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Self-healing bacterial mortar with calcium lactate and improved properties

***V.V. Galishnikova*[a], *S.H. Elroba**[b], *M. Nassar*[c], *A. Sakna*[d]**

[a] *National Research Moscow State Civil Engineering University, Moscow, Russia*

[b] *Peoples' Friendship University of Russia, Moscow, Russia*

[c] *Renders Engineering Consultants, Dubai*

[d] *Egyptian Russian University, Badr City, Egypt*

**E-mail: ERU.SHERIF@yahoo.com*

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Abstract. The advancing technologies of self-healing concrete focus on reversing and repairing the micro-cracks that form in concrete at early ages, increasing the durability of the structure. The utilization of the metabolic properties of bacteria in bacteria-based self-healing mortar has yielded promising results in the reduction of cracks. Two species of bacteria Bacillus sphaericus (BS), Bacillus pasteurii (BP) were used in this study and patched at a ratio of cement weight in addition to Calcium Lactate being added as nutrition of bacteria. Setting time test was performed to measure the effect of bacteria on fresh mortar properties. The bacterial mixtures were compared to control mix to study their behavior under the influence of permeability test, compressive strength test, flexural strength test, sulfate resistance test and acid resistance test. Also, the restoration of bacterial mixes was tested. Furthermore, advanced technique were employed to evaluate the influence of bacteria addition, e.g. Scanning Electron Microscope (SEM). The cell concentration of Bacillus sphaericus (BS) and Bacillus pasteurii (BP) of all bacterial mortar mixes were counted. The bacteria nutrition acts as accelerator of cement pastes for initial setting time for all bacterial mortar compared to control mortar, while acts as a retarder of cement pastes for final setting time for all bacterial mortar compared to the control samples. Rate of water Absorption decreased with time and became nearly impermeable for BS60 and BP60 at the age of 120 days. Compressive strength of BS60 at 120 days age increased by 124 % compared to the control specimens. Flexural strength value of BS30 at the age of 120 days increased by 168 % compared to control mortar. Results showed that BP60 had a high ability to resist salts and acids. SEM proved that the bacterial mortar had less voids than that of control mortar. Microbial Induced Calcite Precipitation (MICP) is responsible for filling up the pores in mortar and hence decreased the rate of water absorption and the capillary permeability coefficient, while increasing the compressive and flexural strength for bacterial mortar.

1. Introduction

The past decade, researchers around the globe have addressed the problem of micro cracks forming in concrete and studied its effect on the future durability and service life of structural elements. Cracks at early ages in concrete are usually the result of rapid temperature changes causing shrinkage stresses during the setting process for concrete, in addition to the loading of reinforced elements [1]. Even though the formation of micro-cracks is not an immediate threat to the integrity and strength of the element, it usually leads to permeability and durability issues, especially in humid or wet environments. Formation of micro-cracks usually result in the exposure of the embedded steel rebar to external conditions, thus, resulting in corrosion and needing costly maintenance and repair procedures [2]. Epoxies and other

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synthetic materials have been previously used to remediate these cracks but alternatives for them are being considered because of their incompatibility with cementitious materials in addition to their higher costs.

Mineral formations such as limestone in concrete can be of use and favorable to the concrete's durability. As they are concrete-compatible materials, they work on closing up and sealing the cracks, making the concrete less permeable and protecting the steel rebar from compounds such as chlorides and other corrosion-inducing chemicals that would otherwise reach the rebar through the cracks. Over the past decade various researchers experimented with various concepts of self-healing using a biological base. Albeit each species of bacteria experimented on has different metabolic process, they all share the same principal: an encased specie of bacteria is added to the fresh concrete mix and when cracks occur in the hardened concrete the changing environmental conditions works on activating the bacteria which precipitates calcium carbonate and closes the crack [3–7]. The metabolic process for the bacteria creates limestone depositions, thus, recovering the functional properties of concrete as the aim of this research. The properties regained with the healing of cracks in concrete include reduced permeability, strength or aesthetics. The 'self-healing agent' needed to enhance the healing ability for concrete is encased bacteria in addition to the required mineral and chemical compounds which are added to the concrete mixture. This increased healing ability would reduce the cost of repairs, but it would also enhance the longevity of the structure. Multiple species of bacteria were used to produce calcium carbonate and other inorganic minerals [8]. This process, being a biological process, depends on specific environmental conditions, therefore, the bacteria can make use of certain metabolic pathways in order to change the environmental conditions to facilitate the production of inorganic materials.

Previous research has been done on adding certain bacteria that would result in the desired mineral disposition on concrete with recycled aggregate and has shown an improvement in the crack width and depth in addition to the increase in compressive and flexural tensile strengths [9], [10]. Other researchers has studied the effect of bacteria spores encapsulated in hydrogel on cement mortar cracks and has shown the ability to heal cracks up to 0.5 mm in width [11]. As for the problem with the increased water absorption of cementitious materials due to the formation of micros-cracks, a research in 2008 has shown that the self-healing effect of bacteria can reduce the water absorption by 60–90% thus reducing chloride migration by up to 40 % [12]. In addition, a more recent 2018 study has been performed on S. Pasteurii bacteria in addition to calcium chloride and urea-calcium lactate as nutrition for the bacteria and it showed that the self-healing properties of the bacteria has improved the compressive strength by 60 % at 28 days of age and reduced the water absorption by up to 55 % in cement mortar specimen [13]. It was also revealed that the maximum crack healing occurs at the maximum bacterial concentration [14], [15].

This process, in addition to recovering the original performance of concrete by repairing its cracks [16], [17], it is also is more compatible and environmentally friendly than the traditional repair materials [18]. The goal of this study is to demonstrate bacteria's potential in enhancing and affecting the mechanical properties of concrete. Setting time test was performed to measure the effect of bacteria on fresh mortar properties. The bacterial mixtures were compared to control mix to study their behavior under the influence of permeability test, compressive strength test, flexural strength test, sulfate resistance test and acid resistance test. Also, the restoration of bacterial mixes was tested. Rate of Water Absorption test and compressive strength test were performed at the ages of 3, 7, 28, 90 and 120 days. Flexural Strength test was performed at the ages of 28, 90 and 120 days. Sulfate Resistance Test and acid Resistance test were performed at the ages of 28 and 90 to determinate the rate of water absorption and the capillary permeability coefficient. Also sulfate resistance test and acid resistance test were performed at the ages of 28, 90 and 180 days to determinate compressive strength. In addition to the mechanical and physical, advanced technique was employed to evaluate the influence of bacteria addition, e.g. Scanning Electron Microscope (SEM).

2. Methods

2.1. Materials

2.1.1 Cement

The properties of mortar are dependent on the ratios and qualities of its constituent. Since cement is the main binding component of mortar and is usually the key cost item, its selection and proper use is crucial in obtaining the most economic mortar mixture with the desired properties. Ordinary Portland (Type 1) grade 42.5 N confirming to the ASTM Standard Specifications was used [19].

2.1.2. Fine aggregate

The fine aggregate used was natural sand. The sand was clean free from impurities. Medium well-graded sand of fineness modulus 2.2mm was used for mortar.

The main physical and mechanical properties of the used sand were measured according to ASTM concrete aggregate specifications [20].

2.1.3. Water

Clean tap drinking water was used for mixing and curing in this work, tap water that used was free from impurities [21].

2.1.4. Bacteria

Two types of bacteria were used in this work (Bacillus Pasteurii DSM 33, Bacillus Sphaericus DSM 396). The pH for these bacteria is usually neutral and germinates at a pH of 7.0 [22]. The percentages of added bacteria were 0.6 % and 0.3 % by the weight of cement.

Both bacteria were cultured in "Luria-Bertani" (LB) broth medium containing, g/L (12g Yeast Extract, 12g peptone and 6g sodium chloride) [23]. The pH was adjusted to 7.0 and cultures were aerobically incubated in 2L Erlenmeyer flasks using a rotary shaking incubator at 150 rpm for 7 days at 30 °C. Growth and sporulation yield of bacteria was regularly checked and quantified using microscopic analysis and pour-plate count method.

2.1.5. Calcium lactate

Calcium lactate is also known as calcium salt pent hydrate and the chemical formula is (Ca(C3H5O3)2). This calcium lactate powder is produced by reacting lactic acid with calcium-based water-soluble compounds such as calcium carbonate or calcium hydroxide.

2.2. Mortar mixes

The dry mortar mixture was weighed and mixed using concrete mixer machine for ten minutes. The water was poured afterwards, and mixing process lasted for another ten minutes. Mortar proportions were chosen according to Ferro cement Model Code [24]. Sand/cement ratio was 1:3 by weight. Water/cement ratio was 0.45. Specimens were prepared for mortar mix with (bacterial mortar) addition of calcite-producing Bacillus pasteurii or Bacillus sphaericus. Control mortar mix was prepared as well to study the effect of bacteria addition.

Bacteria were added to mortar with two ratios (0.6 % and 0.3 % of cement weight). Calcium Lactate was added as nutrition to bacteria by 0.3 % and 0.15 % of cement weight. Experimental mortar mixes proportions are shown in Table 1. The mortar was cast in moulds for different tests. Test specimens were remolded after 24 hours and kept in a wet case. The specimens were kept moist till testing time.

Table 1. Experimental mortar mixes proportions with bacteria and calcium lactate.

Mix	Samples	Sand/ Cement	Water/ Cement	Bacteria /Cement	Calcium Lactate/ Cement
1.	Control			0.0	0.0
2.	*B. sphaericus* (BS50)			0.6 %	0.3 %
3.	*B. sphaericus* (BS25)	3:1	0.45	0.3 %	0.15 %
4.	*B. pasteurii* (BP50)			0.6 %	0.3 %
5.	*B. pasteurii*(BP25)			0.3 %	0.15 %

2.3. Specimen preparation and testing

2.3.1. Preparation of bacteria

Both bacteria were cultured in liquid media containing (1000.0 ml distilled water, 5.0 g Peptone, 3.0 g yeast extract, 15.0 g Agar) [25]. Adjust pH to 7.0. For Bacillus strains the addition of 10.0 mg $MnSO_4$ x H_2O is recommended for sporulation, In addition to the above-mentioned 24 g/l Urea only should be added to bacteria of Bacillus Pasteurii during preparation. Media is added in to conical flask. It is then made air tight by sealing the flask with a paper and a rubber band. The solution is then sterilized using a flame burner for 10-20 minutes. Now the solution should be free from any contaminants and the solution is a clear orange color before the addition of the bacteria [26].

Later the flasks are opened up and an exactly 1 ml of the bacterium is added to the sterilized flask and is kept in a shaker at a speed of 150–200 rpm overnight at 30 °C. After 24 hours the bacterial solution was found to be whitish yellow turbid solution. Bacterial cultures were incubated for 7 days to ensure sporulation then put it within falcon tube 50mm inside centrifugation at 10000 rpm for 10 minutes. Finally,

the cell pellets were re-suspended in sterile solution of 0.9% NaCl to harvest the vegetative cells and spores. Optical density of the bacterial cultures and pure plate count method were used to prepare culture suspensions with a final cell density of 10^9 CFU/mL, then used in two concentrations including, 0.6 % and 0.3 % of the cement weight, respectively.

2.4. Mortar tests

2.4.1. Bacterial count

After cement mortar preparation and solidification both control and treated cement mortar samples were collected for bacterial counts after 3, 7, 14, 28, 60, 90 and 120 days. Ten gram of each sample was added to 90 mL of sterile 0.9 % NaCl solution and left over night to release the bacterial cells and spores from the cement mortar [27], then serial dilutions of each sample were done by the addition of 1 mL of sample suspensions to 9 mL of sterile 0.9 % NaCl solution, afterwards 1 mL of the final dilution was added to petri dish containing about 20 mL of solid LB medium dispensed over each plate in triplicates for each dilution as shown in Figure 1. Finally, the plates were incubated at 30 °C for 6 days and the CFU/mL in each plate [27] was counted during the time course for Bacillus sphaericus (BS) and Bacillus pasteurii (BP).

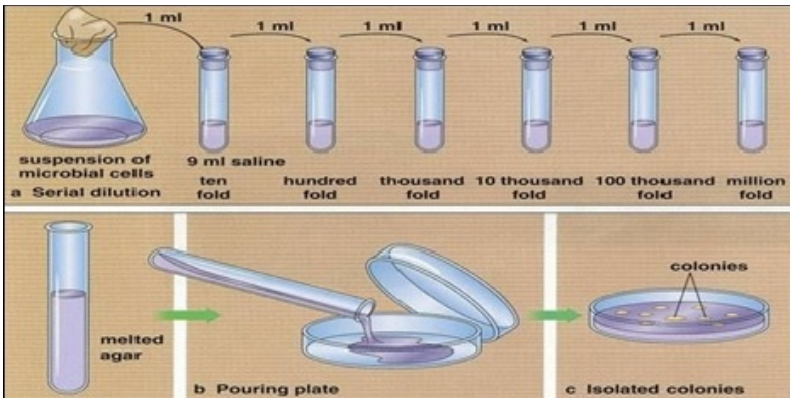

Figure 1. Serial dilution method for bacterial count [29].

2.4.2. Fresh mortar properties

Initial and final setting time tests were carried out by Vicat's apparatus for cement paste [19].

The cement was used without any addition. Standard water/cement ratio was experimented on cement only prior to setting time tests [29].

Another four cement pastes were mixed using the same water ratio with the addition of bacteria and calcium lactate as shown in Table 1 to investigate the effect of bacteria on setting time.

2.5. Hardened mortar properties

2.5.1. Physical properties

2.5.1.1. The rate of water absorption

Speed of water absorption is a measure of the capillary forces caused by the porosity of the structure, thus, forcing liquids to flow into the body of the structure. In this experiment, the flow of water into the body of the samples increased due to the water absorption at times when only one face of the sample was exposed to water [30]. Mortar samples were oven dried at 70 °C for 24 hours and then cooled after 3, 7, 28, 90 and 120 after allowing their moisture to slowly evaporate using the moist curing method. The flow of water in one direction was confirmed by covering the sides of the mortar specimen in silicone pastes. The top and bottom of the samples were insulated using plastic sheets fixed using plastic bands. The initial masses of the samples were recorded after which they were kept partially immersed to a depth of 10 cm in water. The new readings for masses were taken after 2 hours from first contact with water, the samples were removed, and excess water was blotted off using paper towel and then weighed [31]. The gain in mass (Δm, kg/s) at time t (s), exposed area of the specimen (a, m²), and density of water (d), were used to obtain the rate of water absorption (I, m/s$^{1/2}$) as per the equation [31], [32]:

$$I = \frac{\Delta m}{(a.d)} \quad (1)$$

2.5.1.2 Capillary permeability coefficient

For capillary water absorption tests, specimens were taken from the water curing pan at age of 3, 7, 28, 90 and 120 days, and kept in the drying oven for 24 hours at 70 °C. Afterwards, specimens are taken

out of the oven to be cooled down and immersed in water making sure that only 0.5 cm of the cut surface is submerged in water. Other surfaces were sealed with impermeable tape to prevent exposure to water. After the first 24 hours, the specimens were taken out of the water, dried, weighed, and finally the capillary permeability coefficient was calculated from the following formula [33]:

$$K = \frac{Q^2}{(A^2 \times t)} \quad (2)$$

where Q is the amount of water absorbed (cm³)

K is capillary permeability coefficient (cm²/s)

A is the area of the specimen in contact with water (cm²)

t is the time elapsed (s)

2.5.2. Mechanical properties

2.5.2.1. Compressive strength test

The compression test was conducted for the prepared mortar cubes. Test specimens with dimensions of 70×70×70 mm were used. All specimens were given 24 hours to harden and to be cured. Three specimens were used for each age. After the study period of 3, 7, 28, 90 and 120 days, all the specimens were tested up to determine the failure load in a compression stress apparatus of 3000 kN capacity [4].

2.5.2.2. Restoration of bacteria

Samples were loaded after 14 days from casting date with half of failure load at 7 days. Samples were kept moisturized. Then, samples were put to the test to determine the compressive strength at (28 and 90 days). All specimens were tested for its maximum load in the compression testing machine for each age. Average for three tested specimens for each age was taken.

2.5.2.3. Flexural strength test

Test specimens with dimensions of (160×40×40 mm) were casted. The flexural specimens were subjected to three point loading test. The flexural strength was determined for 28, 90 and 120 days, using a flexural testing machine with a capacity of 15 ton to determine the maximum load before failure.

Average of three tested specimens for each age was taken. The flexural strength is calculated using the formula given below [34], [35]:

$$\text{Flexural strength} = \frac{3PL}{2 \times d_1 \times d_2^{\,2}}$$

where P is the maximum applied load to the specimen (N);

d_1 is the width of the specimen (mm);

d_2 is the depth of specimen (mm).

2.6.3. Durability of cement mortar

2.6.3.1. Sulfate resistance test

In this test samples were taken after a month from the date of casting and immersed in sodium sulfate solution with 5 % concentration [36]. Samples were tested to determine the rate of water absorption and capillary permeability coefficient at (28 and 90 days). Two samples of each age were prepared for each test separately.

Other samples were tested to determine the compressive strength at (28, 90 and 180 days). Three samples of each age were prepared for each test separately.

Average of two tested specimens for each age were taken to determinate the rate of water absorption and capillary permeability coefficient, while average of three tested specimens for each age were taken to determinate the compressive strength. Before the measurements, the specimens were removed from sodium sulfate solution then wiped and cleaned.

2.6.3.2. Acid resistance test

In this test samples were taken after a month from the date of casting and immersed in sulfuric acid solution with 1.5 % concentration [37]. Samples were tested to determine the rate of water absorption and

capillary permeability coefficient at (28 and 90 days).Two samples of each age were prepared for each test separately. Other samples were tested to determine the compressive strength at (28, 90 and 180 days). Three samples of each age were prepared for each test separately. Average of two tested specimens for each age were taken to determinate the rate of water absorption and capillary permeability coefficient, while average of three tested specimens for each age were taken to determinate the compressive strength. Before the measurements, the specimens were removed from sulfuric acid solution then wiped and cleaned.

2.6.4. Advanced techniques of self-healing investigation

2.6.4.1. Stopping of the hydration

The stopping of hydration was accomplished by using alcohol-acetone method at age of 120 days of curing then inspected using Scanning Electron Microscopy (SEM). The stopping solution was prepared as (1:1 v/v) of methyl alcohol and acetone. About 10 g was taken from the sample, ground and stirred with 100 ml of stopping solution, then filtered through sintered glass funnel (G4), washed with the same solution three times and finally with Ether. Each sample was dried at 90 ºC for 24 hours and then kept in air-tight bottles inside desiccators till the time of testing.

2.6.4.2. Scanning electron microscopy (SEM)

The scanning electron microphotographs were taken with an Energy Dispersive X-ray Analyzer (EDAX). The device is used to scan the microstructures on the surface of a sample using a tightly focused electron beam to produce an image from the interaction of the beam with the microstructures on the surface of the specimen interactions detected by a wide array of sensors. There are a wide range of detectors from secondary electron detectors to give surface information to backscattered detectors for compositional information that work in high or low vacuum modes. Also, it is used to examine the microstructure of the fractured composites with accelerating voltage 30 K.V., magnification14x up to 106 and resolution for Gun.1n. These samples that were used to conduct SEM observation are first dried at 70 ºC until the constant weight is reached, then bonded on the sample holders with conducting glue carbon [38]. The morphology of the mortar specimens after 120 days curing were observed using Inspect S Scanning Electron. Four magnifications of 150, 1500, 5000 and 7500 were selected for the imaging [39].

3. Results and Discussion

3.1. Bacterial count

The statistical analyses of the given data using one-way ANOVA with post-hoc Tukey HSD Test with Scheffé, Bonferroni and Holm multiple comparison, indicated that, there was no significant difference in the bacterial count between B.Pasteurii and B.Sphaericus during the time course as shown in Figure 2. It is concluded that no significant effects between B.Pasteurii and B.Sphaericus on bacterial counts during the time course.

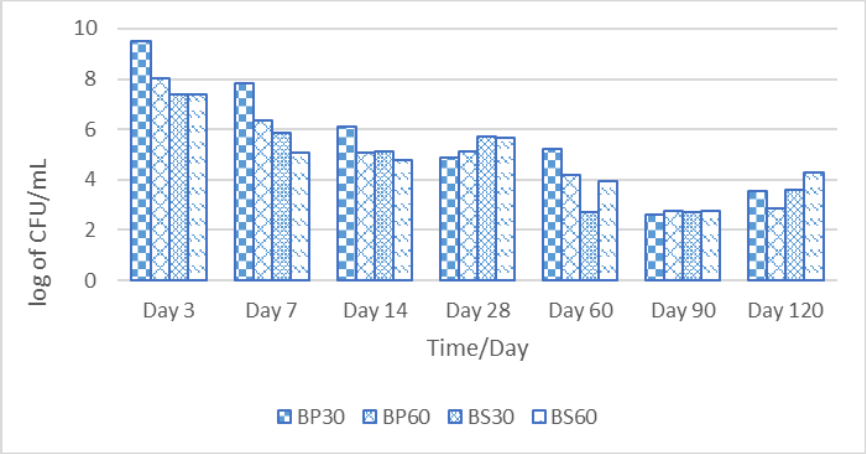

Figure 2. Log of bacterial count CFU/mL of each bacterium (BP = Bacillus Pasteurii, BS = Bacillus Sphaericus) in 0.25 and 0.5 % concentration, respectively for 3, 7, 14, 28, 60, 90 and 120 days. (Blank = LB Agar Medium without Any Samples, Control = Is Untreated Concrete Sample).

On the other hand, the bacterial count decreased during the time course and this can be suggested as a result of depletion of the nutritional elements found in the mortar. Also, both species follow the same growth trend and were adapted in the same rate with the new environmental conditions inside the mortar.

3.2. Fresh mortar properties

Results showed that initial setting time decreased in all bacterial pastes compared to control paste. Initial setting time of all bacterial pastes were more than 60 minutes (within limit time). The final setting time increased in all bacterial pastes compared to control paste. This may be due to nutrients of bacteria added or to the media of bacteria. Final setting time for all pastes was less than 10 hours. (Within limit). The obtained results from the initial and final setting times of bacterial and control pastes are shown in Figure 3. This means that adding bacteria and calcium lactate to cement paste play an important role in accelerating the initial setting time and relating the final setting time compared to control paste. Initial setting time and final setting time for all pastes were with in limit according to ASTM Specifications [19].

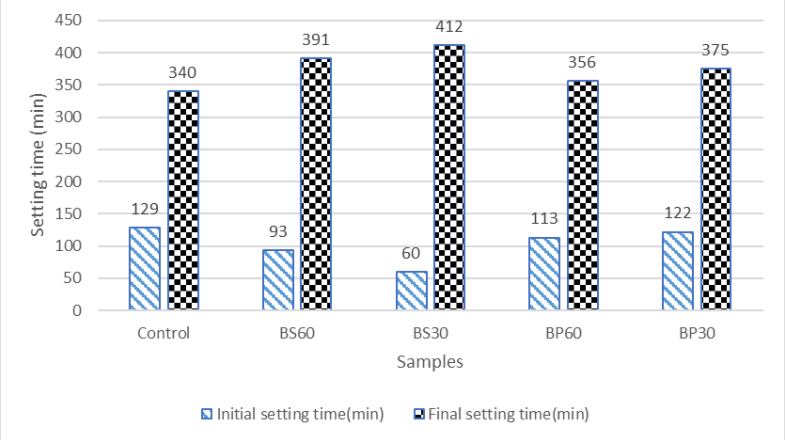

Figure 3. Initial and final setting times of bacterial and control cement paste.

3.3. Hardened mortar properties

3.3.1. Physical properties

3.3.1.1. The rate of water absorption

The influence of bacteria and calcium lactate on the water absorption of mortar after 2 hours was investigated. It was observed that with the inclusion of bacteria, the rate of water absorption of mortar decreased as shown in Figure 4.

At age of 3, 7 and 28 days, it was observed that the rate of water absorption of all bacterial specimens have smaller gain of water absorption than that of control mixture which aligns with what previous literature indicated [9], [12], [13]. At age of 90 days, the relation between rate of water absorption and time after 2 hours showed that BS30 has higher gain of water than that of control mixture. All other bacterial specimens except for BS30 have smaller gain of water than that of control mixture. At the age of 120 days, it was observed that rate of water absorption of all bacterial specimens after 2 hours have smaller gain of water absorption than that of control mixture and became semi-impermeable for BS60 and BP60.

Microbial Induced Calcite Precipitation (MICP) is responsible for filling up the pores in mortar and hence decreasing water absorption of bacterial mortar specimens.

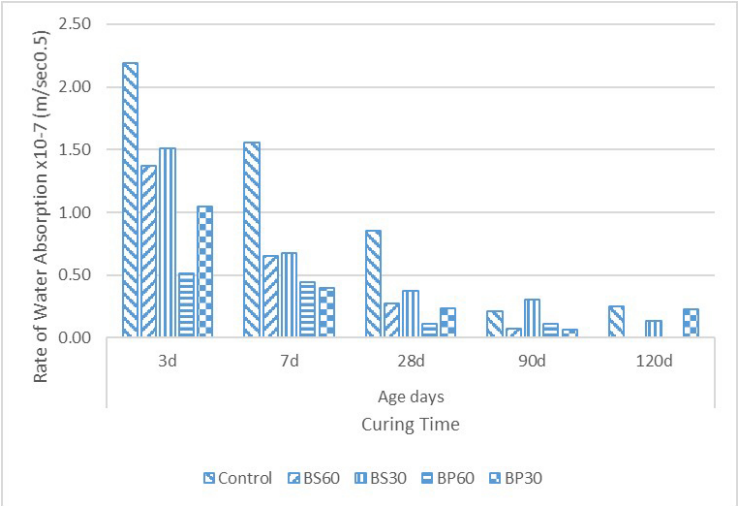

Figure 4. Rate of water absorption (I) for bacterial and control mortar after 2 hours.

3.3.1.2. Capillary permeability coefficient

The influence of bacteria and calcium lactate on permeability of mortar was investigated after 24 hours. At the age of 3, 7, and 28 days capillary permeability coefficient for all bacterial mortar specimens decreased compared to control mortar. At the age of 90 days, capillary permeability coefficient for all bacterial mortar specimens decreased except BS30. At the age 120 days capillary permeability coefficient for all bacterial mortar specimens decreased and became a semi- permeable for BS60 and BP60, as shown in Figure 5. This proves that metabolic activities by bacteria lead to the precipitation of calcium carbonate, which in turn decreases capillary permeability coefficient.

This is in line to previous work that showed a drastic decrease in the relative capillary index by similar amounts indicating a drastic decrease in water absorption [5].

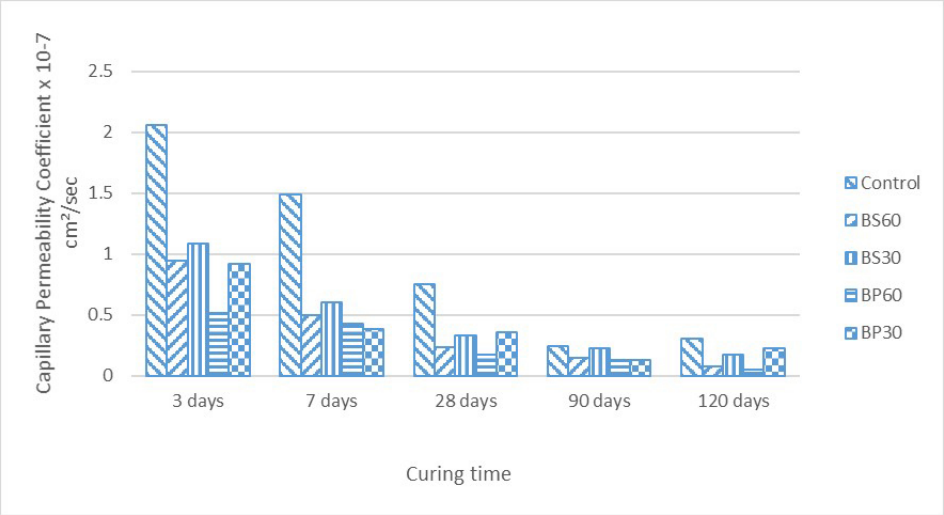

Figure 5. Capillary permeability coefficient (K) for bacterial and control mortar after 24 hours.

3.3.2 Mechanical properties

3.3.2.1 Compressive strength test

The results from the compressive strength test have shown an increase in strength for the bacterial mortar when compared to control mortar. It was noticed that compressive strength values of BS60 were greater than that of compressive strength values of BS30 and control mortar as illustrated in Figure 6. Also, compressive strength values of BP30 were higher than that of compressive strength values of BP60 and control mortar as illustrated in Figure 7.

At the same ratio of bacteria, it was noticed that compressive strength values of BP60 were higher than compressive strength values of BS60 at the age of 3, 7 and 28. At the age of 90 and 120 days, it was noticed that compressive strength values of BS60 became higher than compressive strength values of BP60 as shown in Figure 8. Compressive strength values of BP30 recorded increment in all ages compared to BS30 as illustrated in Figure 9.

Figure 10 showed a significant increase in strength of control and bacterial mortar over time. At the age of 3 days, the compressive strength value of BS60, BS30, BP60 and BP30 were 120 %, 117 %, 134.6 % and 129.1 % of compressive strength of control mortar, respectively. At the age of 7 days, the compressive strength value of BS60, BS30, BP60 and BP30 were 118.4 %, 130.20 %, 123.39 % and 150.0 % of compressive strength of control mortar, respectively. At the age of 28 days, the compressive strength value of BS60, BS30, BP60 and BP30 were 112.30 %, 109.20 %, 115.61 % and 124.88 % of compressive strength of control mortar, respectively. At the age of 90 days, the compressive strength value of BS60, BS30, BP60 and BP30 were 116.51 %, 108.24 %, 113.92 % and 110.76 % of compressive strength of control mortar, respectively. Noting that the increase in compressive strength in bacterial mortar at the ages of 28 and 90 days is remarkably lower that previously mentioned in the literature, having scored an increase of almost 50 % [12], [13].

At the age of 120 days, the compressive strength value of BS60, BS30, BP60 and BP30 were 124.70 %, 112.57 %, 115.79 % and 114.74 % of compressive strength of control mortar, respectively. BS60 had maximum increment in compressive strength. This proved significant activity of bacteria until age of 120 days. Calcite Precipitation Induced by bacteria is responsible for filling up the pores in mortar and hence increasing bonds in the micro structure which resist loads significantly and hence compressive

strength was increased compared to of control mortar. After 120 days, bacterial mortar proves to have higher compressive strength.

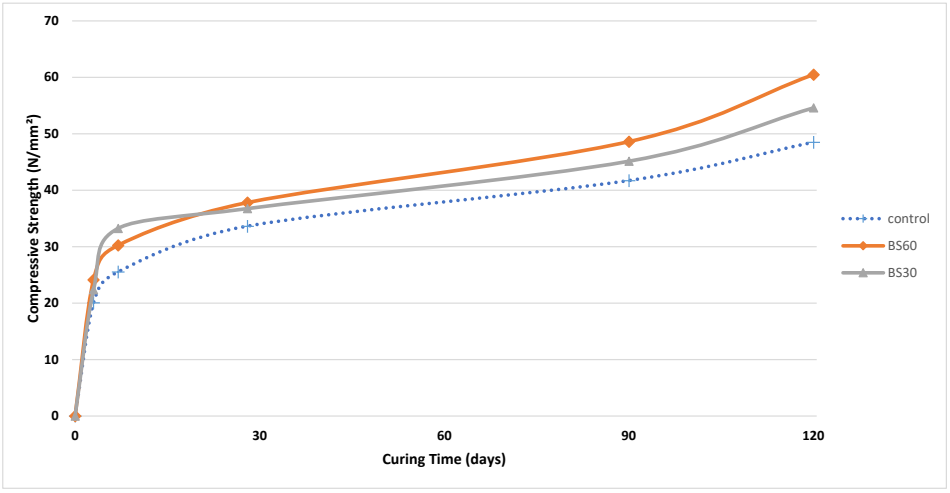

Figure 6. Compressive strength for bacterial mortar specimens (BS60, BS30) and control mortar specimens.

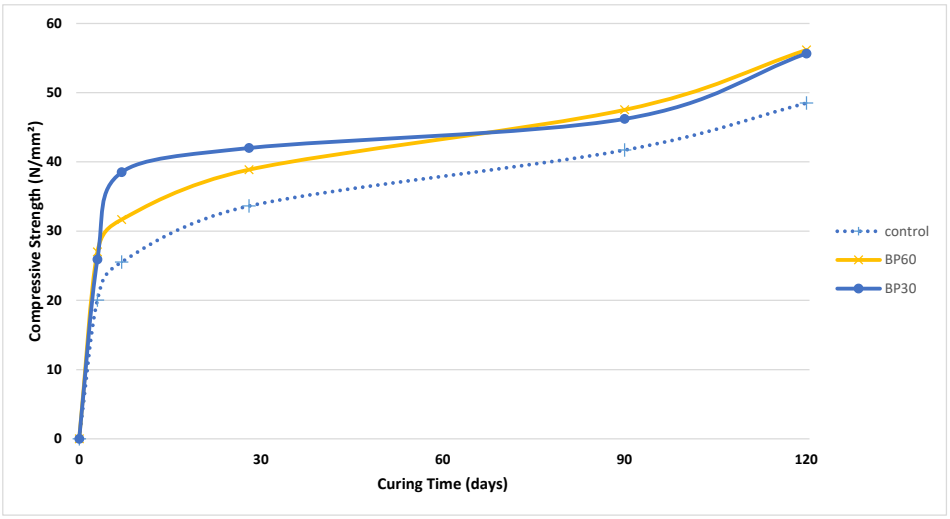

Figure 7. Compressive strength for bacterial mortar specimens (BP60, BP30) and control mortar specimens.

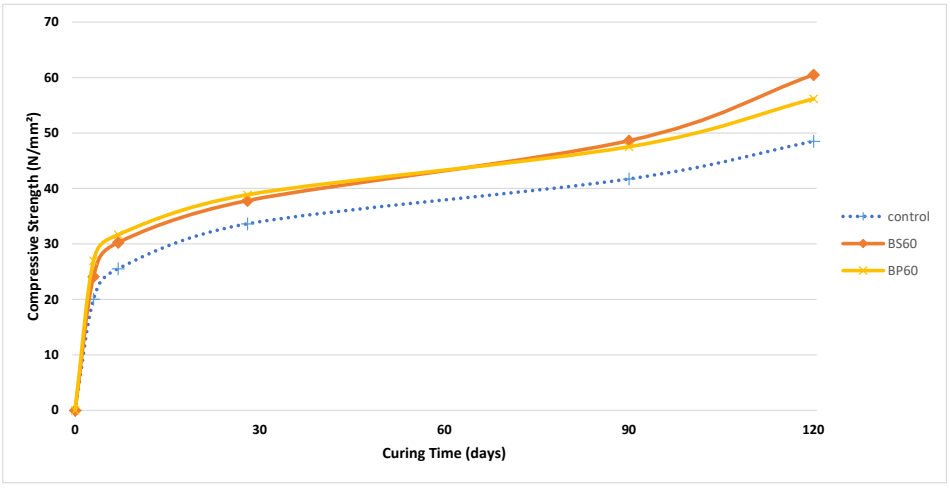

Figure 8. Compressive strength for bacterial mortar specimens (BS60, BP60).

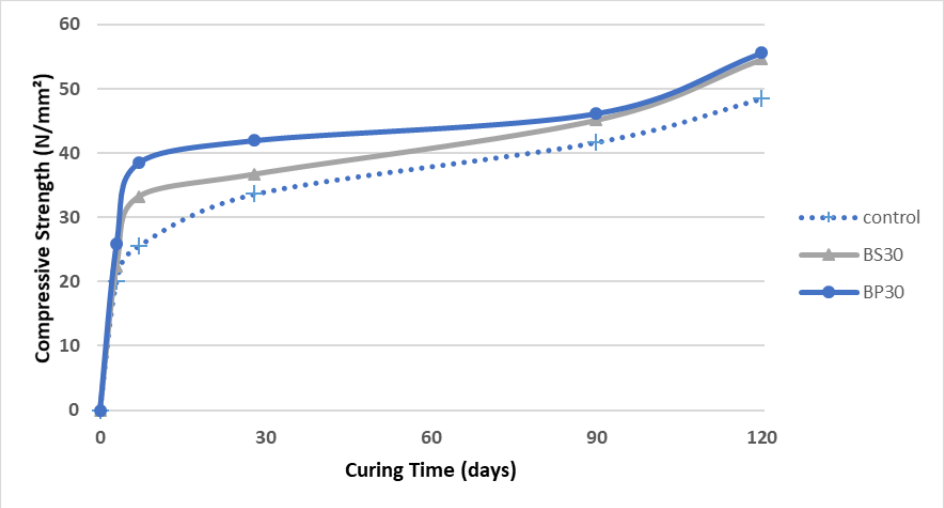

Figure 9. Compressive strength for bacterial mortar specimens (BS30, BP30).

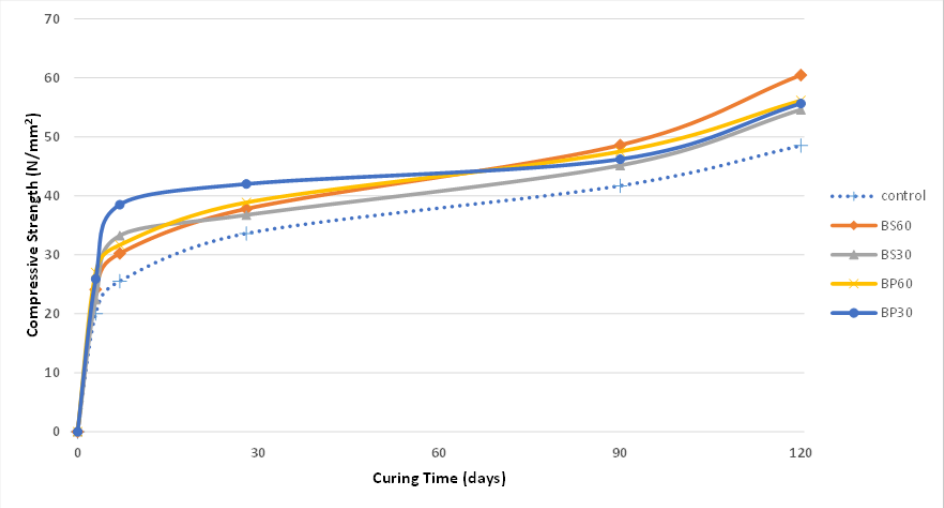

Figure 10. Compressive strength for bacterial and control mortar specimens.

3.3.2.2 Restoration of bacteria

Samples were loaded after 14 days from casting date with 60 % of failure load at 7 days and reloaded at 28 and 90 days. Results of compressive strength test has shown an increase in the strength for all bacterial mortar when compared to its original samples at the age of 28 and for BS30 and BP30 at the age of 90 days as illustrated in Figure 11.

Reloaded samples were compared to original samples. At the age of 28 days, the compressive strength value of Control, BS60, BS30, BP60 and BP30 were 98.06 %, 108.83 %, 121.58 %, 115.49 % and 105.2 % compared to compressive strength of its original state which was determined in compressive strength test. At the age of 90 days, the compressive strength value of Control, BS60, BS30, BP60 and BP30 were 64.87 %, 89.68 %, 103.54 %, 89.92 % and 111.17 % compared to compressive strength of its original state which was determined in compressive strength test. Increasing in compressive strength value of all bacterial mortar at the age of 28 and for BS30 and BP30 at the age of 90 days and decreasing in compressive strength values of control mortar specimen at the age of 28 and 90 days, this means that self-healing in mortar occurred as illustrated in Figures 11, 12.

Increasing in compressive strength value for all bacterial mortar at the age of 28 and for BS30 and BP30 at the age of 90 days and decreasing in compressive strength values of control mortar specimen at the age of 28 and 90 days.

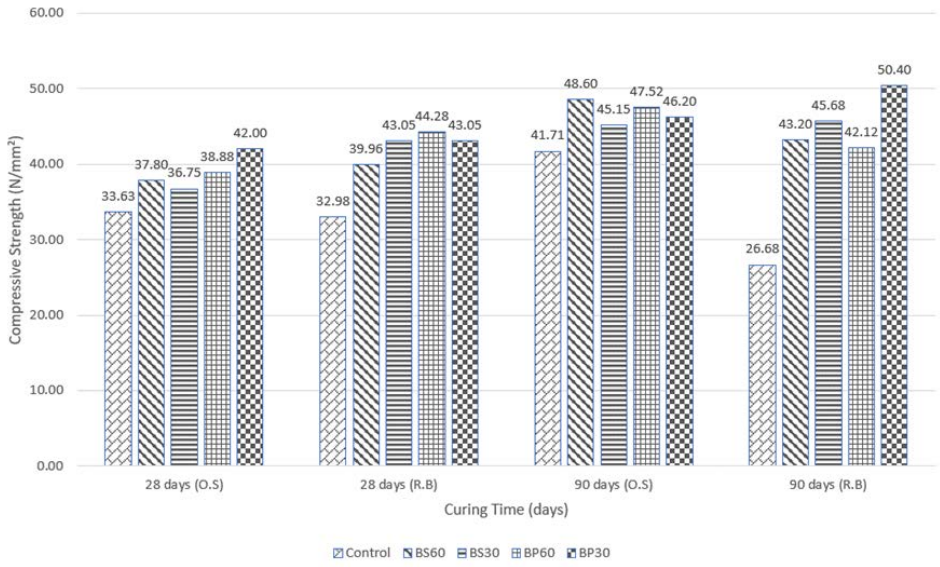

Figure 11. Compressive strength (restoration of bacteria) (R.B) vs. (original samples) (O.S).

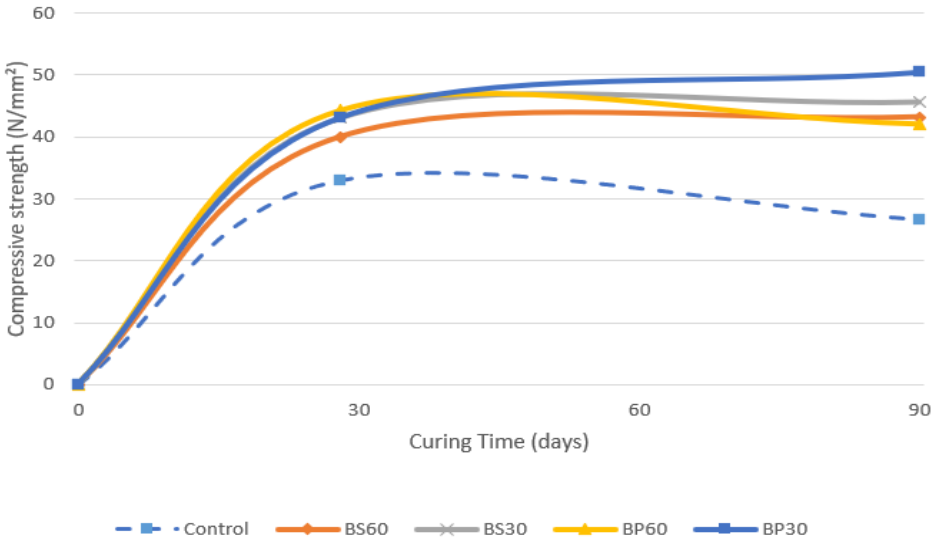

Figure 12. Compressive strength (restoration of bacteria) for bacterial and control mortar specimens.

This means that bacteria of Bacillus Sphaericus and Bacillus Pasteurii (BS30 and BP30) have the ability to restore the mortar mechanical properties to its original state. Also it is stronger than that of its original state before cracking.

3.3.2.3 Flexural Strength Test

Bacterial and control mortar were tested. It was noticed that flexural strength value of BS60 was higher than that of flexural strength value of BS30 at age of 28 and 90 but it decreased at the age of 120 days as illustrated in Figure 13. Flexural strength value of BP60 was higher than that of flexural strength value of BP30 at age of 28 but it decreased at the age of 90 and 120 days as illustrated in Figure 14.

At the same ratio of Bacteria, it was noticed that flexural strength value of BS60 was higher than that of flexural strength value of BP60 at age of 28, 90 and 120 days as illustrated in Figure 15 and flexural strength value of BP30 recorded increment at age of 28, 90 days but it decreased at age of 120 days compared to BS30 as illustrated in Figure 16.

Results of flexural strength test revealed that there is an increase in the strength for the bacterial mortar when compared to the control mortar as illustrated in Figure 17. At the age of 28 days, the flexural strength value of BS60, BS30, BP60 and BP30 were 127.22 %, 115.37 %, 119.15 % and 119.49 % of flexural strength of control mortar, respectively. At the age of 90 days, the flexural strength value of BS60, BS30, BP60 and BP30 were 132.01 %, 117.51 %, 113.15 % and 121.36 % of flexural strength of control mortar, respectively. At the age of 120 days the flexural strength value of BS60, BS30, BP60 and BP30 were 159.89 %, 167.66 %, 118.06 % and 124.39 % of flexural strength of control mortar, respectively.

Microbial Induced Calcite Precipitation (MICP) is responsible for filling up the pores in mortar and hence increased the flexural strength as observed in previous research [9], [13]. Generally, bacterial mortar proved to have a higher flexural strength.

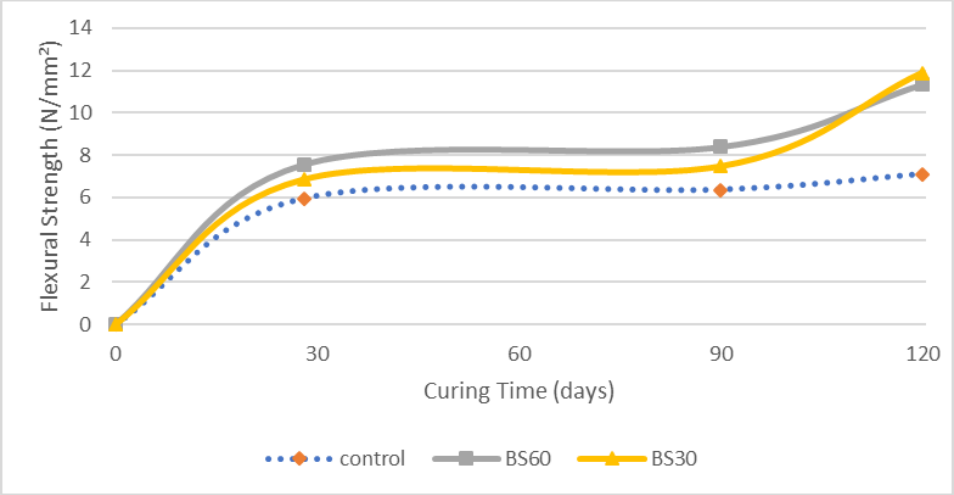

Figure 13. Flexural strength for bacterial mortar specimens (BS60, BS30) and control mortar specimens.

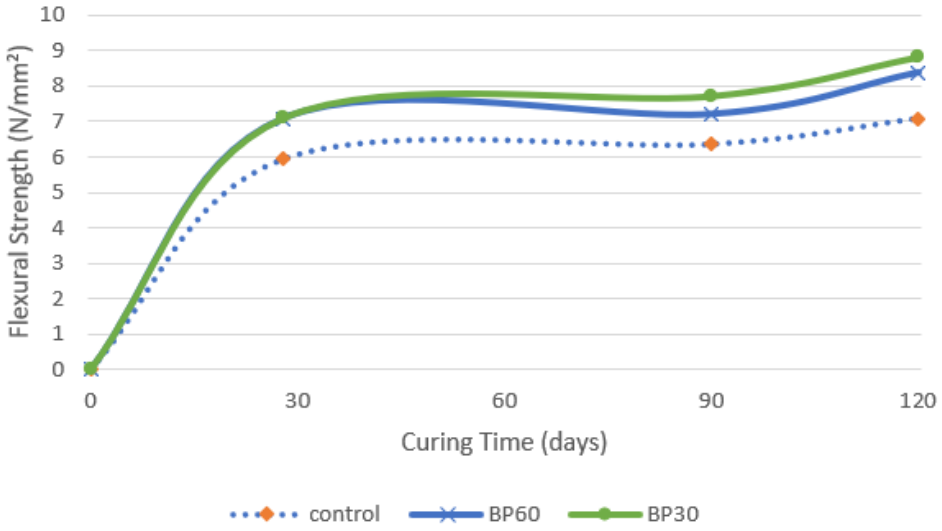

Figure 14. Flexural strength for Bacterial Mortar Specimens (BP60, BP30) and Control Mortar Specimens.

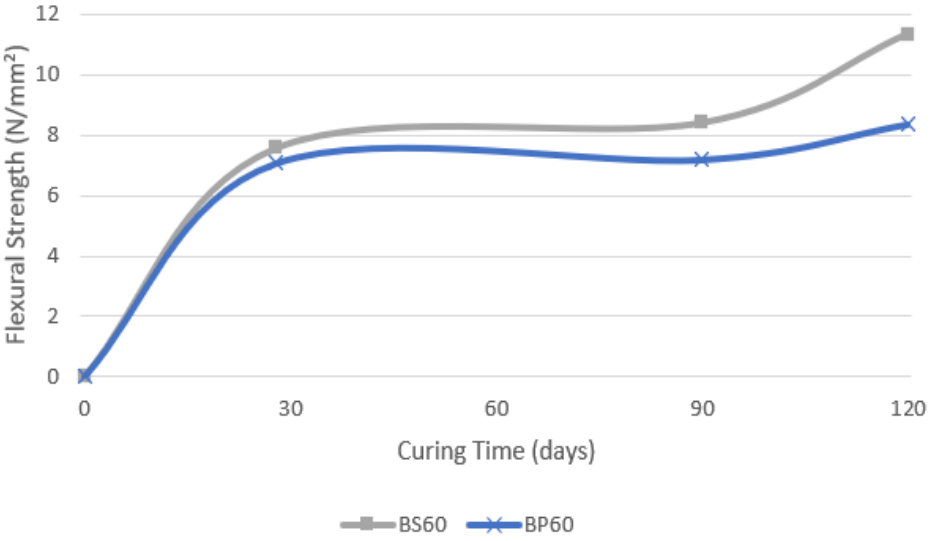

Figure 15. Flexural strength for Bacterial Mortar Specimens (BS60, BP60).

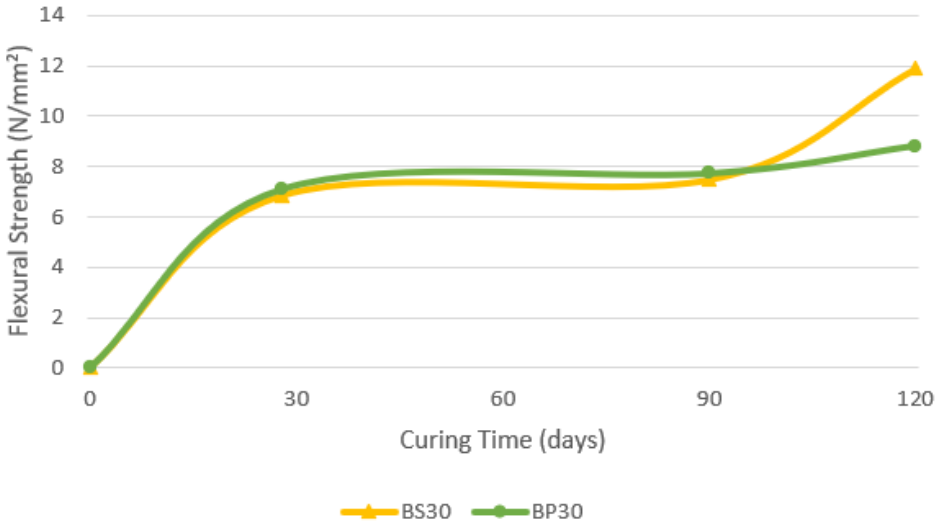

Figure 16. Flexural strength for Bacterial Mortar Specimens (BS30, BP30).

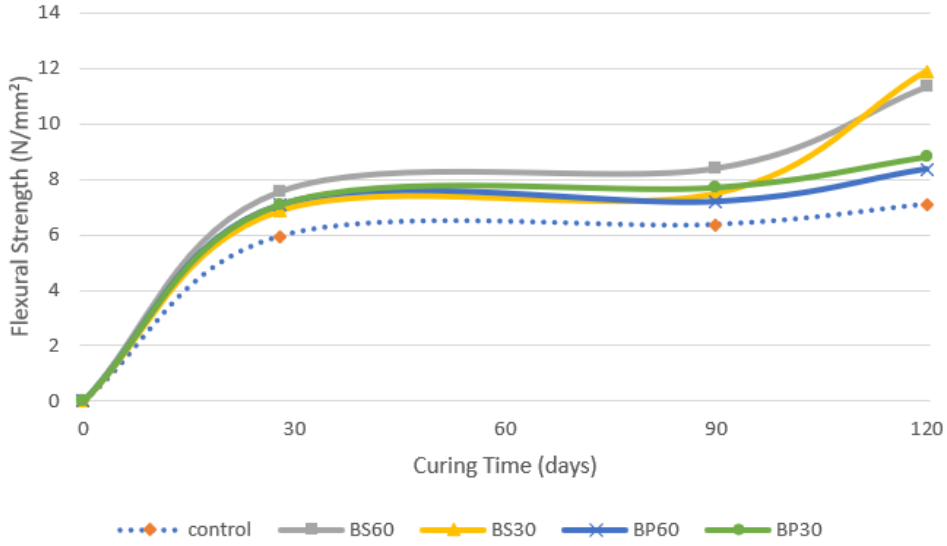

Figure 17. Flexural strength for Bacterial and Control Mortar Specimens.

3.3.3 Durability of Cement Mortar

3.3.3.1 Sulfate Resistance Test

Results of compressive strength test revealed that there was an increase in the strength for the bacterial mortar when compared to the control mortar as illustrated in Figure 18. At the age of 28 days all bacterial mortar increased except BP60 was 98.49 % of compressive strength of control mortar. Also, compressive strength value of BS60, BS30 and BP30 were 134.18 %, 111 % and 113.8 % of compressive strength of control mortar, respectively. At the age of 90 days, all specimens increased for BS60, BS30, BP60 and BP30 and became 139.18 %, 118.1 %, 111.34 % and 118.08 %, respectively.

At the age of 180 days all bacterial mortar increased except BS30 and BP60 were 100.22 % and 96.91 % of compressive strength of control mortar. Also, compressive strength value of BS60 and BP30 were 115.46 %, 116.26 % and of compressive strength of control mortar, respectively.

This means that the compressive strength for bacterial mortar specimens increases in the case of exposure to salts at the age of 28, 90 and 180 days except BS30 at the age of 180 days and BP60 at all ages compared to control mortar at the same ages. Also compressive strength value of control and all bacterial mortar increased with time during exposure to salts compared to them initial statues after immersion in sulphuric acid 98 %.

Comparison between exposed and non-exposed in compressive strength were taken at the age of 28 and 90 days.

At the age of 28 days, compressive strength value of Control, BS60, BS30, BP60 and BP30 were 109.11 %, 145.03 %, 120 %, 103.5 % and 107.63 %, respectively compared to exposed and its non-exposed at the age of 28 days as illustrated in Figure 19.

At the age of 90 days, compressive strength value of Control, BS60, BS30, BP60 and BP30 were 99.26 %, 132 %, 117.21 %, 108 % and 114.55 %, respectively compared to exposed and its non-exposed as illustrated in Figure 20. This showed that BS60 had maximum increment in compressive strength after immersion in sodium sulphate compared to its non-exposed specimens at the age of 28 and 90 days.

On the other side, results showed that the effect of bacteria on the water absorption of mortar after 2hours was investigated. At the age of 28 days, the rate of water absorption for all bacterial mortar specimens decreased compared to control mortar. At the age of 90 days, the rate of water absorption increased for all bacterial mortar specimens except for BP60 increased than of the rate of water absorption for control mortar as illustrated in Figure 21. This means that BP60 had the ability to resist salts absorption.

Figure 22 shows capillary permeability coefficient for control and bacterial mortar specimens after immersion in sodium sulfate. At the age of 28 days, the capillary permeability coefficient for all bacterial mortar specimens decreased compared to control mortar. At the age of 90 days, the capillary permeability coefficient increased for all bacterial mortar specimens except for BP60 decreased than of the capillary permeability coefficient for control mortar. This means that BP60 had the ability to resist salts absorption.

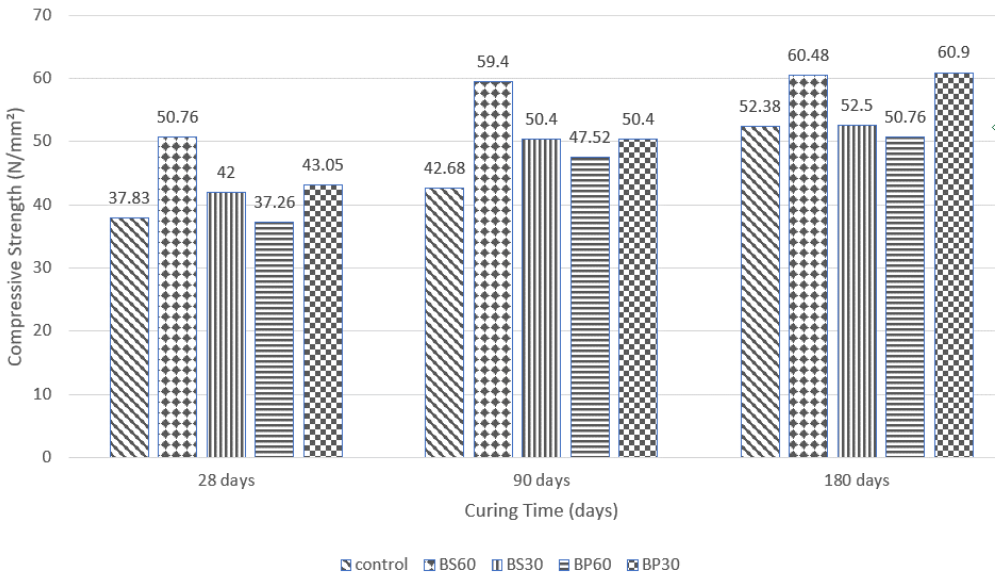

Figure 18. Compressive strength for Bacterial and Control Mortar Specimens after immersion in sodium sulfate.

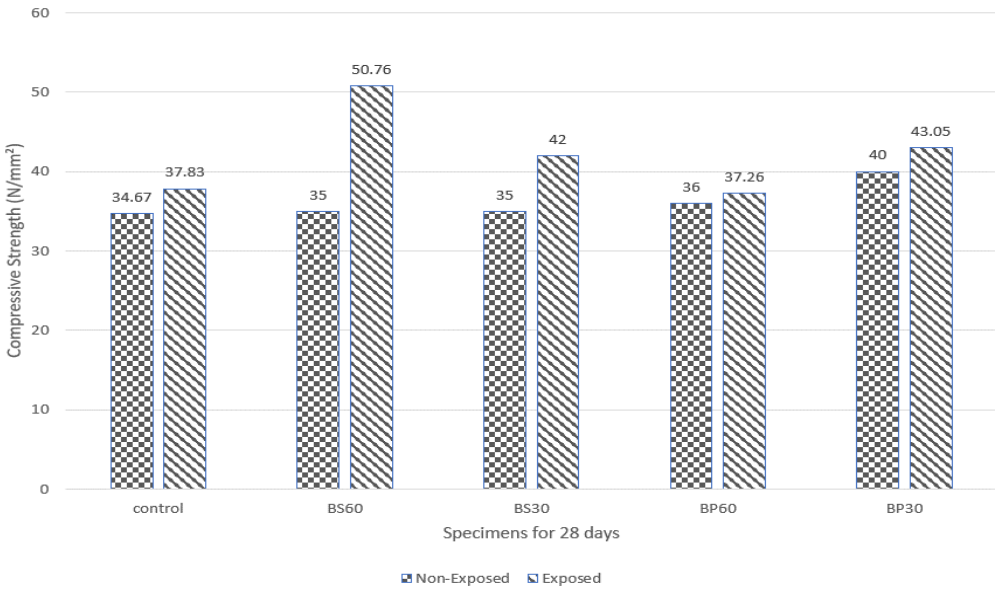

Figure 19. Compressive strength for exposed and non-exposed specimens after immersion in sodium sulfate for (28 days).

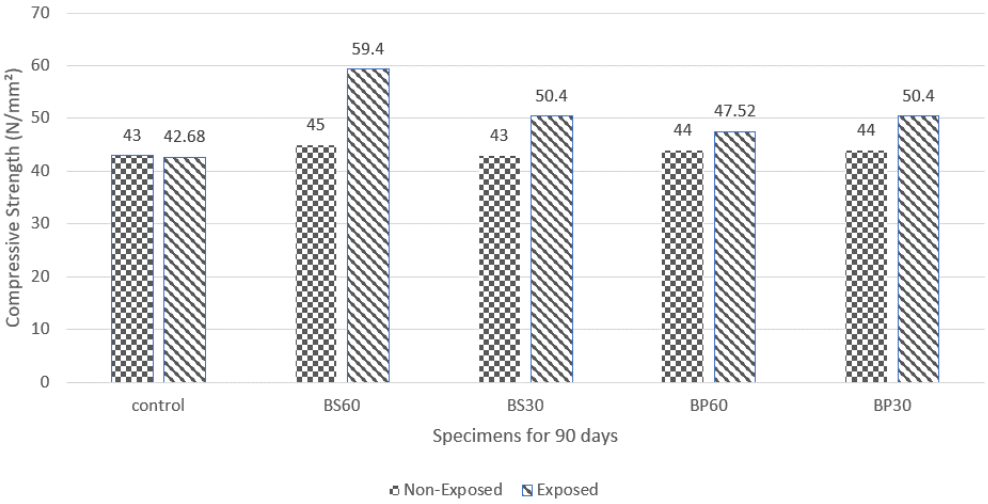

Figure 20. Compressive Strength for exposed and non-exposed specimens after immersion in sodium sulfate for (90 days).

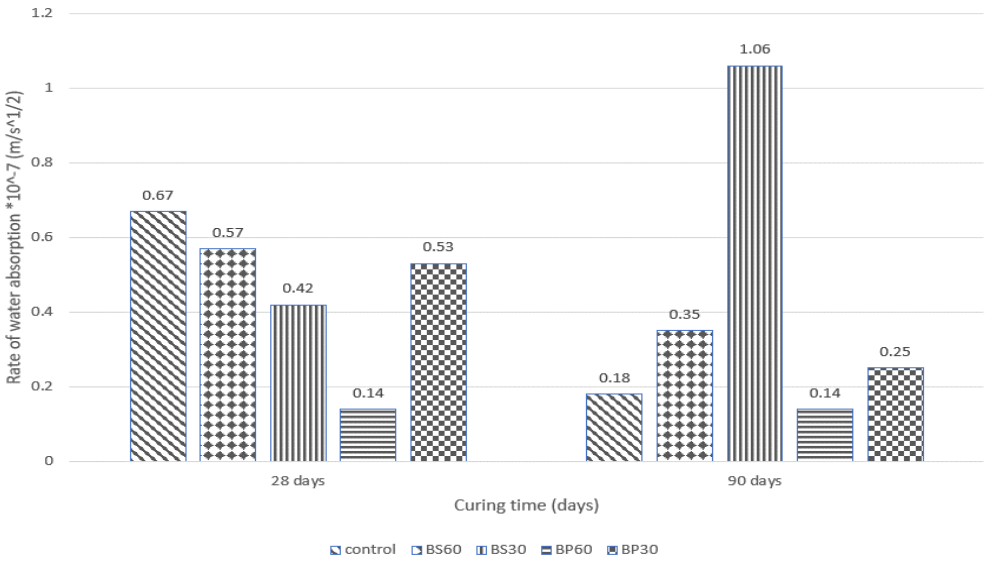

Figure 21. Rate of Water Absorption (I) for Bacterial and Control Mortar Specimens after immersion in sodium sulfate.

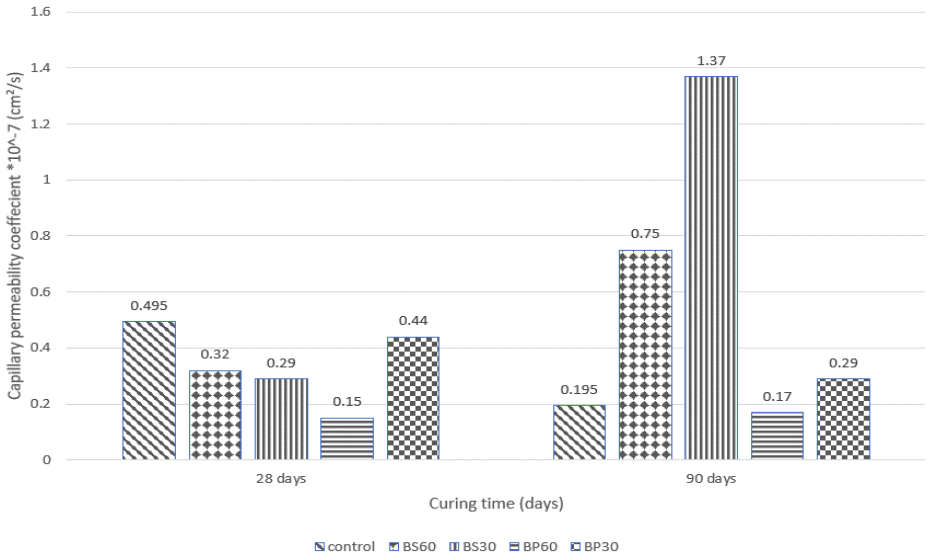

Figure 22. Capillary permeability coefficient (K) for Bacterial and Control Mortar Specimens after immersion in sodium sulfate.

3.3.3.2 Acid Resistance Test

Results of compressive strength test revealed that there was an improvement with time in the strength for the bacterial mortar when compared to the control mortar as illustrated in Figure 23. At the age of 28 days all bacterial mortar decreased except BS60 was 124.92 % compared to compressive strength of control mortar. Also, at the age of 90 days, compressive strength for all bacterial mortar decreased except BS60 was 121.24 % compared to compressive strength of control mortar.

At the age of 180 days the compressive strength value of BS60, BS30, BP60 and BP30 were 121.46 %, 114.8 %, 109.65 and 121.37 % compared to compressive strength of control mortar, respectively.

This means that the compressive strength for bacterial mortar specimens decreases at the early ages (28 and 90 days) in the case of exposure to acids except BS60, but there are improvements in compressive strength with time for all bacterial mortar specimens compared to compressive strength of control mortar. Also compressive strength value of control and all bacterial mortar decreased with time during exposure to salts compared to them initial statues after immersion in sulphuric acid 98 %.

Comparison between exposed and non-exposed in compressive strength were taken at the age of 28 and 90 days.

At the age of 28 days, compressive strength value of Control, BS60, BS30, BP60 and BP30 were 114.71 %, 141.94 %, 105 %, 57 % and 94.50 %, respectively compared to exposed and its non-exposed at the age of 28 days as illustrated in Figure 24. At the age of 90 days, compressive strength value of Control, BS60, BS30, BP60 and BP30 were 101.51 %, 117.6 %, 97.67 %, 78.55 % and 100.23 %, respectively compared to exposed and its non-exposed as illustrated in Figure 25. This showed that BS60 had maximum increment in compressive strength after immersion in sulphuric acid 98 % compared to its non-exposed specimens at the age of 28 and 90 days.

On the other side, results showed that the effect of bacteria on the water absorption of mortar after 2 hours was investigated. At the age of 28 days, the rate of water absorption for all bacterial mortar specimens decreased compared to control mortar. At the age of 90 days, the rate of water absorption increased for all bacterial mortar specimens except for BP60 equaled the rate of water absorption for control mortar as illustrated in Figure 26. This means that BP60 had the ability to resist acids attacking.

Figure 27 shows capillary permeability coefficient for control and bacterial mortar specimens after immersion in sulphuric acid 98%. At the age of 28 days, the capillary permeability coefficient for all bacterial mortar specimens decreased compared to control mortar. At the age of 90 days, the capillary permeability coefficient increased for all bacterial mortar specimens except for BP60 decreased than of the capillary permeability coefficient for control mortar and BP30 equaled the capillary permeability coefficient for control mortar. This means that BP60 and BP30 had the ability to resist acids attacking.

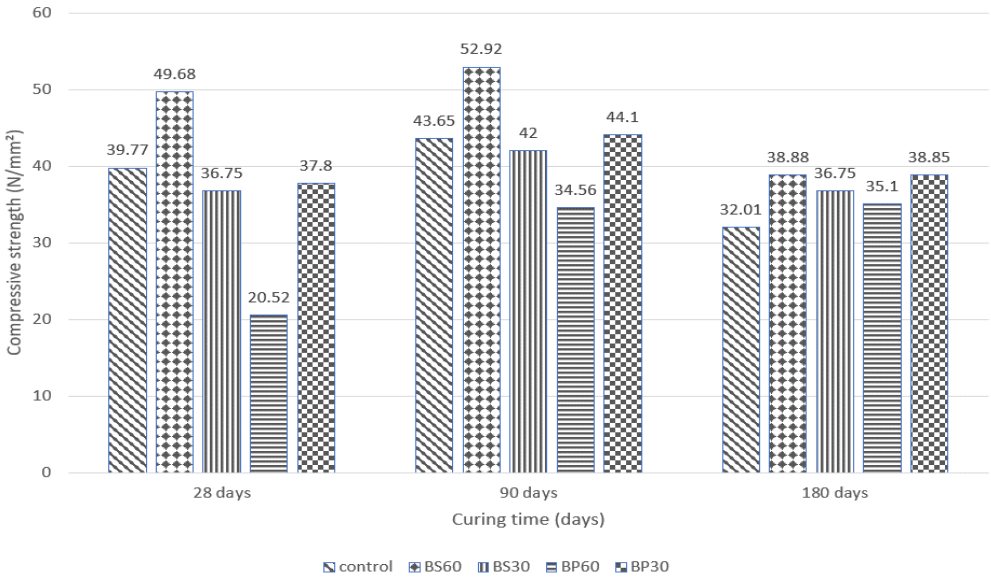

Figure 23. Compressive Strength for Bacterial and Control Mortar Specimens after immersion in sulphuric acid 98 %.

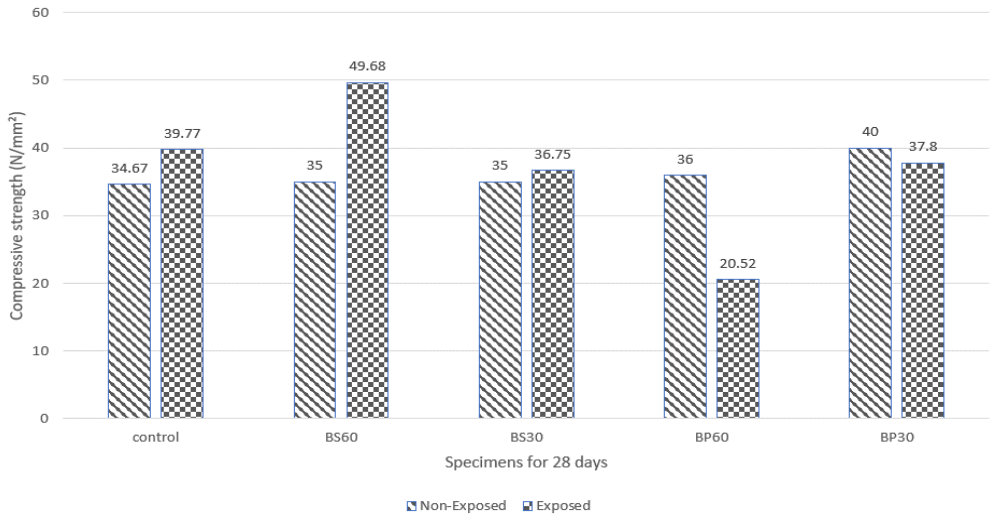

Figure 24. Compressive Strength for exposed and non-exposed specimens after immersion in sulphuric acid 98 % for (28 days).

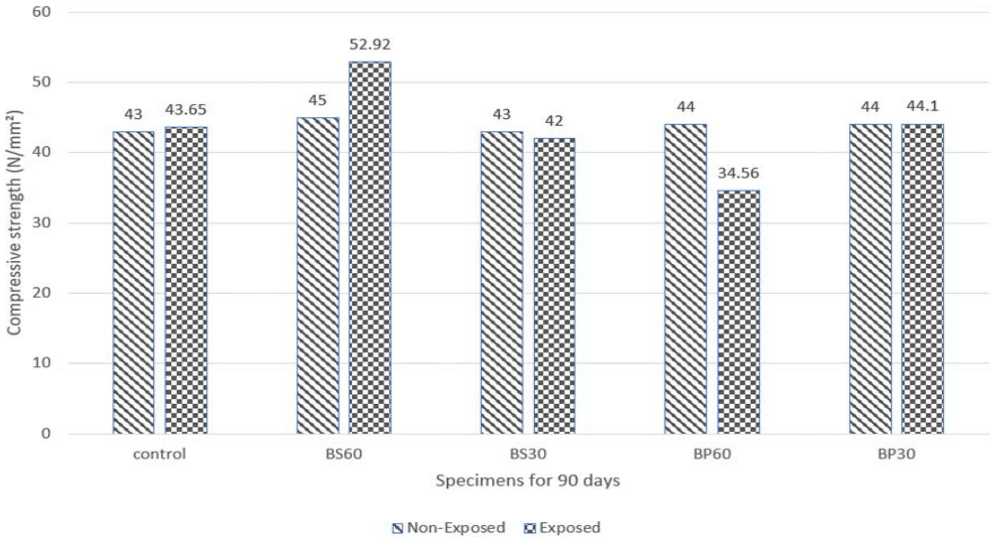

Figure 25. Compressive strength for exposed and non-exposed specimens after immersion in sulphuric acid 98 % for (90 days).

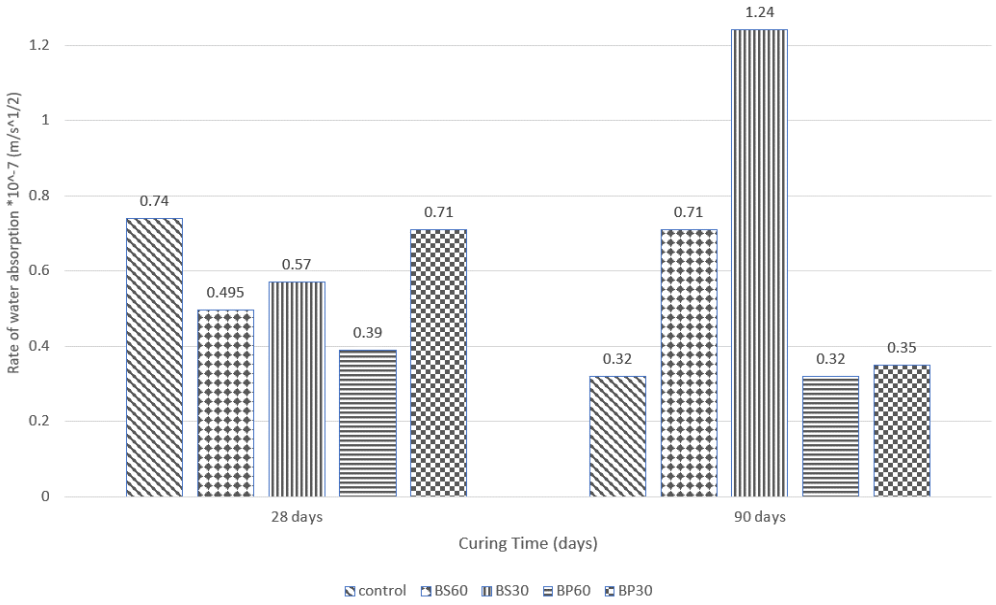

Figure 26. Rate of Water Absorption (I) for Bacterial and Control Mortar Specimens after immersion in sulphuric acid 98 %.

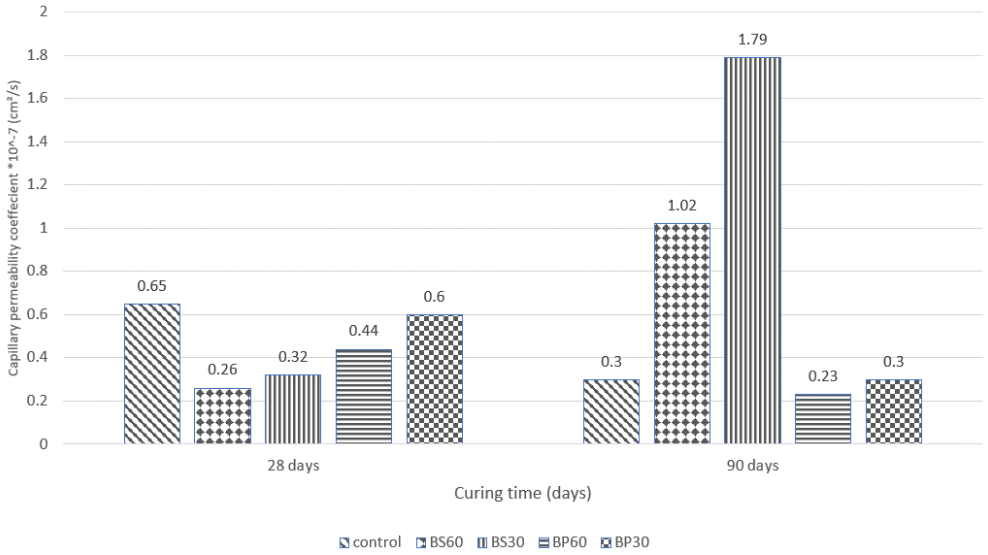

Figure 27. Capillary Permeability Coefficient (K) for Bacterial and Control Mortar Specimens after immersion in sulphuric acid 98 %.

3.3.4 Advanced Techniques of Self-Healing Investigation

3.3.4.1 Scanning Electron Microscopy (SEM)

The SEM micrographs were carried out after 120 days of curing with the magnification of 150, 500, 1500, 5000 and 7500. Figures 28–32 showed SEM pictures for Control (no additions of bacteria) and Bacterial mortar specimens, it is showed that calcite crystals are precipitated by bacterial cells, leading to fill pores. This indicates that the bacterial cells act as nucleating sites for precipitation of calcium carbonate.

The three mortar specimens were compared using SEM pictures at the same magnifying.

Figure 28 at magnifying X150 showed control mortar specimens had many voids compared with BS50, BS25, BP50 and BP25 mortar specimens. Figure 29–32 at magnifying X500, X1500, X5000 and X7500 showed that there are depositions of calcite within voids of bacterial mortar specimens. Calcite crystals are precipitated by bacterial cells leading to fill pores and making good bonds within bacterial mortar specimens. Calcium carbonate precipitation was clear and their shape is random similar to the figures and images shown in previous works [9]. The figures below show bacterial depositions and crystal formations identical to that previously found in literature concerning bacterial mortar [5], [17].

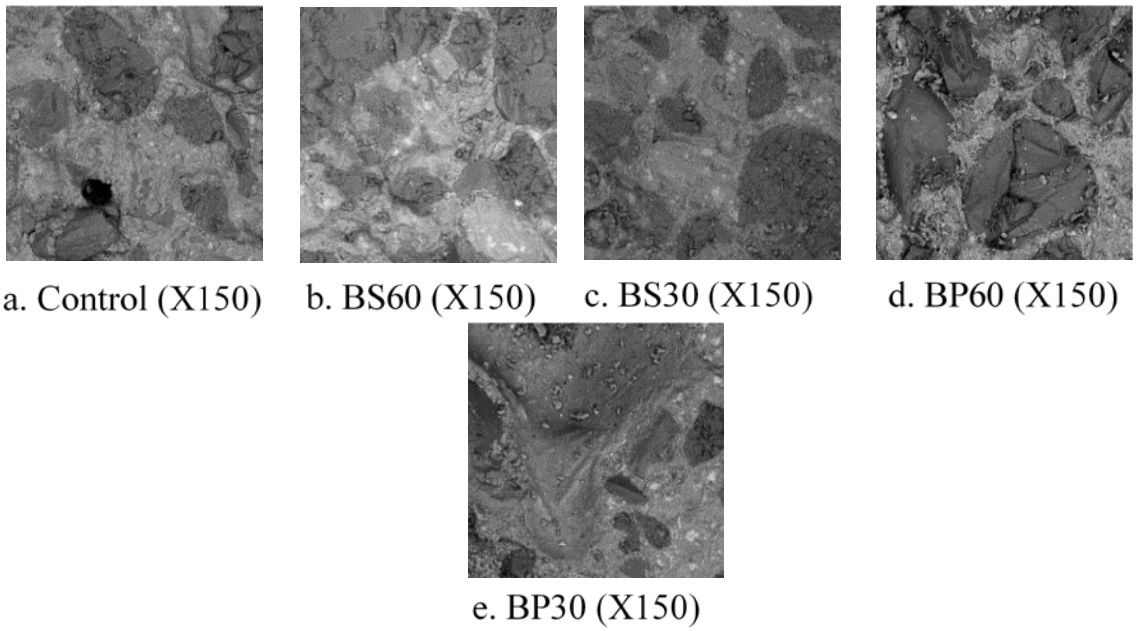

Figure 28. SEM Photographs of Control and Bacterial Mortar Samples (B.Sphaericus and B.Pasteurii) (150X) after 120 days of Curing.

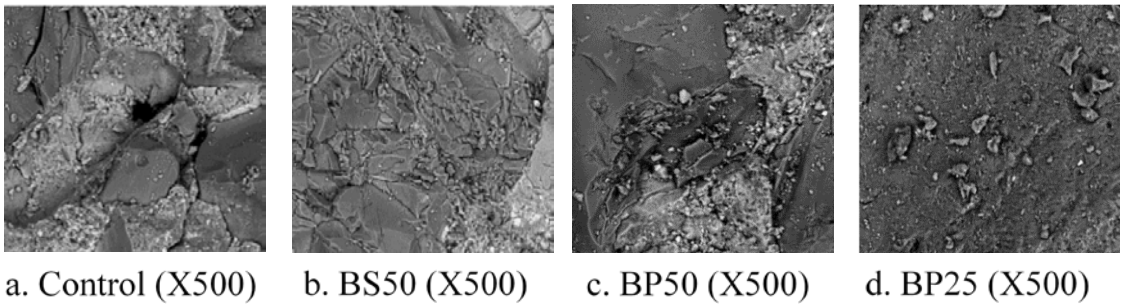

Figure 29. SEM Photographs of Control and Bacterial Mortar Samples (B.Sphaericus and B.Pasteurii) (500X) after 120 days of Curing.

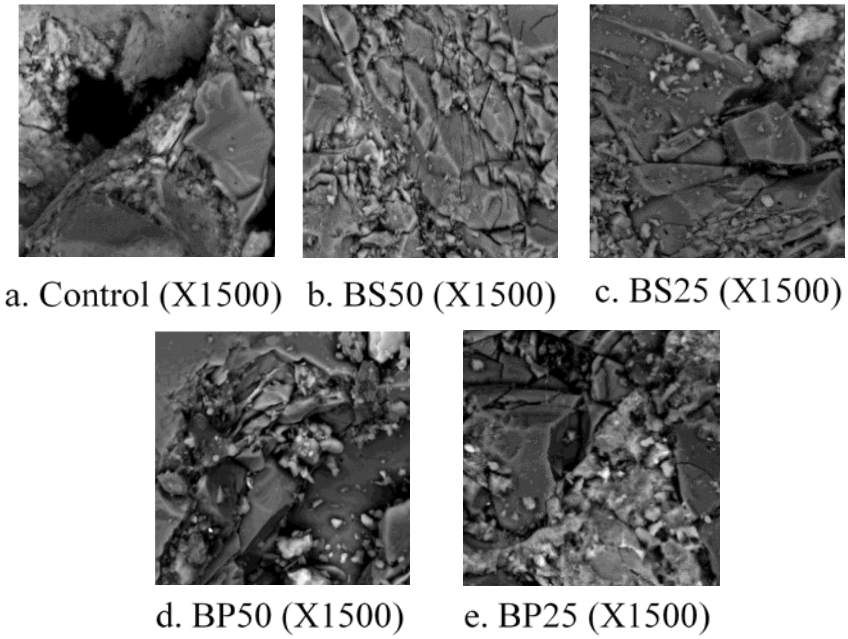

Figure 30. SEM Photographs of Control and Bacterial Mortar Samples (B.Sphaericus and B.Pasteurii) (1500X) after 120 days of Curing.

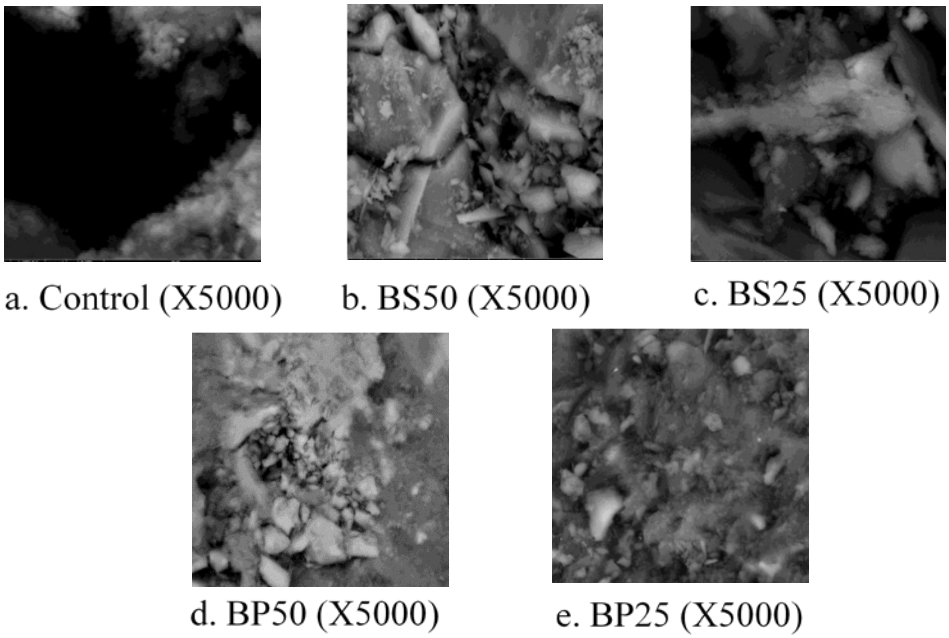

Figure 31. SEM Photographs of Control and Bacterial Mortar Samples (B.Sphaericus and B.Pasteurii) (5000X) after 120 days of Curing.

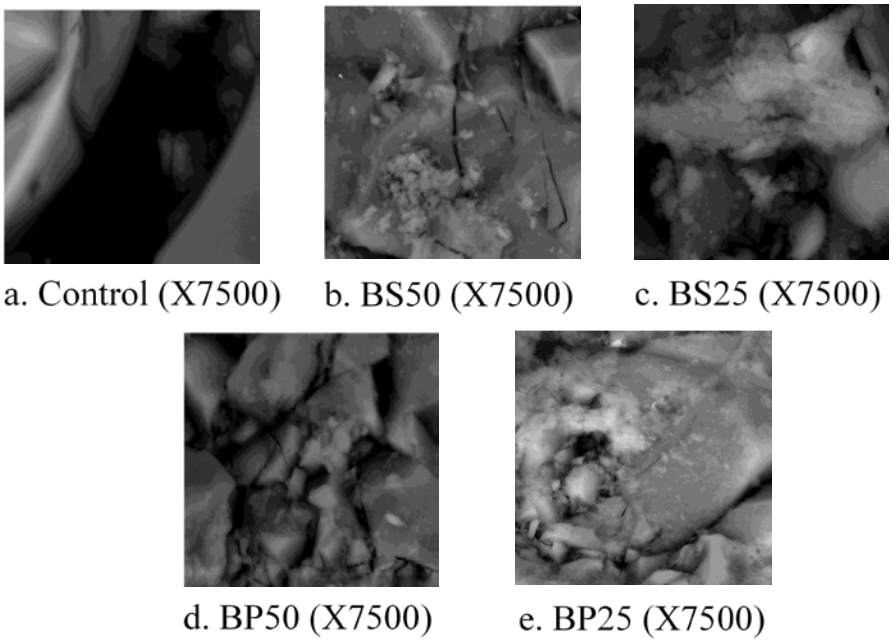

Figure 32. SEM Photographs of Control and Bacterial Mortar Samples (B.Sphaericus and B.Pasteurii) (7500X) after 120 days of Curing.

4. Conclusions

Several conclusions could be derived from the results obtained in this investigation as follows:

1. No significant variation between the two bacterial species (Bacillus sphaericus, Bacillus pasteurii) in cell count during the time course which indicate that both species can adapt in the same rate with the new environmental conditions inside the concrete.

2. The bacteria nutrition acts as an accelerator for cement pastes for initial setting time for all bacterial mortar compared to control mortar, while acts as a retarder of cement pastes for final setting time for all bacterial mortar. Initial and final setting for all mortar were within limit according to ASTM specifications.

3. The rate of water absorption of all bacterial specimens after 2 hours have smaller gain of water absorption than that of control mixture and became semi-impermeable for BS60 and BP60 after 120 days which aligns with previous results that showed a decrease in water absorption and reduced chloride migration.

4. Significant activity of bacterial mortar, biochemically induced calcium carbonate precipitation is responsible for filling up the pores in mortar which in turn decreases rate of water absorption of bacterial mortar and decreases capillary permeability coefficient as shown in previous literature.

5. Compressive strength for all bacterial mortar increased compared to the control specimens compressive strength. Compressive strength of BS60 at 120 days age increased by 124.7 % compared to the control specimens.

6. Bacillus sphaericus, Bacillus pasteurii with the ratio (0.6 % and 0.3 %) showed high restoration of compressive strength when loaded with 60 % of ultimate load of 7 days at the age of 14 days.

7. Compressive strength for reloaded samples after 28days from curing improved by 108.83 %, 121.58 %, 115.49 % and 105.2 % from compressive strength of original samples for BS60, BS30, BP60 and BP30, respectively.

8. Also Bacillus Sphaericus, Bacillus Pasteurii with the ratio (0.30 %) showed high restoration of compressive strength when loaded with 60 % of ultimate load of 7 days at the age of 14days. Compressive strength for reloaded samples after 90days from curing improved by 103.54 % and 111.17 % from compressive strength of original samples for BS30 and BP30, respectively.

9. Flexural strength value of BS30 at the age of 120 days increased by 167.66 % compared to flexural strength of control mortar.

10. Increase in compressive strength and flexural strength are mainly due to the closing of pores inside of the cement mortar due to the bacteria disposing calcite precipitation.

11. Results showed that BP60 had a high ability to resist salts and acids.

12. The SEM micrograph of bacterial mortar shows less voids compared to control mortar. The bacterial cells precipitate calcium carbonate.

Therefore, the calcium-producing microbes are responsible for filling the pores in the cement mortar, thus reducing the rate of water absorption and the permeability of the capillary, while the compressive strength and flexural strength of the bacterial cement mortar have increased, as well as the ability of some types of bacterial cement mortar to resist salts and acids. Thus, improving the properties of cement mortar containing bacteria and calcium lactate as a nutrient for bacteria.

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Contacts:

Vera Galishnikova, GalishnikovaVV@mgsu.ru
Sherif Elroba, ERU.SHERIF@yahoo.com
Mohamed Nassar, nasefmohamedo@gmail.com
Ahmed Sakna, ahmadsakna96@gmail.com