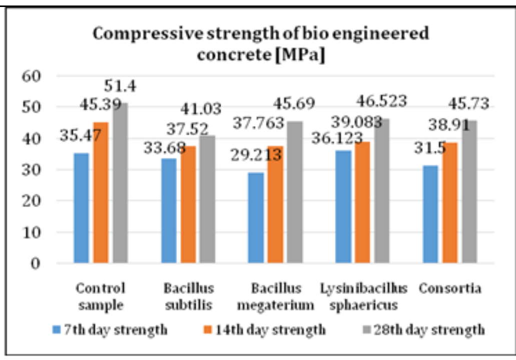


Fig. 1. Compressive strength of bioengineered mortar specimens in contrast with control specimens.

The compressive strength of the samples gives a basis for the performance evaluation of the bacteria. From the above tabular column, we can observe that the initial strength at 7 days is greater for *Bacillus subtilis* and *B. sphaericus* which shows that the calcite precipitation might have initiated early enough in these two bacteria compared to the other two samples. Also, 28th-day compressive strength depicts that the bacteria *Bacillus sphaericus* have a great role in enhancing strength through catalyzing the precipitation of calcite.

4.2. Ultrasonic pulse velocity test

UPV test was conducted on the bioengineered mortar specimens to assess the rate of healing or precipitation of calcite into the gaps and micro-cracks which in turn indicates the densification of microstructure resulting in transmitting Ultrasonic pulses with good velocity in comparison with the specimen unhealed. Table 2 depicts the Ultrasonic pulse duration before cracking, after cracking and after healing the specimens. The observation is made on similar three samples. Figure 2 represents these variations in graphical interpretations. The observations depict that the UPV variations are basically due to calcite deposition by bacteria.

Table. 2. UPV test results on bioengineered concrete.

Cube No.	Before cracking (μs)	After cracking (μs)	After Healing (μs)
1	20.50	62.14	31.20
2	20.00	59.48	28.12
3	20.00	63.90	27.01

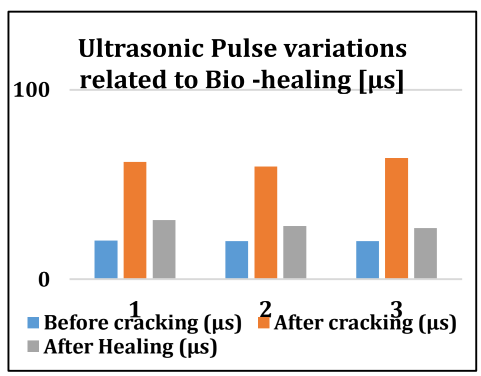


Fig. 2. Variation in Ultrasonic Pulse duration[μs] in bioengineered mortar specimen.

4.3. Scanning electron microscopy

In Scanning Electron Microscope, a beam of electrons incident on the surface of the sample. The electrons interact with the atoms in the sample. The scattering or absorption of the electrons creates the images of the sample. The images can be used to study the shape and texture of the particles of the samples. The electron gun used in the microscope isTungstenheated cathode.

The SEM image is shown in Figure 3 a] is from the pure calcite precipitation on the free surface of the specimen treated with *Lysinibacillus sphaericus* which depict the rhombohedral calcite crystals. Keeping these patterns as reference the powdered bioengineered mortar samples were examined through SEM images as shown in figure 3 b], 3 c] 4 a] and 4 b], which indicates the calcite patterns appeared in all these samples, hence proving the calcite precipitation by the bacteria.

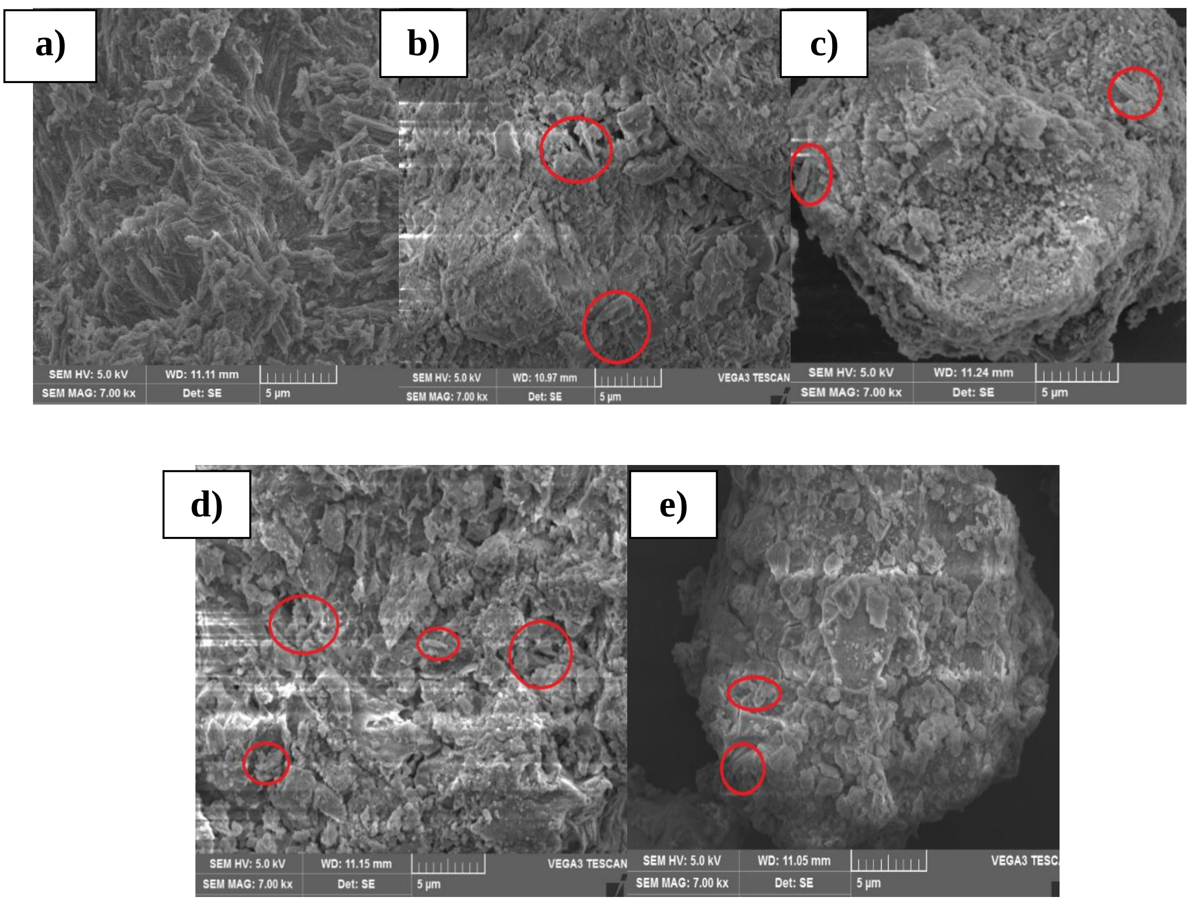


Fig. 3. SEM image at 5.0kx magnificationa] Isolated Calcite precipitation sample b) Bacillus subtilis induced mortar c] Bacillus megaterium induced mortar d) Lysinibacillus sphaericus induced mortar and e) consortia induced mortar.

X-Ray Diffraction (XRD) Analysis: X-Ray Diffraction is performed to analyze the image at its microstructural level. It gives an idea about the crystal structural composition, amorphous content of the sample. The X-ray diffractometer works on Bragg'slaw. The scanning range of the machine is, 2ɵ = -40º to + 220º. Calcite precipitate by *Lysinibacillus sphaericus* in cracks induced in mortar discs is as shown in figure 4.

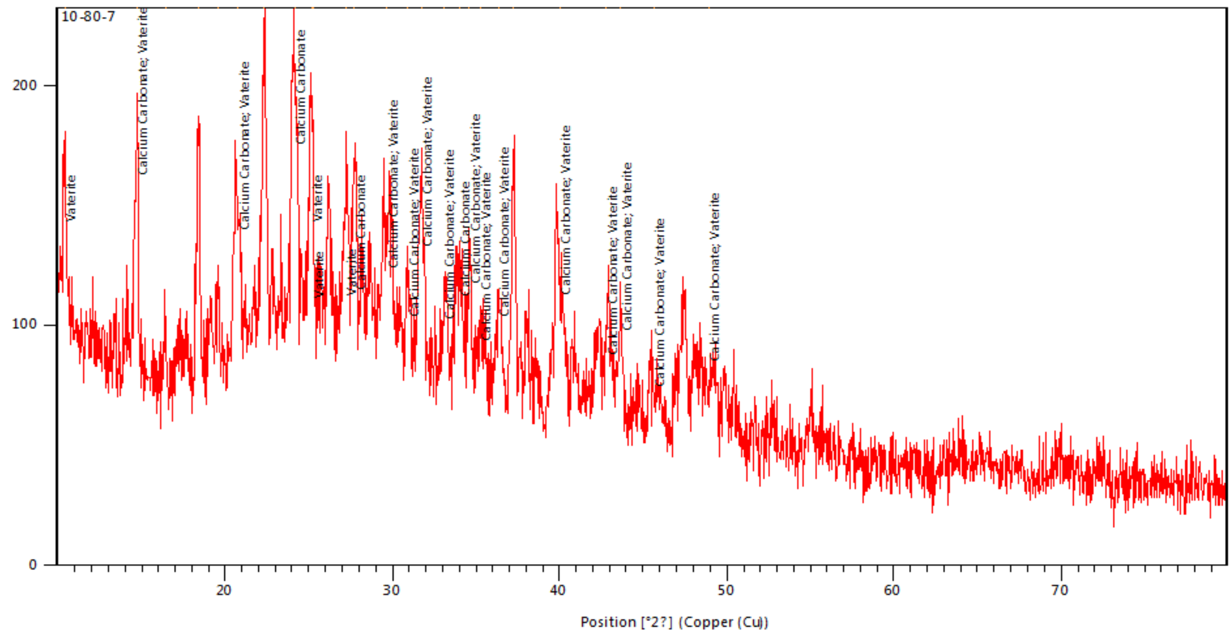
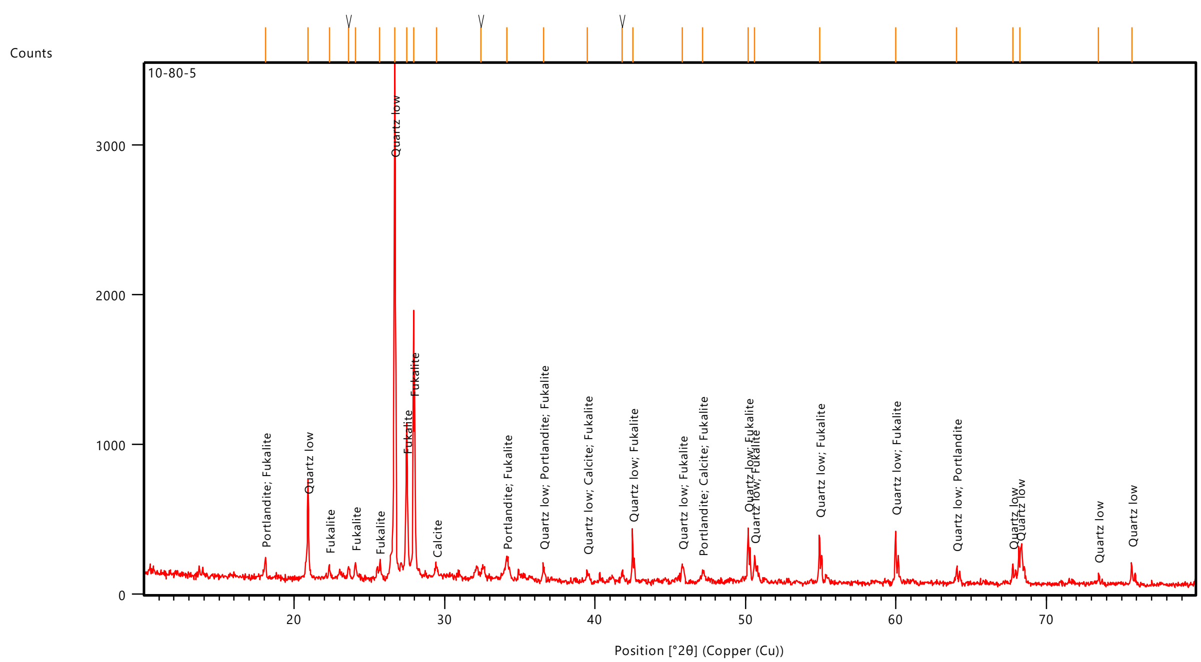


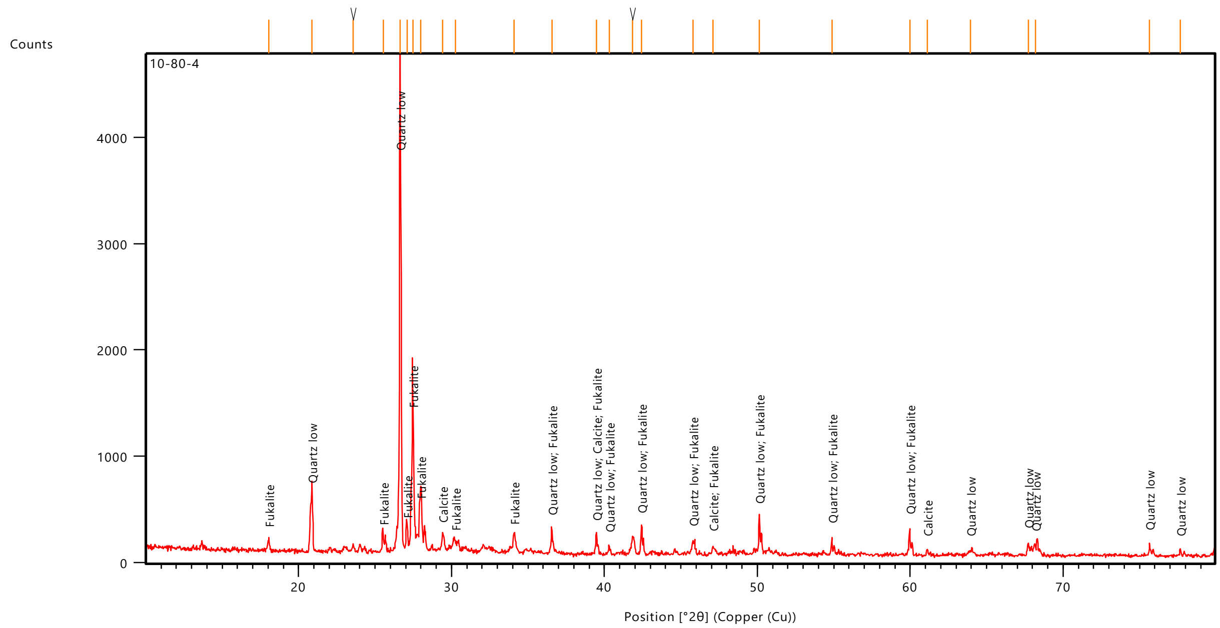
Fig. 4. Calcite precipitate by Lysini bacillus sphaericus in cracks induced in mortar discs.

XRD graph of Calcite precipitation in cracks in mortar disc by *Lysinibacillus sphaericus*. The above XRD image depicts several peaks of calcium carbonate and vaterite which also is a polymorph of calcite hence proving the presence of $CaCo_3$.

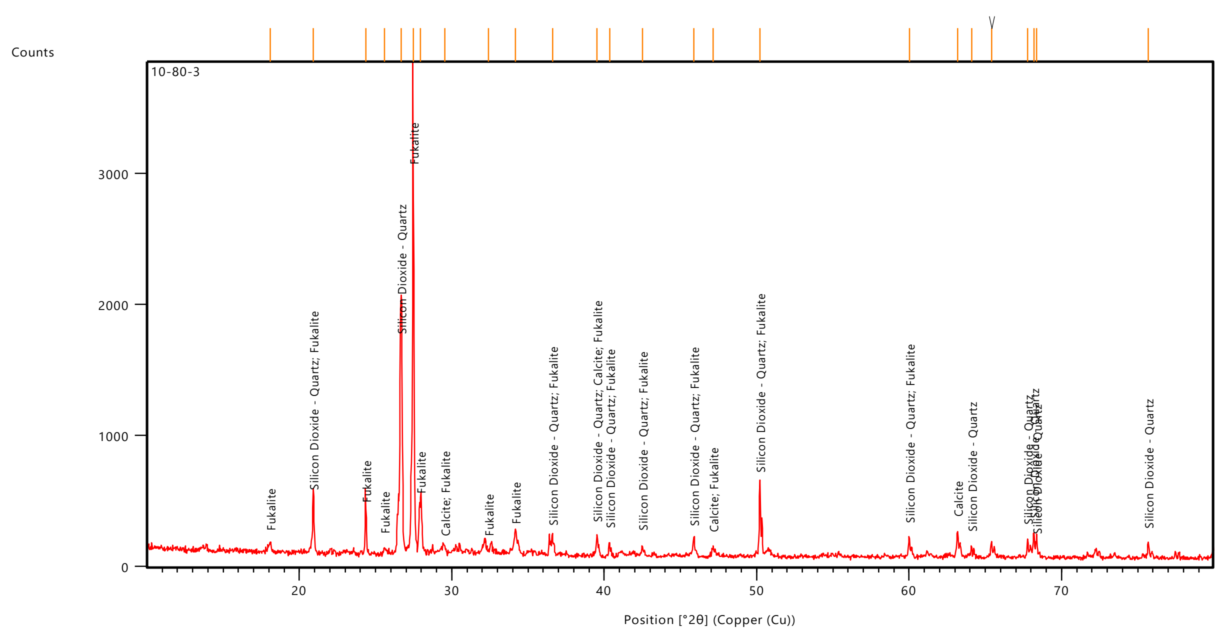
a. Bacillus subtilis



b. Bacillus megaterium



c. Lysinibacillus sphaericus



d. *Consortia*

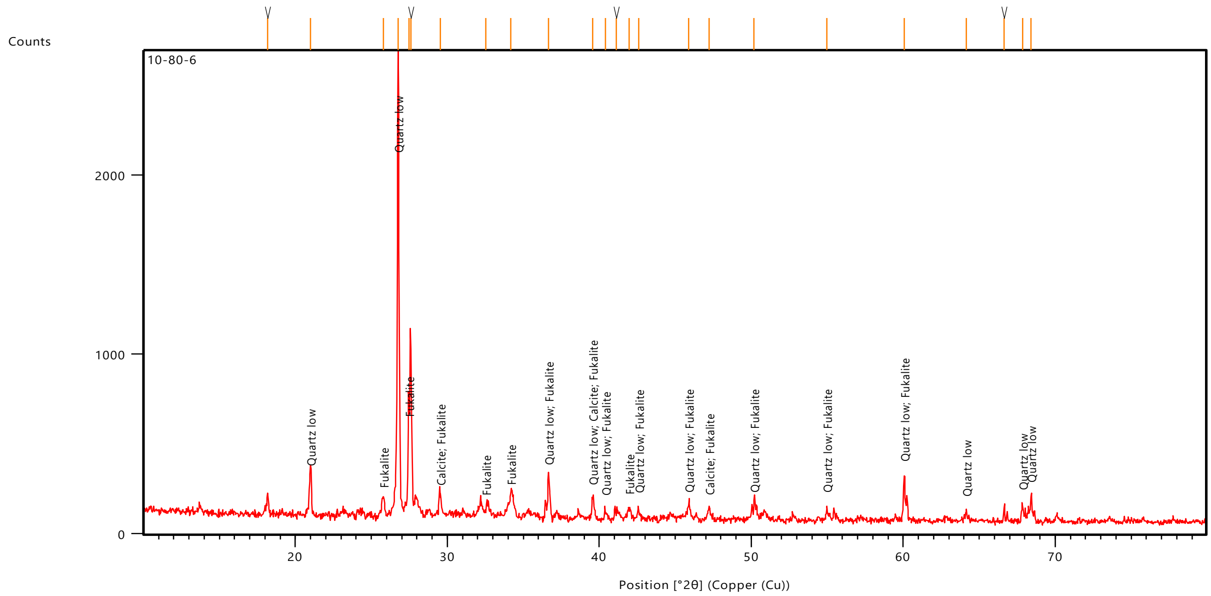


Fig. 5. XRD graphs of bacteria-induced mortar with a] Bacillus subtilis b] Bacillus megaterium c] Bacillus sphaericus d] consortia.

The above XRD images (2,3, 4 and 5) of bacterial-induced mortar on the 28thday of curing, possess the three major compounds as Fukalite, Calcite and Quartz. Though, the primary, secondary and tertiary peaks of these compounds are varying in all 4 samples. *Bacillus subtilis, Bacillus megaterium* and consortia induced mortar samples have the strongest peaks as Quartz which depict the presence of intact sand. The mortar cube with *Lysinibacillus sphaericus* has the strongest peak of Fukalite [$Ca_4Si_2O_6CO_3(OH)_2$] which roughly might indicate the formation of C-S-H gel as a result of hydration.

Energy dispersive x-ray (EDX) analysis: EDX analysis is an analytical method to find out the elemental composition and quantification of the given sample.

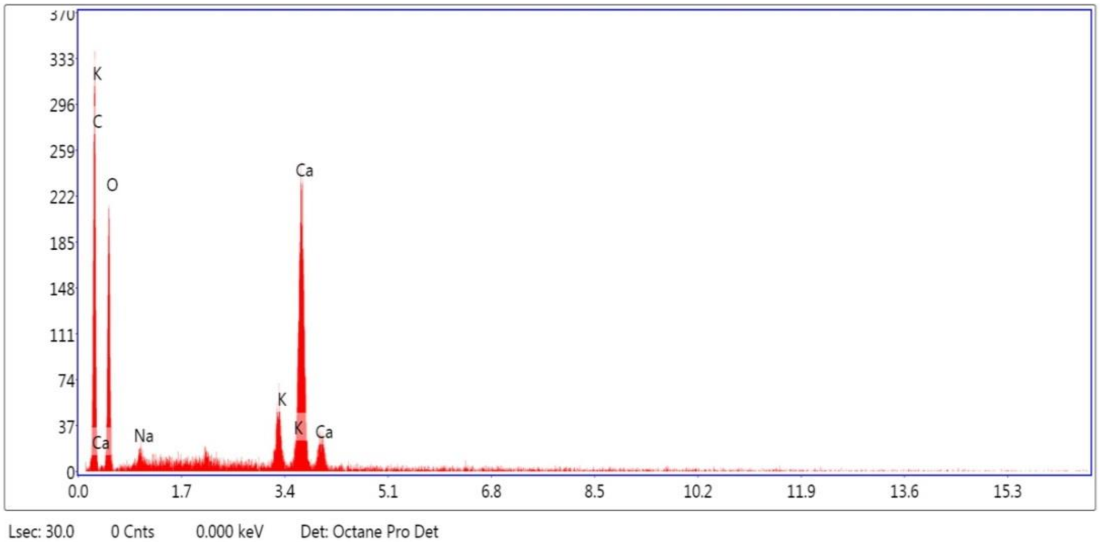
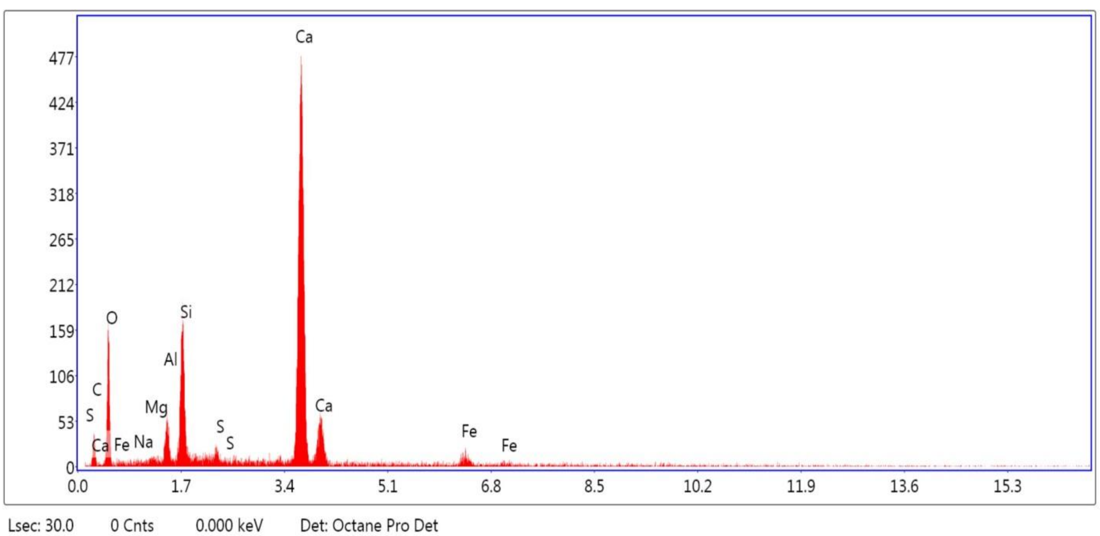
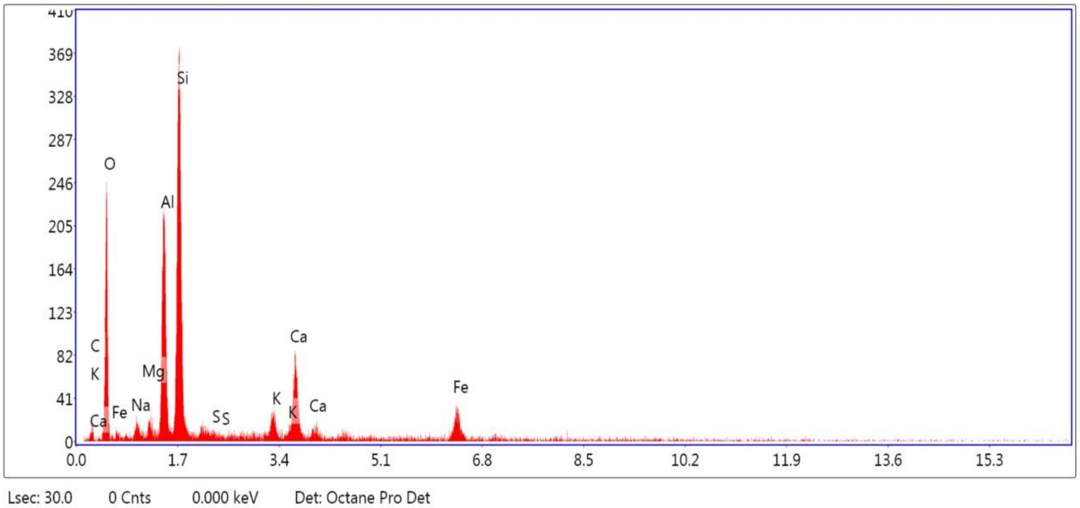


Fig. 6. EDAX graph of calcite precipitation in cracks by Lysinibacillussphaericus.

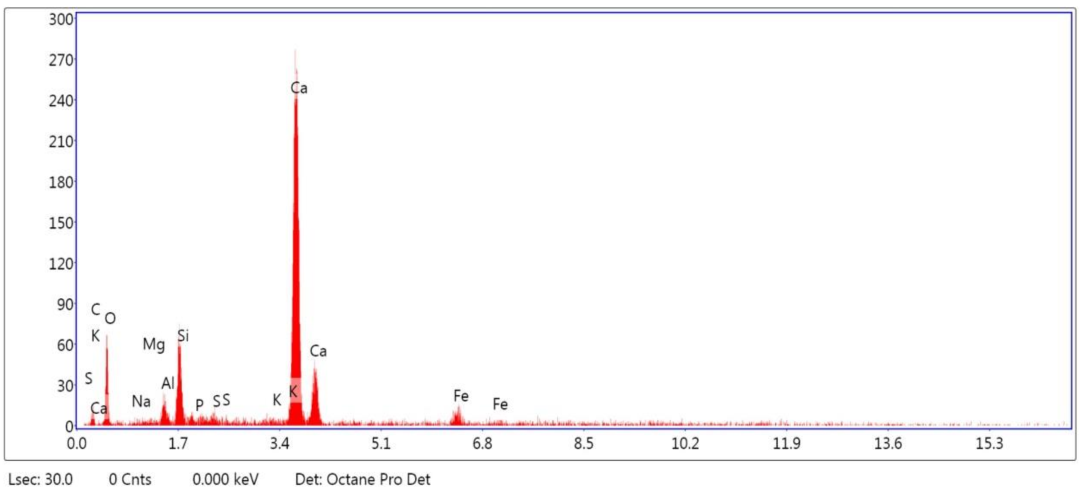
The XRD graph from figure 6 shows strong peaks of Oxygen, Carbon and Calcium. This series also shows the highest percentage presence by weight, which might indicate the presence of the compound $CaCO_3$.



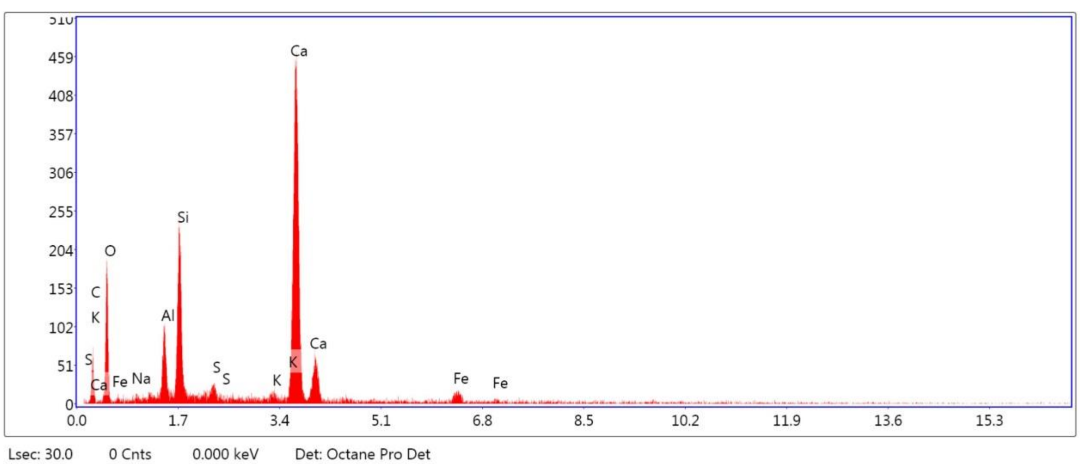
(a)



(b)



(c)



(d)

Fig. 7. EDAX graph of sample a] Bacillus subtilis induced mortar b] Bacillus megaterium induced mortar c] Lysinibacillussphaericus induced mortar d] consortia induced mortar.

Table. 3. Atomic % and Weight % by EDAX test.

Bacteria	Element	Weight%	Atomic %
B sub	Cao	31.84	16.22
B megaterium	Cao	5.53	2.71
B sphaericus	**Cao**	**39.5**	**21.86**
consortia	Cao	27.07	13.21

As itis observed, the XRD images as shown in Figure 6 and 7 of all 4 samples have high calcium peaks indicate the possibility of calcite precipitation. Calcium peaks are one of the strongest peaks in *Bacillus subtilis*, *Lysinibacillussphaericus* and consortia induced mortar samples**.** A lower peak of calcium and a strong peak of silica are observed in *Bacillus megaterium*, which indicates the selected area had more amount of sand intact and the calcite precipitation in the given area was not achieved in full capacity yet. The atomic % and weight% by EDAX test indicate the maximum Cao presence by *Bacillus sphaericus*.

Microscopic observations on healing: The cracks induced mortar discs injected with bacterial solutions as shown in figure 8 a] when photographed in the due course of healing produced the following images. The cracked mortar discs are injected with the 4 different bacterial solutions and the cracks are visually observed under a USB digital microscope at 7 and 21days as shown in figure 9.

Fig. 8. Represents the mortar discs being injected with a bacterial solution.

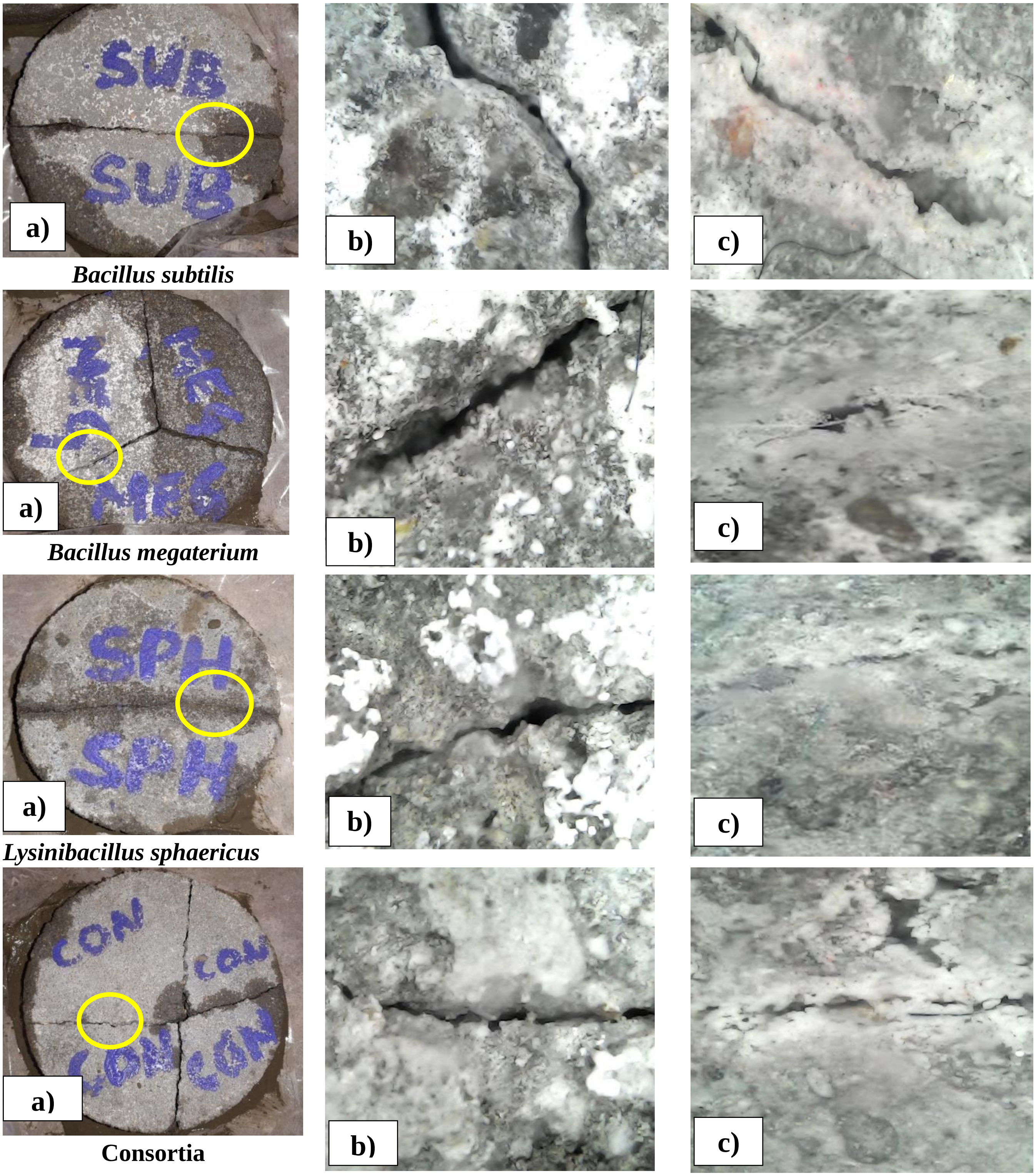


Fig. 9. a) represents the initiated crack and fig b) Represents the initiation of crack bridging at 7 days c) Represents the 90% bridging of cracks at 21 days.

The above microscopic images of the cracks in the mortar discs show their bridging by a white colour compound deposit that is expected to be calcite precipitation. Comparing the a) series pictures with b) series, it's evident of bacteria being able to remediate cracks by inducing calcite precipitation. The white colour deposit seemed pronounced on the surface leading to an almost complete closure of cracks. However, it is expected to have similar actions throughout the depth of cracks induced, as the bacterial solution was injected uniformly throughout the depth of the cracks.

5. Concluding remarks

The entire study was mainly focused on the role of bacteria in the healing of cracks by microbial precipitation and also densification of the microstructure of mortar matrix which was accomplished by the microstructure analysis by various tests such as XRD, SEM, EDAX and also digital microscopic observations. These tests have delivered a clear statistic to arrive at the conclusions on the performance of bacteria in the enhancement of strength properties and durability properties. The work not only proved the effective bacterium is *Lysinibacillus sphaericus* but also the role of calcium lactate as a nutrient. The healing observed was quicker, proving the catalyst activity of bacteria. ***Bacillus sphaericus*** proved to be a better one in achieving strength and also has faster healing ability indicated by the fastest closure of crack injected with it by digital microscopic observations. The microstructure analyses with the help of SEM, E-DAX and XRD proved the Microbial Induced Calcite Precipitation with the help of magnified images and elemental compositions. The study proves the effective use of bacillus bacteria in self-healing concrete which will have a wider application and acceptance in the field.

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